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The Committee on Problems of Drug Dependence, Inc.

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Problems of Drug Dependence 1990:

Proceeding of the 52nd Annual Scientific Meeting The Committee on Problems of Drug Dependence, Inc.

Editors: Louis Harris, Ph.D.

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Foreword

     Once again NIDA is proud to publish the scientific
presentations from the annual meeting of the Committee on
Problems of Drug Dependence (CPDD). These papers and posters
were presented at the 52nd such meeting of CPDD held in Richmond,
Virginia in June 1990. The Committee is to be commended for
organizing an outstanding program and bringing together a group
of premier scientists to report on what is truly state-of-the-
field research from the broad array of scientific disciplines
relevant to the study of drug abuse. Indeed, there are few areas
of scientific endeavor which cover such a broad spectrum of
research focus - from the molecular level to behavioral to the
social. Nor do many areas impact our lives so profoundly.   No
one can deny that our efforts, through research, to find
effective drug abuse prevention and treatment strategies and to
understand the nature of addiction is critical to our society's
health.

     I am especially enthusiastic about the roles of NIDA and
CPDD as we enter the Decade of the Brain. The study of illicit
drugs and their effects on brain and behavior have and will
continue to provide important insights into our understanding of
the most basic features of biology. The study of addiction has
taught us about endogenous opiates, led to the discovery and
characterization of a variety of brain receptors, and recently
resulted in the first cultured cortical neurons. These
developments make me optimistic that continued research on drug
addiction will bring an explosion of new knowledge about the
brain - knowledge that we need to understand drug addiction where
it begins, at the interface of brain and behavior.

                    Charles R. Schuster, Ph.D.
                           Director
                National Institute on Drug Abuse

ACKNOWLEDGMENT

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TABLE OF CONTENTS

POSTER SESSION I

POSTER SESSION II

ORAL COMMUNICATIONS II

ABUSE POTENTIAL EVALUATION

ORAL COMMUNICATIONS III

EPIDEMIC AND PUBLIC POLICY

ORAL COMMUNICATIONS IV

HIV INFECTION AND AIDS

ORAL COMMUNICATIONS V

TREATMENT OF COCAINE ABUSE AND DEPENDENCE

ORAL COMMUNICATIONS VI

OPIOID PHARMACOLOGY I

ORAL COMMUNICATIONS X

CLINICAL PHARMACOLOGY OF ABUSED DRUGS

ORAL COMMUNICATIONS XI

TREATMENT OF OPIOID DEPENDENCE

ORAL COMMUNICATIONS XII

DIAGNOSIS, ASSESSMENT AND SYMPTOMATOLOGY

ORAL COMMUNICATIONS XIII

COCAINE BEHAVIORAL PHARMACOLOGY

ANNUAL REPORTS

Problems of Drug Dependence, 1990

Thomas Burks

I welcome all of you to Richmond, Virginia, for the 52nd annual scientific meeting of the Committee on Problems of Drug Dependence. We look forward to four days of important and stimulating research reports in poster sessions, slide presentations, symposia and workshops.

We are especially pleased to hold our 1990 meeting in this charming city and I want to thank the local organizing committee, chaired by the Past-Chairman of CPDD, Dr. William Dewey, and Ms. Debra Mallory, for all of their hard work and thoughtful arrangements for this meeting. Bill and Debbie, and all members of the committee, thanks for having us as your guests in Richmond.

I hope attendees will have a chance to savor the special attractions of Richmond, although leisure time will be limited because this week's meeting is packed with action. Our Program Committee, chaired by Dr. Louis Harris, has put together a most exciting scientific program. One measure of a superb meeting is when you find so many good things happening simultaneously that you cannot decide which session to attend. I believe we have that kind of meeting this week. Members of the Program Committee, thanks for this outstanding scientific program.

We are pleased that associated groups with affinity for CPDD have chosen to meet with us here in Richmond. These groups include ISGIDAR, the International Cannabinoid Study Group, and a number of functions sponsored by the National Institute on Drug Abuse. I welcome these groups to the CPDD meeting.

You probably wonder who puts all of this fairly complex meeting together. It is our Executive Secretary, Dr. Marty Adler, along with Ellen Geller and Marie McCain. During my term as Chairman of CPDD, my already high opinion of Dr. Adler and his associates has risen even higher. It has been a joy to work with them. Travel and other arrangements for this meeting were performed smoothly, as usual, by Brad Meyer and his associates at Sailair Travel of Nashville, TN.

CPDD has had a busy year. After our very successful annual meeting in Keystone, Colorado, last June, the Board of Directors took up the challenge of working out structural changes to transform CPDD to a membership organization. We have made significant progress and have agreed in principle on a plan for providing membership privileges to qualified persons with interests in aspects of chemical dependence research. We are now addressing specific changes in the Bylaws to meet these objectives and provide orderly transition to a membership structure. We hope to complete these changes in the coming year, under the leadership of Dr. Lou Harris, who will become Chairman of the CPDD.

We have also been active in testifying on behalf of ADAMHA and NIH budgets before Congress, helping address strategies for Congressional funding of research directed at problems of drug and alcohol abuse, and analysis of proposed NIH, ADAMHA and U.S. Department of Agriculture proposed rules and guidelines concerning conflicts of interest, scientific misconduct and animal care and use regulations.

CPDD sponsored, along with ASPET, a public forum on methamphetamine in Washington this past April. The scientific program was well arranged by Dr. Scott Lukas. Joe Brady, Marilyn Waranch and Marty Adler handled media relations and logistics of the conference for CPDD. We received good media coverage and have reason to believe that we reached significant portions of the medical community and some portion of the lay public concerning demystification of methamphetamine, including its use in the form of "ice."

A number of present and previous members of the CPDD Board of Directors have been in the news in the past year for specific scientific accomplishments and for taking on important positions in government agencies. We are proud of those achievements and feel that it reflects positively on the overall calibre of our Board members.

We publish a newsletter, "Newsline," that serves as a medium of communication among those interested in drug dependence research. We regard "Newsline" as an effective vehicle for exchange of information relevant to our scientific and professional activities.

Our major publication each year is the NIDA Research Monograph containing the Proceedings of each CPDD scientific meeting. The 1989 Proceedings, "Problems of Drug Dependence 1989," contains an up-to-date collection of reports and position statements of top government officials in the area of substance abuse, Leo Hollister's Eddy Award Lecture, reports from the CPDD Drug Evaluation Program, and many important research reports from laboratories in the U.S.A. and abroad. It is pleasing, in fact, that we have persons from Europe, Asia and Australia attending and presenting data at our annual meetings. Problems of drug dependence are international in scope. I observe with pride the fact that each annual NIDA Monograph on Problems of Drug Dependence is a little thicker with research reports than that of the previous year. This 1990 meeting in Richmond is our largest ever and I expect the resulting Proceedings will be larger still.

CPDD continues to pursue its goal, enunciated in 1929, of ridding the world of illicit drug use by decreasing demand through research. We are addressing this goal by means of several specific objectives:

1.   We will hold excellent scientific meetings, such as this annual meeting, and special meetings, such as the methamphetamine conference I mentioned.

2.   We will continue our drug testing program, a unique activity of CPDD for evaluation of abuse liability of drugs.

3.   We will continue to serve in a consultant capacity to government agencies, providing scientific information of importance in the formulation of public policy relative to substance abuse and chemical dependence.

Everyone who participates in any of these activities, including researchers who share their findings at this scientific meeting, epidemiologists and sociologists who tell us about patterns of drug use, and persons responsible for treatment programs that translate research to practice, all contribute to the goals of CPDD.

Those that serve on the CPDD Board of Directors make a very special contribution. As we move to a membership structure, we will increase the number of persons directly involved in carrying out the objectives of the organization.

I invite everyone present to attend the 1991 annual meeting of CPDD at the Breakers, near West Palm Beach in Florida, June 15-21, 1991. Plans for that meeting are already progressing well; it will be terrific.

We are privileged to have with us this week some key government officials in drug and alcohol policy and research. These include Dr. Herb Kleber, Deputy Director of Demand Reduction, White House; Dr. Fred Goodwin, Administrator of ADAMHA; Dr. Beny Primm and Dr. Loretta Finnegan, Office of Treatment Improvement; Dr. Enoch Gordis, Director of the National Institute on Alcohol Abuse and Alcoholism; and of course Dr. Bob Schuster, Director of the National Institute on Drug Abuse and a number of scientists from NIDA.

Three persons have been selected for special recognition at this meeting for their contributions to CPDD and drug abuse research: Dr. Charles R. Schuster will receive the Nathan B. Eddy Memorial Award, Dr. Arthur E. Jacobson will receive the J. Michael Morrison Award, and Dr. Thomas R. Kosten will receive the Joseph Cochin Award.

The first CPDD Media Recognition Award will be presented to Kathie McCabe for her article in the February, 1990, Washingtonian, entitled "Beyond Cruelty," an insightful description of the philosophy and state of the animal activist movement in the U.S.

We watched last year with great interest the nomination and confirmation process by which present and former members of the CPDD Board of Directors became directly involved in overall substance abuse policy for our entire government. Dr. Herbert Kleber, now a White House official, is responsible for Demand Reduction. Dr. Beny Primm, also a member of the CPDD Board of Directors, has been appointed Associate Administrator of ADAMHA and Director of the Office of Treatment Improvement of ADAMHA. Another member of the CPDD Board of Directors. Dr. Loretta Finnegan, is now Associate Director of the Office of Treatment Improvement.

Finally, we want to recognize the outstanding contributions of Drs. William Dewey, Beny Primm and Edward Senay to the structure and function of CPDD during their terms as members of the Board of Directors. We are indebted to these fine scientists for all they have done to promote the objectives of CPDD.

The CPDD is healthy and growing as an organization. Its scientific underpinnings are strong. We have crucially important problems to solve, and the scientific talent to have an impact. We are making progress.

Thanks for contributing to the most exciting and largest meeting ever in the history of CPDD.

Thomas F. Burks, Ph.D.
Department of Pharmacology
University of Arizona
College of Medicine
Tucson, AZ 85724

Introduction of the Nathan B. Eddy Memorial Award

Louis S. Harris

It gives me great personal pleasure to introduce Dr. Charles Roberts Schuster, this year's recipient of the Nathan B. Eddy Award. Dr. Schuster was born in Woodbury, New Jersey and attended the public school system in Camden. He received his Bachelor of Arts degree from Gettysburg College, and a Master of Science degree from the University of New Mexico. After a brief teaching stint at Temple University, he was employed as a Junior Scientist at the Smith, Kline and French Laboratories. He left Smith, Kline and French to return to graduate school at the University of Maryland, where he received his Ph.D. in 1962. During this period, he also held teaching positions at the University rising to rank of Assistant Professor. In 1963, he began his academic association with the University of Michigan where he had appointments both in Pharmacology and Psychology, rising to the rank of Associate Professor. In 1968, he accepted a position as Associate Professor of Psychiatry at the University of Chicago with a joint appointment in Pharmacology. He is currently Director of the National Institute on Drug Abuse on leave of absence from his position at Chicago as Professor of Psychiatry, Pharmacological and Physiological Sciences and Behavioral Sciences.

It is difficult to summarize Dr. Schuster's research accomplishments since they are so extensive. They may, however, be characterized by their breadth and elegance of design and execution. For instance, Dr. Schuster pioneered in the development of the techniques of drug self-administration, drug discrimination, neurotoxicology and the study of psychoactive drugs in man. His broad contributions to the field of drug abuse research, including the training of a host of students many of whom are present, alone makes him uniquely qualified for the Eddy Award. He has, however, made many other contributions to the field. These include his service to professional societies and many national and international boards and committees.

For instance, he served as President of Section 28 of the American Psychological Association, the Behavioral Pharmacology Society and ISGIDAR, which he also helped to found. Also of great note is his service on national and international regulatory committees. He should be especially commended for his role on the World Health Organization's Expert Committee on Drug Dependence. His efforts in this regard have aided immeasurably the successful international implementation of the Psychotropic Convention of 1971. Finally, since 1978, Dr. Schuster has maintained a close relationship to the Committee on Problems of Drug Dependence.

Since assuming his position as Director of the National Institute on Drug Abuse in 1986, Dr. Schuster has been at the center of the national and international maelstrom created by the enormous public health problem of drug abuse.

The huge impact of AIDS and "crack" have emerged during his tenure. The public, and the scientific community in particular, owe him a great debt for steering the Institute through rough political and practical seas while preserving the basic research mission of NIDA.

I would be amiss if I did not mention Bob's lifetime interest in music and the arts, both as a performer and collector. This, added to his intense interest in people and his quiet modesty, make him one of the few individuals of our day who can be characterized as a "Renaissance Man". His career may be summed up by the following citation, "Charles R. Schuster", brilliant behavioral scientist, astute administrator, and distinguished public servant, your work has greatly benefitted mankind".

The Committee on Problems of Drug Dependence is honored to name you the 1990 recipient of the Nathan B. Eddy Award.

AFFILIATION:

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613

Nathan B. Eddy Memorial Award Lecture

Charles R. Schuster

I am deeply honored to be the recipient of the Nathan B. Eddy Award and extremely pleased that my family and so many of my friends can be here to share this joyous occasion with me. There are so many people to whom I am personally and intellectually indebted that I hesitate to give names in the short space of time that I have for delivering this speech. I must take this opportunity, however, to thank my mother and sister for their guidance and teachings--both have contributed mightily to whatever I have achieved. To my wife and scientific colleague, I can honestly say that I could not have achieved what I have if it had not been for her nor would it have been nearly so much fun.

I am very proud to be the first behavioral pharmacologist to receive the Nathan B. Eddy Award. In that regard, I am deeply indebted to my intellectual mentor and close personal friend, Dr. Joseph V. Brady. He remains a source of amazement and a role model for me as he continues through the years to broaden the scope of his research activities but always with a steadfast commitment to the principles of science.

And finally, I would like to thank the dozen or more graduate students and post-docs that I have worked with over the past 30 years--we have learned a lot together.

I have been very fortunate to have had the opportunity to engage in a wide range of scientific and clinical activities related to the area of drug abuse. My research has ranged from studies of the molecular mechanisms underlying methamphetamine's toxic actions on dopamine and serotonergic neurons to establishing and investigating the efficacy of Pflash Tyre--a teenage intervention center for kids with "blown out minds" from their drug use. Today, however, I am going to emphasize the major theme of my career, which has been the development of laboratory procedures for studying biological, pharmacological, and environmental variables controlling drug-seeking behavior of animals and humans.

Let me begin with a bit of personal history. Because of my early exposure as a young jazz musician to individuals who were addicted to drugs, I became interested, while a graduate student, in determining whether it was possible to develop an animal model of drug dependence. At that time, them had been a number of reported studies using drugs as either conditioned or unconditioned stimuli in Pavlovian conditioning paradigms (e.g., Bykov, 1957). There were,

however, relatively few studies in which drugs had been studied as either discriminative stimuli (Conger, 1951) or as reinforcing stimuli (Spragg, 1940, Headley, Coppock. Nichols, 1955) in operant conditioning paradigms. My thinking, however, was influenced by the psychological theories of that time which postulated that addicts self-administered drugs as a means of resolving some form of neurotic conflict, a self-destructive urge, or as an expression of a psychopathic personality incapable of loftier goals than simple sensual pleasure. There were also other reasons for discouragement with animal models of drug dependence. Leading experts in the field stated unequivocally that drug addiction was a peculiarly human phenomena that was dependent upon verbal and cognitive abilities which exceeded the capacity of non-human organisms (Lindesmith, 1965). In the few studies in which the reinforcing effects of morphine had been studied in animals (Spragg, 1940, Headley et al., 1955), animals were first made physically dependent upon morphine. This approach was based upon the supposition that animals would self-administer a drug only to escape from the physical distress of withdrawal. In his study of morphine dependency in chimpanzees, Spragg (1940) found that the animals chose a subcutaneous injection of the drug over a banana only if they were in withdrawal. If they had recently received an injection of morphine, they would choose fruit. Following detoxification, the chimpanzees showed no interest in obtaining an injection of morphine, thus leading to the conclusion that the reinforcing properties of morphine were dependent upon the animals being physically dependent. Clearly, this differentiated animals from humans, where physical dependence develops as a consequence of people self-administering drugs for their reinforcing effects. Although opiate addicts may claim iatrogenic opiate dependence as the basis of their problem, available data indicate that patients who receive opiates for pain relief and are physically dependent readily stop taking opiates when the pain is no longer present (Schuster, 1989).

Despite authoritative nay-sayers (e.g., Lindesmith) and the results by Spragg (1940), I was convinced that a clever, then young, behavioral pharmacologist, could find ways to get a rhesus monkey to voluntarily introduce a drug into its body. From the principles of behavior analysis, I knew that to maximize the probability that an event would serve as a reinforcer, it had to be presented with minimum temporal delay following the behavior to be strengthened. In practical terms, this ruled out oral administration of drugs and suggested that direct administration of the drug into the venous system would be ideal. Fortunately, my advisor, Dr. Joe Brady, was working at the Walter Reed Army Institute of Research with others who were studying endocrine changes in rhesus monkeys under various environmental conditions. In order to conduct these studies, they needed to be able to unobtrusively obtain blood samples from the monkeys for hormonal analysis. They had developed a surgical procedure for implanting a catheter into the jugular vein of the rhesus monkey which ran underneath the skin to a connector which was attached to the skull of the animal. From there it was possible to run tubing to an automatic withdrawal pump from which blood samples could be obtained. When I saw this experimental preparation, I realized that if these investigators could obtain blood from the system, I could administer drugs intravenously through this cannula system, and further, make the drug delivery contingent upon a learned response by the monkey.

In the initial experiments I conducted, I thought that it would be necessary for the animals to initiate their drug-seeking behavior in order to obtain other reinforcers and that only after the animals had experience with the drug would its reinforcing properties emerge. One of the first experiments conducted

investigated whether animals who were deprived of water could be trained to self-administer saline through the indwelling jugular catheter system I was joined in this endeavor by Dr. Robert Clark and Dr. Joseph V. Brady. We were able to demonstrate (Clark, et al., 1961) that rhesus monkeys could be conditioned to lever-press for intravenous saline under conditions of water deprivation. The robustness of saline as a reinforcer under the conditions of this experiment was, however, extremely low. For example, only very short fixed ratios could be maintained This was, of course., probably attributable to our use of saline, which we did not realize at the time was a poor source of body water. I therefore abandoned this procedure, which I had hoped would be the first stage in getting animals to introduce drugs into their bodies.

From behavior analysis research, I had learned that events which served as discriminative stimuli often acquired conditioned reinforcing properties. Thus, for example, a red light which served as a discriminative stimulus setting the occasion for a lever press to be reinforced with a presentation of food could acquire conditioned reinforcing properties capable of generating new behavior. It thus occurred to me that it would be possible to establish a drug as a conditioned reinforcer by first having it serve as a discriminative stimulus. We had relatively little guidance in those "pre-Don Overton days," and were concerned by the fact that the duration of action of most drugs would allow only one occasion per day in which the drug could be presented as a discriminative stimulus. In order to avoid this problem, we decided to determine whether epinephrine--a substance which is rapidly degraded, hence allowing a number of stimulus presentations each day--could serve as a discriminative stimulus (Schuster and Brady, 1964). In this experiment we used rhesus monkeys that had been prepared with chronic jugular catheters, and conditioned them to respond on a lever for a food reinforcer following an injection of epinephrine but not following an injection of saline. Over the course of 90 or so sessions, animals learned this discrimination as shown by the fact that in the 60 second period following the infusion of epinephrine, the probability of responding was almost 100 percent, whereas following the infusion of saline, the probability of a lever press occurring was close to zero.

Clearly, we had established that epinephrine was serving as a discriminative stimulus. We were encouraged that it might be possible to use this type of procedure for establishing a drug not only as a discriminative stimulus but also a conditioned reinforcer. We were discouraged about the use of this procedure, however, because of the fact that most drugs of abuse had very long durations of action and, therefore, unlike epinephrine, could only be presented once in a daily session as opposed to multiple occasions. We therefore felt it would take an inordinately long period of time to train animals to discriminate the effects of a drug and an even longer period of time to demonstrate that, once established as a discriminative stimulus, the drug had acquired reinforcing properties.

At about this time--1961--I was joined by Dr. Travis Thompson, and we decided to use the already established procedure of making animals physically dependent on morphine prior to attempting to use it as a reinforcer (Thompson and Schuster, 1964). Under these conditions, we had no difficulty in getting physically dependent monkeys to emit a lever pressing response that was followed immediately by an injection of morphine through the chronic jugular catheter delivery system. Although we used this experimental paradigm to study several behavioral and pharmacological variables (which I will report upon in a later section of this paper), again by the very nature of the design of the

experiment, we perpetuated the idea that physical dependence was a necessary antecedent condition for morphine to act as a reinforcer in non-human organisms.

In 1962 I left the University of Maryland and joined the Pharmacology Department at the University of Michigan where Drs. Collins, Deneau, Seevers, and Yanagita had independently developed a very similar procedure for studying the reinforcing effects of intravenously administered drugs in rhesus monkeys. In their experiments they had not made rhesus monkeys physically dependent upon morphine prior to making it available as a reinforcer. In most monkeys, they found that animals who were not physically dependent would learn to lever press for injections of morphine (Deneau, Yanagita. and Seevers, 1969). But assumptions were hard for me to give up. The data which the Michigan study collected were ambiguous in that it took approximately 7 days of morphine access before the monkeys response rates exceeded baseline control values for saline infusions. It seemed entirely possible to me that, at the unit dose used, animals were receiving enough morphine to become physically dependent during this time. In subsequent research by Dr. James Woods and myself, we were able to demonstrate the reinforcing properties of extremely low doses of morphine (10 ug/kg/injection) available for only short periods of time each day. These data convinced me that physical dependence was not a necessary antecedent condition for morphine to act as a reinforcer in the rhesus monkey (Woods and Schuster, 1968). Today, Wise and his colleagues have shown that different brain loci mediate the reinforcing and physical dependence producing properties of morphine (Bozarth and Wise, 1984).

Although the earlier studies clearly showed that physical dependence was not a necessary antecedent condition for morphine to act as a reinforcer, physical dependence can influence drug seeking behavior. For example, Travis Thompson and I showed (Thompson and Schuster, 1964) that in physically dependent rhesus monkeys, the number of lever presses made under a fixed interval schedule of reinforcement maintained by morphine was markedly increased as a function of the number of hours of morphine deprivation. It would thus appear that, although physical dependence is not a necessary antecedent condition for morphine to serve as a reinforcer, once established, the reinforcing strength of morphine is increased by physical dependence.

I think it is important to note that we cannot generalize from these findings to other classes of drugs which produce physical dependence. For instance, Winger (1988) found that there was a disassociation between the rate of responding for intravenous ethanol and the intensity of withdrawal signs. In this study, rhesus monkeys were allowed to self-administer ethanol through chronic venous catheters daily for 3 hours. After intake of ethanol had stabilized, physical dependence was produced by daily 8 hour infusions of additional alcohol. After 1 week of infusions, the monkeys began to show withdrawal signs prior to the 3-hour daily ethanol self-administration sessions. However, responding for ethanol in the sessions was inversely related to the intensity of the withdrawal signs. This experiment suggests that at least under certain conditions the reinforcing efficacy of ethanol is not enhanced by physical dependence, as was the case with morphine.

Animal Models of Drug Abuse

Following the rather unexpected findings that rhesus monkeys would self-administer morphine without any special conditions, psychologists, behavioral pharmacologists, and neurochemists began marching through the pharmacopeia to determine which drugs would and which drugs would not serve as reinforcers in animals. Rats, dogs, baboons, and squirrel monkeys all were shown to have a propensity to self-administer certain drugs. Suffice it to say that from the late 1960s to the present, several hundred drugs have been investigated to determine whether or not they serve as reinforcers in standard animal models of drug self-administration. As has been amply reviewed elsewhere, by and large, those drugs which serve as positive reinforcers in such animal studies are also drugs which are commonly abused by humans (Johanson and Balster, 1978; Young and Herling, 1986). Drugs which produce unpleasant subjective effects in humans such as the phenothiazines, have been demonstrated to serve as negative reinforcers in animal studies. That is, animals will learn to emit an operant response when that response is followed by the termination or the avoidance of injections of phenothiazines (Kandel and Schuster, 1977). Finally, drugs which appear to be neutral in their effects upon subjective states in humans are neither self-administered nor avoided by animals. It has thus been concluded that, with some notable exceptions (hallucinogenic drugs), there is a striking concordance between the drugs which are self-administered by animals and those which are commonly abused by humans (Johanson, 1990).

In recent years, behavioral pharmacologists have been attempting to determine whether or not there are any common neurochemical actions which serve as the mediators of the reinforcing effects of drugs. The most popular current theory states that drugs of abuse either directly or indirectly activate mesolimbic and mesocortical dopamine pathways in the brain. The evidence for and against this view has been amply summarized elsewhere (Koob and Bloom, 1988) and I will not enter into that debate at this time. I would point out, however, that such theories must reckon with the complex interactions between the environmental contingencies governing drug availability and the behavior which they generate. Despite the appeal which the dopamine hypothesis has as a unifying principle, it must be recognized that it does not account for much of the interesting aspects of abuse and dependence.

One of the most striking features of drug dependence is the perseverative, excessive hustling which the addict engages in to obtain drugs. I would contend, and I believe that there is ample evidence to substantiate this claim, that the excessiveness of drug-seeking behavior seen in addicts is not determined by the inherent reinforcing properties of the drug molecule, but rather the environmental contingencies which govern access to the drug. (For a fuller review of this evidence, see Schuster, 1990.) This is seen most clearly in laboratory experiments where animals are given access to cocaine under the conditions of a fixed ratio one schedule of reinforcement (i.e., drug delivery is contingent upon the animals making one response) in comparison to higher order schedules of reinforcement in which animals may be required to emit thousands of responses to receive a similar injection. Clearly, since the drug is the same in both cases, it is the schedule of reinforcement that is responsible for the excessiveness of the drug-seeking behavior in the second case rather than any inherent properties of the drug.

There are also many enigmatic experimental results which do not fit easily within a simplistic pharmacological explanation of drug dependence. In a very interesting experiment by Spealman (1979), squirrel monkeys were allowed to self-administer cocaine by pressing a lever under a variable interval schedule of reinforcement. Responding on a second lever was maintained under a fixed interval schedule which terminated the availability of cocaine for a brief period. Surprisingly, these two seemingly incompatible contingencies maintained both lever pressing for cocaine reinforcement and as well lever pressing for termination of the availability of cocaine. Clearly, these animals were ambivalent about their use of cocaine! Again, I would be forced to say that these enigmatic results cannot be explained on the basis of the pharmacological effects of cocaine per se, but rather on the basis of the environmental contingencies governing access to cocaine and the animal's ability to terminate access to cocaine. Thus, I would add a note of caution to those who would like to reduce the diverse phenomena associated with the addict's behavior to a simplistic theory of activation of specific pharmacological receptor systems in the brain.

Environmental Factors Influencing Drug Self-Administration

I have spent a great deal of my career studying the manner in which environmental variables control drug-seeking behaviors. I believe such studies should be viewed as mechanistic studies every bit as much as those, for example, that manipulate the levels of brain catecholamines or second messenger systems. I think, however, that the Zeitgeist is such these days that those studies which are influencing brain systems through manipulation of environmental variables are not viewed as on the "cutting-edge of science." It must be remembered that the behavior of the integrated organism and its complex interactions with the environment may not be reducible to explanations at more molecular levels of analysis. At the very least, synthesis of the molecular events taking place in the various brain systems which mediate complex behavioral processes is far in the future. This is why I have chosen to emphasize in my own research career the environmental variables which have been shown to influence drug self-administration.

One cannot address the area of environmental variables in controlling drug-seeking behavior without paying homage to the work of Abraham Wikler. Wikler (1965. 1974) argued very cogently for the central importance of conditioning processes in the various behavioral aspects of opiate addiction. For example, he suggested that in opiate dependent persons, environmental stimuli that accompany withdrawal from opiates may come to elicit withdrawal symptoms. Wikler and Pescor (1967) showed that in rats, after a number of temporal associations of an environmental stimulus and the state of opiate withdrawal, the environmental stimulus alone elicited components of the withdrawal syndrome. To the extent that withdrawal from opiates increases drug-seeking behavior, conditioned withdrawal may be of importance in relapse to drug use. This is, of course, the basis for much of the clinical research being conducted to determine the role of learning factors in the interaction, maintenance, and response to drug use (Ray, 1988).

I would like to briefly review some of the animal research my colleagues and I have carried out in this area. In the first experiment we conducted (Goldberg and Schuster, 1967), monkeys were made physically dependent upon morphine by administering 3 mg/kg of morphine every 6 hours for a period of 30 days. After the 30 days, the injections were continued while the animals were trained

to lever press for a food reinforcer delivered under a variable interval schedule of reinforcement. The variable interval schedule of food reinforcement occurred for a l-hour period each day. After responding maintained by food became stable from day to day, a tone of 10 minutes duration was presented to the animal fifteen minutes after the start of the session. For several sessions, a saline injection was given through a chronic indwelling jugular catheter five minutes after the onset of the tone. After the animals adapted to the tone and saline as indicated by the fact that there was no disruption in their ongoing lever pressing maintained by food, nalorphine injections were substituted for saline. The nalorphine injection rapidly produced withdrawal symptoms including increases in heart rate, marked salivation, tremors, irritability, and an immediate cessation of the animal's food-maintained responding. Following a few pairings of the tone and nalorphine, the tone alone elicited suppression of food responding, a change in heart rate, excessive salivation, and vomiting. We interpreted these results as an indication that the symptoms of the withdrawal syndrome had been classically conditioned. Nalorphine served as the unconditioned stimulus and the tone acquired the ability to produce the withdrawal syndrome, and thus was a conditioned stimulus.

The next experiment we conducted on the conditioning of the withdrawal syndrome was designed to determine whether or not the conditioned withdrawal would increase drug-seeking behavior (Goldberg, Woods, and Schuster, 1969). In this experiment, four rhesus monkeys were allowed to self-inject morphine through chronic indwelling jugular catheters under a fixed ratio one schedule of reinforcement, 24 hours per day. After approximately 4 weeks when the animal's intake of self-administered morphine had stabilized, a red light was illuminated in the animal's experimental chamber each day for a period of 30 minutes. After baseline intake of morphine during this 30-minute period had been established over a period of 7 to 10 days, the red light was illuminated simultaneously with an injection of nalorphine. Following the injections of nalorphine, the animal showed a marked increase in their self-administration of morphine compared to base line values. After a number of pairings of the red light and nalorphine, the presentation of the red light in association with an injection of saline also produced significantly increased levels of morphine self-administration over those observed prior to the association of the red light with nalorphine. With continued daily presentation of the red light and saline, the number of self-administered injections of nalorphine returned to baseline levels. It would thus appear that the opiate withdrawal syndrome, including its ability to increase drug-seeking behavior, can be classically conditioned to environmental stimuli. This evidence lends support to the theories put forth by Abraham Wikler stressing the importance of conditioning processes in controlling drug-seeking behavior.

Conditioning of Opiate Effects

Since aspects of the opiate withdrawal syndrome are conditionable, it seemed probable that stimuli associated with opiate injections might also acquire behavioral significance. There were several experiments which I have been involved in that are relevant to this area One of the first such experiments was conducted by Travis Thompson and myself (Thompson and Schuster, 1964). Those of you who attended the Committee on Problems of Drug Dependence Meetings in 1960, in Ann Arbor, may even remember the data. In this experiment, monkeys were conditioned to respond under a multiple schedule consisting of three components: a food-reinforced component; a chain fixed

interval/fixed ratio morphine-reinforced component; and a l-hour shock-avoidance component. This 3-ply multiple schedule was presented every 6 hours (i.e., four times daily). After the behavior in the three components of the multiple schedule had stabilized, the morphine component was omitted for a period of 48 hours. Since the animals had been self-administering a dose of 12 mg/kg/day of morphine, when deprived they began to show signs of opiate withdrawal after approximately 12 hours. This withdrawal produced a disruption of both the food-maintained and shock-avoidance behaviors. By the second 24-hour period of morphine deprivation, the animals were not responding at all for food, and the latencies for avoiding electric shock were quite variable and markedly longer than under baseline conditions. When the morphine component of the schedule was reinstated and the animals self-administered a dose of 3 mg/kg morphine, food-maintaining responding and electric shock avoidance behaviors returned immediately to baseline values.

Subsequently, this experiment was repeated and the monkeys again showed a marked disruption in their food and shock-avoidance maintained behavior. When the morphine reinforced component was reintroduced and saline was substituted for morphine, there was a temporary recovery of the food-reinforced and shock-avoidance behaviors. This experiment would indicate that environmental stimuli associated with presentation of morphine can, through classical conditioning, acquire the ability to produce at least some of the effects that morphine itself can produce, and in this way cause an alleviation of the opiate withdrawal syndrome. With continued presentation of these stimuli in the absence of association with morphine, their ability to produce morphine-like effects rapidly extinguished and the animal's behavior again showed the disruption associated with opiate withdrawal.

A second experiment that I would like to recall today dealt with the conditioned reinforcing properties of stimuli associated with self-administered morphine. This experiment, conducted in association with Dr. James Woods (Schuster and Woods, 1968) and again used rhesus monkeys who were conditioned to respond for morphine injections delivered according to a variable interval schedule of reinforcement. Tbe variable interval schedule was in force for a period of 1 hour every 6 hours, or four times daily. The onset of a visual stimulus indicated that the morphine injections were available. When morphine reinforcement was actually presented under this schedule, a red light was illuminated for the 25-second duration of the injection. After an animal's behavior had stabilized, extinction sessions were begun in which the white light signaling morphine availability was presented every 6 hours. However, the lever pressing either produced no consequences (on odd numbered days of extinction) or the red light and a saline injection (on the even numbered days of extinction). During the extinction phase, on the even numbered days, when the red response-contingent light and saline injection were presented, responding was significantly higher than on the days when responding produced no consequences. Over 10 days of extinction, these differences gradually disappeared.

This experiment indicated that stimuli associated with drug reinforcement (i.e., the red light) had acquired conditioned reinforcing properties which maintained the animal's responding during extinction. It is interesting to speculate that the conditioned reinforcing properties of stimuli associated with injections in human addicts may be responsible for the very commonly observed behavior of so-called "needle freaks" who are often observed to inject themselves with non-

pharmacologically active substances. Such behavior would make sense if it were maintained by the conditioned reinforcing properties of the stimuli emanating from "tying up and booting" the drug intravenously.

Now a second question of great importance was whether the conditioned reinforcing properties of stimuli associated with morphine would be present after a period of time in which the animals were detoxified so that they were no longer physically dependent. In order to study this, Dr. Woods and I reconditioned the animals to respond to morphine but used very low doses to limit the magnitude of physical dependence. After the animals had again stabilized in their intake of morphine and they had a sufficient number of days in which the red light had been associated with morphine, we removed the animals from the experimental situation for a period of 15 days. During this period, they received no morphine and hence underwent withdrawal. After the first week, there were no discernible withdrawal symptoms. After the 15-day period of detoxification, the animals were returned to the experimental situation and the stimuli signaling the availability of morphine were again presented for a l-hour period every 6 hours. Again, on the even numbered days, responding in the presence of the discriminative stimulus previously signaling the availability of morphine, now produced the red light and an injection of saline under the variable interval schedule of reinforcement. On the odd days of extinction, responding had no consequences. As was seen when the animals were physically dependent, the response rates (although lower than when the animals had been undergoing extinction at the same time as they were undergoing withdrawal) were significantly higher on the days when responding produced the stimuli previously associated with morphine than on the days when responding produced no consequences. After approximately 6 to 8 days, these differences in response rates disappeared.

Now why have I bothered today to recount experiments which were done over 20 years ago? In part, because I think that they represent some of the most important research in which I have been involved. Perhaps more importantly, I think this area of research has not enjoyed the popularity which its significance warrants. You will note that all of the experiments I have reported on in this section were done with opiates. It is clear that we need to conduct such experiments with other classes of drugs, both those which produce clear-cut signs of physical dependence (i.e., alcohol, barbiturates, benzodiazepines) and those whose ability to produce physical dependence is not as obvious (i.e., cocaine, amphetamines). We do not know, for example, whether withdrawal from drugs such as barbiturates or benzodiazepines increases drug-seeking behavior. Further, we do not know whether it is possible for such increased drug-seeking behavior, if it occurs, to be conditioned to environmental stimuli, as with opiates. These conditioned withdrawal studies I think would be of the utmost importance for our understanding of relapse to drug use. Further, we know very little about the conditioned reinforcing properties of stimuli associated with drugs--and these may be of major importance in the maintenance of drug-seeking behavior and in relapse for those who are attempting to abstain from drug use. Much remains to be done in this very fertile research area

I would like to end my talk today by mentioning very briefly some research which I did not conduct, but wish I had! Clinical and epidemiological studies have clearly shown that there are considerable individual differences in the susceptibility of humans to the reinforcing properties of addictive drugs. Of the 21 million individuals who have tried cocaine, for example, only 862,000 went

on to use the drug at least once per week and only 245,000 reported daily use of this drug in 1988 (National Institute on Drug Abuse, 1990). Fortunately, the vast majority of individuals who have tried cocaine have done so only on a very limited basis and curtailed their use after only a few experiences despite the fact that this drug has been demonstrated in animal studies and in susceptible human beings to be highly addictive. There are many factors that could lead to such individual differences: genetics, pharmacological and behavioral history, and environmental influences.

I would like to review some recent experiments that illustrate how such individual differences may be produced. Piazza, et al. (1989) studied some pharmacological factors that predict individual vulnerability to the reinforcing effects of amphetamines. An unselected population of Sprague-Dawley rats were tested to determine the extent to which their general activity was increased by exposure to a novel environment, The animals were divided into two groups--those whose general activity was increased above the median of the group and those who responded at a lower level. The two groups did not differ in any other observable characteristics. Both groups were then given a standard dose of amphetamine (1.5 mg/kg of body weight intraperitoneally), after which their locomotor activity was recorded. Those rats previously categorized as high rate responders on the basis of their reponse to a novel environment showed a larger increase in general activity following amphetamine than those who had been characterized as low rate responders. Thus it would appear that the response to the novel environment predicted the magnitude of effect of amphetamine on general locomotor activity.

In a second experiment, rats were first tested to determine their sensitivity to activity increments produced by exposure to a novel environment. These animals were divided into two groups as before. It is well established that daily administration of amphetamine sensitizes animals to the activity increasing effects of this drug (Tilson and Rech, 1973). Thus in this study half the animals in the high rate and low rate response category were given amphetamine and the other half were given saline for 4 consecutive days. High responders showed similar levels of activity across the 4 days. Those animals who initially showed both a smaller increase in their locomotor activity after being introduced into a novel environment, and who also showed smaller activity increases,in response to amphetamine administration, became sensitized over the 4 consecutive days of administration of amphetamine, as indicated by a significant increase in their general activity levels following amphetamine. Therefore, after 4 days, there were no significant differences between the high rate and low rate responders in their locomotor activity response to the administration of amphetamine.

At this point. the animals were given the opportunity to learn to self-administer low doses (10 ug/kg/injection) of amphetamine. Control animals (i.e., those which had received saline) who were categorized as low rate responders did not acquire the self-administration behavior over the course of 5 days, whereas those who were categorized as high rate responders but had not received the four additional injections rapidly acquired the drug self-administration behavior. In contrast, both the initially high rate and low rate responders who had been given the 4 days of amphetamine administration showed comparable acquisition of the drug self administration behavior. Thus, the repeated administration of the amphetamine not only eliminated the differences in response to the locomotor-activity increasing effects of the amphetamine, but also the differences in acquisition of the self-administration of amphetamine. It is of interest to note

that stress also produces sensitization, and that there is cross-sensitization to the effects of amphetamines in animals who have been given periods of daily mild stress (Antelman, 1980). It is, therefore, possible that stress might increase the probability that amphetamines would serve as a reinforcer.

The results of Piazza, et al,(1989) may have relevance to human behavior in two ways. First, it seems conceivable that individuals who have a high threshold for finding amphetamine reinforcing can become sensitized to this effect if they are coerced by peer pressure to try the drug on several occasions. In addition, the finding that stress also sensitizes animals to the actions of amphetamine has great relevance to the numbers of children who are reared in highly stressful environments and whose sensitivity to the reinforcing effects of amphetamine, as well as other stimulant drugs, might thereby be increased.

The second experiment I would like to discuss was by Jim Barrett and his colleagues on the role of behavioral history as a determinant of a drug's effect. These experiments really quicken my pulse! Barrett (1977) was able to demonstrate that the effects of amphetamine on behavior suppressed by punishment could be radically altered by giving laboratory animals a specific behavioral experience. Initially, squirrel monkeys were trained to lever press for a food reinforcer, which was delivered under a variable interval schedule of reinforcement in one component of a multiple schedule. The second component included a punishment component during which responding produced shock, as well as food. When amphetamine was administered at different doses prior to the session, there were dose-related decreases in the rate of responding during the punishment component. During the second phase of the experiment, the squirrel monkeys were trained to lever press to avoid an electric shock. Animals were maintained on this schedule of shock- avoidance until their behavior was stabilized, but no drugs were given to them under this condition. Phase 3 of this experiment consisted of placing the animals back in the original conditions of Phase 1, i.e., animals were exposed to the same food-reinforced variable interval punishment multiple schedule as in Phase 1.

It should be noted that there were no differences in the baseline performance of animals in Phase 3 compared to Phase 1. In other words, exposure to the shock avoidance schedule in Phase 2 did not alter their behavior under the multiple schedule of reinforcement. When doses of the amphetamine, however, were given, a dose-related increase in punished responding was observed. In the final phase of this experiment, animals were again placed under the schedule conditions of the shock-avoidance; in this case, however, no shock was given, so that the animal's shock avoidance behavior ultimately extinguished. Following extinction of the avoidance behavior, the animals were again exposed to the multiple schedule of reinforcement each day. Again, there was no difference in their baseline performance following this period of avoidance extinction; however, the animals' response to the amphetamine was once again a dose-related decrease in the rate of responding during the punishment component.

This experiment, which is just one of a series of experiments conducted by Barrett and his colleagues, clearly shows that the effects of a drug can be radically modified by behavioral experience; in this case, by exposure to a shock avoidance- schedule. It would be of great importance to determine whether the reinforcing effects of drugs could be modified by an animal's behavioral history. I am certain that such research will be forthcoming.

Concluding Remarks

Receiving the Nathan B. Eddy Award is the crowning achievement of my professional career. It has provided me with the opportunity to reflect about my research contributions, and I am convinced more than ever of the importance of behavioral pharmacology to our understanding of drug abuse and dependence. The paramount role of learning in the initiation, maintenance, and relapse to drug use is unquestionable. Increasingly, the insights from animal behavioral pharmacology studies are generating new clinical approaches for the treatment of addictive disorders. Further, behavioral pharmacology provides a conceptual framework for guiding our research activities.

I believe the future will see some fascinating developments in our understanding of how behavior influences brain chemistry and vice versa. Environmental and genetic influences in combination must be investigated for us to understand the genesis and nature of individual differences in susceptibility to drug abuse and addiction. Behavioral pharmacology will continue in its central role in all of these areas of research. I am very proud of the extent that I have contributed to this endeavor. Antelman, S.M. (1980) Interchangeability of stress and amphetamine in sensitization.

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AFFILIATION

Dr. Charles R. Schuster
Director, NIDA
Rockville, MD 20857

Intestinal Contractions: Excitatory Actions of Opioids

Thomas F. Burks, Lane D. Hirning, and Thomas H. Kramer

The effects of opioids on gastrointestinal functions are complex. Depending on the specific endpoint measured, such as effects on contractions, propulsion, acid secretion, or mucosal transport of electrolytes and water, the opioid response is influenced by the route of administration and sites of action, dose, receptor selectivity of the opioid agonist, and even the species studied. In most mammalian species,. including humans, dogs, cats and monkeys, morphine and related μ-preferring opioids given systemically increase the incidence and amplitudes of phasic contractions of circular smooth muscle of the small intestine (Pruitt et al., 1974; Daniel and Bogoch 1959). Small doses of morphine can also initiate premature migrating motor complexes (MMCs) of the small intestine in dogs (Sarna et al., 1982). The overall motility effect of morphine in non-rodent species is stimulatory, characterized by increased contractile activity. Propulsion of contents through the intestinal lumen is retarded despite the increase in contractions because the phasic contractions induced by morphine are essentially nonpropulsive, corresponding in many ways to normal phase II MMC patterns of activity (Carlson et al., 1972). The morphine-induced phasic contractions also increase resistance to flow through the intestinal lumen by generating local regions of elevated intraluminal pressure that retard net aboral flow of luminal contents. These motility effects of morphine result in constipation.

In guinea pigs and rats, however, the predominant motility response to systemic morphine is a decrease in contractions of the small intestine (Pruitt et al., 1974; Galligan and Burks 1983), although contractions of rat small intestine can be elicited by large doses of morphine in vivo (Burks 1976). The antitransit effects of morphine in rats are associated by dosage and temporally with decreased contractions of the small intestine (Galligan and Burks 1983). In rodent species, the antitransit (constipating) effect of morphine appears to be associated with decreased contractions of the small intestine. Longitudinal strips of rat and mouse colon in vitro however, are contracted by morphine (Scheurer et al., 1981). Thus, even in rodents, morphine possesses elements of smooth muscle excitation.

The mechanisms by which morphine and other opioids bring about contractions of intestine in vivo have been explored in a number of investigations. The types of opioid receptors involved in the excitatory responses, neural and non-neural sites of

action, participation of excitatory and inhibitory neurotransmitters, and cellular mechanisms of excitation have been examined.

DOG INTESTINE EX VIVO

Although morphine and related opioids produce contractions of dog small. intestine in vivo longitudinal or circular strips of dog intestine in vitro do not contract in response to morphine (Daniel et al., 1959). However, ex vivo preparations of intact segments of small intestine, arterially perfused with physiological salt solution, display characteristic contractile responses to morphine (Burks and Long 1967a; Burks 1973). Morphine, administered by bolus injection into the arterial cannula of perfused segments of intestine, produced phasic contractions of circular muscle resembling responses to morphine in vivo. Studies with a variety of opioid agonists showed that the amplitudes of the contractions were dose-related, stereospecific and were blocked by naloxone (Burks and Long 1967b; Burks 1973).

The ability of opioids to induce contractions of the dog intestine depend on which opioid receptors they are able to activate. Examination of a large series of natural and synthetic opioid peptides and nonpeptides in ex vivo intestinal segments revealed great quantitative differences among opioid agonists in terms of their ability to initiate intestinal contractions. As shown in Table 1, peptide and nonpeptide compounds with significant μ opioid agonist activity were most efficacious, those with δ activity were less efficacious, and those with κ were least efficacious. U-50,488H, the most κ-selective substance examined, was essentially devoid of stimulatory activity. The mean maximum increase in intraluminal pressure produced by morphine was 72.3 ± 10.8 mm Hg. The mean for U-50,488H was 3.6 ± 2.2 mm Hg (Hirning et al. 1985). All of the stimulatory opioids were antagonized by naloxone.

Table 1. Opioid-induced contractions of canine small intestine ex vivo[a].

Agonist	Relative Effect[b]
Peptides	
β-Endorphin-(1-31)	++++
[Met[5]]enkephalin	+++
[D-Ala[2], Met[5]]enkephalinamide	++++
[D-Ala[2], Leu[5]]enkephalinamide	++++
[D-Ala[2], NMePhe[4], Gly-ol]enkephalin (DAMGO)	++++
Morphiceptin	++++
[D-Pen[2], D-Pen[5]]enkephalin	+++
Dynorphin-(l-13)	+
Non-Peptides	
Morphine	++++
Phenazocine	++++
Bremazocine	+
U-50,488H	0
Ethylkctocyclazocine	++
Nalorphine	+

[a]Data from Burks et al. (1982) and Hirning et al. (1985).
[b]++++ = highly efficacious, 0 = little or no effect

The sensitivity of μ and δ agonist stimulatory actions to tetrodotoxin (TTX) was examined by use of morphiceptin and [D-Ala[2], N Me Phe[4], Gly-ol]enkephalin

(DAMGO) as selective μ agonists and [D-Pen[2], D-Pen[5]]enkephalin (DPDPE) as the selective δ agonist. Perfusion of the intestinal segments with 100 mg/ml of TTX completely obliterated excitatory responses to dimethylphenylpiperazinium (DMPP), a nicotinic cholinergic receptor agonist that induces intestinal contractions by excitation of enteric nerves. The same concentration of TTX nearly abolished contractile responses to the μ agonists, morphiceptin and DAMGO, and greatly reduced, but did not completely abolish, contractile responses to the δ agonist, DPDPE (Table 2). In the presence of TTX, the maximum responses induced by μ and δ agonists were strikingly similar.

Table 2. Effects of tetrodotoxin (TTX) 100 mg/ml on stimulatory responses to μ (DAMGO, morphiceptin) and δ (DPDPE, [Met[5]]enkephalin) opioid agonists in canine intestinal segments ex vivo.

Agonist	Control	TTX
	Maximum Contractions (mm Hg)	
DAMGO	62 ± 3	23 ± 4
Morphiceptin	63 ± 7	25 ± 5
DPDPE	38 ± 6	24 ± 4
[Met[5]]enkephalin	27 ± 8	22 ± 3

These data indicate that both μ and δ agonists, but not κ agonists, induce contractions of canine small intestine at least in part by means of neural mechanisms. The μ agonists were more efficacious than δ agonists and neural effects contributed relatively more to their stimulatory actions than to the stimulatory actions of the δ agonist. The δ agonist DPDPE was approximately equipotent with DAMGO in inducing contractions, but was less efficacious in terms of the total amplitude of the contractions produced, at least in the absence of TTX. These observations were confirmed in vivo in unanesthetized dogs by Vaught et al. (1985).

The mechanisms by which μ and δ opioids induce contractions of canine isolated intestine are not totally clear, but may involve both neural and non-neural mechanisms. Contractile responses to μ opioids are reduced by TTX, but not completely abolished (Burks 1973). Both 5-hydroxytryptamine (5-HT) and vasoactive intestinal polypeptide (VIP) may participate in the response. Opioid-induced contractions are associated with release of 5-HT (Burks and Long 1967a) and 5-HT antagonists, such as cinanserin, diminished the contractile responses to morphine (Burks 1973). Atropine also reduced contractile responses to both opioids and 5-HT, suggesting that the 5-HT released by opioids acts, in part, by stimulation of excitatory cholinergic neurons in the enteric nervous system. It is possible that the neural excitation component of the morphine response results from disinhibition of tonic inhibitory neurons. VIP is an inhibitory neurotransmitter present in enteric nerves of the intestine that innervate circular muscle and mucosa (Costa and Furness 1983). If VIP is involved, it is likely that morphine and related μ opioid agonists block release of VIP from its nerve terminals and thereby release excitatory 5-HT and cholinergic neurons from VIP-mediated tonic inhibition (Daniel 1989; Grider and Rivier 1990; Bauer and Szurszewski 1989). Because VIP neurons project to the intestinal mucosa as well as to circular muscle, the origin of the 5-HT released by opioids could be from mucosal enterochromaffin cells (Burks and Long 1967a; Domoto et al., 1990).

Non-neural mechanisms, however, may also participate in the opioid-induced contractions of canine small intestine. Opioids acting at μ receptors, such as morphine, morphiceptin, and DAMGO, are more sensitive to inhibition by TTX than opioids acting at δ receptors, such as [Met[5]]enkephalin or DPDPE. That is, there is a significant TTX-resistant component of the δ stimulatory effect. In the presence

of TTX, μ and δ opioids are equally efficacious in producing contractions. Moreover, perfusion of the intestinal vasculature with substances that increase formation of intracellular cyclic AMP, such as isoproterenol, epinephrine or theophylline, inhibited contractions induced by opioids to a far greater extent than they inhibited contractions induced by acetylcholine (Grubb and Burks 1975). These data suggested that opioids might exert direct smooth muscle effects that are specifically counteracted by increases in muscle cyclic AMP. Some gastrointestinal smooth muscle cells in primary culture express opioid receptors (Bitar and Makhlouf 1982). However, the presence of opioid receptors on canine intestinal smooth muscle cells in situ has not been demonstrated (Allescher et al., 1989) and alterations in cyclic AMP in enteric nerves could affect the neural component of opioid actions. These possibilities have been examined further in strips of rat colon.

RAT ISOLATED COLON

In vitro strips of longitudinal muscle from rat colon contract in response to μ and δ opioids (Kramer et al., 1988). DPDPE, a selective δ opioid agonist, was approximately 16-fold more potent in inducing contractions than PL017, a selective μ agonist (EC_{50} for DPDPE 5.8nM, EC_{50} for PL017 101nM). DPDPE was found also to be more potent in inducing contractions than either acetylcholine (EC_{50} 43.4 nM) or 5-HT (EC_{50} 45.6 nM).

Opioid-induced contraction in strips of rat colon were modestly sensitive to TTX, indicating that only a small component of the response is neurally mediated. However, responses were very sensitive to inhibition by forskolin and a-melanocyte-stimulating hormone (α-MSH), both of which raise intracellular levels of cyclic AMP, associated with relaxation of intestinal smooth muscle (Scheid et al., 1979). As with canine small intestine, the TTX-insensitive component of opioid-induced contractions in longitudinal strips of rat colon were apparently sensitive to agents that increase intracellular levels of cyclic AMP.

DISCUSSION

The overt response of the intestine in many mammalian species to μ and δ opioid agonists is contraction, a stimulatory effect. The mechanisms responsible for the contractions are complex and clearly involve neural components. The stimulation of excitability nerves supplying smooth muscle in the intestine could be brought about either by direct opioid actions on the nerves or by their release from tonic inhibition. One theory proposes that the neural stimulatory effect of opioids in the intestine results from disinhibition by blocking tonically active VIP inhibitory neurons, possibly by actions at the terminals of the neurons to inhibit release of VIP (Daniel 1989). Several lines of evidence support this concept, including the observation that μ and δ opioids can reduce smooth muscle inhibitory junction potentials evoked by electrical stimulation of the tissue (Bauer and Szurszewski 1989).

However, the TTX-insensitive contractions of intestinal smooth muscle are much more difficult to explain on the basis of disinhibition. TTX in the concentrations employed (μM range) would be expected to block activity of inhibitory as well as excitability enteric neurons. Indeed, preparation of rat and mouse colon generate pronounced contractions in the presence of TTX, indicating that TTX blocks tonically active inhibitory nerves (Wood 1972; Kramer et al., 1988). Nevertheless, TTX only partially inhibits opioid-induced contractions in canine small intestine or rat colon.

Pharmacological agents that increase intracellular levels of cyclic AMP, including isoproterenol, epinephrine, α-MSH and forskolin, inhibit contractile responses to

opioids to a much greater extent than they affect contractile responses to acetylcholine, suggesting some specificity of changes in cyclic AMP. In some cells, δ and probably μ opioid receptors are negatively coupled to adenylate cyclase (Heijna et al., 1989). Increased levels of cyclic AMP in intestinal smooth muscle is associated with relaxation of the muscle (Scheid et al., 1979). Binding of opioid ligands to dispersed, nerve-free, gastrointestinal smooth muscle cells has been demonstrated (Bitar and Makhlouf, 1982), indicating the presence of opioid receptors on at least some smooth muscle cells. It therefore seems likely that the TTX-insensitive component of opioid-induced contractions in canine small intestine and rat colon could result from direct smooth muscle actions of opioids associated with receptor-mediated decreases in activity of adenylate cyclase. This excitatory effect of opioids can be specifically opposed by agents that increase activity of adenylate cyclase.

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AUTHORS

Thomas F. Burks
Department of Pharmacology
College of Medicine
The University of Arizona
Tucson AZ 85724

Lane D. Hirning
NPS
420 Chipeta Way
Salt Lake City, UT 84108

Thomas H. Kramer
Department of Pharmacology
College of Medicine
The University of Arizona
Tucson AZ 85724

Opioids Can Produce A Concentration-Dependent Naloxone-Reversible Enhancement of Inhibition of Evoked Enkephalin Release

Alan R. Gintzler and Hong Xu

INTRODUCTION

The ability of all opiate receptor type selective agonists to inhibit the release of neurotransmitters or decrease the rate at which neurons fire has been considered to be the predominant mechanism by which they produce their central nervous system (CNS) effects (North 1986). Observations of opioid enhancement of transmitter release or cell firing rate have been mostly attributable to a disinhibition mechanism.

The depressant action of opioids has been demonstrated on the release of a wide spectrum of neurotransmitters. There is, however, only minimal data concerning the ability of opiate receptors to regulate the release of their own endogenous ligands. This laboratory has been studying the opiate receptor-mediated regulation of methionine-enkephalin (met-enkephalin) release. Results from these experiments indicate that conventional concentrations of opioids inhibit the evoked release of met-enkephalin (Xu et al, 1989). This is consistent with the prevailing view that the predominant action of opioid peptides is inhibitory, hyperpolarizing neural elements throughout the CNS. At unconventionally low concentrations of opioid, however, the opposite effect has been observed. Pretreatment with nanomolar doses of opioid agonists results in an opiate receptor mediated enhancement of the electrically stimulated release of met-enkephalin (Xu et al, 1989).

This manuscript will summarize results from recent experiments that differentiate the neural requirements and signal transduction process(es) that mediate opioid enhancement or inhibition of met-enkephalin release. In these experiments, the longitudinal muscle, myenteric plexus (LMMP) preparation of the guinea pig was chosen as a convenient source of enkephalin-containing neurons.

METHODS

"Strips" of the IMMP were prepared, mounted and superfused in a stimulating chamber, as described previously (Glass, et al, 1986). Tissue superfusate (0.5 ml) was collected before, during and after a 30 s period of electrical stimulation (40 Hz, 0.2 ms pulse duration) in the presence or absence of the indicated concentrations opiate receptor type-selective agonists (3 min). Sufentanil citrate (SFNC), [D-Pen^2-D-Pen^5]enkephalin (DPDPE) and dynorphin (or U50,488H were utilized as mu-, delta-, and kappa-

27

selective agonists, respectively. Met-enkephalin immuno-
reactivity was quantitated by a radioimmunoassay procedure using
an antibody generated against met-enkephalin sulfoxide (Xu et
al, 1989) and confirmed by HPLC.

The electrically induced % increase in the rate of met-
enkephalin release was calculated in the absence, 3 min
following pretreatment with varying concentrations of opioid
agonist while still in their presence and following a 15 min
washout. The % rise above basal release under each condition
was calculated by subtracting the mean basal release from the
peak release observed during electrical stimulation and dividing
the difference by the mean basal release (stimulated-
basal)/basal).

RESULTS

Figure 1 illustrates that the opioid regulation of enkephalin
release is bimodal. Opiate receptor type-selective agonists can
both inhibit or enhance the evoked release of met-enkephalin,
depending upon the concentration of agonist used. Conventional
concentrations (100 nM) produce a conventional inhibitory
response. In contrast, unconventionally low concentrations
(0.1-l nM) have an excitatory effect on stimulated enkephalin
release. Both the opioid enhancement or inhibition of
enkephalin release can be blocked with naloxone indicating
opiate receptor mediation (Xu et al 1989).
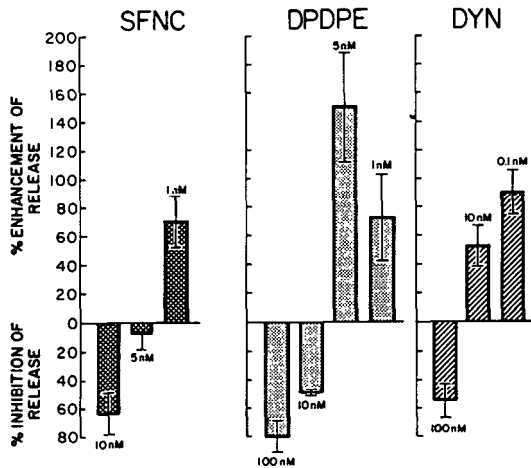
FIGURE 1. The % increase in stimulated enkephalin release was
calculated in the absence and presence of opioid agonist. The
% rise above basal release abserved in the presence of opioid
is expressed as a % of that observed in its absence.

28

Effect of Cholinergic Receptor Blockade

The parameters of electrical stimulation used to induce the release of met-enkephalin also cause the concomitant release of enteric acetylcholine (Ach). Opiates are potent inhibitors of Ach release. Consequently, their inhibitory effect on met-enkephalin release could be secondary to their ability to depress cholinergic function. In order to determine whether or not this was so, the opioid inhibition of met-enkephalin release was determined in LNMP preparations rendered devoid of muscarinic cholinergic function by pretreatment with atropine.

Despite the absence of muscarinic receptor activity, the inhibitory opioid effect on met-enkephalin release was unaltered. In contrast, a different picture emerged for the opioid enhancement of met-enkephalin release. In LNMP preparations devoid of muscarinic cholinergic tone a previously excitatory concentration of SFNC is now without any statistically significant effect on the magnitude of the stimulated release of met-enkephalin ($p > 0.5$).

Effect of Elevating Intracellular cAMP

Forskolin or 8- (4-chlorphenylthio)-cAMP (8-CPT-cAMP) was used to stimulate adenylate cyclase activity or CARP-dependent processes, respectively. Pretreatment with either agent markedly enhanced stimulated enkephalin release (Figure 2).
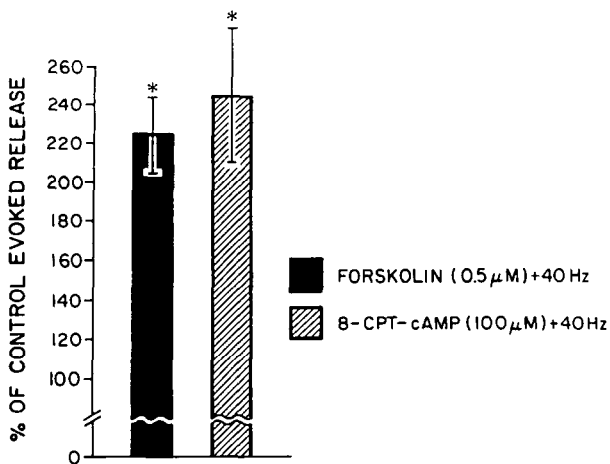
FIGURE 2 The evoked release of enkephalin was quantitated before during and after a 15 minute pretreatment with forskolin (0.5 UM) or 8-CPT-cAMP (10 uM). The magnitude of evoked enkephalin release obtained in their presence is expressed as a percent of that observed in their absence.

Figure 3 illustrates that activation of cAMP-dependent processes
not only enhances the magnitude of the evoked release of met-
enkephalin but is also very effective in blocking the opioid
inhibition of stimulated enkephalin release. Following
pretreatment with forskolin (0.5 uM) or 8-CPT-cAMP (100 uM)
sufentanil (10 nM) DPDPE (10 nM) and dynorphin (100 nM) no
longer produce an inhibition of release. In fact, in these
preparations a previously inhibitory concentration of each
opioid now produces an enhancement of the magnitude of evoked
met-enkephalin release. Excitatory opioid effects remain
unaltered in these preparations. Since all 3 cycles of release
were obtained in forskolin or 8-CPT-cAMP treated myenteric
plexus, the stimulatory effect of these compounds on evoked met-
enkephalin release is not a confounding factor. Moreover, since
excitatory responses. (enhanced release) to lower concentrations
of sufentanil (1 nM) or DPDPE (5 nM) are not affected by
pretreatment with forskolin, the qualitative shift in response
to inhibitory concentrations of opioid that occurs in forskolin-
treated tissue is due to a loss of inhibition and not to an
enhancement of excitatory responses.
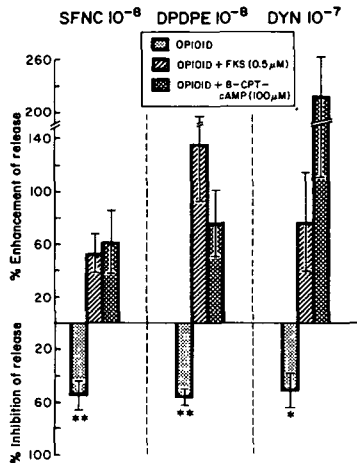
FIGURE 3 Forskolin or 8-CPT-cAMP was added to the superfusate
15 minutes before the start of the first cycle and maintained
for the duration of the experiment. The electrically induced
% rise above basal release observed in the presence of opioid
is expressed as a % (mean S.E.M) of that observed in its
absence.

Effect of Pertussis Toxin and Cholera Toxin on the Opioid
Modulation of Met-Enkephalin Release

The ability of forskolin or 8-CPT-cAMP to enhance the magnitude
of stimulated met-enkephalin release does not prove, but is
consistent with the involvement of the cAMP second messenger
system (and its stimulation by low concentrations of opioid) in
facilitating enkephalin release. Similarly, the reversal of
opioid inhibition to enhancement of release in forskolin or 8-
CPT-cAMP treated preparations suggests that opioid inhibitory
effects on enkephalin release could also derive from their
interaction with this second messenger system. In order to test
this more directly, the effects of toxins that ADP ribosylate
selective G proteins on the opioid regulation of enkephalin
release was investigated. Cholera toxin (CTX) and pertussis
toxin (PTX) were selected because of their ability to
selectively ADP-ribosylate G_s or G_i, respectively, and thereby
alter their activity.

CTX penetrates tissue relatively rapidly. Therefore, segments
of ilea were incubated for 3 hrs in vitro with this toxin
(10^{-11}M) before analyzing the opioid modulation of met-enkephalin
release. In contrast, PTX penetrates tissue very slowly.
Therefore, it was necessary to pretreat guinea pig ilea in vivo
for 5 days (50 ug i.p./500g) prior to testing its effect on
opiate receptor-mediated regulation of met-enkephalin release.

In preliminary experiments, mu, delta, or kappa selective
opioids fail to inhibit the evoked release of met-enkephalin in
PTX-treated tissue despite the fact that excitatory responses
to nanomolar concentrations of opioid persist unaltered.
Conversely, in CTX-treated tissue, opioid inhibitory responses
remain intact but opioid excitatory responses are abolished.

DISCUSSION

The present results clearly indicate that the evoked release of
met-enkephalin is subject to opioid regulation. One novel
aspect of this regulation is that it is biomodal; excitatory or
inhibitory modulation can be observed depending on the
concentration of opioid agonist. This opiate receptor-mediated
bimodal modulation could represent an autoregulatory mechanism
by which the range over which synaptic met-enkephalin
concentration varies is kept within strict limits.

The presence of excitatory as well as inhibitory opioid affects
on enkephalin release has an electrophysiological counterpart.
Morphine (1-100 nM) can both enhance and depress Ca^{2+}-dependent
potentials in a nodose ganglion in vitro (Higashi et al, 1982).
More recently, it has been shown that low (nm) concentrations

of opioids produce a naloxone-reversible prolongation of the Ca^{2+} component of action potentials of mouse dorsal root ganglion neurons grown in tissue culture; higher concentrations of these some agonists shorten the action potentials of the same cell (Shen and Crain 1989).

The opioid enhancement or inhibition of enkephalin release have different neuronal requirements and are mediated by a different signal transduction processes. Muscarinic cholinergic tone is an absolute prerequisite for opioid enhancement but not opioid inhibition of enkephalin release. Since muscarinic receptors are coupled to the hydrolysis of phosphatidylinositol (4,5)-bishphosphate and the generation of inositol (1,4,5)-triphosphate and diacylglycerol this suggests that the intracellular concentration of one or both of these second messengers or the consequence(s) of their action (such as mobilization of intracellular Ca^{2+} and/or activation of protein kinase C) could be essential for opioid excitatory effects.

Preliminary experiments with PTX and CTX support the hypothesis that the enkephalin release process involves a cAMP second messenger system and that the opioid modulation of release results from their interaction with that system. These data also emphasize the divergence of the biochemical components that underly opioid enhancement or inhibition of enkephalin release. The opioid excitation or inhibition of enkephalin release requires a different G protein that can be differentiated on the basis of their sensitivity to CTX or PTX. It remains to be determined whether the substrate for these G proteins is an ion channel or the catalytic unit of adenylate cyclase.

The present data cannot differentiate between a direct facilitation of enkephalin release or enhanced release via disinhibition. Since it seems unlikely that different inhibitory opioid responses (direct inhibition or disinhibition) would be mediated via different biochemical processes, the simplest explanation to explain opioid-mediated enhanced enkephalin release is to postulate a direct facilitation of release. This is supported by the striking similarities between the present results and the electrophysiologic studies demonstrating that nanomalor concentrations of opioid elicit direct excitatory effects on sensory neuron perikarya devoid of synaptic inputs from inhibitory neurons (Shen and Crain 1989).

In summary, their appears to be two parallel pathways that mediate the action of opioids on the electrically stimulated release of met-enkephalin; one mediating excitation, the other inhibition. The presence of a previously unrecognized additional opioid excitatory pathway may be of considerable relevance to understanding acute and longterm effects of narcotics.

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AFFILIATION:

Department of Biochemistry
State University of New York
Health Science Center
Brooklyn, New York 11203

Opioids Can Evoke Direct Receptor-Mediated Excitatory as well as Inhibitory Effects on Sensory Neuron Action Potentials

Stanley M. Crain and Ke-Fei Shen

INTRODUCTION

Over 100 years ago, Claude Bernard (1864) observed that low doses of morphine in dogs evoked excitatory effects, e.g., salivation, retching. vomiting and defecation, in contrast to the depressant sedating effects that occur after higher doses. After a more systematic study of opioid effects on dogs, cats and monkeys, Tatum et al (1929) proposed that *"morphine simultaneously stimulates certain parts of the CNS and depresses others..Abstinence symptoms are plainly due to the fact that stimulation of the nervous system, or increased irritability, outlasts the depression"* which may initially mask the excitatory effects. This "dual action" hypothesis of morphine physical dependence was modified by one of the co-authors of the 1929 paper in a critique presented by Seevers at the 23rd meeting of the CPDD, in 1961. Although Seevers & Deneau (1962) still favored the basic concept of dual stimulatory and depressant effects of morphine they asserted that the symptoms of abstinence could not be due simply to the direct stimulant actions of morphine because *"at the time of maximal intensity of abstinence, 48 to 72 hours, only traces of morphine remain in the body"*. However, this argument is weakened by our recent evidence that excitatory subtypes of opioid receptors on sensory ganglion neurons, which appear to be increased in efficacy after chronic exposure to opioids (Crain et al, 1988), can be activated by *extremely* low concentrations of opioid agonists (Shen & Crain, 1989, and in preparation). Furthermore, Seevers & Deneau (1962) argued that *"simultaneous administration of morphine antagonists...during prolonged administration of morphine should not prevent development of physical dependence to the now unopposed* [putative] *stimulant effects but should in fact enhance it"*. However, they noted that co-administration of morphine and an opioid antagonist does, in fact, block physical dependence.

This criticism of Tatum et al's (1929) *"dual action"* hypothesis is also no longer compelling in view of our electrophysiological evidence that the opioid antagonists; naloxone or diprenorphine can block the *stimulatory* as well as the inhibitory effects of opioids on sensory ganglion neurons (Shen & Crain, 1989; Crain & Shen, 1990; see below). Finally, Wikler's (1980) review of Tatum et al's (1929) hypothesis noted that *"one problem with this theory is identifying the*

stimulant actions" (p. 143) of opioids on the nervous system; in addition concerns were raised similar to those emphasized in Seever & Deneau's critique (1962).

Our electrophysiologic studies demonstrating dual *stimulatory* as well as inhibitory opioid modulation of the action potentials of sensory dorsal-root ganglion (DRG) neurons in culture provide experimental evidence at the cellular level that appears to eliminate the major concerns expressed by Seevers and Deneau (1962) and Wikler (1980) and is remarkably consonant with the original *"dual action"* hypothesis of Tatum et al (1929) based on studies of dual stimulatory and inhibitory effects of opioids in animals.

DIRECT OPIOID EXCITATORY EFFECTS ON SENSORY NEURON ACTION POTENTIALS

In a review of electrophysiological analyses of opioid modulatory effects on neurons, North (1986) noted that *"activation of all types of opioid receptors seemed to...inhibit transmitter release [or] to slow cell firing [rate]"*. Both these inhibitory effects have been shown to be mediated by either increases in specific membrane K+ conductances or decreases in Ca^{2+} conductances, resulting in hyperpolarization of nerve cells or in shortening of the Ca^{2+} component of the presynaptic action potential duration (APD) (see also Mudge et al, 1979; Werz & Macdonald, 1985). However, recent electrophysiologic studies of opioid effects on mouse sensory DRG neurons in culture have shown that specific μ, δ and κ opioid receptor agonists can evoke naloxone-reversible *prolongation* of the APD in many of these cells when applied at low (1-10 nM) concentrations (Shen & Crain, 1989; Chen et al, 1988) (Fig.$1_{1.2)}$. These excitatory effects are generally masked by higher opioid concentrations that shorten the APD (Fig. $l_{1.3}$ and $l_{5,6,7}$; see revs. by North, 1986; Crain & Shen, 1990). Shortening of the APD of DRG perikarya by opioids has generally been considered to be a useful model of their inhibition of Ca^{2+} influx and transmitter release at presynaptic DRG terminals. Similarly, if opioid-induced prolongation of the APD occurs at presynaptic DRG terminals as well as at DRG perikarya this would result in *enhanced Ca^2+* influx in these terminals and increased transmitter release (Shen & Crain, 1989; Crain & Shen, 1990). Furthermore, since DRG neurons are devoid of synaptic inputs, excitatory modulation by opioids of the APD is clearly a direct action (Cram et al, 1988), distinct from disinhibitory mechanisms that may mediate some of the excitatory effects of opioids in the brain (e.g., Zieglgansberger et al, 1979).

Similar naloxone-reversible prolongation of the APD by nM concentrations of morphine, and shortening by μM levels, was observed in freshly isolated adult rabbit visceral sensory (nodose) ganglion cells (Higashi et al, 1982), indicating that the concentration-dependent, dual excitatory and inhibitory opioid effects observed in cultures of DRG cells can also occur in some types of neurons in *situ*. Opioids prolong the APD of DRG neurons by decreasing voltage-sensitive membrane K^+ conductances (via μ, δ or κ/dynorphin receptors) or by increasing Ca^{2+} conductances (via κ/U-50,488H receptors) (Shen & Crain, 1989, 1990a). Thus opioid excitatory modulation of the APD of DRG neurons appears to be mediated by high-affinity receptor subtypes that produce the

1. BSS
2. DADLE 10nM
3. DADLE 1uM

10mV
5msec

4. BSS
5. CTX-A 1ng/ml
6. CTX-A 1ng/ml
   +DADLE 10nM
7. CTX-A 1ng/ml
   + DADLE 1uM
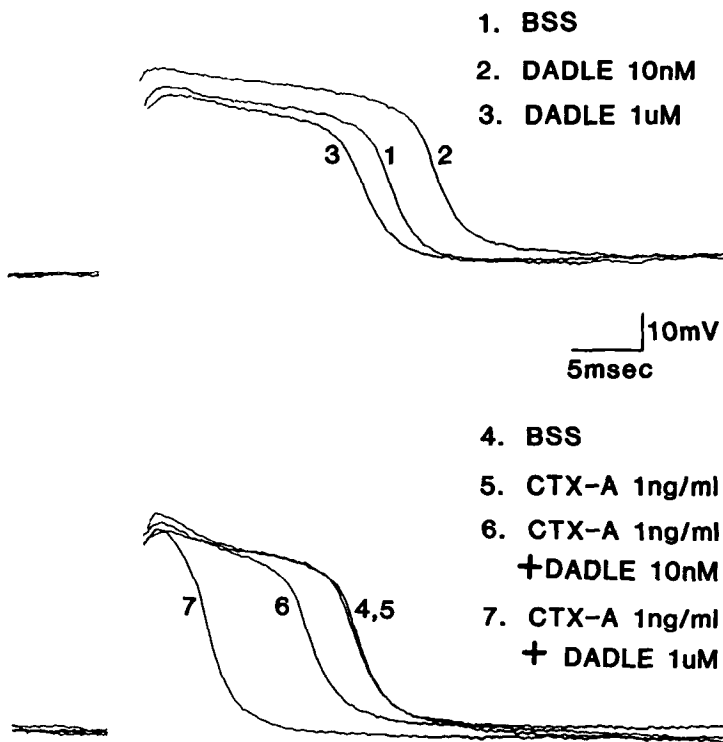
FIGURE 1. Pretreatment of a DRG neuron with a low concentration of cholera toxin-A subunit (CTX-A) blocks opioid-induced APD prolongation. l):AP generated by a DRG neuron in balanced salt solution containing 5 mM Ca^{2+} and 5 mM Ba^{2+} (BSS), in response to a brief (2 msec) intracellular depolarizing current (same 2 msec stimulus used in all subsequent records). 2,3): APD of DRG neuron is prolonged within 3 min after addition of 10 mM DADLE (2) and shortened below control value within 3 min after increasing DADLE concentration to 1 uM(3). 4,5): After control period in BSS (10 min), addition of 1 ng/ml CTX-A did not alter the APD (10 min teat). 6): Addition of 10 nM DADLE in the presence of CTX-A no longer elicits APD prolongation; instead, an opioid-induced APD shortening is unmasked. 7): Increasing the DADLE concentration to 1 uM elicits a further shortening of the APD, notwithstanding the presence of CTX-A (whereas opioid-induced APD shortening is selectively blocked by treatment with pertussis toxin: Shen & Cram, 1989; see this paper for details of electrophysiologic and culture techniques). (From: Shen and Crain 1990b).

opposite effects on K+ and $Ca^{2}+$ conductances as those mediating opioid-induced APD shortening (Cram & Shen, 1990).

These interpretations based on current-clamp recordings of opioid-induced APD prolongation in DRG neurons have recently been confirmed by cell-attached, tight-seal, patch-clamp analyses showing decreased whole-cell K+ currents and attenuation of specific single-unit K^{+} channel openings during bath application of opioids (Fan, Shen & Crain 1989, 1990).

SPECIFIC RECEPTOR MEDIATION OF OPIOID EXCITATORY EFFECTS

Additional evidence that different receptor subtypes mediate opioid excitatory and inhibitory modulation of the APD in DRG neurons is that opioid-induced prolongation is selectively blocked by cholera toxin-A subunit (Fig. 1 $_{5,6}$ vs. Fig. 1.$_{-1,2}$; Shen & Grain, 1990b) [which ADP-ribosylates G_s and attenuates ligand activation of associated receptors], whereas opioid-induced shortening is blocked by pertussis toxin [which ADP ribosylates G_i and G_o and interferes with inhibitory receptor functions; see refs. in Cram & Shen, 1990]. Furthermore, intracellular injection of an inhibitor of cyclic AMP-dependent protein kinase in DRG neurons blocks [D-Ala^2-D-Leu^6]-enkephalin (DADLE)-induced prolongation but not shortening of the action potential (Chen et al, 1988). These data suggest that excitatory subtypes of opioid receptors are positively coupled via a G_s-like protein to adenylate cyclase and to cyclic-AMP-dependent voltage-sensitive ionic conductances (resembling, for example, β-adrenoceptors).   By contrast, inhibitory effects are mediated by opioid receptors linked to G_i/G_o (resembling a$_2$ adrenoceptors).

PHYSIOLOGICAL ROLES OF DIRECT OPIOID EXCITATORY EFFECTS

Our in vitro results are remarkably consonant with single-unit recordings from dorsal-horn neurons in adult rats showing that application of low concentrations of μ or k opioids to the spinal cord produces facilitation of C-fiber-evoked nociceptive responses, whereas higher concentrations results in inhibition (Knox & Dickenson, 1987). Furthermore, in an animal model of persistent pain (arthritic rats), Kayser et al (1987) found that "exceedingly low doses *of morphine...elicit a naloxone-reversible paradoxical hyperalgesia* [whereas increased doses are] *highly effective* in *producing analgesia"*. In addition, opioids have recently been shown to enhance transmitter release from some types of DRG terminals in the spinal cord (Pohl et al, 1989; Sawynok et al, 1989 and this volume) and from myenteric ganglia (Xu et al, 1989; Gintzler & Xu, this volume).

These and related data (Crain & Shen '90) suggest that the effects mediated by excitatory subtypes of opioid receptors may provide a novel mechanism to account for some of the previously unexplained, hyperalgesic, aversive (e.g., itching: Ballantyne et al 1988; see also van der Kooy, 1986) and euphoric effects of opioids in the central and peripheral nervous systems.

ROLE OF OPIOID EXCITATORY MODULATION IN TOLERANCE AND DEPENDENCE

Dual modulation by opioids of the APD in DRG neurons may also provide insights into tolerance/dependence and plasticity in opioid networks. In a study of DRG-cord explants after chronic exposure to 1 μM DADLE, we found that most ganglion neurons became tolerant to the usual APD-shortening effects of high concentrations of DADLE (10 μM), consonant with the tolerance that develops to the depressant effects of opioids on DRG-evoked dorsal-horn post-synaptic network responses in these explants after chronic exposure to opioids (Crain, 1988; Crain et a1 1988). In addition, a remarkably high proportion of the treated cells showed *APD prolongation* even in response to *high* (10 μ M) test concentrations of DADLE. These results after chronic opioid exposure of DRG neurons resemble some of the alterations observed after pertussis toxin or forskolin treatment, i.e., attenuation of inhibitory opioid effects and an increase in the expression of excitatory opioid receptor-mediated functions in the treated neurons (Shen & Crain, 1989). All three treatments result in elevated adenylate cyclase activity and cyclic AMP levels. Biochemical assays of DRG-cord explants after chronic exposure to morphine or pertussis toxin showed marked enhancement of basal and forskolin-stimulated adenylate cyclase activities, decrease in opioid inhibition of forskolin-stimulated cyclase activity, and *increase* in *opioid-stimulated* basal cyclase activity (Makman et al, 1988). In addition to clarifying possible direct uncoupling or down-regulation of inhibitory opioid receptors, this *in vitro* model system may provide clues to compensatory processes [possibly mediated by enhanced adenylate cyclase activity and cyclic AMP levels (Crain, 1988; Crain et al, 1988; Makman et al, 1988; Sharma et al 1975; Collier, 19801 that could attenuate inhibitory effects of opioids on primary afferent synaptic networks in the spinal cord and account for some of the hyperexcitability properties associated with dependence and addiction.

Recent studies demonstrate that opioid prolongation of the APD of DRG neurons can also be selectively blocked by acute treatment with the *B subunit* of cholera toxin, which binds selectively to GM1 ganglioside (Shen & Crain, 1990c), independent of the more potent blocking effects elicited by the A subunit, which acts specifically on G_S, as noted above (Fig. 1 $_{5,6}$). These and related data suggest that G_B-coupled excitatory subtypes of opioid receptors, but not G_i/G_O-coupled inhibitory receptors, on DRG neurons may be allosterically regulated by GM1 ganglioside binding sites that induce conformational changes in these receptors which enhance the efficacy of their coupling to the G_s/adenylate cyclase second messenger system (Shen & Crain, 1990). Preliminary evidence indicates that modulation of the levels of GM1 ganglioside in DRG cell membranes may play an important role in mediating some of the plastic changes in opioid networks that occur during the development of tolerance, dependence and addiction (Cram & Shen, in preparation).

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AUTHORS

Stanley M. Crain, Ph.D.
Ke-Fei Shen, M.D.
Department of Neuroscience
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461

Adenosine Release by Morphine and Spinal Antinociception: Role of G-Proteins and Cyclic AMP

Jana Sawynok, Donna J. Nicholson, Marva I. Sweeney, and Thomas D. White

THE ADENOSINE RELEASE HYPOTHESIS OF OPIOID ACTION IN THE SPINAL CORD

The release of adenosine from capsaicin-sensitive small diameter primary afferent neurons has been proposed to mediate a significant component of the spinal antinociceptive action of morphine (reviewed Sawynok et al., 1989). Thus, spinal antinociception produced by morphine is antagonized by methylxanthines (Jurna 1984; DeLander and Hopkins 1986; Sweeney et al., 1987) at doses which block antinociception produced by intrathecal (i.t.) administration of adenosine analogs. In addition, morphine has been shown to induce a Ca2+-dependent, naltrexone-sensitive release of adenosine from spinal cord synaptosomes in vitro (Sweeney et al., 1987), and to release adenosine from the spinal cord in vivo (Sweeney et al., 1989). In both paradigms, release is reduced by i.t. pretreatment with capsaicin (Sweeney et al., 1989).

The capsaicin-sensitivity of release of adenosine by morphine suggests adenosine originates from central terminals of small diameter primary afferent neurons with their cell bodies in the dorsal root ganglion (DRG). Biochemical markers for neurons in which adenosine may play a significant physiological role also are localized in capsaicin-sensitive terminals in the spinal cord (Geiger and Nagy 1985; Nagy and Daddona 1985). Recently, opioids have been shown to prolong the duration of-the-action potential in cultured DRG neurons, and this increase has been proposed to account for stimulatory effects of opioids (Crain and Shen 1990). At higher doses, opioids shorten the action potential duration, and this may cause inhibition of neurotransmitter release (Crain and Shen 1990). Interestingly, the increase in release of adenosine seen in vitro occurs at l-100μM morphine (Sweeney et al., 1987), doses at which morphine **inhibits** release of substance P from spinal cord slices in vitro (Pang and Vasko 1986). The ability of morphine to promote release of a

neuromediator which results in pain suppression (adenosine) and to inhibit release of a neuromediator which facilitates pain (substance P) appear to, represent parallel but separate actions within the spinal cord. Thus. adenosine analogs do not inhibit release of substance P and methylxanthines do not block the ability of morphine to inhibit release of substance P (Vasko et al., 1986). Although most studies examining opioid effects on substance P release in vitro and in vivo have demonstrated inhibitory effects on release (Aimone and Yaksh 1989), there has been a report that μ agonists **increase** substance P release while δ agonists **inhibit** release (Maughborne et al., 1987). The receptor subtypes mediating morphine-evoked release of adenosine have not yet been characterized.

ROLE OF CYCLIC AMP IN SPINAL ACTIONS OF ADENOSINE AND MORPHINE

Adenosine A1 agonists decrease cyclic AMP production in the spinal cord, while A2 agonists increase cyclic AMP production (Choca et al., 1987). Inhibition of cyclic AMP production by opioids has been demonstrated in cultured DRG preparations and in adult spinal cord tissue (Makman et al., 1988; Attali et al., 1989). In addition, opioids have been reported to increase cyclic AMP production in cultured DRG preparations (Makman et al., 1988). Thus, both purines and opioids could exert pharmacological actions within the spinal cord by actions which are mediated by changes in cyclic AMP. These alterations may result in regulation of ion channel activity and a decrease or an increase in action potential duration, with consequent alterations in release of neuromediators from DRG neurons (Crain and Shen 1990). We have examined the potential involvement of the adenylate cyclase system in spinal antinociception produced by purines and morphine, and in morphine-evoked release of adenosine in the spinal cord by pharmacological manipulation of the cyclic AMP system. Agents used were **pertussis toxin** which initially was understood to ADP ribosylate and inactivate G_i Linked to inhibition of adenylate cyclase (more recent data suggests involvement of G_1 with ion channels and additional second messenger-systems, Rosenthal et al., 1988) as well as G_0 linked to ion channels, **forskolin** which directly stimulates adenylate cyclase, and the phos-**phodiesterase inhibitors** Ro 20 1724, rolipram, 3-isobutyl-1-methylxanthine (IBMX). The underlying assumption was that if inhibition of cyclic AMP production mediated a pharmacoloqical effect of aden-osine or morphine, these treatments would reduce their actions. Conversely, if stimulation of adenylate cyclase was involved, these treatments would enhance their actions.
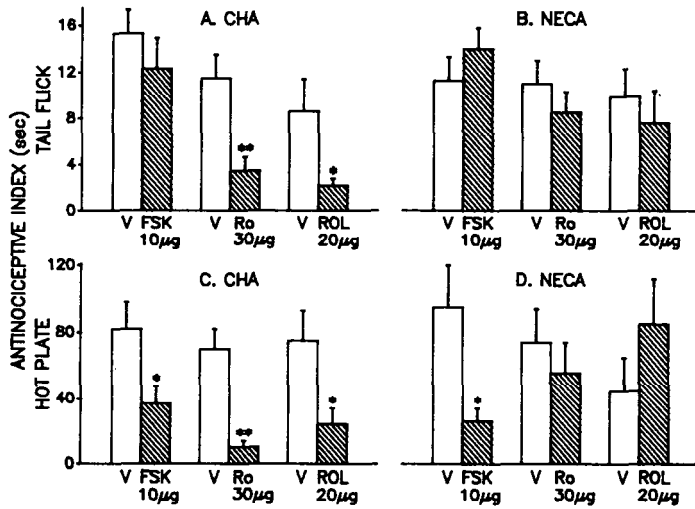
FIGURE 1. Effects of forskolin (FSK) and the phosphodiesterase inhibitors Ro 20 1724 (Ro) and rolipram (ROL) on the spinal antinociceptive action of N^6-cyclohexyl adenosine (CBA) and 5'-N-ethylcarboxamide adenosine (NECA). V indicates vehicle control response. * $p<0.05$, ** $p<0.01$. (Summarized from Sawynok and Reid 1988.)
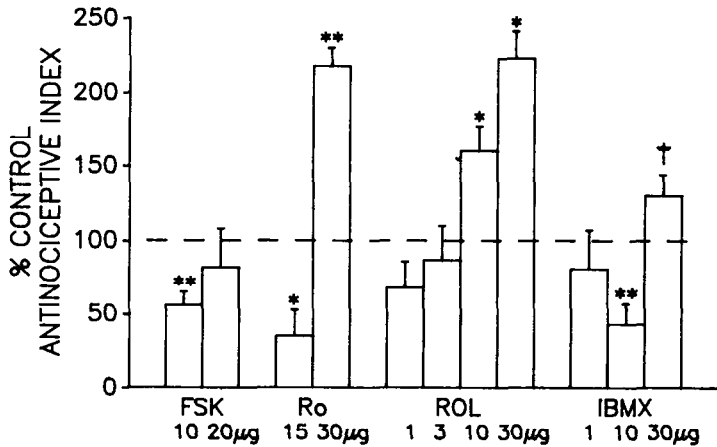
FIGURE 2. Effects of forskolin (FSK) and the phosphodiesterase inhibitors Ro 20 1724 (Ro), rolipram (ROL), and IBMX on the spinal antinociceptive action of morphine in the tail flick test. Doses of morphine were selected to produce a control Antinociceptive Index score of 8-12 sec. * $p<0.05$, ** $p<0.01$ (†$p<0.01$ in time course only). (Summarized from Nicholson et al., 1990.)

42

TABLE 1. Effects of forskolin and phosphodiesterase inhibitors on basal and morphine-evoked release of adenosine from dorsal spinal cord synaptosomes.

Drug Addition	Total	ADENOSINE RELEASE (pmoles/mg protein/15 min) Increase by treatment[a]	Morphine-evoked[b]
Ethanol control	312.2±17.8	-	-
Morphine (50μM)	347.3±17.4	-	35.1±11.0
Ro20-1724 (1μM)	327.7±19.1	15.5± 2.6	-
Morphine (50μM) + Ro 20-1724 (1μM)	329.0±20.8	-	1.3± 3.6*
Rolipram (1μM)	335.3±13.8	23.1±10.8	-
Morphine (50μM) + Rolipram (1μM)	337.7±16.4	-	2.4±10.3*
Ethanol control	289.3±24.5	-	-
Morphine (50μM)	322.9±26.8	-	33.7±10.5
Forskolin (1μM)	316.2±29.4	26.9± 7.8	-
Morphine (50μM) + Forskolin (1μM)	301.9±23.8	-	-14.3± 5.5*
Basal	204.0±11.5	-	-
IBMX (500/μM)	254.4±11.3	50.4±16.8	-
Morphine (50μM)	251.0±16.6	-	47.0± 8.4
Morphine (50μM) + IBMX (500μM)	271.0±11.2	-	16.6± 3.5*

Synaptosomes were incubated for 15 min in the absence or presence of drugs indicated, Values are mean ± s.e.m. for n=4-5. [a]Values calculated by subtracting basal release in presence of vehicle from release in presence of test agent. [b]Values calculated by subtracting adenosine release in presence of forskolin or phosphodiesterase inhibitors from that in the presence of morphine plus these agents. * p<0.05 (Nicholson, White and Sawynok, unpublished).

Pretreatment,with,pertussis toxin in vivo results in a significant inhibition of antinociception produced by i.t. administration of analogs of adenosine and morphine (Hoehn et al., 1988; Sawynok and Reid, 1988). Pertussis toxin also reduces release of adenosine induced by morphine both in vitro and in vivo (Sawynok et al., 1990). In behavioural experiments, i.t. preteatment with forskolin produces an inhibition of the action of CHA and NECA in the hot plate test (figure 1) and of morphine in the tail flick test (figure 2). The influence of phosphodiesterase

inhibitors on the antinociceptive effects of these agents is more complex. The non-xanthine phosphodiesterase inhibitors Ro 20 1724 and rolipram reduce the action of CHA but not NECA (figure 1). When tested against morphine (figure 2), Ro 20 1724 produced a biphasic effect, inhibiting the action of morphine at the lower dose and potentiating it at the higher dose. With rolipram, however, only an increase in effect was observed. IBMX, a methylxanthine phosphodiesterase inhibitor which also antagonizes adenosine receptors, reduces the action of morphine at a low dose (probably due to adenosine receptor antagonism) but increases it at a higher dose. In release experiments, forskolin, Ro 20 1724, rolipram and IBMX all enhance the basal release of adenosine (table 1) by releasing a nucleotide which is subsequently converted to adenosine (Nicholson, White and Sawynok, unpublished). Each of these agents appears to reduce the release of adenosine (which originates as adenosine per se rather than as nucleotide, Sweeney et al., 1987) evoked by morphine. However, the apparent reduction in release could indicate non-additivity between the two classes of agents even though the adenosine originates from a different source (nucleotide vs adenosine) in each instance.

CONCLUSIONS

Given the assumptions on which this series of experiments was based, the reduction in antinociception by morphine and in morphine-evoked release of adenosine produced by manipulating the adenylate cyclase system is consistent with the hypothesis that certain effects of morphine are due to inhibition of cyclic AMP production. However, potentiation of the antinociceptive action of morphine observed with the phosphodiesterase inhibitors also is consistent with stimulation of adenylate cyclase contributing to spinal actions of morphine (Crain and Shen 1990). Inhibition of the antinociceptive action of purines by agents which interact with the adenylate cyclase system suggest inhibition of cyclic AMP production may be involved, particulariy with CHA, the A1 selective agonist. However, the effects of these agents on purines do not appear to be reflected directly in a parallel alteration in the action of morphine, suggesting release of endogenous adenosine from the spinal cord by morphine is only one component of the spinal antinociceptive action of morphine. Thus, while methylxanthines reduce the spinal antinociceptive action of morphine in the tail flick and hot plate tests (Jurna 1984; DeLander and Hopkins 1986; Sweeney et al., 1987), methylxanthine-insensitive effects of morphine on transmission of afferent information in the spinal cord also have been observed (Jurna 1984). Opioid receptors are located on both pre- and post synaptic elements in relation to primary afferent nerve terminals in the spinal cord

(Gamse et al., 1979; Ninkovic et al., 1981), and actions at both sites are implicated in the spinal pharmacology of morphine. At presynaptic sites, opioids may stimulate (adenosine) or inhibit (substance P) the release of neuromediators which influence signalling of pain within the dorsal horn of the spinal cord.

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AUTHORS

Jana Sawynok, Ph.D.   Marva Sweeney, Ph.D
Donna Nicholson, M.Sc.   Dept. Pharmacology and
Tom White, Ph.D.   Clinical Pharmacology
Dept. Pharmacology   St. George's Hospital
Dalhousie University   Medical Scholl
Halifax, Nova Scotia   Cranmer Terrace
Canada, B3H 4H7   Tooting, London
                           UK SW17 ORE

Metabolic Studies of Drugs of Abuse

Nora D. Volkow, Joanna S. Fowler, Alfred P. Wolf, and Hampton Gillespi

INTRODUCTION

Positron emission tomography (PET) is a nuclear imaging technique that allows to measure the concentration of positron-labeled radiotracers in the human brain (Phelps et al., 1985). Since there are positron isotopes for the elements of life, it can be used to label compounds of physiological and chemical relevance without affecting their pharmacological behavior (Fowler et al., in press). In the investigation of brain function, the use of tracers that reflect brain metabolism and/or cerebral blood flow (CBF) can provide with indices of brain activity, since in the normal brain regional brain activation is accompanied by an increase in regional brain energy metabolism and cerebral blood flow (Greenberg et al., 1981; Silver, 1979). The most widely utilized tracers to assess brain function have been fluorine-18 and carbon-11-labeled deoxyglucose, an analog of glucose, to measure regional brain glucose metabolism (Reivich et al., 1982, 1985) and oxygen-15-labeled water to measure regional cerebral blood flow (Raichle et al., 1983).

Measurements of regional brain glucose metabolism and CBF have been used to assess brain dysfunction in various psychiatric and neurological diseases (Volkow et al., 1988a; Andreasen 1988). Since regional brain dysfunction is not necessarily paralleled by a change in regional anatomy or morphology, this strategy is more sensitive in detecting brain pathology than other available techniques such as CT scan and MRI (Volkow et al., 1987). In the investigation of toxic actions of drugs of abuse this is of relevance, since chronic drug exposure can lead to regional brain dysfunction without necessarily changing brain structure. Furthermore, because of the short half life of these tracers and the relatively low radiation dose, the studies can be repeated on the same individual permitting to monitor differences in brain function during drug addiction, drug withdrawal, and drug rehabilitation.

Measurement of the changes in glucose metabolism or CBF after

acute drug administration can be used to evaluate the areas of the brain that are activated by a given drug and thus can provide with information about the mechanisms of actions of the drug. Since these studies can be carried out in living subjects where the subjective emotional responses can be recorded, it allows to start to establish the relation between activation or deactivation in a given region and a specific behavioral response. Differences in regional brain activation secondary to acute drug administration between non-dependent and dependent individuals can be of use in investigating phenomena,such as drug predisposition, tolerance, and/or sensitization.

In this chapter we will describe the different studies done with PET to measure changes in brain glucose metabolism and/or CBF secondary to cocaine, alcohol, and marijuana.

Cocaine

We have measured regional brain glucose metabolism and CBF with PET in cocaine abusers to investigate the toxic properties of cocaine in the human brain.

We measured CBF using oxygen-15-water in a group of 20 chronic cocaine abusers and 24 normal controls (Volkow et al., 1988b). The cocaine abusers were tested twice during their hospitalization: initially within 72 hours of admission and 10 days after they have been withdrawn from cocaine. The cocaine abusers showed decreased CBF throughout the brain except in cerebellum. Decreases in CBF were more prominent in the frontal cortex and in the left parietal and left temporal cortex. The decreases in CBF in the cocaine abuser remained 10 days after cocaine withdrawal. The defects in CBF were interpreted as reflecting the vasoactive properties of cocaine. It has been shown that cocaine is a very powerful vasoconstricting agent (Isnert and Chokoshi 1989) and can lead to cerebral vessel vasoconstriction (Altura et al., 1985). The deleterious actions of cocaine on cerebral circulation is also documented by the clinical reports describing vascular strokes and hemorrhages secondary to cocaine intoxication (Levine and Welch 1987; Lichtenfeld et al., 1984).

Despite widespread decreases in CBF, most of these patients did not have evidence of neurological symptoms or neuropsychological impairment. This discrepancy could reflect the ability of the brain to increase nutrient extraction during decreased perfusion (Powers et al., 1985; Wide et, al., 1983). Deficits in nutrient delivery would occur only when CBF is severely impaired. To investigate the consequences of impaired CBF in brain energy utilization, we investigated 10 cocaine abusers both with [18]FDG and with oxygen-15-water (Volkow 1988). The [18]FDG scans showed less defects than those seen in the oxygen-15-water scans. Only 4 patients showed similar metabolic and CBF defects. Four patients showed no evidence of metabolic abnormalities despite defects in CBF. The discrepancies between the metabolic and the CBF images are probably due to the direct

actions of cocaine on cerebral vessels. The findings from this PET study demonstrate that chronic use of cocaine can lead to vascular pathology that, if severe, can lead to tissue ischemia and necrosis.

Alcohol

PET studies investigating the effects of chronic alcohol on brain have shown mixed results with two studies showing evidence of decreased metabolism in the brain of alcoholics (Sachs et al., 1987; Wik et al., 1988) and two failing to demonstrate changes in absolute metabolic values (Samson et al., 1986; Volkow et al., 1989). Discrepancy in results among investigators is probably a reflection not only of the differences in the clinical population (three studies excluded patients with neurological symptoms; whereas one did not (Wik et al., 1988)), but also to the period after alcohol withdrawal at which they were investigated. This is particularly relevant since animal studies have demonstrated increased metabolic activity during early alcohol withdrawal (Eckardt et al., 1986).

We have investigated 6 alcoholic patients and 6 normal controls with [18]FDG under baseline condition and upon challenge with acute alcohol administration (Volkow et al., 1990). Both the normals and the alcoholics showed decreased brain glucose metabolism during alcohol intoxication. Decreases in brain glucose metabolism were heterogeneous. The pattern of regional changes secondary to alcohol paralleled the pattern of the regional concentrations of benzodiazepine receptors in the human brain. The areas of the brain with the highest density of benzodiazepine receptors showed the largest reduction in brain glucose metabolism. It was, therefore, hypothesized that the decreases in regional brain glucose metabolism secondary to alcohol were due to the actions of acute alcohol on the benzodiazepine-Gaba receptor complex. When comparing the normal subjects with the alcoholics, we found no differences in the average metabolic values for both groups when tested during baseline conditions. However, three of the patients showed evidence of marked decreases in brain glucose metabolism in the cortex and cerebellum. Differences between normals and alcoholics were also demonstrated when comparing the response to acute alcohol administration. The alcoholics showed a much marked decrease in glucose metabolism after alcohol administration than did the normals. The mean difference between baseline-intoxication for the whole brain was 6±6 mg/l000g/min for the normals and 12±4 ng/l000g/min for the alcoholics. Since the decrease in brain glucose metabolism from alcohol was related to its action on the benzodiazepine-GABA receptor complex, we postulated that the increased responsivity to alcohol in the alcoholic was due to increased sensitivity of the benzodiazepine-GABA receptor complex.

A regression analyses revealed a relation between reductions in cortical metabolism and subjective response to alcohol

r = -0.8253 p ≤.01) (Volkow et al., 1989). A relation between
the behavioral actions of acute alcohol and the regional
changes in brain glucose metabolism was also documented by
another group of investigators (DeWit et al., 1989) who tested
8 normal controls during baseline and after 0.8g/kg of ethanol
p.o. This study reported a positive correlation between the
degree of negative moods during intoxication and increases in
regional brain glucose metabolism in the left temporal cortex,
left thalamus, and left cerebellum.

Marijuana

We have started to investigate the effects of acute marijuana
administration on regional brain glucose metabolism. We have
completed studies in 3 normal controls and 3 marijuana abusers.
Subjects were tested with FDG during baseline conditions and 40
minutes after intravenous administration of 2 mg of 9-delta-
tetrahydrocannabinol (THC). Acute marijuana administration
increased brain glucose metabolism in 4 subjects and decreased
it in two. All of the subjects showed increased metabolism in
the cerebellum after THC administration. Differences in average
metabolic values during intoxication were significant in
prefrontal cortex, cerebellum, and left basal ganglia (Volkow
et al., in preparation). The increased metabolic activity in
cerebellum from THC correlated with the subjective sense of
intoxication (r = 0.899 p ≤ .0l). The marijuana abusers
reported less subjective effects (on a scale ranging from 0-10)
from marijuana (x = 5±1) than normal controls (x = 7.5±3).
Their metabolic images also showed less changes in regional
brain metabolism than any of the three normal controls.
Predominance of effects from marijuana in cerebellum is in
accordance with the autoradiographic data which have shown
localization of the cannabinoid receptor in the cerebellum
(Herkenham et al., 1989).

SUMMARY

Although still very preliminary, these studies exemplify how
metabolic brain measurements can address questions of relevance
in the investigation of drugs of addiction such as:

1. Mechanisms of drug toxicity, i.e., vascular pathology
demonstrated from chronic use of cocaine.

2. Neurotransmitters that may be involved in the
pharmacological actions of drugs, i.e., alcohol and the
benzodiazepine-Gaba receptor complex, marijuana, and the
cannabinoid receptor.

3. Mechanisms of drug withdrawal, i.e., hyperresponsivity of
the brain to alcohol in the alcoholic, possibly as a
consequence of increased sensitivity of the benzodiazepine-Gaba
receptor complex.

4. Knowledge about brain function, i.e., the relation between

activation of the cerebellum by marijuana and alcohol and the
mood-changing effects of these drugs, suggests that the
cerebellum may play a role in the mood-disturbing actions seen
with these drugs (a role which is different from the classical
one which associates the cerebellum to motor regulation).

Future work will provide more definitive answers to the above
questions and will similarly provide information about
mechanisms of toxicity, addiction, withdrawal, and
reinforcement of other drugs of abuse.

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51

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AUTHORS

Nora D. Volkow, M.D., Medical Department, Brookhaven National
Laboratory, Upton, NY 11973
Joanna S. Fowler, Ph.D., Chemistry Department, Brookhaven
National Laboratory, Upton, NY 11973
Alfred P. Wolf, Ph.D., Chemistry Department, Brookhaven
National Laboratory, Upton, NY 11973.

Mapping the Metabolic Correlates of Drug-Induced Euphoria

Edythe D. London, Michael J. Morgan, Robert L. Phillips, June M. Stapleton, Nicola G. Cascella, and Dean F. Wong

Recent efforts using positron emission tomography (PET), a noninvasive approach to imaging biochemical processes in the brain, have been directed at understanding the biochemical and neuroanatomical correlates of drug-induced euphoria. Studies on the acute effect of psychoactive compounds on brain metabolism have used the $[^{18}F]$fluorodeoxyglucose (FDG) procedure (Phelps et al., 1979; Reivich et al., 1979), an extension of the $[^{14}C]$deoxyglucose method, which employs quantitative autoradiography in rats (Sokoloff et al., 1977). Both methods quantitate regional cerebral metabolic rates for glucose (rCMRglc). the major substrate for oxidative metabolism in the adult brain (Sokoloff, 1972; Siesjo, 1978). Although it was thought that glucose consumption directly reflects oxidative metabolism in the brain, recent findings have suggested that transient metabolic needs are met with nonoxidative glucose metabolism, adding to the utility of the deoxyglucose method in cerebral activation studies (Lear and Ackermann, 1989).

The deoxyglucose procedure has been used in numerous investigations of drug effects on rCMRglc in rats (McCulloch, 1982; Weissman et al., 1987; Wilkerson and London, 1989; Kimes and London, 1989). These studies have demonstrated unique patterns of response, which vary with the agents administered. For example, analgesic doses of mu opioid agonists reduce glucose metabolism primarily in thalamic nuclei associated with somatosensory processing (Fanelli et al., 1987). Cocaine stimulates glucose utilization in the extrapyramidal motor system and the nucleus accumbens, but reduces rCMRglc in the lateral habenula (London et al., 1986; Porrino et al., 1988). In general, these effects reflect the

distribution of relevant receptors (μ opiate receptors in the thalamus mediating effects of morphine; dopamine receptors in the extrapyramidal motor system, lateral habenula and nucleus accumbens, mediating the effects of cocaine). The most striking relationship between the density of receptors and the distribution of the metabolic response to specific agonists is seen in the case of nicotine, in which the drug stimulates rCMRglc in a pattern which is strikingly similar to the distribution of receptors identified with [³H]1-nicotine (London et al., 1985, 1988).

Metabolic studies with PET generally have not revealed anatomically discrete responses to psychoactive compounds in human volunteers. One reason for this discrepancy between studies in rats and humans may be the lack of resolution of PET as compared with quantitative autoradiography in animals. Human PET studies have been performed with barbiturates (Theodore et al., 1986), benzodiazepines (Buchsbaum et al., 1987; Foster et al., 1987), amphetamine (Wolkin et al., 1987), ethanol (de Wit et al., in press), morphine (London et al., 1990a), and cocaine (London et al., 1990b). The remainder of this chapter will focus on studies of human volunteers, who had a history of substance abuse, and participated in studies on the acute affects of morphine or cocaine.

Two studies were performed to test the acute effects of euphorigenic treatments with morphine and cocaine on rCMRglc in human volunteers (London, 1989: London et al., 1990a, 1990b). Subjects were right-handed males, between the ages of 21 and 45, with a history of use of opioids, cocaine, marijuana, and alcohol. At the time of the studies, none of the subjects were dependent upon any drugs other than nicotine (all but one were smokers). DSM III-R criteria were applied; the only diagnoses which were allowed were substance abuse and/or dependence, anti-social personality disorder, and borderline personality. Although the subjects manifested no frank pathology in medical or neurological tests, this population of subjects exhibited greater than normal levels of brain atrophy, as measured by the ventricle:brain ratio, which was significantly correlated with age in the second and third decade of life, reflecting alcohol binging behavior and not the use of other psychoactive compounds (Cascella et al., 1988).

Both studies used a double-blind crossover design. The subjects received either placebo or active drug (30 mg morphine i.m. or 40 mg cocaine i.v.) in random order at the time of PET studies. Prior to the PET studies, subjects participated in simulations in which they received placebo and two ascending doses of the active drug. The purpose of the simulations was to acquaint the subjects with the various questionnaires and to eliminate those subjects who had abnormal responses to placebo or active drugs.

TABLE 1. Morphine effects on rcmRglc (mg/lOOg/min)

Brain Region	Saline		Morphine Sulphate	
Cortical	Left	R i g h t	Left	Right
Superior frontal (11)*	8.8 ± 1.2	9.1 ± 1.0	7.5 ± 1.0	8.2 ± 0.9
Precentral (11)	8.8 ± 1.0	8.7 ± 0.9	8.2 ± 1.1	8.2 ± 1.0
Postcentral (11:)[*]	8.2 ±1.1	8.0 ± 0.9	7.4 ± 0.8	7.9 ± 1.0
Ant. cingulate[*]	9.7 ± 0.9		8.8 ± 0.9	
Superior temporal	6.4 ± 0.7	6.4 ± 0.7	5.8 ± 0.8	5.5 ± 0.8
Primary visual	9.0 ± 1.0	9.1 ± 1.4	8.6 ± 1.4	8.6 ± 1.3
Gyrus rectus[*]	8.3 ± 1.0		7.5 ± 1.1	
Subcortical				
Caudate nucleus	8.8 ± 1.2	8.5 ± 1.4	7.8 ± 1.2	7.7 ± 1.2
Hippocampus	5.7 ± 1.0	5.8. ± 0.9	5.2 ± 1.0	5.1 ± 0.7
Thalamus	7.4 ± 0.9	8.0 ± 1.0	7.2 ± 1.1	7.8 ± 1.0
Cerebellar cortex (9)	7.8 ± 0.9	1.4 ± 0.8	7.0 ± 0.6	6.9 ± 0.6
Cerebellar vermis (9)	7.0 ± 0.1		6.5 ± 0.6	
Whole Brain[*]	6.5 ± 0.6		5.9 ± 0.6	

Each value is the mean rCMRglc ± SD for n = 12, except as indicated by the number in parenthesis. Significant main effect of morphine (after partialling out the contribution of $PaCO_2$) by 2-way ANOVA, uncorrected for the number of comparisons, with drug and hemisphere as repeated measures, $p < 0.05$.

TABLE 2. Cocaine effects on rCMRglc (mg/lOOg/min)

Brian Region	Saline		Cocaine HCl	
Cortical	Left	Right	Left	Right
Superior frontal (11)[*]	7.3 ± 1.0	7.6 ± 1.1	6.5 ± 1.3	6.7 ± 1.2
Precentral	8.9 ± 1.2	9.9 ± 1.5	7.6 ± 1.6	8.3 ± 1.2
Postcentral*	8.1 ± 1.3	8.4 ± 1.3	6.8 ± 1.2	7.3 ± 1.1
Ant. cingulate*	9.7 ± 0.9		8.1 ± 1.1	
Superior temporal*	6.5 ± 1.3	6.5 ± 1.5	5.4 ± 1.1	5.1 ± 1.0
Primary visual	8.7 ± 1.4	9.1 ± 1.6	7.4 ± 1.2	7.8 ± 1.1
Gyrus rectus (11)*	8.3 ± 1.1		7.4 ± 0.9	
Subcortical				
Caudate nucleus (11)*	8.2 ± 0.9	7.7 ± 1.0	6.7 ± 1.3	6.7 ± 1.2
Hippocampus (11)*	5.8 ± 1.1	5.2 ± 0.5	5.0 ± 0.1	4.6 ± 0.5
Thalamus	6.8 ± 0.8	7.2 ± 1.0	5.8 ± 1.4	6.3 ± 1.5
Cerebellar cortex (11)*	7.7 ± 0.9	7.5 ± 0.8	7.1 ± 1.1	6.9 ± 0.8
Cerebellar vermis (11)	6.3 ± 0.6		6.3 ± 1.0	
Whole brain[*]	7.4 ± 0.8		6.7 ± 0.5	

Each value is the mean rCMRglc ± SD for n = 12, except as indicated by the number in parenthesis. Significant main effect of cocaine by 2-way ANOVA, uncorrected for the number of comparisons, with drug as the repeated measure, $p < 0.05$.

Morphine produced a significant euphorigenic effect, as indicated by several measures, including scores on the morphine benzedrine group (MBG) scale of the Addiction Research Center Inventory (Haertzen, 1974) and on visual analogue scales. Morphine also significantly reduced the global cerebral metabolic rate for glucose (CMRglc) by 10% in whole brain and rCMRglc in individual regions by about 5 to 15%, assuming no contribution of hypercapnia (Table 1). When the contribution of $PaCO_2$ was partialed out, significant morphine-induced reductions in glucose utilization persisted in whole brain and in six cortical areas. The results indicated that morphine-induced euphoria is associated with a reduction of cortical activity.

Cocaine also produced significant euphorigenic effects, as indicated by several measures, including scores on the MBG scale, visual analogue scales and components of the cocaine sensitive scale, which have been used in previous studies with i.v. cocaine (Sherer, 1988; Muntaner et al., 1989). Subjects reported being high and feeling the drug for at least 20 min after cocaine injection. They also reported rush and a pleasant feeling immediately after the injection. Cocaine significantly reduced glucose utilization by 9% in whole brain and in the majority of specific regions of interest (Table 2). These results indicated that, like morphine, cocaine-induced euphoria is associated with a reduction of cortical activity. In contrast to the effect of morphine, however, decrements in rCMRglc occurred in both cortical and subcortical regions.

The effect of cocaine on mood and CMRglc was negatively correlated with cerebral atrophy (ventricle:brain ratio). Subjects with the most cerebral atrophy showed the least cocaine-induced euphoria (Morgan et al., in press) and the smallest decrements in CMRglc. This finding agrees with a report that the effect of amphetamine on rCMRglc was blunted in normal and schizophrenic subjects with cortical atrophy (Wolkin et al., 1987), and is in line with our preliminary finding that a history of past cocaine usage is negatively correlated with cerebral atrophy. In contrast, the effects of morphine on mood and glucose utilization were not significantly related to cerebral atrophy.

Although cocaine and morphine produce vastly different behavioral effects (Grabowski, 1984; Jaffe and Martin, 1985), they produce a similar diffuse reduction in cerebral metabolism. This similarity suggests that a reduction of brain metabolism is a fundamental component of drug-induced euphoria. Support for this hypothesis comes from observations that every abused drug tested so far in human subjects has been shown to reduce rCMRglc, primarily in the cortex. Such findings have been obtained with barbiturates (Theodore et al., 1986), benzodiazepines (Buchsbaum et al., 1987; Foster et

al., 1987), amphetamine (Wolkin et al., 1987) and ethanol (de wit et al., in press) as well as morphine and cocaine. This hypothesis is also in concert with the theory of Cannon (1927) that affective states, which are of subcortical origin, can only be appreciated when sufficient release from cortical inhibition is achieved.

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AFFILIATION:

Addiction Research Center
National Institute on Drug Abuse
Baltimore, Maryland 21224

Effects of Ethanol, Diazepam and Amphetamines on Cerebral Metabolic Rate: PET Studies Using FDG

Harriet de Wit, John Metz and Malcom Cooper

This series of studies was designed to assess changes in cerebral metabolic rate after administration of psychoactive drugs, in particular drugs with some potential for abuse. The technique used to study cerebral metabolic activity was positron emission tomography, or PET, with a radio-labelled glucose analog (18-F-fluoro-deoxyglucose; FDG) as the tracer. With this technique, FDG is taken up preferentially in regions that are most metabolically active. The labelled substance becomes trapped in the cells, where it can be detected by the scanning device. In addition to identifying which regions are metabolically activated by psychoactive drugs, we sought to relate regional changes in metabolic activity to the drugs' mood-altering, or euphorigenic effects.

PET has several features that make it well suited for psychopharmacological studies. It is safe, relatively non-invasive, and subjects can be monitored in the awake, conscious state. Repeated testing of the same individuals yields highly reproducible estimates of regional cerebral metabolic activity of glucose (CMRglu; Bartlett et al, 1988). This makes it possible to test the same individuals under various conditions, such as while they are perceiving stimuli, experiencing drug effects, or performing tasks. In the studies reported here, normal volunteers were tested using a placebo-controlled, within-subjects design, in which we simultaneously measured both metabolic rate and mood.

Three studies, involving ethanol (ETH), diazepam (DZP) and amphetamine (AMP), are presented. These drugs were selected because they are known to have some potential for abuse while differing in many of their pharmacological effects. They are also drugs which have been studied extensively in our behavioral pharmacology laboratory, providing us with methodological experience and a source of comparative data. Because the behavioral and subjective effects of drugs are known to be influenced by the environment in which they are experienced, we were initially concerned that the environmental setting of the PET studies would attenuate or otherwise alter the subjective effects of psychoactive drugs. This concern could be addressed by comparing the subjective drug effects obtained in the PET setting to their effects in the more naturalistic settings used in other studies. Our experience also showed that there are wide individual differences in subjects' responses to psychoactive drugs (de Wit et al, 1989a,b), which might also occur in the PET studies. Relatively little attention has been given to individual differences in behavioral pharmacology, and they are often considered a hindrance. However, they can be used as a unique experimental tool with which to explore mechanisms of drug effects. One of our original goals in undertaking these experiments was to study individual differences in psychopharmacological effects using PET.

The PET studies reported here utilized normal healthy volunteers, most of whom had little or no history with illicit drugs. The rationale for using relatively drug-naive subjects, rather than individuals with histories of drug abuse, is that drug use history is known to influence subsequent responses to drugs, often in unknown or uncontrolled ways. Since normal volunteers exhibit orderly, drug-typical subjective and behavioral responses to relatively low doses of a variety of abused drugs, they provide a sensitive assay for the biological and psychological effects of drugs in humans (de Wit and Johanson, 1987). In the studies reported here, three groups of healthy males (n=8 per group) were tested with placebo and two doses of ETH, DZP or AMP.

METHODS
Subjects: Normal healthy males (21-29 years old) were recruited from the university and surrounding community. All subjects reported at least occasional alcohol use, but their prior experience with illicit drugs was minimal. They were screened for major medical or psychiatric disorders, including history of drug or alcohol abuse.

Procedure: Each subject underwent three PET scans at one week intervals, receiving, on each occasion, placebo, a low or a moderate dose of drug. The drugs were administered double-blind and in counterbalanced order across subjects. Subjects were told they might receive a stimulant, tranquilizer, placebo or alcohol. The drug doses were selected to be low enough to minimize potential non-specific, global drug effects on CMRglu (e.g., those associated with sleep), but high enough to produce modest euphoriant or mood-altering effects. Subjects in Study I ingested a beverage containing 0.5 g/kg or 0.8 g/kg ETH in sugar-free tonic and lime mix, or mix alone (placebo). Subjects in Study II ingested capsules containing DZP (5 or 10 mg) or placebo, and subjects in Study III ingested capsules containing d-AMP (5 or 10 mg) or placebo. Drugs were administered double-blind, and in Studies II and III placebo beverages were administered as well as capsules to maintain blind conditions. Drug pretreatment times were selected so that the drugs' onset and/or peak effects would coincide with the period of highest FDG uptake: ETH was administered 10 min before FDG injection, DZP 1 hr and AMP 2 hr before.

Subjects reported to the laboratory in the morning and a transmission scan was performed. They completed a pre-drug (baseline) mood questionnaire (Profile of Mood States; POMS; McNair et al, 1971) before taking any capsule or beverage. The POMS was also completed at intervals later in the sessions, together with a second questionnaire measuring drug liking, "high", and drug identification. POMS scores were analyzed by comparing pre-to-post-drug change scores on drug sessions to equivalent change scores on placebo sessions. On the drug effects questionnaire, scores after placebo administration were compared to scores after drug administration.

Intravenous catheters were inserted for administration of the isotope and collection of arteriolized blood samples. Blood samples were analyzed for glucose, tracer and drug levels. After insertion of the catheters, subjects briefly practiced a visual monitoring test (VMT), which they would be performing throughout the 40 min scanning period. The VMT consisted of brief bright and dim light presentations; subjects were instructed to press a hand-held button when the dim light was presented but not when the bright light was presented. The VMT provided an indication of gross drug-induced psychomotor impairment (accuracy and reaction time), and served to stabilize the subjects' behavioral and attentional state throughout the period of FDG uptake. Without a standardized

task to perform, subjects may vary widely in their cognitive activity during the scanning period, ranging from full wakefulness to sleep. Duara et al (1987) reported that within-subject variability across repeated scans was reduced by 60-70% when subjects were tested in an activated, as compared to resting, state.

After practicing the VMT task subjects completed the POMS. (In Study I this POMS determination provided the baseline (pre-drug) mood measure.) Subjects then ingested a 250 ml beverage, which contained ETH or placebo (Study I) or only placebo (Studies II and III). Immediately after consuming the beverage, subjects were placed in the scanner, using an individualized plastic face mask to stabilize head position. When positioned they began performing the VMT, and 5-10 mCi of FDG was injected. Subjects continued to perform the VMT for the 40 min period of the scan, pausing briefly at 20 min to complete the mood and drug effect questionnaires. They also completed mood and drug effect questionnaires after the scan was completed.

PET studies were performed in the "dynamic" mode (Huang et al, 1980), but the data reported here used the autoradiographic method (Phelps et al, 1979; Reivich et al, 1979). A 3-ring PETT VI scanner (Ter-Pogossian et al, 1982) was used. This provides five transaxial image slices with an intraslice spatial resolution of 8 mm (full width half maximum) and an interslice distance of 14 mm. CMRglu was assessed bilaterally in seven regions of interest, including four cortical regions (temporal, parietal, occipital and frontal), basal ganglia, thalamus and cerebellum. The regions were identified for each subject on the reconstructed images by a technician who was blind to drug condition. Global CMRglu was calculated by averaging all 14 regions. Regional CMRglu was calculated as a ratio of CMRglu in each region to CMRglu in the whole brain. Regional ratios from drug sessions were compared to regional ratios obtained after placebo to determine the effect of the drug.

RESULTS
Drug Effects on CRMglu
Global CMRglu (relative to placebo) was significantly decreased after one or both doses of ETH and DZP (Fig 1). Wide individual differences were observed in CMRglu AMP study, both on placebo sessions and after drug administration. Although there was an apparent decrease in CMRglu after the moderate dose, this was not representative of the group. Both doses of AMP increased CMRglu in half of the subjects and decreased it in the other half.

In all three studies, the drugs had similar effects in the 14 brain regions (Fig 2). Only the thalamus was relatively less affected than other brain regions in the ETH and DZP (but not the AMP) experiments. Regional changes in CMRglu were also examined as a ratio of changes in the rest of the brain. For each subject, normalized z-scores were calculated for each region (i.e., relative to the mean of all regions). The z-scores on placebo sessions were subtracted from z-scores on drug sessions to determine whether the drug treatment changed relative regional CMRglu. There was a good correspondence between the direction of relative regional changes produced by the low and those produced by the moderate doses of both ETH and DZP: In the ETH study, 10/14 regions changed in the same direction after the two doses, and in the DZP study, 11/14 regions changed in the same direction. Correspondence between the two doses of AMP, however, was only 5/14 regions. The relative regional changes in CMRglu were also compared across drugs by comparing relative regional ratios of the moderate dose of the three drugs. There was no consistent pattern across the three drugs,
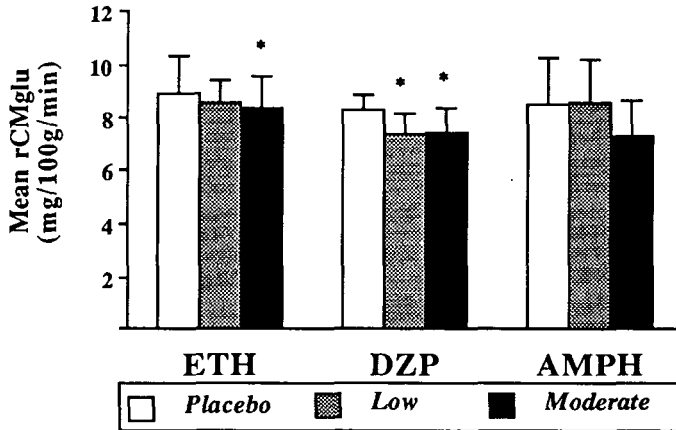
FIGURE 1 Mean global CMRglu in ethanol (ETH), diazepam (DZP), and amphetamine (AMPH) studies. Standard deviations are shown, and asterisks indicate significant difference from placebo.

although DZP and AMP appeared to have opposite effects on regional CMRglu in several regions: Relative to the rest of the brain and compared to placebo, CMRglu in the left basal ganglia and left and right thalamus was higher after DZP than AMP, and CMRglu in the left temporal cortex was lower after DZP than AMP.

Drug Effects on Visual Monitoring Task
None of the three drugs significantly impaired performance on the psychomotor task, as measured by reaction times, percent hits and false alarms. Thus, subjects remained alert and in a uniform behavioral state throughout each of the scanning periods.

Drug Effects on Mood
In all three drug studies, subjects reported increasing sedation over the course of the session, regardless of drug treatment. That is, even on placebo sessions their scores on Vigor, Friendliness and Elation scales decreased from pre-drug levels to 20 min into the scan, and scores on Confusion during this period increased. Moreover, subjects identified the substance they received on placebo sessions as "tranquilizer" on 50% of occasions.

The mood effects of the drugs relative to the subjects' mood changes after placebo are portrayed in Fig 3. ETH produced subjective effects typical of this drug in other settings (i.e., increases in POMS Positive Mood, Friendliness, Elation and Vigor scales). However, neither DZP nor AMP produced the full profile of subjective effects typically seen in more naturalistic settings. DZP decreased Anxiety, as is typical of this class of drugs, but relative to placebo sessions, it failed to increase sedation. AMP produced slight decreases in Confusion and increases in Vigor, but failed to produce significant effects on any POMS scale. Consistent with these modest subjective changes, subjects could not identify DZP or AMP.

Relationships between CMRglu and Behavioral Measures
Drug-induced changes in whole brain CMRglu were not related to other dependent measures, including subjects' demographic or drug use variables, or drug effects
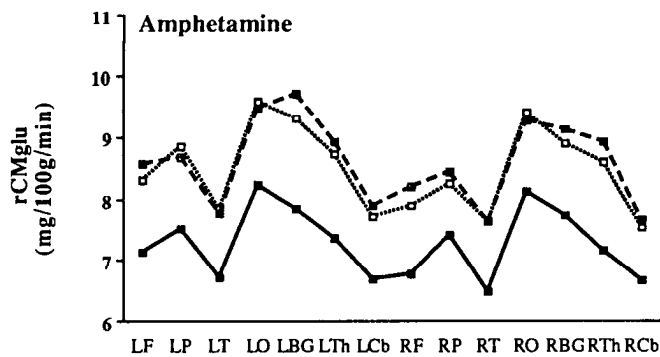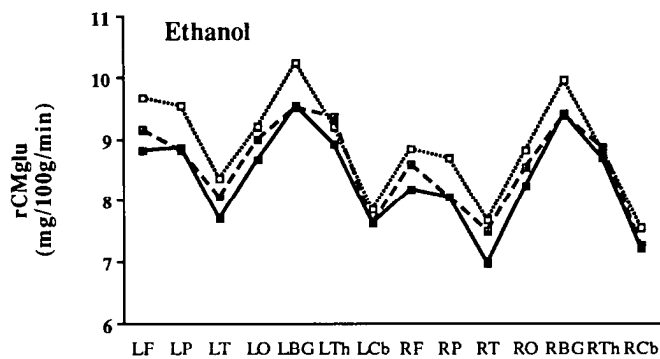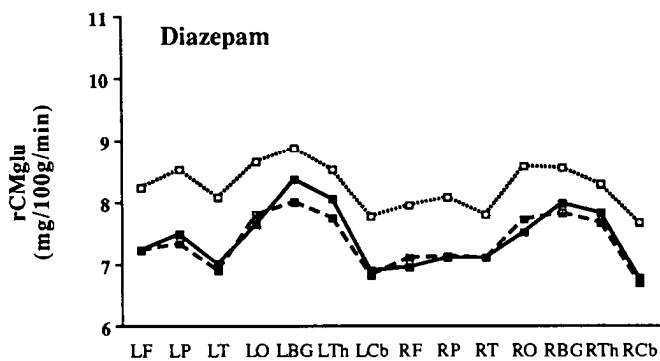
FIGURE 2 Mean regional CMRglu after placebo and low and moderate drug doses. Brain regions are, in the left hemisphere: frontal cortex (LF), parietal cortex (LP), temporal cortex (LT). occipital cortex (LO), basal ganglia (LBG), thalamus (LTh) and cerebellum (LCb), and corresponding regions on the right.
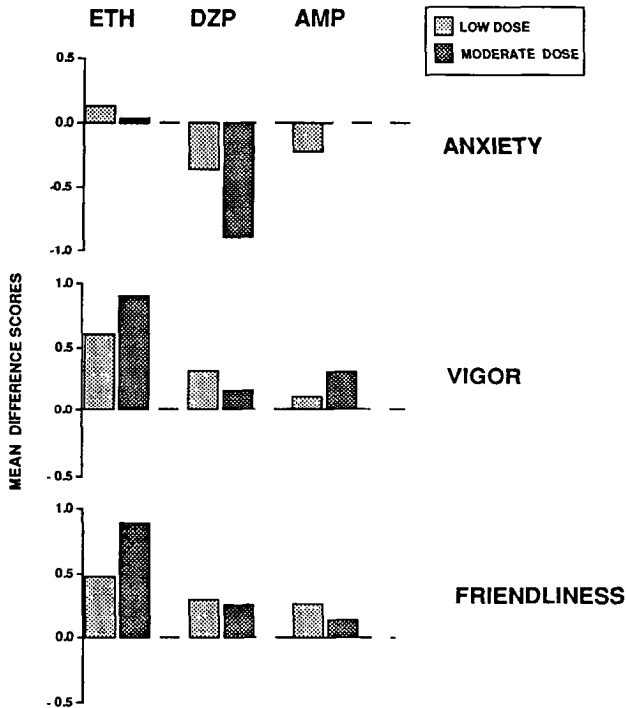
FIGURE 3 Effects of drugs on three POMS scales. Mean difference scores are pre-versus-post drug change scores on drug sessions, minus pre-post scores on placebo sessions.

on psychomotor performance or mood. The relationship between changes in regional CMRglu and changes in mood states and psychomotor performance were examined in correlational analyses for each dose of each drug. Although individually these correlational analyses suggested potentially interesting relationships between CMRglu and subjective drug effects, the nature of these correlations (i.e., direction and regions involved) varied across doses and across drugs, such that overall there was no consistent pattern. Changes in regional CMRglu after drug treatments were also not related to psychomotor performance.

DISCUSSION
ETH, DZP and AMP, three drugs which differ in mechanism of action as well as pharmacological, subjective and behavioral effects, all either lowered global CMRglu or had no consistent effect. Global decreases in CMRglu have been observed previously after ETH (Volkow et al, 1989), morphine (London et al, 1989), DZP (Foster et al, 1987), cocaine (London et al, 1988), barbiturates (Theodore et al, 1986) and AMP (Wolkin et al, 1987). Why these apparently dissimilar drugs decreased CMRglu and why there have been so few reports of pharmacological agents that increase CMRglu in humans is not clear. Interestingly, most of the drugs listed above are drugs with some potential for abuse, and the one centrally-acting drug without abuse potential that has been

tested in our laboratory, ACTH (Metz et al, 1989), produced an increase in CMRglu. There is clearly a need for research with drugs from a broader range pharmacological classes.

Whereas regional drug effects on CMRglu were obtained in some of the previous studies, (i.e., decreases in CMRglu reached statistical signficance in some regions and not in others), we did not find significant differences in magnitude of the decrease among the regions. The only exceptions were that the thalamus was relatively less affected by ETH and DZP than other brain regions (an effect previously reported by others (Volkow et al, 1989; London et al, 1989) and the left temporal cortex was relatively more affected by DZP. In both the ETH and the DZP studies, there was consistency in the direction of regional changes in CMRglu after the low and the moderate doses, suggesting that these drugs' regional effects, while small, were replicable. In contrast, in the AMP study there was no relationship between the regional effects of the low and the moderate dose, making interpretation of these results difficult.

In the present studies the drugs' mood-altering effects were measured during the PET scans. We found that while ETH produced mood-enhancing effects typical of this drug in other settings, the subjective effects of DZP and AMP were less than those observed in more naturalistic settings. Relative to placebo, DZP decreased anxiety but did not produce sedation typically seen with this drug, and AMP produced only slight increases in vigor. The attenuated subjective effects may have been related to the restricted PET environment, which appeared to have sedating effects in itself. Whether more robust relationships between CMRglu and mood would have been observed if these drugs had produced more drug-typical subjective effects remains to be determined.

The existence of some intersubject variability in both CMRglu and subjective drug effects enabled us to conduct correlational analyses between regional changes in CMRglu and mood. These analyses, however, revealed no consistent relationships between CMRglu and mood across the three drugs. It is possible that stronger drug effects or more heterogeneous subject populations would yield more consistent relationships.

In sum, our results indicate that at these doses, ETH and DZP produce global, but not regional changes in CMRglu. Further, while both drugs produced some subjective changes typical of their classes, these did not bear any obvious relation to the changes in CMRglu. AMP neither consistently changed CMRglu, nor produced significant subjective effects. Our findings may have been influenced by aspects of the methodology such as the doses of drugs tested, the behavioral task or the type of subjects used. Alternatively, it may be that PET using FDG as the tracer is not yet a sensitive enough technique to detect the anatomically and temporally limited effects produced by psychoactive drugs. Technological advances such as image correlation techniques and improved scanners currently under development will greatly enhance the power of imaging techniques.

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AFFILIATION Departments of Psychiatry and Radiology, The University of Chicago, Chicago IL 60637

Measurement of Brain Ethanol Concentrations in Humans With In Vivo Proton Magnetic Resonance Spectroscopy

Jack Mendelson, Bryan T. Woods, Tak-Ming Chiu, Nancy K. Mello, Scott E. Lukas, Slew Koon Teoh, and Pradit Sintavanarong

INTRODUCTION

Recent technologic advances in Magnetic Resonance Imaging and Spectroscopy have made possible more precise detection and measurement of the ethanol molecule methyl proton group. Figure 1 presents a summary of procedures employed for the measurement of alcohol in human brain with in vivo proton magnetic resonance spectroscopy. During basal conditions protons in atomic nuclei spin in a random manner. When placed in a strong magnetic field (1.5 T) the spin of nuclei are aligned parallel to the magnetic field. Radio waves are then broadcast into the magnetic field in order to perturbate the atomic nuclei. When the radio waves are terminated the nuclei realign in the magnetic field and release energy which can be subsequently recorded and quantified. Computer programs are used for generating a spectra and these spectra are subsequently converted by appropriate mathematical procedures for precise display and analysis. The manner in which radio waves are broadcast to perturbate nuclei in the magnetic field including the frequency or sequence of radio wave broadcast (TR-repetition time) as well as the techniques for collecting and recording released energy following cessation of radio brain wave broadcast (TE-echo time) are important parameters for determining the specificity and quality of spectral acquisition.
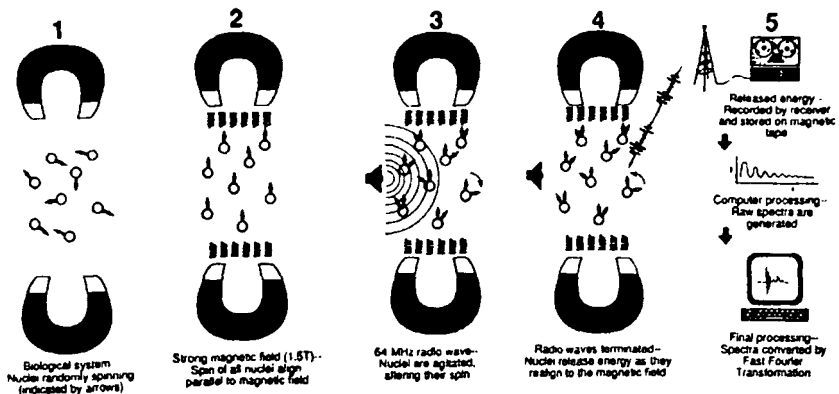
Figure 2 shows the structural formula for N-acetyl aspartate (N-AA) and ethanol. The methyl protons of each compound (shown circled) permit in vivo MRS determination of these compounds in brain. The spectrum displayed at the bottom of Figure 2 shows four major peaks: creatine/phosphocreatine (3.0 ppm), choline (3.2 ppm), N-AA (2.00 ppm) and ethanol (1.2 ppm). Because the concentration of N-AA (Hetherington et al., 1989) in grey and white matter of brain is relatively constant (7 mM) and stable, it can be used as an internal standard for determining the concentration of ethanol in brain.
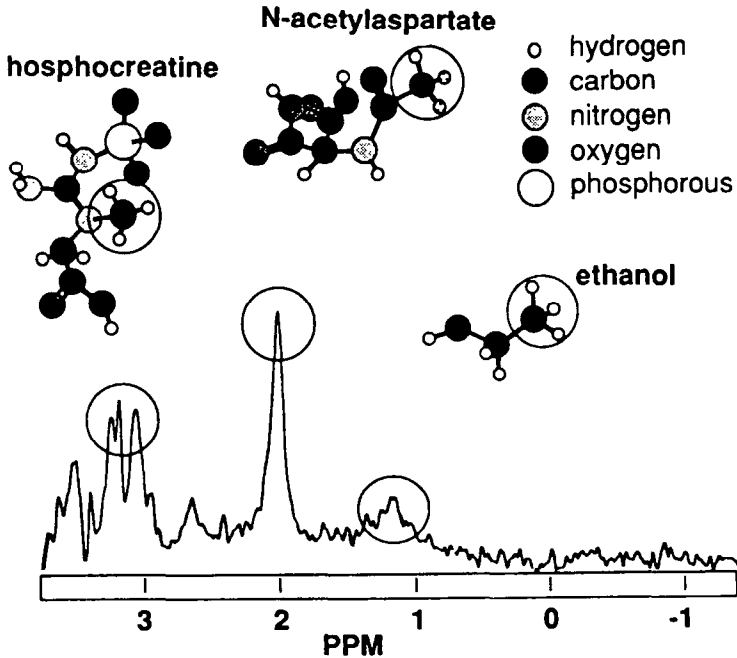
Figure 2

We have employed in vivo proton magnetic resonance spectroscopy for measuring alcohol concentrations in regional portions of the human brain. Brain ethanol concentration as well as gas chromatographic measures of ethanol levels in plasma were obtained during the ascending, peak and descending blood ethanol curves. Blood plasma samples were also obtained for determination of ethanol effects on plasma levels of anterior pituitary, gonadal and adrenal hormones. During these procedures alcohol-induced changes in mood states were measured simultaneously utilizing a nonferrous instrumental mood report device.

METHODS

Six healthy adult males between the ages of 23 and 26 (mean age 24.3 ± 0.4 years) provided informed consent for participation in this study. All subjects were of normal weight (77.5 ± 3.7 kg). All subjects reported occasional social alcohol use (2 to 6 drinks week). No subject had a history of alcohol or drug abuse and dependence nor any family history of alcoholism. All had normal physical examinations, blood hemogram and blood chemistry studies. All urine drug screens were negative at initial evaluation. Following an overnight fast, subjects reported to the McLean Hospital Brain Imaging Center. On arrival, subjects provided a urine specimen for drug screening and all were negative. A Korwarski-Cormed butterfly catheter was inserted into the antecubital vein for blood sampling. Blood alcohol levels were determined with gas chromatographic procedures.

Mood Report Measures

Subjects were given a nonferrous instrumental device to report changes in mood states following alcohol intake (Lukas et al., 1990). The device is activated by air pressure changes, and specific details of its construction and operation have been described elsewhere (Lukas et al., 1989). Subjects held the device in their right hand and were asked to depress one of three air modulated switches when they experienced the following effects of alcohol: initial detection of an alcohol-induced change in feeling state; feelings of euphoria; feelings of dysphoria. Subjects were told to depress the switch for as long as they experienced the particular change in mood state. All responses were registered on a cumulative recorder which was located approximately 6 meters from the whole body imager.

^{1}H Spectroscopy

Magnetic resonance imaging (MRI) and spectroscopy (MRS) procedures were carried out with a G.E. Signa 1.5T (General Electric, Milwaukee, Wisconsin) whole body imager. Subjects were supine on the MRI table and a reference position (the intersection of the axial and sagittal light beams) was located at the glabella. The chosen voxel of interest (VOI) was localized based on a series of Tl weighted (TE = 30 ms, TR = 600 ms) coronal and sagittal images. The VOI utilized in all studies included the medial frontal and cingulate gyri, the ventricles, the medial portion of the basal ganglia, the centrum semi ovale at the level of the anterior commissure, and the splenium of the corpus calosum. Voxel localization was achieved with the STEAM pulse sequence (Frahn et al., 1989; Narayana et al., 1989), and the magnetic field homogeneity was optimized by shimming on the water signal. Typically, a water line width of 4-8 Hz can be achieved over a voxel size of 4 x 4 x 4 cm in less than 10 min. Water suppression was achieved with a pre-sat pulse and an additional sat pulse between the second and third slice selective RF pulses of the STEAM sequence.

After a control spectrum was obtained for the chosen VOI, the subject sat up and drank the alcohol solution, a mixed drink of orange juice and vodka (0.7 gm alcohol per kg body weight) over a period of approximately 15 min. After completion of alcohol intake, the subject was repositioned, using the reference markings at the glabella, and the VOI was re-shimmed. Typically 128 scans were collected at an echo time TE = 270 ms, mixing time TM = 90 ms and repetition time TR = 1500 ms. The free induction decays (FID) were processed on the 1280 spectroscopy data station (General Electric, Milwaukee, Wisconsin) with

exponential line broadening (1 Hz), zero-filled to 2K points and Fourier transformed. Brain ethanol (ETOH) concentration was determined by measuring the areas under the N-acetyl aspartate (N-AA) and ethanol peaks, and then multiplying the ratio ETOH area/ N-AA area by 7 (the reported brain N-AA concentration in mM).
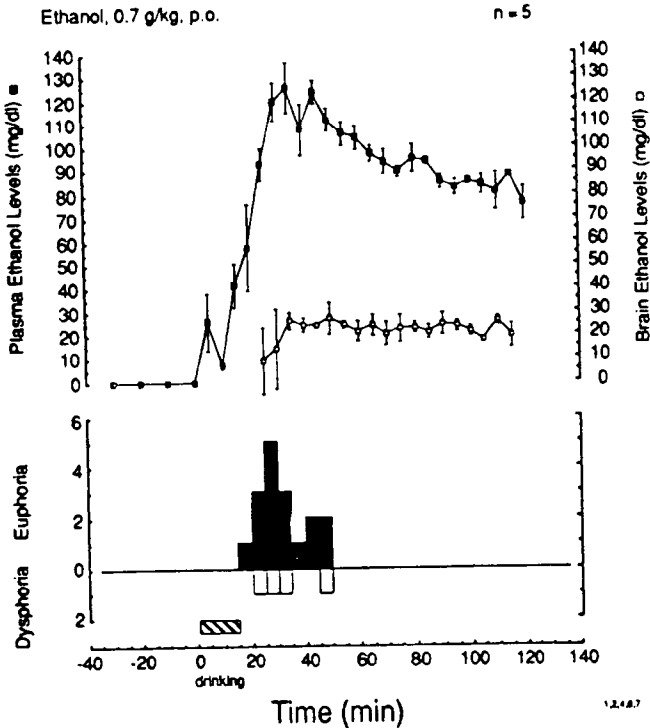
Figure 3

Figure 3 shows mean (±. S.D.) plasma alcohol and brain alcohol levels for 5 subjects based upon the N-AA concentration as an internal standard. However, the calculated brain alcohol levels as revealed by MRS were 4 to 6 fold lower than blood alcohol levels. Peak blood alcohol levels of 125.67 ± 10.91 mg/dl occurred within 35 min after initiation of drinking. The blood alcohol peak occurred 15 min earlier than the peak level of alcohol measured in brain. Figure 3 also shows the time and frequency of reports of euphoria and dysphoria by this group of 5 subjects (bottom panel). Euphoria reports began during the ascending limb of the brain and blood alcohol curve. The most frequent reports of euphoria occurred within 25 to 30 min after alcohol consumption began, just before peak alcohol concentrations were measured in plasma and in brain. Episodic reports of dysphoria also occurred primarily during the ascending and peak phases of the blood alcohol curve.

71

Effects of Altered Echo Time (TE) on Ethanol Spectra
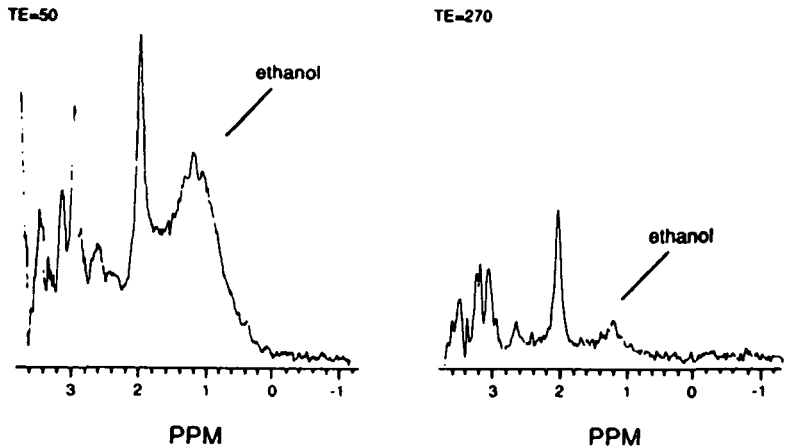
Figure 4

Figure 4 shows the effects of altering the echo time (TE) on alcohol spectra. The echo time initially used for determination of alcohol concentration in brains of the 5 subjects who participated in this study was 270 ms. The TE 270 spectrum for an additional subject is shown on the right hand panel. When the TE was reduced to 50 ms, a significantly enhanced recovery of the ethanol peak in relation to the N-AA peak was observed.

DISCUSSION

The findings obtained in this study are in agreement with previous reports of the feasibility of employing MRS for *in vivo* determination of alcohol levels in regional portions of the brain (Hanstock et al., 1988; 1990, Hetherington et al., 1989). Our findings are also consistent with previous reports that a considerable portion of alcohol in brain following drinking may be *invisible* during MRS (Hetherington et al., 1989; Moxon et al., 1989). If this problem were not resolved, the potential application of MRS for examining *in vivo* brain alcohol concentrations quantitatively would be severely limited. However, partial recovery of the total ethanol peak may be achieved at a shorter echo time (TE) through reducing signal loss by spin relaxation (T_1 and T_2). Although it is conceivable that some ethanol molecules in brain are situated (e.g. between inner and outer lipid membrane layers) so as to be undetectable by MRS, it is also possible that varying acquisition parameters (e.g. shortening TE) may lead to a considerably greater recovery of the ethanol signal. We are currently investigating means to achieve fuller signal recovery and more precise quantitative measurement. With more accurate and reliable measures of regional brain ethanol concentrations via MRS, it will now be possible to compare and contrast brain alcohol concentrations and blood alcohol levels over relatively short time intervals during the ascending, peak and descending blood alcohol curves.

Our data also provide new evidence that concurrent assessments of mood states and behavior prior to, during and following alcohol intake are possible during sequential measurements of *in vivo* alcohol concentrations in brain with MRS. The occurrence of <u>both</u> euphoria and dysphoria reports during the ascending phase of the brain and blood alcohol curve illustrates the often contradictory and complex subjective effects of alcohol intoxication (Mello 1983). Rapid and transient changes in mood states during the ascending phase of the blood alcohol curve correlated with measurements of alcohol-induced changes in neurophysiologic and neuroendocrine function in our previous study (Lukas and Mendelson 1988). These data converge to suggest that it will be feasible to concurrently assess changes in mood state and neuroendocrine function during measurement of alcohol concentrations in regional portions of the central nervous system. It will also be possible to examine alcohol-induced changes in regional brain electrical activity similar to studies previously reported by our Center (Lukas et al., 1989) by using nonferrous EEG electrodes when subjects are within the whole body imager. The simultaneous measurement of behavioral, neurophysiologic and neuroendocrine function should permit a more precise understanding of how alcohol affects brain function and behavior. This multidisciplinary approach may also provide new insights into mechanisms underlying alcohol-induced tolerance and dependence.

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AUTHORS

Jack H. Mendelson, M.D.; Bryan T. Woods, M.D.[1]; Tak-Ming Chiu, Ph.D.[1]; Nancy K. Mello, Ph.D.; Scott E. Lukas, Ph.D.; Siew Koon Teoh, M.D.; Pradit Sintavanarong, M.D. Alcohol and Drug Abuse Research Center/Department of Neurology and Brain Imaging Center[1], Harvard Medical School/McLean Hospital, 115 Mill Street, Belmont, MA 02178

Immunological Function in Active Heroin Addicts and Methadone-Maintained Former Addicts: Observations and Possible Mechanisms

Mary Jeanne Kreek

From the mid-1960's multiple observations suggesting altered immune function, including lymphadenopathy, lymphocytosis, elevated levels of immunoglobulins and altered T-cell function as measured by T-cell rosette formation, were made both in heroin addicts and in former heroin addicts entering the newly developed methadone maintenance treatment programs for heroin addiction (Kreek 1972; Kreek et al 1972; Kreek 1973a; Kreek 1973b; Kreek 1978, Kreek 1987). These findings have been extensively reviewed recently (Kreek 1989; Kreek 1990; Novick et al 1990). Early prospective studies of patients in methadone maintenance treatment suggested some improvement in immune function with respect to each of the indices mentioned. It was shown that although chronic liver disease is extremely common in heroin addicts, both due to the sequelae of hepatitis infection, (including hepatitis B, non-A, non-B, and probably C, and since the mid-1970's, also delta), and also alcohol abuse, with over 90% of addicts in many studies having markers of hepatitis B infection and from 25 to 50% a history of alcohol abuse, nevertheless, the abnormalities of immune function could not (and still in recent studies cannot) be attributed to liver disease alone (Kreek 1972; Kreek et al 1972; Kreek 1973a; Kreek 1973b; Kreek 1978; Novick et al.,1985; Kreek et al 1986; Novick et al 1986a; Novick et al 1986; Kreek 1987; Novick et al 1988a; Novick et al 1988b; Novick et al., 1989a; Kreek et al 1990).
Studies of immunologic function in heroin addicts and in former heroin addicts have become much more difficult to perform and more complex to interpret since the advent of the AIDS epidemic, first identified in 1981, but which, based on retrospective studies of sera banked in our laboratory and subsequently in the laboratory of others, began in the mid-1970's, with rapid escalation between 1978 to 1983 in New York City (Des Jarlais et al 1984; Novick et al 1986; Novick et al 1988; Des Jarlais et al 1989; Novick et al 1989; Kreek et al 1990).

From the time of the delineation of the specific opioid receptors in 1973, and the discovery of the endogenous opioids in 1975, speculations arose as to the possible role of the endogenous opioid system in a wide variety of physiological functions and pathological states, including possibly in normal and abnormal immune function. In these studies, questions were raised about the possible role of exogenous opiates and also of other drugs of

abuse (and possibly also of pharmacotherapeutic agents used in the treatment of drug abuse or addiction) in altering immune function. Also the related questions of whether any of the observed effects were direct opiate effects on cellular or humoral immune function or possibly due to indirect effects of opiates have been raised by our group and others.

Many studies have documented that the pharmacokinetic properties of methadone are significantly different from those of short-acting narcotics such as heroin and morphine in humans, though not necessarily significantly different in other species such as the rat (Kreek 1973; Kreek et al 1976; Hachey et al 1977; Kreek 1979; Kreek et al 1979; Nakamura et al 1982) Since racemic methadone has a half-life of over 24 hours in humans and its active enantiomer a half-life of over 48 hours, a steady state of opioid at specific receptor sites of action may be achieved. This may contribute to the normalization observed during methadone maintenance treatment of many aspects of physiological function, such as neuroendocrine function which is usually significantly abnormal during cycles of heroin addiction and sometimes remains deranged during other modes of treatment for heroin addiction (Kreek 1972; Kreek 1973; Kreek 1978; Kreek et al 1981; Kreek et al 1983; Kosten et al 1986a; Kosten et al 1986b; Kreek 1987). This different pharmacokinetic profile of methadone may also contribute directly or indirectly to the normalization of immune function which has been observed to occur partially or even completely in short or long-term methadone maintenance treatment patients (Kreek et al 1986; Kreek 1989; Kreek et al 1989; Novick et al 19891 Ochshorn et al 1989; Novick et al 1990; Kreek 1990).

Many studies from our laboratory have focused on natural killer cell (NK) activity. Since natural killer cells have cytotoxicity activity against many types of tumor cells and some viral infections, without prior exposure to foreign agents required for activity, they may provide the first line of defense in many pathological settings. Early (1978) unpublished work from our laboratory (Lavie, Franklin and Kreek) suggested that natural killer cell activity is significantly reduced in heroin addicts, a finding subsequently confirmed by several groups including our own (Kreek 1989; Ochshorn 1989; Kreek et al 1989; Novick et al 1989; Kreek 1990; Novick et al 1990). In a very carefully controlled study, natural killer cell activity was shown to become normalized, along with normalization with other indices of immune function, during very long-term (11 years or more) methadone maintenance treatment (Novick et al 1989; see Figure) Similar findings of normalization of natural killer cell activity along with normalization of some other indices of immune function in some, but not all, patients in methadone maintenance treatment for varying periods of time, with or without ongoing alcohol, cocaine, or polydrug abuse, have also been found (Ochshorn et al 1989; Kreek et al 1989; Kreek 1990)

Laboratory studies from our group published, or in progress, suggest that pharmacological agents and opioid antagonists, do not have any direct effects of lowering (or increasing) natural killer cell activity at pharmacological concentrations as studied in vitro: also it has been shown that endogenous opioids bound to

natural killer cell apparently do not exert a significant modulating effect as studied in vivo (Ochshorn et al 1988, Kreek, 1989, Kreek 1990; Ochshorn et al 1990). Studies of possible effects of alcohol as well as other natural substances on natural killer cell activity are in progress in our laboratory. Recent studies from our laboratory conducted in normal healthy volunteer subjects, support our earlier hypothesis that neuroendocrine function especially function of the hypothalamic-pituitary- adrenal axis, may modulated natural killer cell activity normally and that any disruption may contribute to abnormalities of natural killer cell activity such as those observed in heroin addicts (Bodner et al 1990).

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NK activity and lymphocyte subsets

Results are mean ± S.E.M. Normal ranges: lymphocyte count, 1.5 to 4.0 × 10^9/l; CD2-positive, 0.9 to 2.7 × 10^9/l; CD4-positive, 0.4 to 1.55 × 10^9/l; CD8-positive, 0.15 to 0.7 × 10^9/l. Normal ranges not established for other parameters.

	Current Heroin Abusers	Long-Term Methadone Maintenance Patients	Apparently Healthy Individuals
NK activity (100:1)[a]	36.4 ± 3.1*	56.9 ± 4.1	63.7 ± 3.6
NK activity (50:1)[a]	26.6 ± 2.3*	47.3 ± 4.9	51.6 ± 4.2
NK activity (25:1)[a]	17.2 ± 1.6*	34.8 ± 4.6	38.3 ± 4.3
No. of lymphocytes (×10^9/l)	3.68 ± 0.49*	2.08 ± 0.15	2.21 ± 0.16
No. CD2-positive cells (×10^9/l)	2.70 ± 0.39*	1.36 ± 0.12	1.43 ± 0.13
No. CD3-positive cells (×10^9/l)	2.61 ± 0.36*	1.18 ± 0.10	1.26 ± 0.13
No. CD4-positive cells (×10^9/l)	1.50 ± 0.17*	0.89 ± 0.06	0.82 ± 0.10
No. CD8-positive cells (×10^9/l)	0.97 ± 0.17*	0.47 ± 0.04	0.48 ± 0.05
No. Leu-7 positive cells (×10^9/l)	0.40 ± 0.17	0.21 ± 0.06	0.26 ± 0.06
No. Leu-11a positive cells (×10^9/l)	0.27 ± 0.08	0.26 ± 0.09	0.41 ± 0.09

[a] Values are percentage of cytotoxicities. Numbers in parentheses, effector-target ratios.
* Significantly different from the other two groups (P < .01).

Immunomodulatory Activity of Kappa-, Mu-, and Delta-Selective Opioid Compounds

Thomas J. Rogers, Dennis D. Taub, Toby K. Eisenstein, Ellen B. Geller, and Martin W. Adler

INTRODUCTION

Opioids have been found by several investigators to exert significant immunomodulatory activity when measured either in vivo or in vitro. Both exogenous and endogenous opioid agonists have been shown to alter immune function, and opioid-like receptors have been described on both murine (Carr *et al.,* 1989) and human (Wybran *et al.,* 1979) lymphoid cells. These findings support the notion that regulatory pathways exist between the immune system and the central nervous system.

In the present study, we have attempted to identify the nature of the immunomodulatory activity of a variety of well characterized opioid compounds. The results from these experiments provide valuable information concerning the type of opioid receptor involved in the interaction of opioid compounds with the cells of the immune system.

MATERIALS AND METHODS

Animals. BALB/cByJ, C57BL/6J, B1O.BR, B1O.A(5R), C3H/HeJ, C3H/FeJ and CBA/J mice were obtained from the Jackson Laboratories, Bar Harbor, ME. Mice were used between the ages of 8 and 12 weeks. All mice, except the C3H/FeJ strain, were male.

Proliferative response in vitro. Single cell suspensions of splenocytes, at a final concentration of 8×10^5 cells per culture, were placed in culture in a tissue culture medium consisting of minimal essential medium supplemented with 10% fetal calf serum. Designated agonists or antagonists were added at culture initiation along with a mitogenic stimulus. The mitogenic stimulus consisted of staphylococcal enterotoxin B (SEB) at 1 µg/ml, or phorbol myristate acetate with A23187 at 2 µg/ml and 1 µM, respectively. Cells were cultured for two days prior to the addition of ^{3}H-thymidine, and the cells were harvested for analysis on day 3.

Generation of antibody in vitro. The technique for generation of antibody *in vitro* has been described in detail elsewhere (Donnelly and Rogers 1983). Briefly, splenocytes were obtained from mice

which were Immunized two weeks previously with sheep erythrocytes. These immune splenocytes were placed in culture with the specific antigen (sheep erythrocytes), along with the designated opioid compound, and the antibody response was measured on day 5. The assay for antibody production involved the use of a complement-mediated plaque assay. This assay permits a determination of the number of specific antibody-producing cells at the termination of the culture.

Treatment with designated opioid antagonists. Studies were carried out with either naloxone or norbinaltorphimine (norBNI) by first treating splenocyte suspensions with the antagonist for a period of 1 hr at 37°C, followed by addition of the designated opioid agonist. Control cultures containing the antagonist in the absence of agonist were also established.

RESULTS AND DISCUSSION

Experiments were carried out to determine whether opioid agonists which interact with the mu receptor have, any effect on the capacity of murine T cells to respond to a mitogenic signal. These experiments were conducted with PMA, an analog of diacylglycerol which triggers the translocation of protein kinase C. PMA activates a variety of immune cell populations, but primarily stimulates the proliferation of T cell populations. Experiments were also carried out with the polyclonal T cell mitogen SEB. This compound stimulates virtually all T cells which bear certain, but not all, T cell-receptor classes. The activity of SEB is much more limited, in that only T cells appear to be activated and, as expected, only T cells proliferate. The capacity of splenocytes to respond to these mito-genic agents in the presence of the mu agonists morphine and DAMGE was determined in several experiments. The results from a representative experiment (Table 1) show that both morphine and DAMGE inhibit the proliferative response to either PMA or SEB. The degree of inhibition of the response to PMA was typically greater than that to SEB, and in addition, these opioid agonists were at least 100 times more potent inhibitors of the PMA response.

Experiments were also carried out to determine the capacity of the kappa-selective agonist U50,488H to modulate the proliferative response. The results (Table 1) show that the proliferative response to both PMA and SEB is inhibited by U50,488H and, moreover, that this kappa-selective agonist is much more potent than either of the mu-selective agonists. The inhibition of the response to SEB is particularly striking in this regard.

In an effort to characterize further the inhibitory activity of the mu- and kappa-selective agonists, experiments were carried out to determine the capacity of opioid antagonists naloxone and norBNI to reverse the activity of morphine and U50,488H. The results from representative experiments (Table 2) show that naloxone, a rather non-specific antagonist, reverses the activities of both morphine and U50,488H. On the other hand, the kappa-selective antagonist norBNI reverses the activity of U50,488H but not morphine. These results suggest that the inhibitory activity of morphine is mediated largely via opioid receptors other than the kappa receptor. The results represent the first evidence that kappa-receptor agonists mediate

significant immunomodulatory activity. In addition, there is also evidence to suggest that interaction with one or more of the other opioid receptor types results in an immunosuppressive effect.

A caveat in the interpretation of the proliferative response results described above is that the response of lymphoid cells to mitogenic agents *in vitro* is rather artificial. While this caveat is inherent in carrying out studies *in vitro,* there is also the possibility that the process of response to these mitogenic agents may in some way be artificial. Partly for these reasons, we have carried out a series of experiments to determine the effect of opioid compounds on the antibody response. Our results (Table 3) show that kappa-receptor agonists U50,488H and U69,593 are potent inhibitors of the antibody response and that these agents are at least 100 times more potent than the mu-receptor agonists morphine and DAMGE. The delta-receptor agonist DPDPE failed to exhibit any modulatory activity for the antibody response in several experiments.

Studies carried out to characterize the modulatory activity of these opioid agonists show that, as expected, the inhibitory activity of both morphine and U50,488H is reversed by naloxone (Table 4). On the other hand, the kappa-selective antagonist norBNI exerts an effect only for U50,488H. In addition, the effect of U50,488H exhibits stereospecificity. in that the (-)isomer is at least 100 times more potent an immunomodulator than the (+)isomer (Table 5).

All of the experiments described above were carried out with the BALB/c mouse strain. This strain was selected because it is a particularly good responder in both the PMA- and SEB-driven proliferation and antibody-forming responses. Evaluation of several mouse strains has revealed a remarkable degree of variability in the response to the opioid agonists morphine and U50,488H. These experiments have provided results which suggest that these mouse strains can be classified into two groups (Table 6). The first group, composed of BALB/c, C57BL/6 and B10.A(5R), are particularly sensitive to the effects of these opioid agonists. The second group, composed of B10.BR, C3H/HeJ, C3H/FeJ, and CBA/J, are much less sensitive. Of particular interest is the fact that B10.BR and B10.A(5R) fall into different groups, since these strains are congenic and only differ within the major histocompatibility complex.

Our strain analysis also included the CxBK/ByJ mouse strain, which is known to be deficient in mu opioid receptors (Moskowitz *et al.,* 1985; Vaught *et al.,* 1988). The results showed that both U69,453 and U50,488H exhibited significant immunomodulatory activity in both the CxBK/ByJ and the parental C57BL/6 mouse strains. On the other hand, the mu-receptor agonists morphine, PLO17 and DAMGE all failed to exert any immunosuppressive effect in the CxBK/ByJ strain. Additional experiments showed that the immunosuppressive activity observed with the kappa agonists U50,488H and U69,453 was reversed by norBNI in both the CxBK/ByJ and C57BL/6 mouse strains (data not shown). We also assessed the immunomodulatory activity of DPDPE in the CxBK/ByJ and C57BL/6 strains. The results (Table 7) are consistent with data obtained with BALB/c mice in that this delta agonist exerts little immunosuppresssive activity under these experimental conditions.

SUMMARY

1. Morphine, DAMGE and U50,488H each inhibit the *in vitro* prolifera-
tive response of murine splenocytes to the mitogenic agents PMA or
SEB. The kappa agonist U50,488H is much more potent than either of
the mu-receptor agonists. The immunosuppressive activity of U50,488H
is reversed by the opioid antagonists naloxone or norBNI. On the
other hand, the immunomodulatory activity of morphine is reversed
only by naloxone.

2. The mu-receptor agonists morphine and DAMGE also inhibit the
development of an antibody response *in vitro.* Much more potent
inhibitory activity was also observed for the kappa agonists U50,488H
and U69,593.   The delta agonist DPDPE failed to exert measurable
immunomodulatory activity under these experimental conditions.

3. The immunosuppressive activity of the kappa agonists was reversed
by both naloxone and norBNI. In addition, the activity of these
opioid compounds exhibited stereospecificity.

4. Strain analysis has revealed the existence of two groups of mouse
strains. The relatively sensitive mouse strains appear to include
the BALB/c, C57BL/6 and BlO.A(5R) strains. Four relatively less sen-
sitive strains have also been identified.

5. Immunomodulatory activity has been detected for the kappa-
selective agonists in the mu receptor-deficient strain CxBK/ByJ.
Both mu and delta agonists fail to exert immunosuppressive activity
in this mouse strain.

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AUTHORS

Thomas J. Rogers, Ph.D., Dennis D. Taub, B.S., and Toby K. Eisenstein, Ph.D., Department of Microbiology and Immunology

Ellen Geller, M.A., and Martin Adler, Ph.D., Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140.

TABLE 1. The Effect of Opioid Agonists on the Proliferative Response of Murine Splenocytes. *Results represent the analysis of data obtained from the titration of opioid compounds ranging from 10 µM to 1 nM. Individual data points wet-e obtained from at least 4 replicate cultures.*

Mitogen	Morphine ID_{50} (µM)	DAMGE ID_{50} (µM)	U50,488H ID_{50} (µM).
PMA	0.1	0.1	0.04
SEB	10	>10	0.8

TABLE 2. The Effect of Naloxone and norBNI on the Immunosuppressive Activity of Morphine and U50,488H. *Cultures of splenocytes were treated with a constant concentration of morphine or U50,488H, and the antagonists naloxone and norBNI were titrated over a range of 10 µM to 1 nM. The concentration of antagonist required to reverse 50% of the activity of the agonist is reported as the ID_{50}.*

Agonist	Mitogen	Naloxone ID_{50} (nM)	norBNI ID_{50} (nM)
Morphine	PMA	0.1	No effect
(1 µM)	SEB	1	No effect
U50,488H	PMA	ND	ND
(0.1 µM)	SEB	0.1	0.08

TABLE 3. The Effect of Various Opioid Agonists on the *In Vitro* Antibody Response. *Cultures of splenocytes were treated with the designated opioid agonist over a range of 10 µM to 1 nM. Each data point is obtained from at least 3 replicate cultures.*

Compound	ID_{50} (µM)
Morphine	5
DAMGE	10
U50,488H	0.02
U69,593	0.4
DPDPE	>10

TABLE 4. The Effect of Naloxone and norBNI on the Immunosuppressive Activity of Morphine and U50,488H. *The experiment was carried out to determine the capacity of antagonists to reverse the inhibition of antibody responses by morphine or U50,488H. The experimental design is* as *described in Table 2.*

Agonist	Naloxone ID_{50} (nM)	norBNI ID_{50} (nM)
Morphine (1 µM)	0.08	No effect
U50,488H (0.1 µM)	0.6	0.6

TABLE 5. *Stereospecificity* of the U50,488H Effect on the Antibody Response. *Cultures of splenocytes were exposed to a titration of either the (-) or (+) isomer over a range of concentrations from 10 μM to 1 nM.*

Compound	ID_{50} (μM)
U50,488H ([+]isomer)	8
U50,488H ([-Iisomer)	0.04

TABLE 6. The Analysis of the Effect of Opioid Compounds on the Antibody Response of Various Mouse Strains. *Splenocyte cultures from each mouse strain were exposed to a titration of morpine or U50,488H over a range of concentrations from 10 μM to 1 nM.*

Strain	Morphine ID_{50} (μM)	U50,488H ID_{50} (μM)
BALB/c	0.1	0.004
C57BL/6	0.06	0.008
B10.A(5R)	0.06	0.004
B10.BR	0.8	>10
C3H/HeJ	0.8	5
C3H/FeJ	0.9	>10
CBA	0.9	>10

TABLE 7. The Effect of Opioid Compounds on the Antibody Response of Mu-Deficient CxBK/ByJ Mice. *Designated opioid agonists were titrated over a range of concentrations from 10 μM to 1 nM. The CxBK/ByJ and C57BL/6ByJ mice were age and sex matched.*

Agonist	CXBK ID50 (μM)	C57BL/6 ID_{50} (μM)
U69,453	0.07	0.01
U50,488H	0.08	0.09
Morphine	>10	2
PLO17	>10	10
DAMGE	>10	6
DPDPE	>10	>10

Immune Effects of Opiates in Test Tubes and Monkeys

Robert M. Donahoe

INTRODUCTION

We have reviewed the connection between opiate abuse and altered
immune function previously (Donahoe and Falek 1988; Donahoe 1988).
From this information, it is clear that heroin addicts frequently
experience abnormally high incidences of opportunistic diseases
like infectious disease (Sapiral 1968) and cancer (Harris and Garret
1972; Sadeghi et al ., 1979) which are indicative of diminish
immune capacity. The question, therefore, has naturally arisen as
to what the causes for this diminished immune capacity might be.
Investigations into this issue have intensified considerably over
the past 10 years as the result of the knowledge that i.v. drug
abuse is associated epidemiolcgically with the spread of AIDS. In
this regard, it is not able that immune deficiencies of drug abuse
appear to be distinguishable from those associated AIDS.

Since there are numerous factors associated with the addiction
milieu with the potential to alter immune function (Donahoe and
Falek 1988), the role of the drugs themselves in the linkage
between drug abuse and immune deficiency remains ambiguous.
Definition of this role is made even more difficult by the fact
that the drugs involved affect many nonimmunological aspects of
host physiology that influence immune function secondarily.
Accordingly, no obvious single experimental approach is available
to determine the way drugs of abuse influence host immunocompetency
in humans. Focusing mainly on opiates, the present report briefly
considers knowledge of the immune modifying effects of drugs of
abuse with the aim of specifying strategies that may lead to better
definition of the immunomdifying properties of such drugs.

ARE DRUGS OF ABUSE CAPABLE OF MODIFYING HOST IMMUNE COMPETENCY?

In vivo clues to the immunodifying properties of drugs of abuse
are available from a variety of studies. Clinical. observation and
epidemiolcgical assessments of street opiate addiction have clearly
shown that addicts suffer immunologically. Still, as mentioned
previously, the association of drug addiction with immune
deficiency (Donahoe and Falek 1988; Donahoe 1988) is only

suggestive that a given drug may be a causal factor, per se, because other immmunodifying factors common to the addiction milieu also may be involved. Notably, therefore, studies using rodents as study subjects have clearly demonstrated the potential of opiates to alter host immune competence (Tubaro, et al., 1983). Yet, because the physiological response of rodents to opiates differs in many ways from that seen with humans, as does the ability to mimic drug-dosing conditions inherent to human street addiction, the relevance of findings with rodents to the human circumstance is more general than specific.

Since prospective studies of the effects of chronic exposure to opiates in human addicts is logistically impossible, study of animal models remains an important means to determine how opiates influence immue function. In an attempt to increase the relevancy of animal studies to the human circumstance we have been studying the immunological effects of opiate dependency in a rhesus monkey (Macaca mulatta) model. Monkeys and man have very similar physiological responses to opiates (morphine) as well as nearly identical immune systems. Studies are ongoing still, but current findings indicate that certain immune changes are related directly to opiate dependency. In many ways these changes are similar to those encountered with heroin addicts: (1) immune effects appear to segregate on the basis of whether exposure to opiates is acute or chronic; (2) immune changes are manifest in T-cell responsiveness and NX-cell cytoxicity; (3) the immune changes seen appear to be consistant with the phenomenon of pharmacological tolerance since monkeys that are chronically exposed to morphine appear to become tolerant to its initial immunological effects. The pattern of responses seen also suggest that the immunological effects of morphine are to some extent interdependent on coincident effects of stressors inherent to the experimental addiction milieu.This latter observation emphasizes the notion that the effects of opiates on the entire neuroimmune network must be determined before the immunological effects of opiate exposure can be properly gauged.

In the monkey studies, we have also been examining the effect of morphine dependency on pathological and virological progression of infection with simian immunodeficiency virus (SIV/smm). After nearly two years of infection with SIV/smm coincident with opiate dependency, the course of development of pathology resulting from the infection does not seem to have been altered by the experience of opiate dependency. Pending completion of this study and final data analyses, a tentative conclusion from the current evidence might be that opiates are not cofactors in simian AIDS and, by analogy, human AIDS. However, for humans, addiction of long duration appears to be a requirement for immune breakdown to occur. Thus, it is likely that an observation of no exacerbation of SIV/smm infection under the current study conditions is best considered as an indication that the duration of addiction is insufficient to exacerbate infection. Several other facts about this study also support this latter conclusion.

One such fact relates to our data discussed above,and to data from other investigators (Shavit et al., 1984) that stress factors may be important cofactors with opiates in alteration of immune responses in vivo. Indeed, stress is a well know immunomodulating force (Keller et al., 1988). Certainly, the stresses of street heroin addiction associated with the social-stigmas involved and, particularly,with the abstinence syndrome accompanying drug withdrawal, could be powerful factors affecting the immunolqical balance in street addicts. Indeed, in our ongoing study of virally-infect, opiate-dependent monkeys, a number of the animls involved became latently or persistently infected with SIV, much as happens with HIV infections (Greene 1990). When these animals were exposed to the relatively stressful rigors of a brief episode of naloxone-induced opiate withdrawal, preliminary evidence indicates that both immune changes and viral induction occurred. Such findings indicate the potential importance of stress cofactors in AIDS among addicts. If a similar situation occurs for HIV infections in street addicts, it would add emphasis to the appropriateness of using methadone maintenance in treatment of street opiate addiction because the minimization of the abstinence withdrawal syndrome that is inherent to this treatment modality would have the important potential of reducing stress and, thereby, potentially, the spread of virus.

Taken together, the preliminary monkey data further emphasize the notion that immunological changes effected by opiates should be considered within the context of their effects on the entire neuroimmune network. This conclusion is also harmonious with the fact that opiates have direct effects on cells of the immune system (see following discussion) as well as a variety of systemic effects with indirect impact on the immune system as suggested by Shavit et al. (1984), and Weber and Pert (1989). These investigators have shown that morphine-induced and stress-induced alterations in immune function of laboratory mice are centrally mediated through the actions of the endogenous opioid system (Shavit et al., 1984), and that certain immunological activities of morphine are centrally mediated through neurons in the cerebral periacqueductal gray region (Weber and Pert 1989).

IN VITRO DATA SUPPORT THE NOTION THAT OPIATES MODULATE IMMUNE FUNCTION

Numerous studies have shown that both alkaloid and peptide opioids can modulate immune response parameters in vitro (Donahoe and Falek 1988; Donahoe 1988). By showing that cells of the immune system are influenced directly by opioids, such studies complement results of in vivo investigations in helping to define the opioid role in modulation of immune function. However, data from in vitro investigations of the immunological effects of opiates do not certify how this effects is manifest in vivo because other factors also need to be considered. In particular the indirect immunological effects of opiates that are encountered in vivo must be a part of any consideration in this regard. Furthermore, the longstanding ambiguities that exist in regard to the in vivo

relevance of in vitro immunological assays toward predicting host immunocompetence add to the interpretive problems with any data collected from in vitro analyses of immune parameters.

Because of these circumstances, definition of the means by which opiates alter immune function through in vitro studies will ultimately require approaches aimed at determining the nature of interactions between both direct and indirect effects of opiates on host immune parameters. There are various ways that this maybe done. A primary site of the immunological influence of opioids, whether through direct means or indirectly through effects of neuroendocrine and immunoendocrine products induced by the opioids, is at the surface of the cells involved, presumably occurring largely through receptor-mediated events. For this reason, we have focused our in vitro efforts at trying to understand how opiates and other drugs of abuse influence such events.

We have employed two main tactics in examining effects of opioids and other drugs of abuse on T-cells: 1) analysis of their ability to modulate receptors on the surface of T-cells that are relevant to the regulation of T-cell function; 2) analysis of pharmacologically relevant binding sites for the drugs involved. The results of our efforts in this regard have been reported in several reviews and monographs (Donahoe 1988; Madden and Donahoe 1990) and other published articles (Madden et al., 1987; Donahoe et al., 1988).

We (Madden et al., 1987) and other investigators (reviewed by Madden and Donahoe 1990) have identified opiate-binding sites on T-cell lymphocytes. Presumably, therefore, many of the effects of opiates on T-cell activity are mediated through such binding sites. Still, other evidence suggests that opioids exert effects through binding sites that do not fit classical definitions as opiate receptors (Madden and Donahoe 1990). Therefore, more work is needed to specify the nature of receptor sites for opiates on cells of the immune system.

Another aspect of our efforts to characterize the effects of opiates at the T-cell surface relates to the conceptualization that both peptide and alkaloid opiates--as well as other drugs of abuse and behaviorally active substances in general--influence immune function through a common ability to modulate expression of T-cell receptors involved in immune regulation (Donahoe and Falek 1988). In this regard, we have focused cur attention, primarily, on the ability of opiates to modulate expression of various T-cell receptors, but most particularly, The E-receptor/CD2 molecule as well as CD4 and CD8 molecules.

Then nature of the interaction of opiates with T-cell CD2 molecules is particularly noteworthy. It has been known for over a decade that, in vitro,morhine depresses expression of E-receptors as measured by the use of active T-cell E-rosetting (Wybran et al., 1979). Similarly, our molecular approach employing monoclonal antibodies to CD2 has also proven effective in monitoring the

effects of opiates on expression of the E-receptor (Donahoe et al., 1988). Since CD2 molecules (Bernard et al., 1982; Donahoe et al., 1985) and opiate receptors share (Fantozzi et al., 1981) the property of residing within the membrane as a large pool of dormant receptors and since CD2 interacts in various ways in controlling expression of a variety of T-cell receptor molecules (Bierer et al., 1988; Donahoe et al., 1988; Schraven et al., 1990), understanding the basis for opiate effects on CD2 appears to be an essential part of understanding immunological effects. This conclusion is bolstered by the fact that CD2 expression is apparently critical for maintenance of immunocopetence (Kerman and Geis 1976) and that it is modulated in vivo after opiate exposure as it is in vitro (McDonough et al., 1980).

Since CD2 expression can be modulated by all manner of behaviorally active substances including the peptide opioids (Wybran et al., 1979), study of the modulatory processes involved are pertinent to the immune outcome of opiate exposure as both a primary event of the interaction of opiate with T-cells as well as a secondary event resulting from the interaction of the opiate with the neuroimmune network leading to induction of immunoactive neuroendocrine and immunoedocrine product. By focusing on the ability of opiates to modulate CD2, it will be possible to examine not only direct effects of opiates on expression of CD2 and other interdependently modulated T-cell receptor molecules but also the coordinate effects of opiates with the secondary products of opiate interaction within the neuroimmune network. We feel this approach will contribute materially to knowledge about the way the neuroimmune system regulates immune function in circumstances of opiate exposure.

In conclusion, better definition of the immunological consequences of opiate addiction and, in fact, drug addiction in general, requires a combination of in vivo and in vitro strategies. A central objective of such research must be to delineate primary from secondary immunological effects of drug exposure and to understand how these direct and indirect effects combine to influence the immune response. As much as possible, these issues should be pursued in studies of street heroin addiction and other forms of human opiate exposure and dependency. However, studies with both animal models and in vitro approaches will also be needed to determine how drugs affect immune parameters. Because of the complexities of the intersystemic independencies that exist in animals exposed to drugs that have multi-systemic effects, it is important to see that the animal models that are used are as close to the human circumstances as possible such as with the use of nonhuman primates. In vitro studies can profit by focusing on studies with human tissues and by emphasizing efforts directed at delineating the nature of signal reception and transduction events that occur in response to the drug of interest. Finally, these efforts will have to be joined with a better understanding of the immunological interdependencies that are important to the host in mounting immune responses--e.g., how the interaction between monocytes and T-cells are effected by drugs, etc. Obviously, a great deal of research is yet required before the final chapter on

how drugs of abuse affect host immune capacity is written.

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AUTHOR:

Robert M. Donahoe, Ph.D.
Laboratory of Psychoimmunology
Emory University School of Medicine
Georgia Mental Health Institute
1256 Briarcliff Road, N.E.
Atlanta, GA 30306

Neural Control of Immune Function: Opioids, Opioid Receptors and Immunosuppression

Richard J. Weber, Linda C. Band, Brain deCosta, Agu Pert, and Kenner C. Rice

INTRODUCTION

In Vitro Actions of Opioids. Opioids have been shown to exert profound effects on several immune parameters (Weber and Pert, 1984; Yahya and Watson, 1987; Sibinga and Goldstein, 1988) through direct and indirect interactions with various components of the immune system. *In vitro* studies, for example, have found that opioid agonists alter antibody production (Johnson et al.,1982; Jankovic and Marie, 1988; Munn and Lum, 1989), produce changes in the ability of leukocytes to respond to mitogenic stimuli (Gilman et al.,1982; McCain et al.,1982; Bryant et al.,1987), increases cytotoxic activity of natural killer (NK) cells (Mandler et al., 1986) and T-cells. (Carr and Klimpel, 1986), and alter monocyte chemotaxis (Simpkins et al.,1984; Van Epps and Saland, 1984; Ruff et al.,1985). Opioids alter interferon gamma production (Peterson et al., 1987; Mandler et al., 1986; Brummitt et al.,1988), stimulate the production of interleukin-2 (Gilmore and Weiner, 1988), and have mixed effects on leukocyte superoxide release (Sharp et al.,1985; Willems et al., 1988; Peterson et al.,1987; Diamant et al.,1989; Seifert et al. , 1989). The identification of opioid receptors on leukocytes (Wybran et al., 1979; Madden et al.,1987; Mehrishi and Mills, 1983; Schweigerer et al.,1985; Lopker et al., 1980; Carr et al., 1988; Stefano et al.,1989; Borboni et al., 1989; Carr et al.,1989), provides a structural basis for the effects of opioid agonists on parameters of immunocompetence *in vitro*.

In Viva Actions of Opioids. Not surprisingly, opioid agonists alter immune function *in vivo* (Weber and Pert, 1984;

Yahya and Watson, 1987). Clinical observations first implied that the immune system could be affected by systematically administered opioids (Hussey and Katz, 1950; Louria et al.,1967; Sapira, 1968) (see Williams et al., this volume). More recent *in vivo* investigations have shown that parenteral heroin users, but not methadone maintenance patients have reduced NK cell activity (Novick et al.,1989); however, patients maintained on methadone have-impaired superoxide anion production (Peterson et al.,1989). Certain opioid agonists suppress antibody production (Weber et al.,1987), alter leukocyte mitogenic responses (Bryant et al.,1987), and decrease delayed-type hypersensitivity responses and NK cell activity (Shavit et al., 1986). High-dose narcotic analgesia-mediated suppression NK cell activity can be reversed by pretreatment with poly I:C, presumably by inducing interferon production and increasing NK cell activity prior to opioid administration (Beilin et al.,1989). Furthermore, opioids result in decreased survival in tumor-bearing animals (Lewis et al., 1983) and increased susceptibility to bacterial infections (Watson and Nguyen, 1990), effects thought to be related to the immunosuppressive potential of this class of drugs.

Effects of (-)-morphin on Antibody Production IN VIVO. Recently, we (Hagan et al.), have extended earlier findings (Weber et al.,1987), and evaluated the *in vivo* effects of opioid agonists on the primary and secondary antibody responses of mice to trinitrophenyl 40-ovalbumin (TNP-ova). Single or multiple acute injections of (-)-morphine were ineffective in suppressing Ab production to TNP-ova. Time course studies suggested that chronic (-)-morphine administration affects an early event in the primary Ab response to TNP-ova. Interestingly, chronic (-)-morphine administration did not suppress the secondary Ab response to TNP-ova. In addition, no suppressive effect of (-)-morphine on the Ab response to the T-independent antigen, TNP-Ficoll, was observed, suggesting that chronic (-)-morphine-induced suppression of Ab production to TNP-ova is mediated directly or indirectly through T-cells or macrophages. These actions of chronic (-)-morphine were pharmacologically specific since they were abolished in animals implanted concurrently with (-)-naloxone pellets and Only the active enantiomer (-)-morphine and not the inactive enantiomer (+)-morphine was effective in producing immunosuppression. The time course of

administration of (-)-morphine suggests that opioids act early to suppress the primary Ab response to TNP-ova. The chronic (-)-morphine-induced suppression of Ab production is blocked by concurrent administration of (-)-naloxone and exhibits enantioselectivity, suggesting that the effect is opioid receptor-mediated. These findings indicate that the effects of (-)-morphine on the primary Ab response to TNP-ova are pharmacologically specific and receptor-mediated and relate to the kinetics of T-dependent Ab production *in vivo.*

NEURAL CONTROL OF IMMUNE FUNCTION

Central Actions of Opioids. Although a number of opioid effects on immune function are undoubtedly mediated through the peripheral actions on immune system components, it has become apparent that opioids also can alter immunocompetence by initial actions in the central nervous system. For example, injections of (-)-morphine into the lateral cerebral ventricles of rats have been found to suppress NK cell activity in this species (Shavit et al., 1986). Conversely, the same investigators demonstrated that peripheral administration of N-methyl morphine which has reduced capacity to cross the blood brain barrier, had no effect on NK activity. We have been able to localize a precise neural structure through which these opioid effects are mediated (Weber and Pert, 1989). Rats were prepared with intracerebral cannulae guides aimed for a variety of structures having the potential to participate in immunoregulation and high concentrations of opioid receptors. Bilateral injections of (-)-morphine (6.8 nmoles) into the anterior hypothalamus, arcuate nucleus, medial amygdala and dorsal hippocampus had no effect on NK cell activity, whereas injections of (-)-morphine into the periaqueductal gray matter of the mesencephalon produced a dramatic suppression of NK cell activity when compared to control animals. This effect of (-)-morphine was pharmacologically specific and mediated through an interaction of (-)-morphine with opioid receptors in the PAG, since the suppression of NK cell activity was blocked by prior intraperitoneal injections of the specific opioid antagonist naltrexone (10 mg/kg). Vehicle injections into the PAG also failed to exert an effect on NK cell activity. These findings suggest that certain central actions of (-)-morphine can induce changes in NK cell function, and that these effects are mediated in part through opioid receptors in the PAG.

Stress, Immunosuppression, and the PAG. The PAG has been previously implicated in modulating some aspects of immune function (Weber, 1988, Weber and Pert, 1989, Weber and Pert, 1990b). While the PAG appears to be the primary neural focus for the action of exogenous opioids in regulating immune function, it is tempting to speculate that endogenous opioid action in this brain region may have relevance for understanding the ability of some forms of stress to alter immune function through opioid-dependent mechanisms. Opioid receptors and endogenous opioid peptides are present in the PAG, and it has been shown that endogenous opioids are released in the PAG during foot-shock stress. Also, the PAG also appears to be one of the primary sites of action of opioids in eliciting analgesia. Shavit and his colleagues (1986), have demonstrated that certain forms of foot-shock stress in rats which produce an opioid dependent analgesia also produce suppression of splenic NK cell activity, which can be blocked by naltrexone. It is conceivable that stimuli associated with foot-shock stress gain access to the PAG, possibly through spino-reticular pain pathways, and enhance the activity of endogenous opioid containing neurons in this structure resulting in analgesic activity as well as suppression of immune function. Electrical stimulation of this region has been shown to enhance metastatic tumor growth, presumably through alterations in immune function. Interestingly, electrical stimulation of the PAG also has been reported to produce an opioid mediated analgesia in a variety of species including man. We have shown that electrical stimulation of the ventral/caudal PAG, but not stimulation in other more rostral PAG regions causes suppression of NK cell activity (Weber and Pert, 1990a). Indeed, electrical stimulation of this region produces analgesia. The precise mechanisms underlying the ability of (-)-morphine injections in the PAG to alter NK cell activity are not entirely clear. CNS signals to the immune system are relayed primarily through the hypothalamic-pituitary-adrenal axis (HPA) or via sympathetic innervation of primary lymphoid organs. Thus, enhanced opioid activity in the PAG could be translated into effects on NK cell activity either through hypothalamic efferents and activation of the HPA axis, or increases in peripheral sympathetic output.

Central Effects of Selective mu, delta, and kappa Opioid Agonists on NK Cell Activity. Based on the observations cited above the following studies were conducted in order to determine Whether specific opioid receptor-subtype(s) mediating opiate-induced suppression of NK cell activity (Table 1). The selective mu, kappa, and delta agonists [D-Ala^2, NMe-Phe^4,Gly-ol]-Enkephalin (DAGO), (S,S)-U50,488, and (D-Pen^{2,5}]-Enkephalin (DPDPE) were microinjected into the lateral ventricle of Fisher 344N male rats. Three hours foilowing microinjection, spleens were removed and assayed for NK cell activity (see Williams et al., this volume). Microinjection of DAGO (60-200 nmol), reduced NK cell activity, while microinjection of (S,S)-U50,488 or DPDPE (20-200 nmol) was ineffective (Table 1).

TABLE 1.
Effect of ICV Injection of Selective Opioid Agonists on Rat Natural Killer Cell Activity

Receptor	Agonist	[nMOL]	NK Activity
Mu	DAGO	6-200	decreased
Kappa	S,S-U50,488	20-200	no effect
Delta	DPDPE	60-200	no effect

The selective mu, kappa, and delta agonists [D Ala^2, NMe+phe^4, Gy-ol]-Enkephalin (DAGO), (S,S)-U50,488, and [D Pen^{2,5}]-Enkephalin (DPDPE dissolved in 5 ul 9%saline were microinjected into the lateral ventricle of Fisher 344N male rats. DAGO (60-200 nmol) reduced, whereas (S,S)-U50,488 and DPDPE(20-200 nmol) had no effect on splenic NK activity measured 3 h after injection (see Williams et al., this volume).

These findings suggest that opiate-induced suppression of NK cell activity is mediated primarily through mu-type opioid receptors. Information on the opioid receptor-subtype involved is important in predicting and controlling the outcome of opioid

administration on immune function *in vivo.* Research in this area could ultimately lead to the logical design and development of potent opioid analgesics which do not compromise NK cell function. Such agents could be useful in the treatment of pain in burn victims and cancer patients, as well as many other clinical situations Where suppression of the immune system would not be desired.

Opioids and AIDS: Could Opioid-Induced Immuno-suppression and Morbidity Result in Augmentation of Viral Production? We and others have previously hypothesized that drug abuse may contribute to the high incidence of AIDS in users. In addition to transmission of the virus through contaminated needles, heroin abuse could result in a further suppression of an already compromised immune system and therefore would serve as a co-factor in the disease. Recent evidence concerning the molecular biology and pathology of HIV-1 and similar retroviruses has prompted the following hypothesis.

Hypothesis. It has been well established that T-cell activation is required for HIV gene expression (Lowenthal et al., 1988; Lowenthal et al.,1989; Böhnlein et al.,1989). This occurs via a mechanism which involves synthesis of inducible nuclear proteins during mitogenesis which regulate both the expression of the interleukin-2 receptor (IL-2R) alpha and HIV-1 gene elements. These proteins bind to a similar 12 base pair sequence in the regulatory region of the IL-2R-alpha gene and the enhancer element of the HIV-1 long terminal repeat (LTR) (Hoyos et al.,1989; Böhnlein et al.,1988; Malim et al.,1989). The result is enhanced HIV-1 gene expression due to mitogen activation of HIV-1 infected T-cells, increasing the level of HIV-1 replication. Induction of viral expression therefore occurs through a normal immunological event. One might hypothesize that individuals who are HIV seropositive and harbor the virus in a latent form, may convert to a productive viral infection if they are prone to frequent acute or chronic infections.

Heroin addicts suffer from a variety of immunopathological features, including suppression of both humoral and cellular immunity, IgM hypergammaglobulinemia (Brown et al.,1974), as well as other immunological abnormalities, which result in an increased susceptibility to infectious disease. An increased incidence of acute and chronic infections among HIV positive heroin addicts could lead to activation of infected cells through normal immunological mechanisms, and may result in expression of HIV-1 genes and augmentation of viral production. This hypothesis may partially explain the increased number of HIV seropositive IV drug users who convert to ARC (AIDS related complex) and AIDS.

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AFFILIATION

R.J.W., L.C.B., B.D. and K.C.R. Neuroimmunology Unit, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, and
A.P. *Biological Psychiatry Branch, National institute of Mental Health, Bethesda, MD 20892.

Opiate Binding Sites on Cells of the Immune System

John J. Madden, Arthur Falek, Robert Donahoe, David Ketelson, and Curtis Len Chappel

The modulation of the human immune system by in vivo opiate use has been an established experimental fact for almost 100 years (Archard et al., 1907); but despite its continuing rediscavery in recent times (Brown et al.,1974; McDonough et al.,1980; Kreek,1988), no one has yet defined the pharmacologic and biochemical mechanisms by which these processes occur. Indeed, it has still not yet been determined whether the modulation is a direct one cause by an interaction between the drug and the target cell; or whether the drug first binds to another target cell, e.g. in the central nervous system (CNS),producing the release of a hornmal secretion which in turn modulates the immune target system (Weber and Pert 1989). In vitro experiments have clearly demonstrated that opiates can have direct effects on cells of the immue system at drug concentrations that are comparable to those which activate the CNS. Some of these effects have also keen shown to be stereospecific and reversible by appropriate antagonists (Wybran et al.,1979). However, it remains to be proven that the direct effects monitored in vitro and the in vivo effects seen in opiate users and abusers have the same cause, i.e. direct opiate action; but in vitro models at least provide the limits for what is possible via direct opiate interaction with cells of the immune system.

One approach to understanding and defining the limits of opiate action in the immune system has been to attempt to measure the specific binding of various opiate 1igands to cells and elements of the immune system. In this paper, we report for the first time on a high affinity mu opiate binding site which is accessible to both mu agonists and antagonists as well as further characterize a low affinity site previously reported (Madden et al., 1987). Previous binding studies, reviewed in Sibinga and Goldstein, 1988, and Madden and Donahoe, 1990, have not presented compelling evidence for opiate receptors on lymphocytes completely analogous to those in the CNS,and it is important to

understand the difference between tissues to determine the
degree of exact analogy. The binding studies reported in this
paper suggest that for mu receptor the degree of analogy
may be higher than previously thought.

LOCATION OF NALOXONE RECEPTORS ON HUMAN T LYMPHOCYTES

Human T lymphocytes have a law affinity binding site for
naloxone, K_D 50nM (Madden et al.,1987), from which
the[3]H-naloxone can be displaced only by very high
cocentrations of morphine, the IC_{50} being approx.
300nM (Fig.1). While at first glance the affinity of this
Site would appear to be too low to be physiologically
significant, recent in vitro immunologic results (Rogers et
al., this volume) suggest that this site may in fact play a
role in controlling immune cell function.
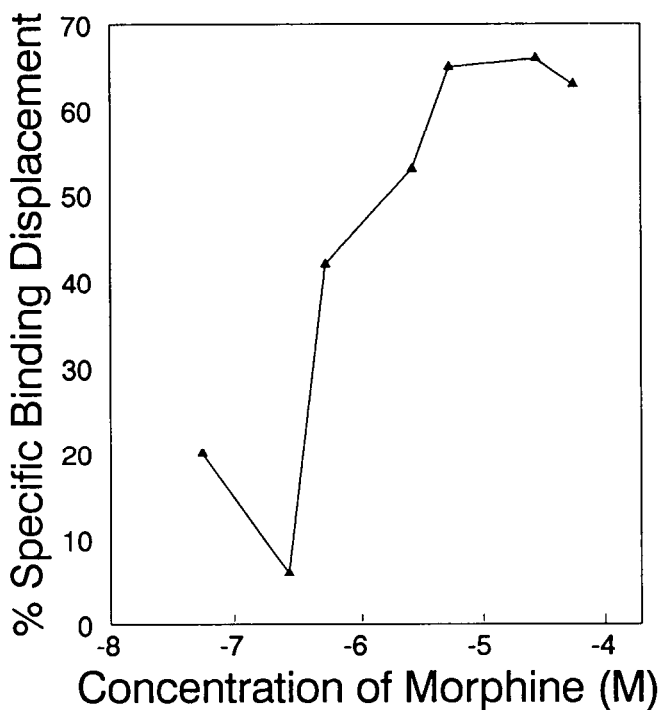
FIGURE 1 - *Displacement of 40 nM ^{3}H-naloxone from
membranes of sonicated lymphocytes (Madden et al., 1987) by
morphine as measured by a centrifugation binding assay.*

Because of suggestive evidence that uptake of naloxone into
the lymphocyte might contribute significantly contribute to
the high levels of "non-specific" binding observed,
autoradiography was used to determine the location of the
naloxone specific binding sites. Whole lymphocytes were fixed
to cleaned glass slides by centrifugation in a Cytospin II
and incubated with 40 nM ^{3}H-naloxone in the absence or
presence of 40 uM naloxone at 4°C for 1 hr. The slides were
washed rapidly with buffer dipped in Kodak NTB3 emlsion and
incubated for 2 weeks at 4°C. After development, cells were
screened at 125x under oil. All of the specific birding of
the naloxone could be attributed to sites on the cell
membrane and not in the lymphocyte's interior (Fig. 2a).

It was noticed that the ruptured cell on the slide had a
high number of grains in the interior of the cell when
compared with those on the outer membrane. To test whether
these grains represented specific binding sites within the
cell, lymphocytes were permeabilized by treatment with acidic
methanol and autoradiography performed as described. Binding
to these permeabilized cells was found primarily in the
interior of the cells with a lesser amount on the peripheral
membranes (Fig. 2b). In the presence of excess cold naloxone,
essentially all binding was abolished as was the case with
the intact lymphocytes. These results point to 2 possible
locations for the naloxone binding site - the lymphocyte
outer membrane and, surprisingly, the cell's interior.
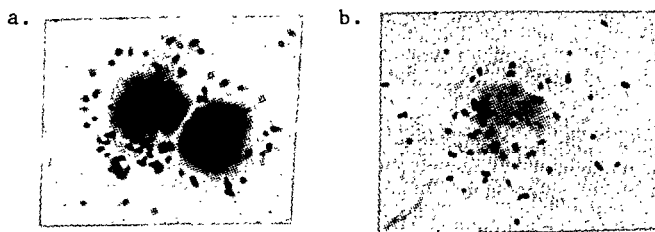
FIGURE 2. Autoradiograms *of naloxone binding to human T
lymphocytes. a - Lymphocyte with membrane integrity intact;
b - Lymphocyte permeabilized with acidic methanol.*

BINDING OF NALOXONE TO PERMEABLE CELLS

Lymphocytes permeabilized by acidic methanol treatment were
tested for their naloxone binding capacity. After methanol
wash,the media was immediately neutralized and the intact
permeabilized cells isolate by low speed centrifugation.
These cells, when tested for specific naloxone binding using
a filter assay, exhibited the expected low affinity,
saturable binding site previously reported. More

interestingly, there appeared to be a saturable naloxone binding site with a k_D < 1 nM. While not robust nor completely reproducible from cell preparation to preparation, there was no doubt that there was specific ,saturable binding below 5 nM which not be exlained by the low affinity site. To further distinguish between the low affinity and this new high affinity site, naloxone displacement by morphine was tested. As described above ,it took an exceedingly high concentration of morphine to displace naloxone from the low affinity site. Displacement of 1 nM naloxone from this new high affinity site by morphine required a very reasonable concentration of morphine yielding an IC_{50} < 1 nM (Fig. 3). Morphine displaced all of the naloxone specific binding at this site, whereas it only displaced roughly 70% of the specific binding at the low affinity site. Since this site would appear to have some of the characteristics of the receptor responsible for morphine's action on the T lymphocyte, at least two very important questions remained to be decided about this site - its stereospecificity and location.
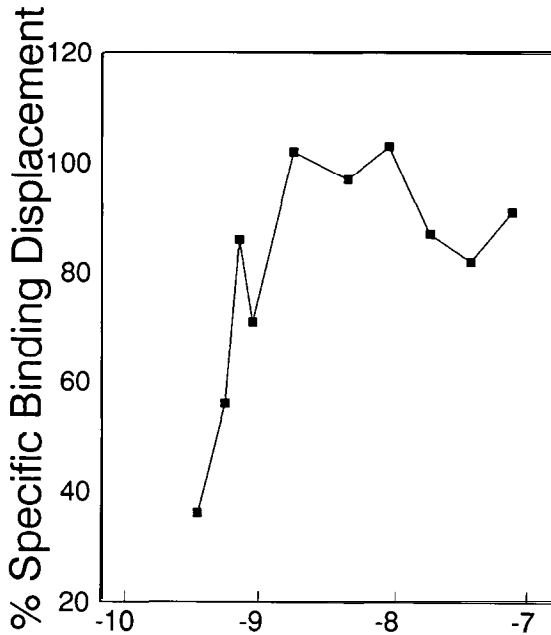
FIGURE 3 – *Displacement of 1 nM ^{3}H-naloxone from acidic methanol permeabilized lymphocytes by morphine as measured by a membrane filter assay.*

In attempting to gauge the stereospecificity of the site levorphanol and dextrorphan were used to displace naloxone as in Fig. 3 where morphine was used. Essentially no displacement was seen for either of these drugs over a wide range of concentrations. Interestingly, these compounds also had little effect on naloxone binding at the low affinity site. This failure of the mu agonist levorphanol to interact with the mu antagonist naloxone is currently inexplicable.

To see if this high affinity site was procduced by the action of acidic methanol on the lymphocyte outer membrane, membranes were first prepared by sonicating the cells and collecting them by centrifugation. These membranes were then washed with acidic methanol and the membranes pelleted. Naloxone bindings curves of these preparation yielded only the low affinity site.

SUMMARY AND CONCLUSIONS

Naloxone binding to T lymphocytes seems to occur both at the outer cell surface and in the interior of the cell. The binding sites on the out membrane appear from previous work to be of affinity and to be displaced by morphine only at very high concentrations. Permeable cells seem to express both high and low affinity binding sites in their interiors. Morphine readily displaces naloxone from this high affinity site, suggesting that this site maybe the long sought after morphine receptor responsible for morphine's naloxone reversible actions on the human T lymphocyte.

Do these results suggest that the opiates need to enter the lymphocyte before finding a receptive binding site to initiate their biological activities? Not necessarily. In isolating the lymphocytes, the surface opiate binding sites may be lost because of the exhaustive washing procedures needed for cell purification. The internal receptors may just represent new receptors on their way to the cell surface or used receptors remaining after endocytosis. Or they may indeed represent the true site of action of the opiates on the lymphocyte. Fractionation experiments are currently underway to distinguish between these possibilities.

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AUTHORS

John J. Madden, Ph.D.; Arthur Falek, Ph.D.; Robert M. Donahoe, Ph.D.; David Ketelsen; and Curtis Len Chappell

Department of Psychiatry, Emory University, Atlanta, GA 30322; and Human and Behavioral Genetics Laboratory, Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta GA 30306.

Prodynorphin Gene Expression in Neuronal and Endocrine Cell Types

James Douglass

INTRODUCTION

Molecular cloning has been used to characterize a wide variety of
neuroendocrine peptide precursor molecules encoding small
biologically active peptides which act as neurotransmitters and
neuromodulators in the central nervous system, and hormones in
the endocrine system (Douglass et al. 1984). One such precursor,
prodynorphin, encodes the dynorphin family of opioid peptides
(Kakidani et al. 1982; Horikawa et al. 1983; Civelli et al. 1985;
Douglass et al. 1989). The prodynorphin precursor contains three
leucine-enkephalin moieties, with at least six additional
biologically active opioids (Dyn A1-8, Dyn A1-17, a-neoendorphin,
β·-neoendorphin, Dyn B1-13 and Dyn B1-28) representing carboxy-
terminal extended forms of the leucine-enkephalin sequences.
Northern blot and in situ histochemical techniques have deter-
mined the rat tissues and cell types in which the prodynorphin
gene is transcriptionally active (Civelli et al. 1985; Akil et al.
1984). Such regions include numerous brain structures (including
the hypothalamus, striatum, hippocampus, midbrain, brainstem and
cerebral cortex), as well as spinal cord neurons. In endocrine
tissues, the prodynorphin gene is transcriptionally active in the
anterior pituitary, adrenal gland, testis, ovary and uterus
(Civelli et al. 1985; Douglass et al. 1987).

The opioid receptor subtype exhibiting specificity for
prodynorphin-derived peptides is the kappa opioid receptor (for
review, see Mansour et al. 1988). The kappa receptor belongs to
the G protein-linked family of receptors, and is also coupled to
calcium conductances. This latter characteristic serves to
biochemically distinguish the kappa receptor from the mu and
delta opioid receptor subtypes which are coupled to potassium
conductances. Studies utilizing radiolabelled ligands specific
for kappa receptor binding have determined that this species of
opioid receptor is widespread throughout the CNS and endocrine
system. Numerous studies have suggested that prodynorphin-
derived peptides serve to regulate a wide variety of physio-

logical and behavioral responses (Mansour et al. 1988). These
roles include the mediation of visceral analgesia, effects on
appetite suppression, mediation of hypotensive cardiovascular
responses, inhibition of ADH secretion and possibly additional
renal functions, involvement in modulation of seizure thresholds
and intensity, and involvement in recovery from spinal cord
injury and stroke. A role for dynorphin peptides in narcotic
tolerance mechanisms has also been suggested.

REGULATION OF PRODYNORPHIN GENE EXPRESSION IN NEURONAL CELL TYPES

Cloning of rat prodynorphin cDNA and genomic DNA (Civelli et al.
1985; Douglass et al. 1989) has afforded the opportunity to study
regulated patterns of prodynorphin mRNA levels in specific neuro-
nal cell types following surgical or pharmacological manipula-
tions. The studies described here represent those in which the
most dramatic alterations in prodynorphin mRNA levels have been
observed to date.

Prodynorphin Gene Regulation in the Rat Spinal Cord

The prodynorphin gene is transcriptionally active in the
mammalian spinal cord. In situ hybridization histochemistry/
immunohistochemistry has localized the majority of rat spinal
cord prodynorphin transcripts and peptides to laminae I-II, and
V-VI (Ruda et al. 1988). The localization of prodynorphin-
derived opioid peptides to these specific regions of the spinal
cord suggests that these peptides play a role in the endogenous
pain recognition/control system. Numerous experimental paradigms
have served to strengthen this belief (Iadarola et al. 1988; Ruda
et al. 1988; Millan et al. 1985; Millan et al. 1987).

In the study described here, prodynorphin mRNA and peptide (Dyn A1
-8) levels were measured in the rat lumbar spinal cord at various
times following inflammation of the hindpaw (Iadarola et al.
1988). An experimental model of unilateral inflammation of
the hindlimb (induced by a single, intraplantar injection of
Freund's adjuvant) produced hyperalgesia to both mechanical and
radient thermal stimulation which was rapid in onset. During
this period, prodynorphin biosynthesis was substantially
elevated in the region of the spinal cord receiving sensory
input from the affected limb. Prodynorphin mRNA levels rose
substantially within the first 24 hour period, and maximal
stimulation (8- to 9-fold increase) was observed between 3 to 5
days after injection. By day 14, prodynorphin mRNA levels
approached control values. This time course of induction and
subsequent decline closely paralleled that of hindpaw edema and
hyperalgesia. Spinal cord Dyn A1-8 levels also rose approximately
3-fold during the inflammatory period. This pattern is consistent
with an increase in both the rate of synthesis and release of
dynorphin peptides from spinal cord neurons. These data suggest

the active participation of dynorphin-containing spinal cord neurons in the modulation of sensory afferent input during peripheral inflammatory pain states.

Prodynorphin Gene Regulation in the Rat Hippocampus

Opioid peptides derived from the prodynorphin precursor are present at high levels in the rodent hippocampus. Dynorphin immunoreactivity exhibits a restricted distribution, being localized to the granule cell/mossy fiber axonal system (McGinty et al. 1983). The hippocampus is a highly excitable structure, unusually susceptible to seizure activity. Numerous studies have documented specific alterations in the levels of hippocampal opioid peptides and opioid peptide mRNAs following the induction of seizure activity by techniques such as electroconvulsive shock (Yoshikawa et al. 1985; Kanamatsu et al. 1986; Xie et al. 1989a) or kindling (Iadarola et al. 1986; Xie et al. 1989b).

Kainic acid (KA) is a cyclic analog of the excitatory amino acid, glutamate, which elicits the induction of hippocampal epilepti-form activity and motor seizures. Over the last decade, a variety of studies have utilized this potent neurotoxin as a model of temporal lobe epilepsy (Ben-Ari, 1985). In the study described here (Douglass et al. 1990), hippocampal prodynorphin mRNA and peptide levels were measured at various times following a single subcutaneous injection of kainic acid (8.0 mg/kg). Prodynorphin mRNA levels rose remarkably quickly, with a 13- to 14-fold induction observed 3 hours after KA administration. Prodynorphin mRNA levels began to decline at 12 hours, and by 48 hours levels were at, or below, control values. Even though mRNA levels were dramatically stimulated, hippocampal Dyn A1-8 levels remained at values below control at all time points monitored. This observa-tion suggests that KA treatment also results in a prolonged stimulation of release of dynorphin peptides from hippocampal neurons.

Interestingly, other paradigms which induce seizure activity, such as electroconvulsive shock, result in a dramatic lowering of hippocampal prodynorphin mRNA levels (Xie et al. 1989a). Administration of a single electroconvulsive shock (85 mA, 50 Hz, 1 ms pulse interval for a duration of 1 s) results in a signifi-cant reduction of hippocampal prodynorphin mRNA levels to 40% of control values by 24 hours. This decrease in prodynorphin mRNA levels is not observed in other brain regions containing prody-norphin transcripts, such as striatum and hypothalamus. Moreover, hippocampal proenkephalin mRNA levels are increased following the identical ECS treatment. Thus, the hippocampal prodynorphin system appears to be capable of both positive and negative transcriptional regulation.

REGULATION OF PRODYNORPHIN GENE EXPRESSION IN ENDOCRINE CELL TYPES

The aforementioned studies demonstrate that cytoplasmic prody-
norphin mRNA levels can be dramatically altered in specific
neuronal cell types. Presumably, these changes reflect altered
rates of synthesis of the prodynorphin transcript in the cell
nucleus. Additionally, signals received at the cell surface,
presumably through the process of receptor activation, mediate
these transcriptional changes. For example, prodynorphin mRNA
levels in the spinal cord may be affected by activation of CGRP
or Substance P receptors on the surface of prodynorphin-express-
ing cells. Thus, in order to understand the molecular mechanisms
which regulate cellular levels of prodynorphin mRNA, it is
necessary to determine the effects of various second messenger
molecules on prodynorphin gene transcription. These studies are
difficult to perform using neuronal cells as a model system.
Accordingly, we have utilized a prodynorphin-expressing endocrine
cell type to study the effects of second messengers, such as cAMP,
on prodynorphin gene expression (Collard et al. 1990).

Northern blot and histochemical analysis have been used to
determine that Sertoli cells are the singular site of synthesis
of prodynorphin mRNA in the adult mammalian testis. Sertoli cells
are the highly secretory somatic cells of the seminiferous
epithelium. Adjacent cells form tight junctional complexes which
help to create a blood-testis barrier, thus resulting in an
adluminal environment whose components are largely defined by
Sertoli cell secretion products. Developing germinal cells are
found deeply imbedded within the invaginated Sertoli cell surface,
and it is generally thought that Sertoli cells provide both phys-
ical and biochemical support to the process of spermatogenesis.

Sertoli cells can be easily purified from 22 day old rat testis,
and maintained in defined primary cell culture conditions for up
to 4 days. Thus, using this controlled cell culture system it is
possible to test various second messengers for their ability to
alter cellular levels of prodynorphin mRNA, as well as secretion
of prodynorphin-derived peptides. The cAMP analog, 8-(4-chloro-
phenylthio) cAMP (cpt-AMP), stimulates Sertoli cell prodynorphin
mRNA levels approximately 6-fold following 48 hours of treatment.
Additionally, levels of secreted Dyn A1-17 are increased
approximately 3-fold following cpt-AMP treatment. Thus, the
second messenger, cAMP, appears to stimulate the production of
both prodynorphin mRNA and prodynorphin-derived peptides in this
endocrine cell.

CHARACTERIZATION OF THE RAT PRODYNORPHIN GENE

The studies described above document changes in prodynorphin mRNA
levels in both neuronal and endocrine cell types. These changes

are presumably the result of alterations in the rate of transcription of the prodynorphin gene. We have isolated and characterized the rat prodynorphin gene in order to begin to understand the molecular events which serve to mediate transcriptional regulation of the gene (Douglass et al. 1989). Such analysis has led to the identification of the rat prodynorphin mRNA cap site, promoter element, and possible regulatory sequences.

We are currently analyzing the promoter and 5' flanking region of the rat prodynorphin gene to determine the specific nucleotide sequence elements which control transcriptional activity of the gene. Various restriction DNA fragments representing specific segments of prodynorphin gene 5' flanking DNA have been ligated to the bacterial reporter gene, chloramphenicol acetyl transferase (CAT), and introduced into various eukaryotic cell lines. Preliminary results suggest that nucleotide sequence elements between -1860 and -1280 appear to dramatically stimulate basal levels of transcription from the prodynorphin promoter region. Additionally, various promoter constructs appear to contain sequence elements which respond positively to cAMP analogs, and negatively to phorbol esters (McMurray et al. 1989).

In summary, the molecular mechanisms underlying dependence, withdrawal and tolerance associated with the compulsive use of opiate drugs are poorly understood. The problem of physical dependence and withdrawal is highly complex, and likely involves a complex cascade of events which begins with the diverse and widely distributed opiate receptors, and extends to complex arrays of autonomic and sensorimotor neural networks. These events are also influenced by genetic, species and environmental factors, which only serve to complicate scientific ventures aimed at characterizing the molecular basis of these narcotic-related syndromes. It is reasonable to assume that the introduction of exogenous opiates (as well as other drugs of abuse) results in the activation of G-protein coupled opiate receptors, followed by changes at the genomic DNA level with regard to the transcriptional activity of specific sets of genes expressed in the CNS. Indeed, administration of morphine appears to significantly decrease striatal levels of proenkephalin mRNA in rat (Uhl et al. 1988), while chronic naloxone treatment increases expression of both proenkephalin and protachykinin mRNA in the rat striatum (Tempel et al. 1990). In order to continue to gain insight into the molecular events associated with substance abuse, we must understand the basic mechanisms which regulate the expression of transcriptionally active genes in the CNS.

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114

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AUTHOR

James Douglass, Ph.D.
Vollum Institute - L474
Oregon Health Sciences University
3181 SW Sam Jackson Park Road
Portland, OR 97201

Tissue-Specific Splicing of Pro-enkephalin mRNA

Richard D. Howells and Saranya Rao

ABSTRACT

A testis cDNA library derived from 4-6 week-old rats was screened using a ^{32}p-labeled 435 base pair (bp) cDNA probe derived from exon 3 of rat brain proenkephalin (PE). Two positive clones were isolated from an initial screening of approximately 20,000 recombinant bacteriophage plaques. The longest insert (1,500 bp) was sequenced and was found to contain a portion of intron A at the 5'-terminus. Intron B was sliced as it is in the brain, therefore, the domain structure of the cDNA was intron A-exon 2-exon 3, reading in the 5'-3' direction. Since the translational initiator codon is located at the 5'-terminus of exon 2, the reading frame of PE is unaltered, however, within intron A are 4 upstream AUG codons which exist in a favorable context for initiation of translation. It is probable that translation of PE would be inhibited by the presence of these upstream initiator codons, which may explain the discrepancy between the levels of PE mRNA and opioid peptides in the testis. Hypophysectomy prior to the onset of puberty was found to drastically reduce the level of PE mRNA in the testis and epididymus, suggesting that pituitary factors affect the expression of the PE gene in these tissues, either directly or indirectly.

INTRODUCTION

The PE gene has been found to be expressed in several tissues outside of the central nervous system such as the testis, ovary, and other reproductive organs of the male and female (Kilpatrick et al., 1985; Howells et al., 1986; Kilpatrick and

116

Rosenthal 1986), helper T-lymphocytes (Zurawski _et al.,_ 1986), and heart (Howells _et al._ 1986). We found that the PE mRNA in the testis is larger than the transcript in the brain and other tissues by several hundred bases. Another difference regarding PE expression in the heart and testis is that there is a large discrepancy between the level of PE mRNA and the levels of opioid peptides derived from PE compared with the brain. It appeared that PE mRNA was under translational control (or not translated at all), or that the opioid peptides were either released or degraded rapidly following translation. We sought to explain the molecular basis for the polymorphism of the PE mRNA in the testis by molecular cloning and sequencing of PE cDNA and, furthermore, hoped that this sequence analysis might shed light on the reason behind the disparate levels of PE mRNA and opioid peptides in the testis. Our progress thus far is described in this paper.

MATERIALS AND METHODS

A testis cDNA library constructed from 4-6 week old Sprague-Dawley rats in Lambda ZAP (Stratagene) was screened using a 32p-cDNA fragment derived from exon 3 of rat brain PE (Howells _et al_., 1984). Sequencing was performed with the chain termination method of Sanger _et al_., 1977.
Male Sprague-Dawley rats were hypophysectomized by the supplier (Taconic) at 2 weeks of age and maintained with 0.9% saline for drinking water for three weeks following surgery. The rats were sacrificed along with sham-operated controls by CO_2 inhalation and the brain, heart, testis, and epididymus were removed and immediately frozen on dry ice prior to storage at -75 degrees Centigrade. RNA was extracted according to the method of Chomczynski and Sacchi (1987). PE mRNA levels were quantified using slot-blot analysis with the same probe used to screen the testis library. Autoradiograms were scanned with a Shimadzu densitometer.

RESULTS AND DISCUSSION

We obtained 2 positive clones from an initial screening of approximately 20,000 recombinant bacteriophage plaques, in good agreement with the abundance of PE mRNA in the rat testis. One clone contained an insert of 1,500 bp while the other was 1,300 bp. Sequence analysis of the longer insert

(Figure 1) revealed that this cDNA had a portion of intron A of the PE gene in the region of the 5'-terminus. At the time that this result was obtained, the entire primary sequence of intron A was not known (Rosen et al., 1984). Our cDNA clone had 106 bp of intron A attached to exon 2, with another 222 bp of unknown origin 5' to the intron A sequence. Subsequently, the entire sequence of intron A has been reported (Garrett et al., 1989). It is now clear that the sequence at the 5'-end of our cDNA is entirely derived from intron A, and is the result of

```
5'-CCCUCGGAAGGACAGG AUG CCAUG CCAUCGGAAGACAGGAC
UCCCCAAGGAGAACAGG AUG CCAUCAGGGAGACAGGACUCCCCC
GUGGAAGAUAGGAUACCUCCAGGAAGACAGA  AUG CCCCCCAGGC
CAGCCCUGGGACAGCGGGAAACACUAGGGACCAAGCUAUCCCUG
GUUCACUCGGGAUUUUGUUGGGGUUGUGGGCGGGGCUGAGGAAA
GAUUG-UCCCUGCUGGUCCUGCUCCACGACCACCCACCCGGCAAG
GUUCCCUCCUAGAGAACCUUGUCAGAGACAGAACGGGUCCCCAC
AGGCGCAUUCUUCUUUCCACAG-CCC AUG G...exon 2...exon 3.-3'
```

FIGURE 1. Sequence of the 5'-terminus of testis PE mRNA based on testis cDNA. DNA sequence is written in the 5'-3' direction. AUG initiator codons are underlined and shadowed. The hyphen in line 6 indicates the boundary of the sequence of lntron A reported by Rosen et al., (1984); the hyphen in line 8 refers to the intron A-exon 2 junction.

a tissue-specific slicing event. Since the start site for translation of PE resides 3 bases downstream of the 5'-terminus of exon 2, the reading frame for the precursor protein is unaltered, so that the opioid peptides derived from PE could still be synthesized in the testis from appropriate processing of PE. However, within the sequence of lntron A there are an additional 4 AUG initiator codons which are upstream (5') to the AUG start site in exon 2. All 4 of these codons are in a favorable context to serve as strong initiators (Kozak 1989), as shown in Figure 2. Each of the initiators contains a purine at position -3 relative to the AUG, which is the most important determinant of whether an initiator codon will serve as a strong initiator of translation. The astute reader will notice that the context of the brain initiator is not favorable with respect to the -3 position, but this is presumably countered by the presence of a G at position +4

According to the scanning model for translational initiation (Kozak 1989), the 40S ribosomal subunit carrying initiation factors and ^{Met}tRNA will bind to the capped 5'-end of the mRNA and then slide linearly in an ATP-requiring manner to

	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4
Consensus	G	C	C	G	C	C	Pu	C	C	A	U	G	G
Brain	G	G	C	A	G	C	C	C	C	A	U	G	G
Testis ORF 1	A	A	G	G	A	C	A	G	G	A	U	G	C
Testis ORF 2	C	A	G	G	A	U	G	C	C	A	U	G	C
Testis ORF 3	G	A	G	A	A	C	A	G	G	A	U	G	C
Testis ORF 4	G	A	A	G	A	C	A	G	A	A	U	G	C
Testis ORF 5	C	A	A	C	A	G	C	C	C	A	U	G	G

FIGURE 2. Context of initiator codons in testis PE mRNA. The contex of the initiator codons in PE mRNA are compared with the concensus sequence reported by Kozak (1989). Positions are numbered relative to the AUG triplet,

the first AUG triplet in the proper context. Thus, it is likely that if the intronic AUG codons are utilized, the first of these would be the preferred site of initiation. Translation beginning with the first AUG encountered would result in a peptide 26 amino acids in length, and would terminate at a UAG stop codon. We have no evidence as yet that this peptide is actually translated from the PE mRNA in the testis, however we are presently investigating if cRNA derived from in vitro transcription of the PE cDNA will synthesize this peptide in the rabbit reticulocyte translation system. In many cases, the presence of upstream initiators severely limits translation from downstream start sites (Kozak 1983; Marth et al., 1988). If this is the case with the PE transcript in testis, it would agree well with the observed paucity of opioid peptides in this tissue. It is possible, however, that subsequent to translation of the first open reading frame, reinitiation might occur at the internal AUG which is the start site for PE synthesis. That reinitiation does not occur is supported by the observation that the testis PE mRNA is not associated with polyribosomes on density gradients as it is in the brain (Kew et al., 1989).
We were interested if the pituitary gland had any influence on PE gene expression, since it is crucial to the processes of spermatogenesis and steroid hormone production in the testis.

We found that when hypophysectomy was performed prior to the onset of puberty in the rat, PE mRNA levels in the testis and epididymus are drastically reduced (Table 1), while the levels in the brain and heart are unaffected. Experiments are

Tissue	Percent Sham Control
Hypox brain	105
Hypox heart	92
Hypox testis	9
Hypox epididymus	3

TABLE 1. Effect of hypophysectomy on proenkephalin mRNA levels in rat tissues. Values represent the percentage of the sham-operated controls

underway to determine whether the PE gene is regulated at the transcriptional level by the anterior pituitary, and whether the PE mRNA levels can be restored in hypophysectomized animals with administration of luteinizing hormone, follicle-stimulating hormone, androgens, or estrogens. It should be noted that we have shown that estrogen can increase the levels of PE mRNA in the hypothalamus of ovariectomized rats (Romano et al., 1988) and that the PE gene contains an element with similarities to the estrogen-response element that is found in genes that are regulated by estrogens in the 5'-flanking region of the PE gene (Romano et al., 1989).
Although the physiological role of peptides derived from PE in the testis is not known, we now know that the PE gene is expressed in testicular germ cells (pachytene spermatocytes and round spermatids) as reported by Kilpatrick and Millette (1986), and in Sertoli cells (Yoshikawa and Aizawa 1988). Expression in these two tissues differ in that the germ cells express the longer transcript described in this paper, while the Sertoli cells express a PE message with the same size as is found in the brain and other tissues. I would propose that the longer testis-specific transcript is not translated into opioid peptides due to the presence of the upstream initiator codons but may express a novel 26 amino acid peptide, as just discussed. The low levels of opioid peptides that are present in the testis probably are derived from the PE transcript in the Sertoli cells which is present in much lower abundance than the germ cell message. Alternatively, it is possible that the

longer PE mRNA is processed further in a developmentally-regulated fashion to remove the inhibitory intron A sequences and allow for normal translation of the PE precursor from a relatively low abundance transcript

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ACKNOWLEDGEMENTS

AUTHORS

Richard D. Howells, Ph.D. and Saranya Rao
Department of Biochemistry and Molecular Biology
New Jersey Medical School and The Graduate School of Arts and Sciences
University of Medicine and Dentistry of New Jersey
Newark, New Jersey 07103

Synaptic Regulation of the Enkephalin Gene and Transcription Factors In Vivo: Possible Roles in Drug Abuse

George R. Uhl, D. Appleby, T. Nishimori, M.G. Buzzi,
and M.A. Moskowitz

INTRODUCTION

Synaptic regulation of opioid peptide genes in vivo, is
ubiquitous; the number of examples of regulated expression of
these peptides is impressive (reviewed in Uh1 and Nishimori,
1990). Enhanced activity in circuits containing
proenkephalin-expressing neurons tends to up-regulate
expression of the enkephalin gene, and diminished activity
tends to down-regulate this expression. This is especially
striking in the case of dorsal horn neurons, to which primary
afferent inputs can be augmented or diminished (see below).

There is a substantial and growing rationale for studying
regulated gene expression in drug abuse. The concept: brain
mechanisms producing tolerance, dependence, craving and other
manifestations of drug abuse are likely to share common
features with molecular mechanisms of memory (Table I). Thus,
both memory and drug tolerance/dependence result in changed
brain function based on prior exposure to specific stimuli.
As molecular mechanisms involved in gene regulation become
increasingly-attractive as candidate repositories for
remembered events (Berridge, 1986, Goelert et al, 1986), so
they may become attractive candidates for the storage of
information relating to drug tolerance, dependence, and
craving (Uh1, in preparation).

TABLE I: Rationale for Exploring Neuronal Neurotransmitter
          Gene Regulation In Vivo in Drug Abuse

1) In addiction, the brain's molecular mechanisms may change
    due to prior drug exposure.
2) Some of these molecular changes may store information
    about the prior drug exposure.
3) The exact molecular mechanisms in which this information
    about prior drug exposure are stored are currently
    unproven (the molecular substrates for tolerance,
    dependence, craving, etc., remain largely unproven).

4) The genes of neurotransmission, including the genes for opioid peptides, are exquisitely regulated in concert with neuronal activity (and drug exposure) in neuronal populations <u>in vivo;</u> these effects can outlast the stimulus that triggers them.

5) This activity-dependent gene regulation is thus one potential molecular mechanism for storing information about prior drug exposure.

6) Some of the factors that mediate gene regulation in general are likely to mediate this match between neural activity and neuronal gene expression (trans-synaptic regulation) and to determine its temporal parameters. These factors are thus also important to study.

7) both the patterns and mechanisms of gene regulation are likely to differ substantially between neurons <u>in vivo</u> and cultured cell models.
Exploration of these mechanisms <u>in vivo</u> may not confirm results obtained in cell culture.

8) Patterns of gene regulation differ from cell group to cell group in the brain.
Exploration of these mechanisms in the appropriate neuronal populations is thus important.

PHARMACOLOGIC ISSUES CONCERNING OPIOID PEPTIDE GENE REGULATION

Opiate drugs, both agonist and antagonist, can produce effects on expression of these genes. We have documented modest effects of high doses of morphine in down-regulating proenkephalin expression in striatal neurons (UH1 <u>et al,</u> 1988). In addition, Zukin and collaborators have found, and we have been able to confirm in some (but not in all) experiments, that opiate antagonists up-regulate proenkephalin expression (Temple et al, 1990; O'Hara et al, in preparation). These effects show substantial variability (Uhl <u>et al,</u> 1988; Lightman and Young, 1987, O'Hara <u>et al,</u> in preparation); how this may reflect animal to animal variability in physiology or drug responses has not been clearly elucidated. These alterations in levels of the opiate peptide mRNAs could reflect changes in transcriptional rate and/or mRNA stability (Uhl and Nishimori, 1990). Conceivable, they could also have importance for the functions of the neurons that express them.

Hints of the possible functional significance of changed levels of opioid peptide mRNA come from consideration of the points at which substantial regulation of peptide production have been frequently documented (Schwartz et al, 1986). Although mRNA levels are dramatically regulated, evidence for neuronal regulation of the other steps in peptide production (including translation; posttranslational processing, peptide packaging, etc.) is much more modest (Uh1 and Nishimori, 1990). Thus, if post-transcriptional events are neither regulated nor rate-limiting, and if gene transcription rates can and do vary, then regulation of mRNA levels could have significant neurobiologic importance in altering neural

abilities to synthesize opioid neurotransmitter/
neuromodulator peptides. One measure of such importance,
indeed, might be the relative stability of peptide
concentrations found in several studies after stimuli known to
change neural firing (Schwartz et al, 1986). Stable peptide
levels, in the face of altered gene expression and neural
firing, point to a functioning homeostatic balance between
rates of peptide synthesis and release. Peptide levels are
not always constant, such a scenario is not invariable. In
many settings, however, more mRNA could make more enkephalin
available for release.

REGULATION OF PREPROENKEPHALIN AND ASSOCIATED
TRANSCRIPTION FACTORS

If gene regulation can play an important regulatory role in
neural responses to synaptic and trans-synaptic events,
interest in the mechanisms by which these genes are regulated
in neurons in vivo is substantially enhanced. A small region
of the proenkephalin gene's five-prime flanking region, for
example, has been suggested as crucial for changes in the
expression of preproenkephalin. This region can bind
transcription factors that can enhance or repress gene
expression in cultured cells (Maniatis et al, 1989, Comb et
al, 1986, 1988). These DNA sequence elements are present on
each copy of the proenkephalin gene, regardless of the cell
type involved. In order to define patterns of regulation in
vivo, one must define which of the factors potentially binding
to this regulatory region "cassette" element are actually
expressed in the cells of interest. Cell culture studies are
thus able to define these "cis acting" DNA binding elements
potentially involved in gene up- and down-regulation, but
cannot identify the "trans acting" genes which code for the
transcriptional factor DNA binding proteins whose expression
may actually control proenkephalin's expression in specific
neuronal populations in brain. Focused study of the
transcription factors regulating enkephalin gene expression,
and the cascades of cytoplasmic and other biochemical events
in turn regulate expression of these presumed transcription
factors in vivo, may have an impact on understanding not only
the mechanisms but the temporal profile of gene expression.
The entire cytoplasmic and nuclear pathways for synaptically-
regulated gene expression may be regarded as likely
biochemical candidates for storage of some of the information
about previous neuronal excitation, or previous neural
exposure to drugs (Uh1, in preparation).

Understanding the possible mechanisms for regulation of opioid
peptides in vivo thus takes on enhanced importance. In an
initial approach to this question, several laboratories have
begun to study the baseline and enhanced expression of the
genes for transcription factors that could bind to the
proenkephalin promoter in neural populations where robust
trans-synaptic regulation of proenkephalin expression has been
documented (Uh1 et al, in preparation, Sonnenberg et al,

125

1989; Wisden et al, 1990; Menetry et al, 1989).

There is substantial activity-dependent regulation of proenkephalin in individual neurons of the pain-modulating lamina I and, II areas of the nucleus caudalis (Uh1 and Nishimori, 1990; Nishimori et al, 1988, 1989, and in press). The nucleus caudalis acts as a primary waystation to modulate nociceptive and other inputs from the face. Receiving inputs from several highly-innovated structures, this nucleus may be one of the most intensely pain-regulating zones in the body. There is a substantial activity-dependent regulation of proenkephalin in these neurons, as documented by in situ hybridization techniques that allow quantitation of the gene expression in individual neurons. These studies provide evidence for up- and down-regulation of proenkephalin expression by adding to and subtracting from the population of expressing neurons noted in control tissues. Further, this regulation is expressed in an activity- and fiber-type specific manner (Table II). Several of these findings have also been made in neurons of the spinal dorsal horn (e.g., Noguchi et al, 1989).

TABLE II: Regulated Dorsal Horn Preproenkephalin Expression:

1) Removing large- and small-caliber primary afferents causes downregulation.
2) Removing small-caliber primary afferents neonatally causes (less marked) downregulation.
3) Brief stimulation of primary afferents causes upregulation with a time-course that depends on the stimulus intensity.
4) Chronically stimulating large- and small-caliber primary afferents causes upregulation.
5) In each of these cases, there are substantial changes in the fraction of dorsal horn neurons that express preproenkephalin.

Recent studies have focused on finding as to which of the transcription factors binding to the proenkephalin promoter might be involved in this up-regulation (Uh1 et al, in preparation; Wisden et al, 1990). These neuronsare favorable sites at which to study the time-dependent and physiologically-relevant regulation of this gene's expression in a neural circuit in vivo, for several reasons.

TABLE III: Advantages of Dorsal Horn for Studying Mechanisms of Preproenkephalin Upregulation:

1) Time course of stimulation can be easily controlled, and thus time course of responses noted.
2) Changes in number of expressing neurons provide a more rigorous test of proposed mechanisms than simple upregulation alone.
3) Possible physiologic relevance, accessible to study.
4) Documented cFOS (protein) upregulation by increases in the number of expressing neurons.

In order to be a candidate for participation in preproenkephalin upregulation in vivo, a factor should be present in nucleus caudalis neurons, should be present in an increased number of these neurons after primary afferent stimulation, and should display enhanced expression with a time-course similar to that required for proenkephalin up-regulation. Such correlative evidence obviously does not constitute proof that such a factor is involved in this sort of regulation. However, such studies can at least provide a number of potential candidates for involvement in the regulation.

The nucleus caudalis displays strikingly different expression of several of the factors that potentially bind to the AP1 site, a powerful enhancer sequence of the enkephalin promoter (Comb et al, 1988). Three members of the Jun family of transcription factors were studied (Uh1 et al, in preparation). Levels of Jun B were enhanced a striking fashion in animals sacrificed immediately after primary afferent stimulation. Further, this enhanced expression was largely due to the increase in the numbers of neurons expressing the transcription factor. Thus, this factor may be a good candidate for involvement in the trans-synaptic regulation of proenkephalin, under these circumstances.

Immunohistochemical studies have documented elevation of a fos-like immunoactive protein with sensory stimulation (Hunt et al, 1987; Menetrey et al, 1989). We have found that c-fos mRNA shows enhanced expression, again largely by increasing the fraction of expressing neurons (Uh1 et al, in preparation).

Using these set of approaches one can identify which of the transcriptional factors might be active in vivo in a specific setting to bind to the regulatory elements of genes encoding opiate peptides, and thus start to work out potential pathways of gene regulation in vivo. However, such studies allow only tentative assignment of relationships between upregulation of a specific factor and enhanced expression of another gene. One approach to testing this relationship more directly involves introducing mutated versions of the gene's regulatory region into transgenic animals, and examining the retention or loss of regulation after a specific regulatory site is altered.

CONCLUSIONS

The pathway from cell-surface to nucleus is an increasingly interesting one for study by many molecular neurobiologists. Given the potential for study of this pathway to elucidate ways in which genes important for drug abuse are regulated in vivo, and even the possibility that this pathway could provide a biochemical means for storing information about previous neural stimuli and drug exposure, understanding the in vivo regulation of genes in this fashion should gain increasing prominence in studies of drug abuse.

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AUTHORS:

George R. Uh1, M.D., Ph.D.*; Donna Appleby, M.S.*; Toshikazu Nishimori, Ph.D.**; M. Gabriella Buzzi, M.D.+; Michael A. Moskowitz, M.D.+

AFFILIATION:

*Laboratory of Molecular Neurobiology, ARC/NIDA and Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

+Department of Neurology & Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

**Current Address: Department of Oral Anatomy, Hiroshima University School of Dentistry, Minami-ku, Hiroshima 734 Japan

IN MEMORIAM: ULRICH WEISS

1908-1989

The "Current Trends in the Chemistry of Medications for the Treatment of Chemical Dependencies" symposium of the 52nd Annual Scientific meeting of the Committee on Problems of Drug Dependence is respectively dedicated to Dr. Ulrich Weiss.

Ulrich Weiss was an extraordinary scientist who worked at NIH from 1957 until his retirement in 1978 when he became Scientist Emeritus in the National Institute of Diabetes, Digestive and Kidney Diseases. He died on July 15, 1989 of cardiac arrest in Denpasar on the island of Bali at the age of 81.

During a career which spanned more than 60 years, Ulrich Weiss made major contributions to natural products chemistry, medicinal chemistry and synthetic methods. He possessed an insatiable interest in organic and natural products chemistry as well as an incredible memory for these subjects. He was particularly fond of alkaloid chemistry and nowhere is this more evident than in his studies of the opium alkaloids. His opium alkaloid work is described in more

than 25 publications and patents and addressed (a) structure activity relationships in the morphine and codeine series and their 14-hydroxy derivatives, (b) new synthetic transformations and spectroscopic studies of opium derivatives and (c) structural elucidation of a number of opium alkaloid derivatives. Although he carried out many synthetic transformations in this area, his most crucial centered on the development of a method in 1955 for the 0-demethylation of 14-hydroxydihydrocodeinone (oxycodone) to 14-hydroxydihydromorphinone (oxymorphone). This process, which had been unsuccessfully attempted by others, provided a potent analgesic which is still valuable today and is marketed as numorphan. Oxymorphone was the starting material which enabled the synthesis (by Fishman et al.) of naloxone and later of naltrexone, both pure narcotic antagonists which serve as life saving antidotes for narcotic overdose and hold promise for the prevention of relapse in former narcotic abusers. Naloxone, in particular proved to be an indispensable research tool in the discovery of the opiate receptor endorphin system and continues to be important for the elucidation of the structure and function of this system. Most certainly had it not been for Weiss's development of oxymorphone and its subsequent transformation to naloxone our knowledge of the opiate receptor endorphin system would not have advanced so rapidly.

During his seminal studies on the biosynthesis of aromatic compounds, he elucidated the structure of prephenic acid, a key intermediate in the biosynthetic pathway to these biologically important building blocks. He also contributed to the structure determination of other intermediates along this pathway and his work was chronicled in a book "The Biosynthesis of Aromatic Compounds" which he coauthored. In addition, much of the earlier work at NIH on the chiral diene rule employing optical rotatory dispersion and circular dichroism was carried out by Weiss and his worldwide array of collaborators.

In 1968, he discovered that the reaction of 1,2-dicarbonyl compounds with dimethyl-3-oxoglutarate resulted in a facile synthesis of the cis-bicyclo[3.3.0]octane-3,7-dione system. This, he realized, was the best method for the preparation of fused cyclopentanoid compounds. In the ensuing years he was heavily involved in the use of this reaction in the synthesis of polyquinanes and polyquinenes. The preparation of the natural product modhephene, the [5.5.5.5]fenestranes, staurane and the staurane tetraene, parrlorane, as well as centrosubstituted triquinacenes including ellacene are only a few of the cyclopentanoid compounds prepared by the Weiss reaction. His love for natural product chemistry and organic chemistry is mirrored in his 130 publications, many of which involved structural determination of new natural products. In fact, he was working on the structures of a number of related alkaloids, some of which exhibited anti-HIV activity, shortly before his death.

Weiss acquired a reputation with his colleagues as an invaluable source of information because of his vast knowledge extending over the entire literature of chemistry, botany, plant physiology and medicinal chemistry. His erudition extended to music, mineralogy, fossils, textiles and history. In the technical environment of NIH, he represented Old World scholarship at its best.

A resident of Bethesda, Maryland for 32 years, Weiss was born in Prague where he studied chemistry and earned his Ph.D. at the then German University. After the Nazis annexed Czechoslovakia, Weiss and his wife, Anna, left for Brussels. It was to be the beginning of a precarious odyssey that included sojourns in Paris, Puy de Dome, Marseille, Martinique and New York, where they arrived with

their daughter, Ruth, on June 2, 1941. Shortly before his death, Weiss, in a 4-hour taped interview, recorded the details of this passage for the documentation center of the U.S. Holocaust Museum in Washington, DC.

Weiss was well known in the international scientific community as an excellent scientist, an ambassador of goodwill for NIH, and was a valued consultant in the medicinal chemistry of narcotic drugs for a number of scientists associated with the Committee on Problems of Drug Dependence.

AUTHORS:

Kenner C. Rice, Ph.D.
Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD.

James M. Cook, Ph.D.
Department of Chemistry
University of Wisconsin, Milwaukee
Milwaukee, WI.

Inverse Agonists, Probes to Study the Structure, Topology and Function of the Benzodiazepine Receptor

James M. Cook, Hernando Diaz-Arauzo, and Michael S. Allen

The identification of stereospecific, high-affinity binding sites for benzodiazepines (Bz) in the mammalian CNS has been described *in vitro* (Squires and Braestrup 1977) and *in vivo* (Mohler and Okada 1977). The benzodiazepines exhibit a wide range of pharmacological actions which include anticonvulsant, sedative-hypnotic, muscle-relaxant, and anxiolytic effects. It has been shown that the benzodiazepine receptor (BzR) is part of a supramolecular complex which also contains discrete but allosterically coupled recognition sites for GABA and barbiturates. The oligomeric units of this supramolecular complex are thought to form a drug and transmitter responsive chloride channel (Skolnick and Paul 1988); moreover, recent results from cloning experiments suggest that at least three homologous $(\alpha, \beta, \gamma^2)$ but distinct proteins are required to effect benzodiazepine potentiated GABA transmission (Pritchett *et al.* 1988). It is generally believed that BzR ligands elicit their pharmacological effects through modulation of this ligand-gated chloride ion channel. The pharmacological properties of these ligands appear to be a continuum (Gardner 1988), ranging from a complete mimicry of the 1,4-benzodiazepines [anxiolytic, anticonvulsant, hypnotic, ataxic (agonists)] to substances that produce actions best described as opposite (convulsant, proconvulsant, anxiogenic, etc.) to the benzodiazepines termed inverse agonists.

Although Nielsen et al. (1979) originally proposed β-carboline-3-carboxylic acid ethyl ester **1** *(βCCE)* as the endogenous ligand for this binding site, subsequent studies have demonstrated this compound is formed during the isolation process. Nonetheless, the demonstration that specific β-*carbolines* (inverse agonists) potently inhibit [³H]diazeparm binding with high affinity and increase neuronai firing in the CNS suggests this group of compounds may be useful both as tools for studying benzodiazepine receptors, as well as for the development of new therapeutic agents. For example, an inverse agonist devoid of convulsant/proconvulsant actions has been proposed as a possible cognitive enhancer. Moreover, ligands which increase neuronal

firing in the CNS, but are not convulsant may be effective in reversal of barbiturate/barbiturate-alcohol induced CNS depression.

Although many β-carboline-3-carboxylic acid esters have been prepared and evaluated for inverse agonist activity, two of the most important are βCCE **1** (anxiogenic, proconvulsant, weak convulsant) and βCCM **2** (convulsant, proconvulsant). While both of these full inverse agonists are short-lived *in vivo* requiring large doses for maximal effect, DMCM **3,** an analog of **1** has been demonstrated to be an extremely potent convulsant at low doses; however, again it is not long-lived *in vivo*.
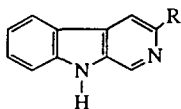
1	R=CO_2Et (5nM)
2	R=CO_2Me (5nM)
4	R=CH_2OH (1470nM)
5	R=CO_2tBu (10nM)
	diazepam (5nM)
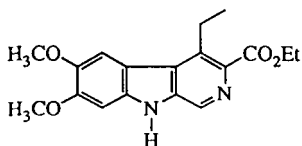
3   (DMCM) < 5nM

Recently, 3HMC **4** as well as DMCM **3** have been shown to reverse the effects of barbiturate-induced CNS depression (Albrecht *et al. 1985,* Havoundjian *et al.* 1987). In a related study, physostigmine is known to increase neuronal firing in the CNS via a cholinergic mechanism. The β-carboline βlCCt **5** was shown to reverse midazolam-induced CNS depression and this reversal was potentiated by physostigmine (Hoffman *et al. 1986).* This suggests that combination therapy with physostigmine and a long-lived inverse agonist may be an effective means in which to safely reverse (indirectly) clinical CNS depression from barbiturate/barbiturate-alcohol overdose. In this regard the search for long lived, potent, safe inverse agonists has continued.

In order to employ rational drug design to prepare selective inverse agonists and/or agonists the pharmacophores for both activities must be defined. There is controversy as to the nature of the binding sites for inverse agonist/antagonist and agonist ligands at BzR. The recent report by Pritchett *et al.* (1989), which showed that at least three distinct cDNA's must be expressed *in vitro* for a fully functional (i.e. BzR modulated) GABA-gated chloride channel, indicates the Bz binding site (cleft) may be constituted by domains formed by the interaction of the extracellular portions of these proteins. For this reason we have continued to treat the pharmacophores for the inverse agonist/antagonist and agonist sites as separate entities, although different interactions in the same domain

(Ehlert) is fully consistent with this approach.

In 1985, the synthesis of 7,12-dihydropyrido[3,4-b:5,4-b']diindole **6** (Figure 1) was realized. This rigid, planar ligand was found to bind (5nM) with high affinity to BzR *in vitro* and to elicit inverse agonist activity *in vivo*. More importantly, however, the rigid planar nature of **6** has provided a basis with which to characterize the topography of the BzR inverse agonist/antagonist site.   A series of diindoles were synthesized and substituents were varied individually at positions 1-7, 10 and 12.

Figure 1. Proposed Pharmacophore of the Benzodiazepine Receptor Inverse Agonist/Antagonist Site (Diindole **6** Template).
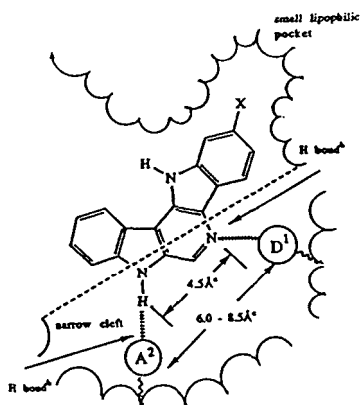
Table 1. *In Vitro* Binding of Selected β-Carboline Ligands at BzR.

Cpd.	R	A	in vitro IC_{50} nM
7	H	H	1620
8	OCH_3	H	124
9	OCH_2CH_3	H	24
10	$OCH_2CH_2CH_3$	H	11
11	$OCH(CH_3)_2$	H	500
12	$OCH_2CH_2CH_2CH_3$	H	98
13	$COCH_2CH_2CH_3$	H	2
14	$CH_2CH_2CH_2CH_3$	H	245
15	$N \approx C = S$	H	8

[a]Average interatomic distance between binding sites on ligand.
[b]Intermolecular bond lengths obtained from crystal structures of ligands.
[c]Distance between binding-site residues on receptor dependent on hydrogen bond lengths.

The SAR of this series was evaluated and the data compared to that determined for 3-substituted β-carbolines (βC). Substituents at C-2 in **6** were well tolerated by the binding site and this region was found to overlap with position-3 of the β-carboline-3-carboxylic acid alkyl esters **1, 2, 3,** and **5.** In addition, other ligands known to elicit inverse agonist action *in vivo* including **1-5,** 3-[(methylamino)carbonyl]-β-carboline (FG-7142) and the pyrazoloquinoline (CGS-8216) were overlayed with **6** to determine the common points of electronic and lipophilic character. These representative structures were rotated until a nearly planar comformation was achieved and then fit to the diindole **6** template. From this analysis two separate sites of electronic interaction were determined. In one region each of the

135

molecules contained a N-H function which interacts with a hydrogen bond acceptor site (A^2) on the binding site, whereas a pair of electrons on a heteroatom (O or N) provided the interaction with the hydrogen bond donor (D^1) site on the receptor protein (see Figure 1). To refine this model, a 3D-QSAR (Cramer *et al.* 1988) was carried out and receptor excluded volumes were determined. The lipophilic regions depicted in Figure 1 for the pharamacophore are qualitatively in good agreement with the CoMFA analysis (R^2=0.59) determined for 37 test compounds (Allen *et al.* 1990). Based on this model of the inverse agonist/antagonist site a number of 3-substituted β-carbolines were prepared and evaluated. It was found that electron withdrawing substituents at position-3 of the βC increased the binding affinity of ligands to the inverse agonist site, presumably, by polarization of the indole N(9)-H bond (Allen *et al.* 1988); moreover, electron releasing substituents (Table 1) at C-3 also increased the *in vitro* affinity at BzR (compare **7** to **8-12**). The alkoxy oxygen atom in **8-12** donates electron density to the pyridine nitrogen atom and increases the interaction at D^1 (Figure 1) in the βC series. From this analysis the synthesis and evaluation of 3-ethoxy β-carboline **9** was designed (Trullas *et al.* 1988). This ligand has now been shown to be a long-lived partial inverse agonist. Although 9 was not a convulsant even at 50mg/kg, it potentiated the convulsant effects of pentylenetetrazole in mice at doses (ED_{50} 7mg/kg) much lower than those required for the same effect with βCCE 1. Moreover, 3EβC•HCl9 is more water soluble than FG 7142 and **9** is more soluble and longer-lived (>4 hr) *in vivo* than **1.** This suggests that **9** or related congeners would be excellent candidates with which to study the mechanisms of sleep or anxiety, as well as the reversal of barbiturate-induced CNS depression (overdose).

The results from molecular modeling indicate that in addition to hydrogen bond donor (D^1) and acceptor (A^2) sites on the BzR, there appears to be a relatively narrow lipophilic pocket in the binding cleft. It can accomodate substituents at position-3 of β-carbolines which have chain lengths of $\leq$5 bonds. This analysis enables one to distinguish between inverse agonists and antagonists based in part on experimental findings which demonstrate that 3-n-propoxy β-carboline **10** is an antagonist at the BzR with low efficacy and is devoid of proconvulsant activity at the highest dose tested (40mg/kg). Essentially, β-carbolines which possess substituents at C-3 of shorter length than **10** (for example **1** and **9**) which are con-strained in the plane of the aromatic ring display inverse agonist activity. However, ligands such as βCCt **5** and **10** which carry longer substituents that can access regions of space above and below the plane of the aromatic rings are expected to elicit antagonist activity.

In regard to the pharmacophore of agonist molecules, several models have

been proposed previously; however, the pharmacophoric descriptors which predict an agonist profile of activity are still ill-defined. The strategies for receptor modeling recently redefined by Cramer (1988) were employed with SYBIL to determine the pharmacophore for the agonist site. Agonists (see Figure 2) including the benzodiazepines, the pyrazoloquinolines and the β-carbolines (ZK-series) were employed for this study. As illustrated in Figure 2, it is proposed that the two electron-rich atoms [termed $\phi 1$ and $\phi 2$ (ϕ=N or O)] are located approximately 3.8Å from each other on the ligand and both are required for an agonist profile of activity. Furthermore, the two atoms must be electronegative enough to form hydrogen bonds with the protein at [H1] and [H2] on the binding site; however, only one interaction is required to exhibit *in vitro* affinity at BzR, as pointed out earlier by Fryer. In addition to the above mentioned electrostatic interactions ($\phi 1$ and $\phi 2$), the pharmacophore of the ligand must fill the lipophilic region termed ¶1 (see Figure 2). The substituents on ligands which fill this region must be at least 6.57Å distant from the center of the line between $\phi 1$ and $\phi 2$, as well as at an $\angle$ of 88.42° with $\phi 1$ For agonist activity the optimal position of the substituents is achieved when these groups are located at a minimum distance of 5.99Å from the median distance of the line between $\phi 1$ and $\phi 2$ at an of 71.30° with $\phi 1$.

FIGURE 2

X=CL, Z=H: diazepam
X=NO₂, Z=F:flunitrazepam

ZK-93423 (6-benzyloxy)
ZK-91296 (5-benzyloxy)

CGS-9896 R=Cl
CGS-9895 R=OCH₃

Á= 88.42°
B= 71.31°
ø= 25.00°

Ø1   5.22 Å
3.8 Å   5.99 Å
6.57 Å
Ø2
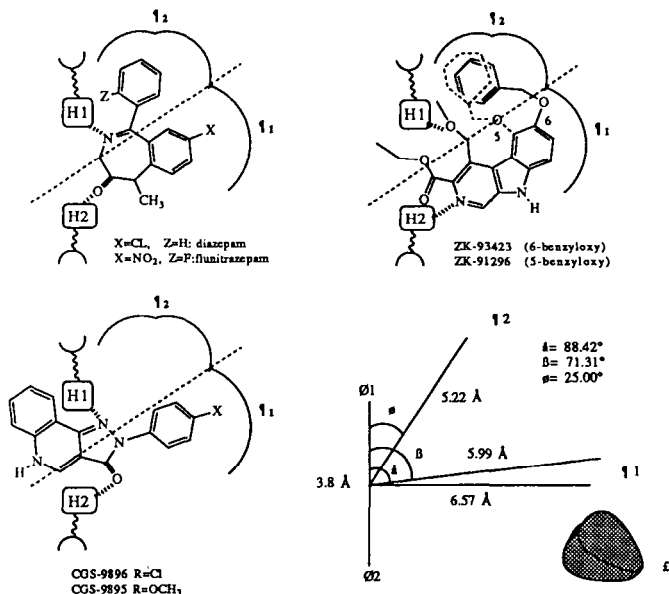
A second lipophilic region was located and labelled ¶2, the center of which is located at 5.22Å from the median distance of the line between Φ1 and Φ'2 at an ∠ of 25° with respect to 1Φ This lipophilic area is not in the same plane as ¶1 but is displaced about 2.179Å above the plane which intersects Φ1 ,Φ'2 and ¶ 1 (Figure 2). This region is not in the same plane as ¶1 because substituents in the plane at this position would interfere with the hydrogen bond of the binding site with Φ1. It is an important lipophilic region with regard to benzodiazepines and ZK-93423. The above inform- ation was employed to establish an alignment rule followed by an excluded volume map of the binding site. The region denoted £ in Figure 2 re- presents a region of the receptor protein that will experience negative interactions with the ligand or substituents. The pharmacophore for agonist activity generated in this fashion is illustrated in Figure 2. Moreover, from this approach the propyl ether **16** (Figure 2) has recently been prepared and has been shown to exhibit agonist activity. Illustrated in Figure 2 is the structure of the rigid, planar benzimidazole **17** whose synthesis is under- way. This molecule is predicted to have agonist activity for at least one of the regions ¶1 and ¶2 is completely occupied. Ligand **17** represents a new series of hybrid ligands related to both inverse agonists (diindoles) and agonists. The synthesis of molecules such as **17** is underway to establish if inverse agonists bind at the same site as agonists but simply possess different bioactive pharmacophores. From examination of Figures 1 and 2, it is clear the ligand-receptor interactions necessary for inverse agonist/ antagonist activity are different from those necessary to elicit an agonist response, although a nydrogen bond acceptor site in one ligand class may overlap with that of another class.

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Affiliation: Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201.

Synthetic and Biological Studies of Potential Affinity Ligands for the Cannabinoid Receptor Based on CP-55, 244

**Scott Richardon, Seid Mirasadeghi, Allison Lynn,
M. Ross Johnson, Lawrence S. Melvin, Miles Herkenham,
and Kenner C. Rice**

INTRODUCTION

Marijuana, of which the major biologically active constituent is Δ^9-tetrahydrocannabinol (Δ^9-THC) (Figure 1), has been used for at least 2500 years as a medicinal agent and in social and religious rituals. Today, marijuana is well known as a recreational drug of abuse (Mechoulam 1986). Centuries of marijuana research, which continues today, have revealed numerous physiologic effects of the drug, however, the the medicinal use of marijuana declined during the early part of this century. Roger Adams identified the basic cannabinoid skeleton in the middle 1940's (Adams 1941), but the modern age of cannabinoid research began with the isolation, purification and structural identification of Δ^9-THC by Mechoulam and Gaoni (1964). They hypothesized at that time that Δ^9-THC was the major biologically. active constituent; their hypothesis was confirmed in 1970 (Mechoulam et al. 1970). Researchers today are working toward elucidating the mechanism of action of the cannabinoids. Recent work provides compelling evidence that its pharmacologic effects are caused by interaction with a specific receptor rather than by influencing membrane fluidity (Devane et al. 1988, Howlett et al. 1988, Herkenham et al. 1990, Matsuda et al. 1990).

The now classic studies of Wilson and May (1976) using 9-nor-9β-hydroxyhexahydrocannabinol (HHC) and the epimeric 9-nor-9α-hydroxyhexahydrocannabinol stimulated research in the nonclassical cannabinoid field. Their findings demonstrated that motor effects were separable from antinociceptive effects and later studies showed that these latter effects were not mediated through opioid receptors. Melvin and Johnson (1987) embarked on a SAR study to determine what structural features were necessary for analgesia; they synthesized the

potent and enantioselective nonclassical cannabinoids CP-55,940 and CP-55,244. They hypothesized the existence of a specific and unique cannabinoid receptor which was first identified by the pharmacologic studies of Devane (1988) and Howlett (1988). Autoradiographic studies later performed by Herkenham and coworkers (1990) have provided highly convincing evidence in support of a cannabinoid receptor. Matsuda et al. (1990) have recently reported their results of cloning and expressing the gene which codes for the cannabinoid receptor.
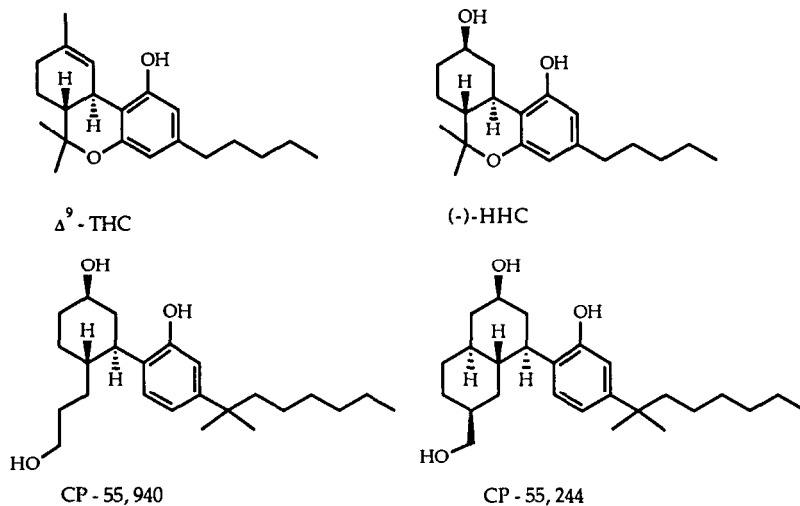
FIGURE 1

We initiated our synthetic studies with the hope of developing an affinity ligand for the cannabinoid receptor. We chose to use CP-55, 244 as a skeletal template, since there are three sites which can easily be manipulated to afford an electrophilic moiety. The isothiocyanate group is our functional group of choice, due to the ease of preparation, its stability to water and hydroxyl substrates, and the high reactivity towards amino and thiol bionucleophiles (Rice et al. 1979). We initially focused on synthesizing the isothiocyanates in the southern quadrant of the molecule and the necessary precursors, followed by the synthesis of the isothiocyanate in the eastern quadrant (Figures 2 and 4).

Our synthesis of the racemic southern isothiocyanates 1a and 1b (Figure

9 $H_1 = \alpha$ R = TBS R' = OH
10 $H_1 = \beta$ R = TBS R' = OH
11 $H_1 = \alpha$ R = TBS R' = Phth
12 $H_1 = \beta$ R = TBS R' = Phth
13 $H_1 = \alpha$ R = H R' = Phth
14 $H_1 = \beta$ R = H R' = Phth

6 R = H R' = O
7 R = H R' = CH$_2$
8 R = TBS R' = CH$_2$

15 $H_1 = \alpha$ R = Bn
16 $H_1 = \beta$ R = Bn
17 $H_1 = \alpha$ R = H
18 $H_1 = \beta$ R = H

19 $H_1 = \alpha$
20 $H_1 = \beta$

1a $H_1 = \alpha$
1b $H_1 = \beta$
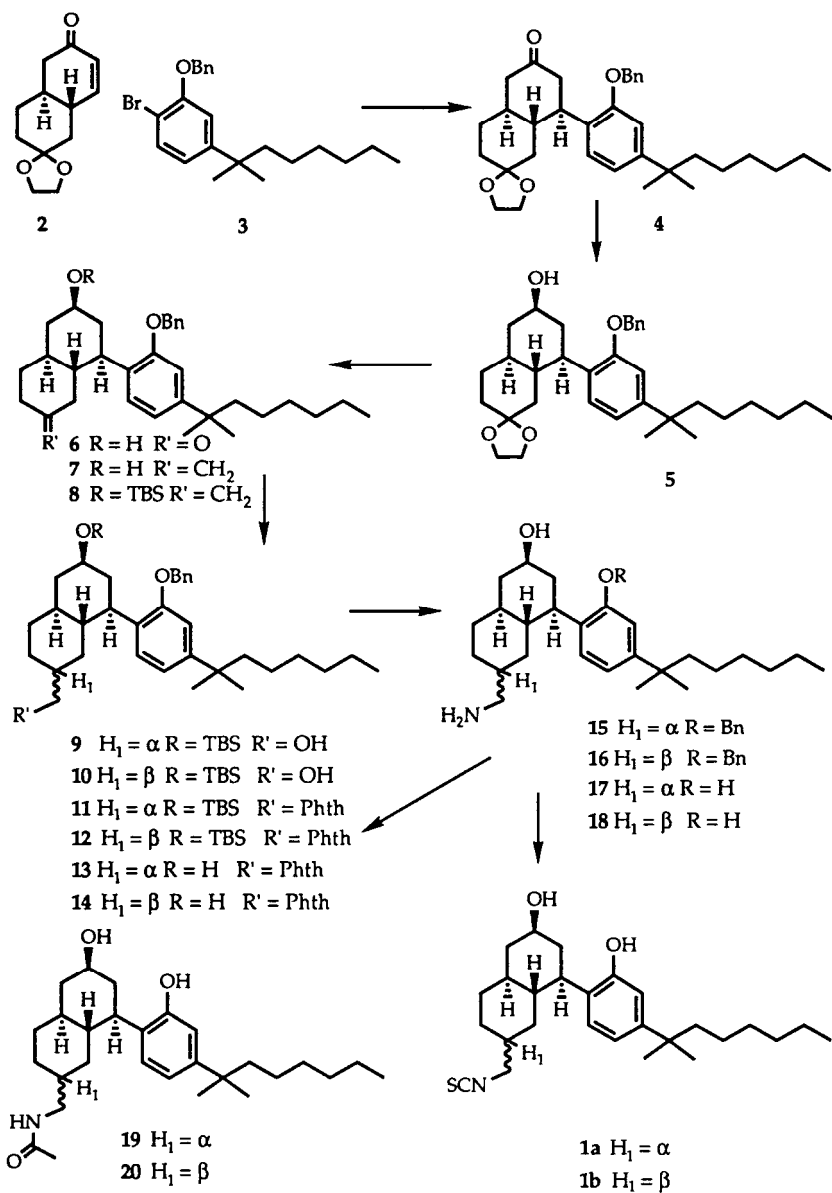
FIGURE 2

2) began by coupling enone **2** with the aromatic bromide **3,** affording the crystalline tetracyclic ketone **4,** which contains the fundamental ACD nucleus. Stereoselective reduction of the ketone with $NaBH_4$ afforded the β--hydroxyketal 5 as a 4.4/ 1 mixture of diastereomers. The ketal was removed under acidic conditions, yielding hydroxyketone **6.** The ketone was modified to the exocyclic olefin **7** using the Wittig reagent methyl triphenylphosphonium bromide. The secondary alcohol was protected as a *t* - butyldimethylsilyl ether (Corey and Venkateswarlu 1972), followed by steroselective hydroboration of the exocyclic olefin. The resulting compounds **9** and **10** were diastereomeric at the hydroxmethyl group (2.6/1) and could not be separated at this stage. Reaction of the hydroxyl group with phthalimide using Mitsunobu conditions (Mitsunobu et al. 1972) afforded phthalimides **11** and **12.** The silicon protecting group was removed using an activated acid resin and the two diastereomers **13** and **14** were separated chromatographically. Both diastereomers were used individually for the remainder of the reaction sequence. The phthalimides were cleaved to the primary amines **15** and **16** using hydrazine hydrate in refluxing ethanol, followed by removal of the benzyl moiety, which protected the phenol, by catalytic hydrogenation. Selective acylation of the phenolic hydroxyamines **17** and **18** was accomplished using pentafluorophenylacetate (Kisfaludy et al. 1979) to yield the N-acetyl derivatives **19** and **20.** Compounds **17** and **18** were also treated with thiophosgene in a $NaHCO_3/$ $CHCl_3$ biphasic solution (Rice et al. 1979) to afford the isothiocyanates **1a** and **1b** in excellent yield.
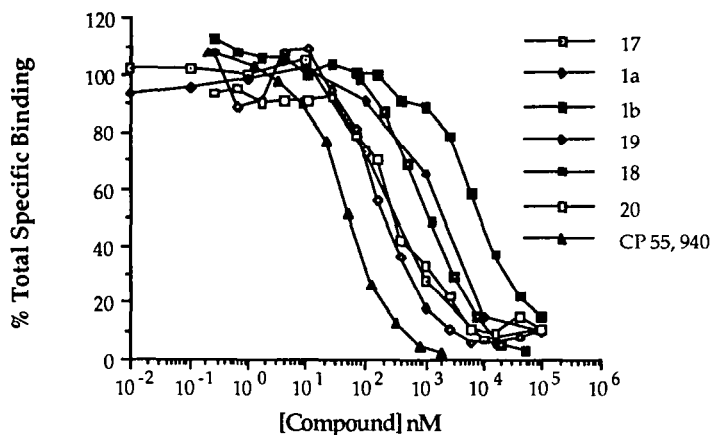
FIGURE 3

143

Compounds **1a, 1b, 17,18,19** and **20** were examined in a competitive binding assay developed by Herkenham (Herkenham et al. 1990), the results of which are shown in Figure 3. These racemic derivatives were all less potent than (-)-CP-55,940 by at least one order of magnitude, with the hydroxyphenolic amines, in general, being less potent than the the corresponding isothiocyanates. We believe this difference is most likely due to the zwitterionic nature of the phenolic amine. The data indicates that the ß-hydroxymethyl group is important for binding, perhaps *via* hydrogen bonding in the receptor cavity.

SYNTHESIS AND BINDING STUDIES OF EASTERN ISOTHIOCYANATE

The synthesis of the racemic eastern isothiocyanate (Figure 4) begins with a cuprate coupling reaction between enone **2** and aromatic bromide **21,** affording the tetracyclic ketone **22.** Stereoselective reduction of the ketone to the secondary ß-alcohol **23** with $NaBH_4$ occurs in good yield and as a 4/ 1 mixture of diastereomers. The ethylene ketal of **23** is removed with aqueous acid to give **24,** followed by a Wittig reaction to afford olefin **25** in excellent yield. Hydroboration yielded the ß-hydroxymethyl **26** in reasonable yield, as a 2/ 1 mixture of diastereomers. Protection of the two hydroxyl groups with acetic anhydride gave **27.** The silicon protecting group was removed with *n*-Bu_4NF affording alcohol **28.** Replacement of the alcohol with the phthalimide group utilized the Mitsunobu reaction (Mitsunobu et al. 1972) as before. The reaction of **29** with hydrazine hydrate in refluxing ethanol afforded the dihydroxyamine **30** in modest yield. Catalytic hydrogenation, followed by formation of the isothiocyanate as previously described afforded the phenolic dihydroxyisothiocyanate **32** in excellent yield.

Examination of **31** and **32** in the reversible, competitive binding assay (Herkenham et al. 1990) gave the following results (Figure 5): the free racemic amine **31** displayed a potency near that of (-)-CP-55,940, while the racemic isothiocyanate **32** was one order of magnitude more potent than (-)-CP55,940, and nearly equal to that of (-)-CP-55, 244 (K_i=1.4 nM vs K_i=6.3 nM, **32**).

In summary, our results indicate that replacement of the southern hydroxyl group with an isothiocynate function, as in **1** and **2,** is detrimental to receptor binding; introduction of an ω-isothiocyanate funtion in the sidechain of CP-55,244 has little effect on the receptor

affinity. We thus believe that preparation of the proper enantiomer of **32** will lead to a highly potent, and quite possibly irreversible ligand for the cannabinoid receptor. Initial studies toward preparing this enantiomer have met with success.
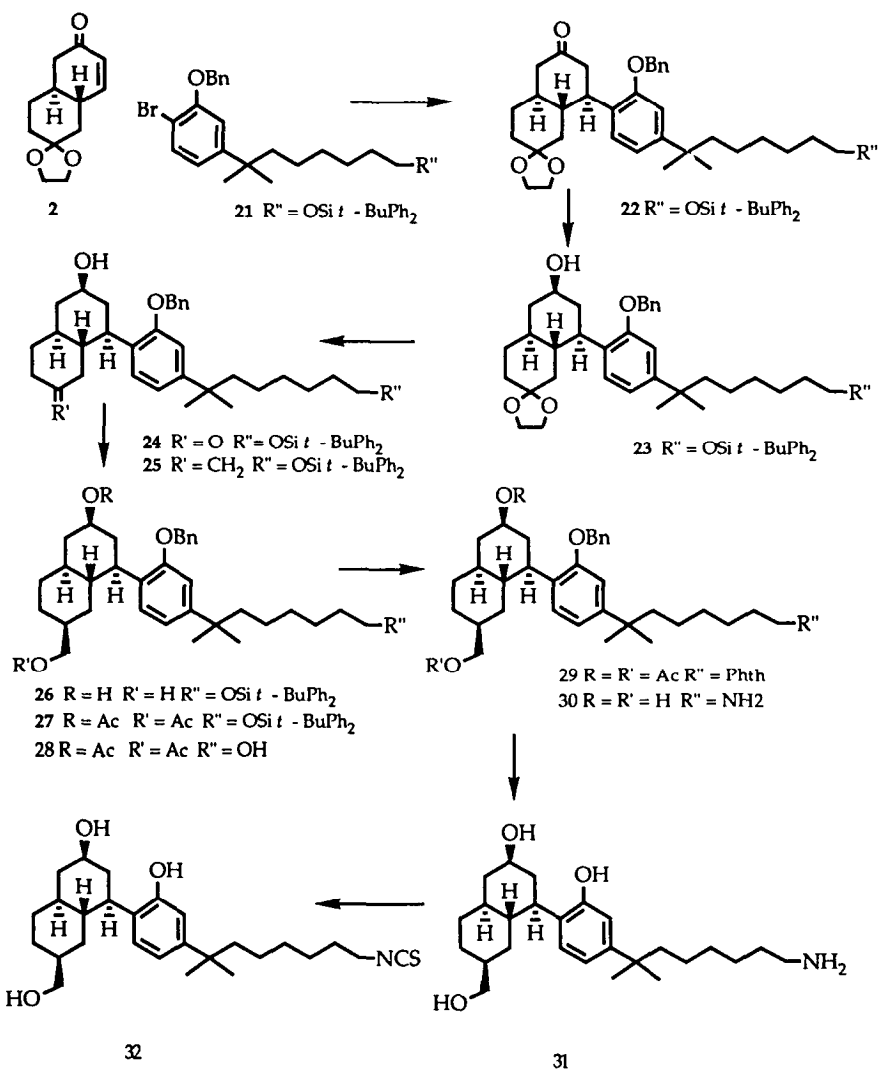
FIGURE 4
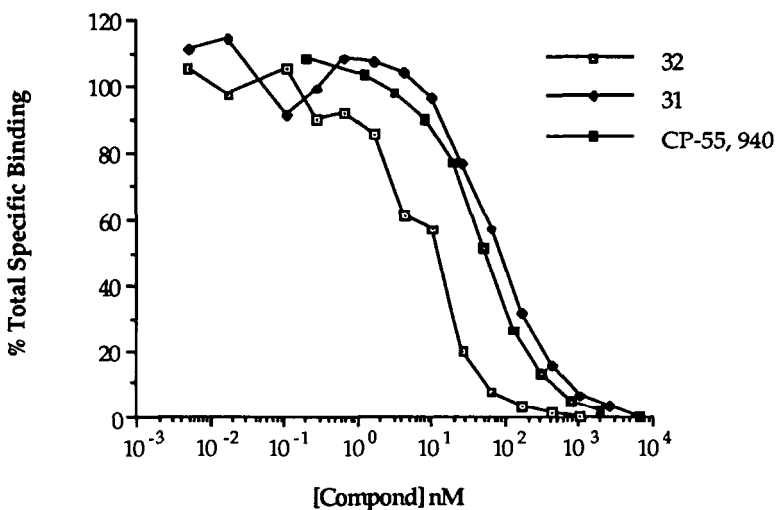
FIGURE 5

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Available from author upon request.

AUTHORS

Scott Richardson, Ph. D.
Seid Mirsadeghi, Ph. D.
Kenner C. Rice, Ph. D.
LMC/ NIDDK
National Institutes of Health
Building 8/ Room B1-23
Bethesda, MD 20892

Miles Herkenham, Ph. D.
Allison B. Lynn
National Institute of
Mental Health
Building 36/ Room 2D-15
Bethesda, MD 20892

M. Ross Johnson, Ph. D.
Department of Chemistry
Glaxo, Inc.
Five Moore Road
Research Triangle Park, NC
27709

Lawrence S. Melvin, Ph. D.
Medicinal Chemistry Research
Pfizer Central Research
Groton, CT 06340

Synthesis and Receptor Binding of Cocaine Analogs

Frank I. Carroll, M. Abdur Rahman, Abraham Philip, Anita H. Lewin, John W. Boja, and Michael J. Kuhar

INTRODUCTION

The apparent correlation between the potency of cocaine and cocaine-like compounds in self administration studies, and their ability to inhibit [3H]mazindol binding to the dopamine uptake site in rat striatum. suggests that this site may be associated with cocaine abuse (Ritz et al., 1987; Bergman et al., 1989). In order to gain insights to the structural and electronic requirements-of this binding site, we have undertaken to design and synthesize cocaine analogs for evaluation of their binding potencies (Carroll et al., 1990). The design of the analogs was based on available data and was aided by molecular modeling. Receptor binding was studied using [3H]WIN 35,428, a close structural analog of cocaine shown to label binding sites associated with the dopamine transporter (Madras et al., 1989). exhibiting higher affinity than cocaine for inhibition of either [3H]cocaine (Reith et al., 1986) or [3H]mazindol (Boja et al., 1990) binding.

RESULTS AND DISCUSSION

Effects of Stereochemistry

To establish the stereoselective nature of the cocaine binding site at the dopamine transporter, the three isomers of natural cocaine--pseudococaine, allococaine and allopseudococaine--as well as the optical antipodes of each of the isomers were investigated. In this report, R and S (Cahn et al., 1966) are used as prefixes to the common names to denote configuration. Thus natural cocaine is named R-cocaine, while unnatural cocaine is designated S-cocaine. The majority of the compounds in this series was synthesized following previously established methodology (Carroll et al., 1982; Lewin et al., 1987), starting from (+)- and (-)-2-carbomethoxy-3-tropinone obtained by resolution

147

of the racemate (Lewin et al., 1987); R-pseudococaine (2) was obtained from natural cocaine (1) by epimerization (Carroll et al., 1982). Only R and S cocaine and pseudococaine have been previously reported (Carroll et al., 1982; Lewin et al., 1987). The remaining four compounds were characterized by comparison of their 1_H NMR spectra to those of the previously reported racemates (Carroll et al., 1982). Synthetic details will be reported elsewhere. Receptor binding assays of R-cocaine and its seven isomers (Table 1) show striking differences between the eight compounds. While all seven isomers of R-cocaine inhibit [^{3}H]WIN 35,428 binding (Table 1) at the dopamine transporter, their potencies vary from 1/60th to 1/600th of that of R-cocaine.

Electronic and Steric Effects at C-2

The data in Table 1 show that the position of the carbomethoxy group at C-2 has a profound influence on binding affinity (compare IC_{50} of R-cocaine 1 and R-pseudococaine 2). However, the effects of steric bulk and electron density were never elucidated. Examination of a series of R-cocaine analogs in which the bulk of the O-substituent was systematically varied showed a remarkable lack of sensitivity to the nature of the O-substituent; only the very long phenylpropyl substituent (cf. 15) showed reduced potency (Table 2).

Effect of the Position of the Nitrogen Atom

The same effect was observed when the substituent at C-2 was removed entirely (cf. 16, Table 3). This information allowed us to investigate the requirements for the location of the nitrogen atom by preparing the 6-aza-analog 17 and comparing the potencies of 17 and 16. The synthesis of compounds 17-19 followed the methodology reported (Philip et al., 1990) for the preparation of azaprophen. Interestingly, the 6-aza-analog exhibited no enantioselectivity, with the two antipodes (18 and 19) possessing the same potency to inhibit [^{3}H]WIN 35,428 binding at the dopamine transporter: the potency of the racemate 17 was essentially identical to that of tropacocaine (16).

Effect of Substitution at C-3

Of particular interest was a modification of cocaine introduced several years ago by Clarke et al. (1973). These researchers replaced the benzoate moiety of cocaine by a phenyl group, essentially excising the carboxyl group at C-3, leaving the phenyl group directly attached to the bicyclic skeleton. This modification resulted in compounds possessing pharmacological profiles analogous to that of cocaine. Our investigation of a series of analogs in which the phenyl ring at C-3 was either substituted at the para position or replaced by an isostere or

by a group of similar lipophillicity, resulted in two compounds with potencies two orders of magnitude greater than cocaine (Table 4). Although no clear cut pattern of activity vs. substitution pattern could be identified at this point, it was noted that "large" substituents seemed to lead to lower potency, and that electron density at C-3 is necessary.

CONCLUSIONS

These results indicate that the cocaine binding site at the dopamine transporter is:
    a.   stereoselective, qualifying as a bona fide receptor.
    b.   sensitive to stereochemistry but fairly insensitive to substitution at C-2.
    c.   tolerant of changes in the position of the nitrogen atom.
    d.   extremely sensitive to substitution at C-3, with high electron density leading to very potent compounds.

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AUTHORS

F. I. Carroll, Ph.D., M. Abdur Rahman, Ph.D., Abraham Philip,
Ph.D., and Anita H. Lewin, Ph.D.
Research Triangle Institute
Research Triangle Park, NC 27709

J. W. Boja, Ph.D., and M. J. Kuhar, Ph.D.
NIDA Addiction Research Center
Baltimore, MD 21224

Table 1

Inhibition of [3H]WIN 35, 428 Binding to Striatal Rat Membranes: Cocaine Isomers
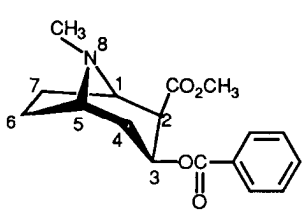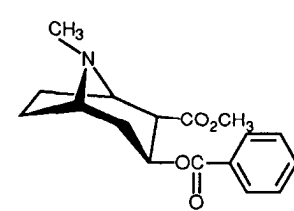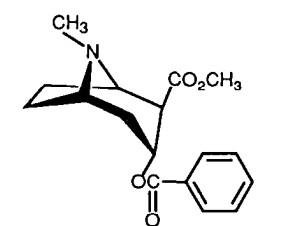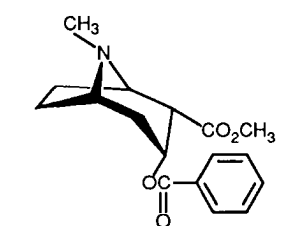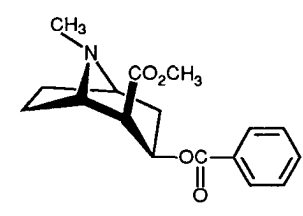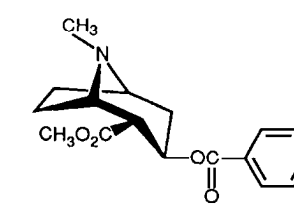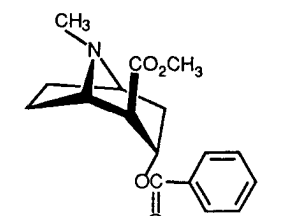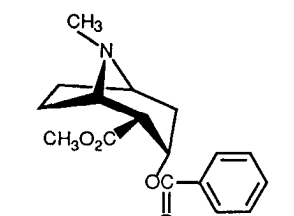
	IC50 (μm)
R - cocaine (1)	0.102
R - pseudococaine (2)	15.80
R - allococaine (3)	6.16
R - allopseudococaine (4)	28.5
S - cocaine (5)	15.8
S - pseudococaine (6)	22.5
S - allococaine (7)	9.82
S - allopseudococaine (8)	67.7

151

Table 2
Inhibition of [³H]WIN-35, 428 Binding in Striatal Rat Membranes: Effect of C-2 Substituent
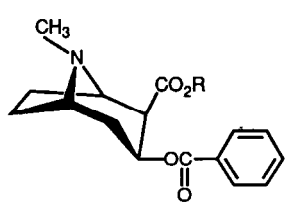
	R	IC_{50} (μm)
1	CH_3	0.102
9	C_2H_5	0.130
10	$CH_3(CH_2)_3$	0.191
11	$(CH_3)_2CH$	0.210
12	C_6H_5	0.112
13	$C_6H_5CH_2$	0.257
14	$C_6H_5(CH_2)_2$	0.264
15	$C_6H_5(CH_2)_3$	5.34

Table 3
Inhibition of [³H]WIN 35,428 Binding in Striatal Rat Membranes: Effect of the Position of Nitrogen

1   0.102 IC_{50} (μm)

17   4.95 IC_{50} (μm)

16   5.18

18   2.85

19   2.94

152
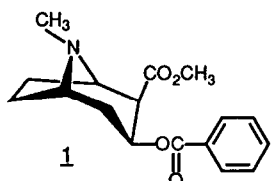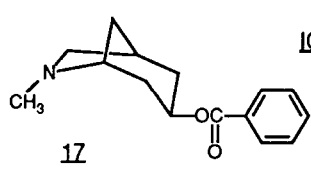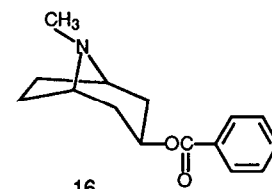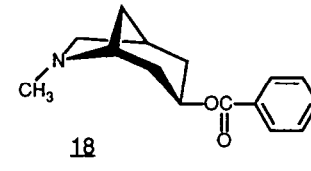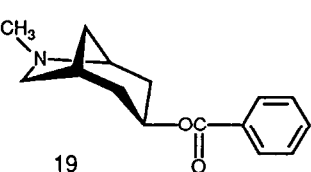
Table 4

Inhibition of [^{3}H]WIN-35, 428 Binding in Striatal Rat Membranes:

Effect of Substituent at C-3
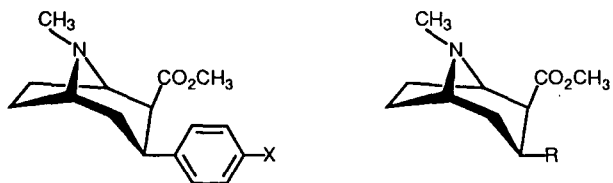
X	R	IC$_{50}$(nm)
H		23.0
F		15.7
Cl		1.2
Br		1.2
CH$_3$		1.7
N$_3$		2.1
NO$_2$		29.0
NH$_2$		48.0
NHCOCH$_3$		46.2
NHCOC$_2$H$_5$		68.0
NCS		165.0
NHCO$_2$C$_2$H$_5$		316.0
	C$_4$H$_3$S	277.0
	C$_6$H$_{11}$	10,700.0

Phenylakylamine Stimulants, Hallucinogens, and Designer Drugs

Richard A. Glennon

The phenylalkylamine (PAA) skeleton is one of the most common structural features found amongst biologically active substances. These substances include many therapeutically useful agents with a wide variety of pharmacological activities. Certain simple PAAs are also drugs of abuse and may be classified primarily as central stimulants or as hallucinogens. We have long been interested in determining how structural modification of the PAAs influences their stimulant and hallucinogenic character and have proposed, from a structural perspective, that such agents exist on a central stimulant-to-hallucinogen continuum. Amphetamine-like agents exist near the stimulant end of this continuum whereas agents such as mescaline and DOM (that is, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) lie closer to the hallucinogen end. 1-(3,4-Methylenedioxyphenyl)-2-aminopropane (MDA) seems to exist near the center of the continuum; MDA produces both stimulant and hallucinogenic effects and these activities are associated primarily (though not necessarily exclusively) with the S(+)- and R(-)-isomers, respectively. In addition to defining the structural requirements for each type of activity, we have investigated the mechanism of action of the PAA hallucinogens and have proposed that these agents act as 5-HT2 serotonin agonists. See Glennon (1989a,b) for recent reviews.

In general, we have employed two different approaches for investigating these agents: behavioral and radioligand binding studies. For the most part, the behavioral work has consisted of locomotor studies using mice and drug discrimination studies using rats. In the drug discrimination studies, animals have been trained to discriminate, for example, either the PAA stimulant S(+)-amphetamine (AMPH) or the PM hallucinogen DOM from saline. By conducting tests of stimulus generalization, it has been possible to classify PAAs as being either AMPH-like or DOM-like. To date, only one agent has been found to produce both AMPH- and DOM-like effects: MDA. Conversely, rats trained to discriminate MDA from saline recognize both AMPH and DOM. Structurally, MDA is a unique PAA in that it possesses a methylenedioxy group. Interestingly, animals trained to MDA recognize certain PAAs, such as 3,4-DMA (i.e., the 3,4-dimethoxy analog of amphetamine), 2,3-MDA (a positional isomer of MDA) and MDMA, that are not recognized by either AMPH- or DOM-trained animals (for a review, see Glennon 1989a). The purpose of this presentation is to summarize some of our more recent findings.

STRUCTURE-ACTIVITY   RELATIONSHIPS (SARs)

Separate and distinct SARs have been developed for AMPH-like and
DOM-like activity. Summarized below are some of the SARs derived from
drug discrimination studies using rats trained to discriminate either
S(+)AMPH or DOM (1 mg/kg, administered via the intraperitoneal route 15
min prior to testing) from saline. The SARs for these agents have already
been described in detail (Glennon 1989b).

Potent AMPH-like activity is typically associated with:
  (1) a primary amine or a monomethylamine,
  (2) an α-methyl group,
  (3) the <u>S</u> optical isomers (though both are usually active),
  (4) an unsubstituted aromatic ring, and
  (5) an unsubstituted, or carbonyl-substituted, benzylic position.

Optimal DOM-like activity is associated with:
  (1) a primary amine (alkyl, even methyl, substitution decreases potency),
  (2) an α·-methyl group,
  (3) the <u>R</u> optical isomers (though both are usually active),
  (4) 2,4- (e.g. 2,4-DMA) and 2,5-dimethoxy (e.g. 2,5-DMA) substitution,
        and, for 2,5-DMA derivatives:
  (5) a substituted 4-position, where the 4-position substituent is, for
        example, methyl (DOM), ethyl (DOET), n-propyl (DOPR), chloro
        (DOC), bromo (DOB) or iodo (DOI).

STUDIES WITH SOME NEWER DESIGNER DRUGS

Over the past several years, several novel PAAs have appeared on the
clandestine market as designer drugs. Whereas certain of these agents are
new, others were first synthesized more than 50 years ago; nonetheless, it is
only recently that their abuse potential is being realized.

<u>MDMA-Related Analogs.</u> MDMA ("Ecstasy, XTC, Adam") is the N-mono-
methyl analog of MDA. As might be expected from the above mentioned SAR
studies, and because MDA possesses both stimulant and hallucinogenic
activity, N-monomethylation should reduce the hallucinogenic properties of
MDA and, at the same time, enhance its AMPH-like effects. Consistent with
this prediction, we have shown that stimulus generalization results upon
administration of MDMA to AMPH-trained animals and that generalization
does not occur in DOM-trained rats. Others have obtained comparable
results using different species of animals trained to discriminate AMPH
from saline (e.g. Evans and Johanson 1986). For an agent with AMPH-like
effects, the S-isomer should be more potent than its R-enantiomer; this was
found to be the case. Interestingly, although AMPH-trained animals
recognize N-ethylamphetamine, they do not recognize the N-ethyl homolog of
MDMA (i.e., MDE; "Eve"). This seemed rather surprising in view of reports
that MDMA and MDE produce similar effects in humans. Consequently,
groups of rats were trained to discriminate MDMA (1 and 1.5 mg/kg) from
saline. In 1986 (Glennon et al, 1986), we demonstrated for the first time that
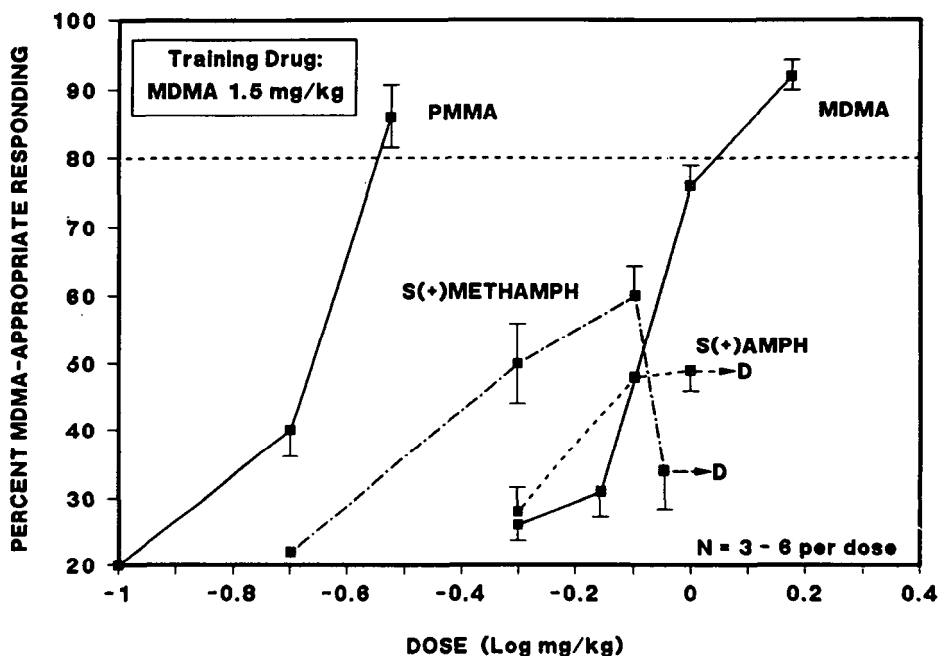
FIGURE 1. *Stimulus effects of PMMA, S(+)AMPH and S(+)methAMPH in rats trained to discriminate 1.5 mg/kg of MDMA from saline.*

MDMA serves as a discriminative stimulus in animals, that both isomers of MDMA produce MDMA-like effects, and that S(+)-MDMA is several times more potent than R(-)-MDMA. Subsequently, we demonstrated that MDE produces MDMA-like effects (Glennon and Misenheimer 1989), but that administration of S(+)AMPH and S(+)methamphetamine result only in partial generalization (Fig 1). This asymmetric generalization, coupled with the above mentioned studies using MDA as training drug, suggest that MDMA and AMPH share some common components of action but that they are also capable of producing effects that are distinct from one another. This is substantiated by the finding that the AMPH-stimulus does not generalize to MDE but that the MDMA-stimulus does. These findings also support the arguments of Nichols and Oberlender (1989) that MDMA may be the prototype of an entirely new class of psychoactive agents. Clearly, MDMA is not simply an AMPH-like agent.

N,N-Dimethylamphetamine. Although there is relatively little pharmacological data on this agent in the literature, it has recently been confiscated from several clandestine laboratories. Because it is the N-methyl analog of methamphetamine, it might be expected to produce AMPH-like stimulus effects. However, in rats trained to discriminate (+)AMPH from saline, neither isomer of N,N-dimethylamphetamine resulted in stimulus generalization at doses of up to 50 times the ED50 dose of(+)AMPH (i.e., 0.42 mg/kg) (Fig 2).
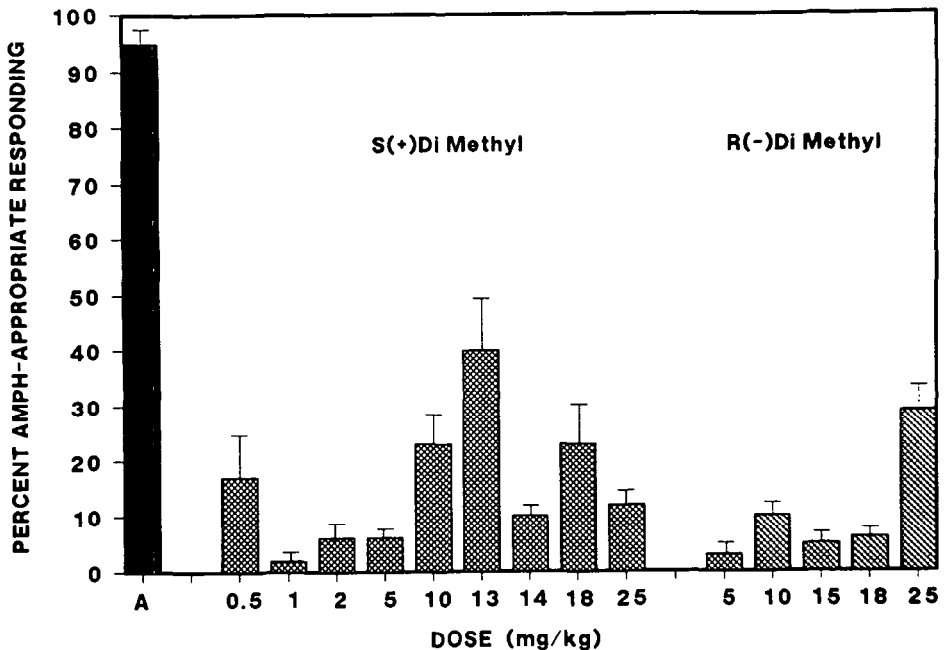
FIGURE 2. *Stimulus effects of S(+)- and R(-)-N,N,dimethylamphetamine in rats trained to discriminate 1 mg/kg of S(+)AMPH from saline.*

4-Methylaminorex. 4-Methylaminorex (4-MA), the 4-methyl analog of the appetite suppressant aminorex, exists as two geometric isomers: cis and trans; each isomer is composed of two optical isomers. Thus, there are four possible isomers of this agent. 4-MA found on the clandestine market has been identified as the racemic cis isomer ("U4Euh"). Using (+)AMPH-trained rats, we conducted tests of stimulus generalization with racemic cis, racemic trans, and all four optical isomers of 4-MA (Glennon and Misenheimer 1990). Both the cis racemate (ED50 = 1.56 mg/kg) and the trans racemate (ED50 = 0.41 mg/kg) produce AMPH-like effects. Although the cis isomer has now been Scheduled, the more potent trans isomer has not.

PMMA. PMMA (or N-methyl-1-(4-methoxyphenyl)-2-aminopropane) may be viewed as the para methoxy analog of methamphetamine, or as the N-methyl analog of PMA (para-methoxyamphetamine). Both PMA and methampheta-mine produce AMPH-like stimulus effects; surprisingly, PMMA does not (Glennon et al, 1988). Because PMMA disrupts AMPH-trained animals at rather low doses (i.e. < 0.2 mg/kg), it may (a) possess a different pharma-cological profile than AMPH, or (b) produce some effect that obscures its AMPH-like effect (that might have been observed at higher doses had it not been for the disruptive effects). Indeed, PMMA produces MDMA-like effects (ED50 = 0.2 mg/kg) in rats trained to discriminate MDMA (ED50 = 0.76 mg/kg) from saline (Fig 1). Because it is several times more potent than
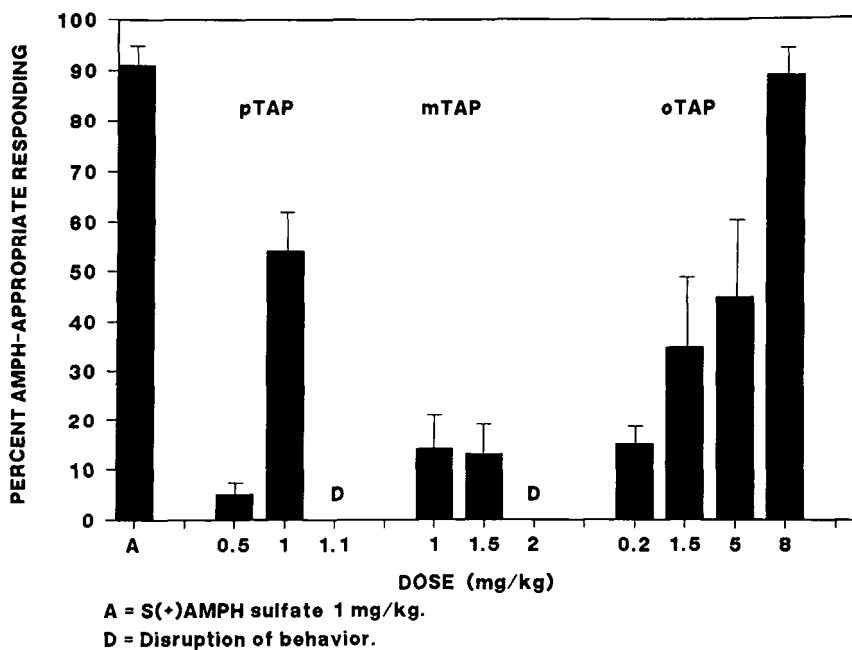
FIGURE 3. *Stimulus effects of o-, m- and pTAP in rats trained to discriminate 1 mg/kg of S(+)AMPH from saline.*

MDMA, and because it is the first non-MDA analog shown to display MDMA-like properties, it suggests that the methylenedioxy group is not essential for this type of activity. As anticipated, PMMA does not produce DOM-like stimulus effects.

α'-Desmethyl DOB. DOB is the 4-bromo counterpart of DOM, and α-desmethyl DOB is the phenethylamine analog of DOB. This agent has appeared on the West coast and, although some human data have been reported in the literature, essentially nothing has been reported concerning its effects in animals. Stimulus generalization occurs between DOB and DOM regardless of which is used as the training drug. On the basis of the above mentioned SAR, it might be expected that α'-desmethyl DOB would produce stimulus effects similar to those of DOM but that it would be somewhat less potent than DOB. This appears to be the case. The DOM-stimulus generalizes to α-desmethyl DOB (ED50 = 0.7 mg/kg) and it is several times less potent than DOB (ED50 = 0.2 mg/kg). Nevertheless, stimulus generalization is accompanied by a significant reduction in the animals' response rates suggesting that α'-desmethyl DOB may produce effects in addition to its DOM-like effects. Indeed, radioligand binding studies show that α-desmethyl DOB, though it possesses an affinity at 5-HT2 receptors similar to that of DOB, does not bind with the same degree of selectivity.

1-(Tolyl)-2-aminopronanes (TAPs). The aromatic nucleus of amphetamine is unsubstituted; incorporation of an aromatic methyl group at either the 2-, 3-, or 4-position results in three positional isomers: oTAP, mTAP, and pTAP. An unidentified tolylamphetamine has been rumored to be available on the clandestine market, and all three tolylaldehyde precursors are commercially available. The three TAPs have been examined as anorectic agents in humans and all reportedly produce some AMPH-like stimulant effects (AMPH > mTAP > oTAP > pTAP) (Marsh and Herring, 1958). In AMPH-trained rats, only oTAP resulted in stimulus generalization (ED50 = 4.2 mg/kg) (Fig. 3); as such, it is about one-tenth as potent as S(+)AMPH. Neither mTAP nor pTAP resulted in complete generalization; the highest non-disruption dose (followed by percent AMPH-appropriate responding): mTAP 1.5 mg/kg (14%), pTAP 1 mg/kg (54%); slightly higher doses resulted in disruption of behavior. In the case of pTAP, 1.1 mg/kg disrupted three of four rats with the one responding rat making 57% of its responses on the AMPH lever. As in the human studies, these agents are less potent than (+)AMPH (ED50 = 0.4 mg/kg).

SUMMARY

Phenylalkylamine derivatives produce several types of behavioral effects including central stimulation and hallucinogenic activity. SAR are being formulated and already (a) it has been demonstrated that each of these types of activities is associated with a distinct SAR, and (b) it is now possible to use these SAR to make predictions as to whether the stimulus effects of certain PAAs are primarily AMPH-like or DOM-like. The AMPH-like nature of PAAs seems to involve a dopaminergic mechanism whereas DOM-like activity involves a serotonergic (in particular a 5-HT2) mechanism. It is apparent, however, that there is an additional type of activity emerging from studies with some PAAs that is neither solely AMPH-like nor DOM-like. MDA seems to produce both types of actions and may even produce this third type of effect. MDMA produces AMPH-like and MDA-like effects, but does not produce DOM-like effects. Other agents, such as MDE and PMMA, produce neither AMPH-like nor DOM-like effects but clearly produce MDMA-like stimulus effects. Thus, there is a third type of SAR that may be formulated. In all likelihood, however, few PAAs will be shown to produce a single "pure" activity and because there are some similarities in the different SARs (even though there are some very clear differences) it is not unreasonable to assume that many PAAs will produce more than one type of effect or will display vestiges of one or more different components of action. Therefore, although a PM may be classified as primarily producing one type of effect, it should be understood that the other types of effects are not necessarily absent. In the future, it will be necessary to examine these different actions and SARs with great care.

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AUTHOR

Glennon, Richard A.
Department of Medicinal Chemistry
School of Pharmacy
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298-0581

Use of Naltrexone for the Diagnosis and Treatment of Reproductive Hormone Disorders in Women

Jack H. Mendelson, Nancy K. Mello, Siew K. Teoh, and James Ellingboe

INTRODUCTION

During 1976 our laboratory reported that acute administration of naltrexone stimulated an increase in plasma luteinizing hormone levels in men who had a past history of opioid dependence (Mirin et al., 1976). In 1977 Bruni and his associates observed that the narcotic antagonist naloxone could induce stimulation of luteinizing hormone in the rat. We have also administered Naltrexone (50 mg. p.o.) to normal men and found a significant increase in plasma LH levels (Mendelson et al., 1979). Experimental animal studies by Cicero and his colleagues (1979) demonstrated that endogenous opioids regulate, in part, hypothalamic control of luteinizing hormone secretion by the pituitary. Since these early publications there have been a number of additional reports which confirm the importance of endogenous opioid systems for modulating gonadotropin secretion in experimental animals and humans (Ellingboe et al., 1978; Morley et al., 1980; Quigley et al., 1980; Stubbs et al., 1978; Volavka et al., 1979). Very recent experimental animal studies have provided anatomical data which indicate that GnRH release is modulated by endogenous opioid peptides (Chen et al., 1989). This report summarizes data obtained in our human clinical research paradigms for the assessment of naltrexone effects on the secretion of luteinizing hormone in men and women.

METHODS

Healthy male and female volunteers between 20 and 32 years of age provided informed consent for participation in our studies. All subjects were in good health and none had any current or past history of alcohol or drug abuse or dependence. No subject had ever received naltrexone prior to the study. Eight men served as their own control and were given naltrexone (50 mg. p.o.) and naltrexone placebo on subsequent days. Four subjects received naltrexone on the first day and four others received placebo on the first day. All subjects reported to the laboratory during the early morning and received either naltrexone or placebo at the same time on each day.

Prior to administration of naltrexone or placebo, an intravenous catheter was placed in the arm vein and connected to a portable nonthrombogenic pump. Integrated plasma samples were collected during consecutive 20 minute periods for a total of eight hours. Plasma samples were frozen and analyzed for

luteinizing hormone with radioimmunoassay procedures as described below. In addition to the eight subjects who received both naltrexone and placebo, 38 other male subjects were studied utilizing identical plasma sampling procedures. However, none of these subjects received either naltrexone or naltrexone placebo.

Eighteen women participated in studies to determine the effects of naltrexone on plasma luteinizing hormone levels. All had normal menstrual cycle function; none were pregnant and none used contraceptive medication or intrauterine devices. All had normal physical, mental status, blood chemistry, urinalysis and blood hemogram studies. No women had any past history of alcohol or drug abuse and none were using any medications at the time of the study. These women reported to the laboratory at 9 a.m. following a 12 hour fast. An indwelling catheter was placed in the antecubital vein and connected to a slow intravenous infusion of 5 percent dextrose in saline. Subjects were recumbent throughout the study and were not permitted to eat solid foods, smoke or drink beverages containing caffeine. Following collection of three to four consecutive blood samples at 30 minute intervals, they ingested one 50 mg. tablet of naltrexone hydrochloride or naltrexone placebo. Blood samples were collected at consecutive 20 to 30 minute intervals for 180 to 240 minutes following naltrexone administration.

Quantitative Analysis of Pulsatile LH Secretion in Men

Secretory pulse parameters were calculated by an IBM 360 computer, using a program previously described (Santen et al., 1973). The specific parameters included: 1) the number of secretory pulses (defined by the increment from nadir to peak of >20%) during the eight hour sampling interval; 2) the absolute maximum LH concentration attained per secretory pulse; 3) the percent increment of LH per secretory pulse; 4) the integrated area under the curve described by LH levels derived over the eight hour sampling period; 5) the arithmetic mean of LH concentrations for the entire sampling interval, and 6) the apparent half-life of LH elimination, estimated by the log-linear decrement of LH after a secretory pulse (regression lines computed by least squares analysis).

Hormone Analysis

Plasma LH levels were measured in duplicate aliquots of plasma by a double antibody RIA using materials provided by the National Pituitary Agency, NIAMDD, as previously described (Mendelson et al., 1978).

RESULTS

Naltrexone Effects on LH Secretion in Men

Figure 1 shows that naltrexone administration produced significantly higher plasma LH levels in all subjects regardless of the sequence of administration of the drug or placebo. When data were normalized and pooled between subjects, naltrexone administration was associated with an approximately 50 percent increment in plasma LH levels above placebo administration levels. Group mean plasma LH concentrations during the entire eight hour collection period on the placebo day were compared with group mean plasma LH concentrations on the naltrexone day with a paired T test (two-tailed) and LH levels following naltrexone administration were significantly higher ($P < 0.0009$).

Naltrexone Effects on LH in Men
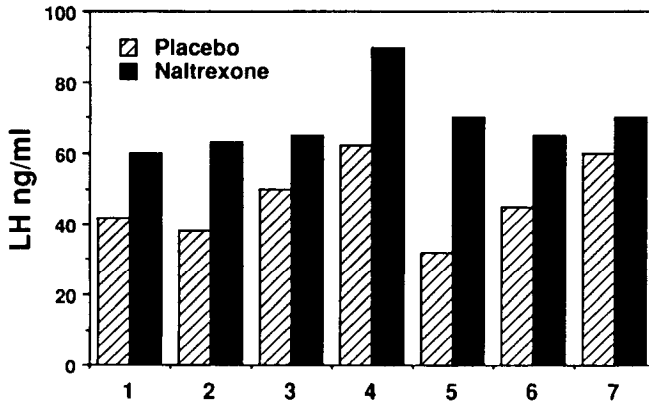
FIGURE 1 from: Mendelson et al., 1979

In order to determine more precisely the effects of naltrexone on pulsatile secretion of luteinizing hormone, a quantitative analysis of pulsatile LH secretion was carried out. Figure 2 shows the integrated mean LH, the absolute peak LH, the number of LH peaks per hour and the area of the LH curve after naltrexone (50 mg. p.o.) administration in subjects who served as their own control when they received placebo naltrexone. In addition, data are shown for 38 normal males who did not receive naltrexone or naltrexone placebo.
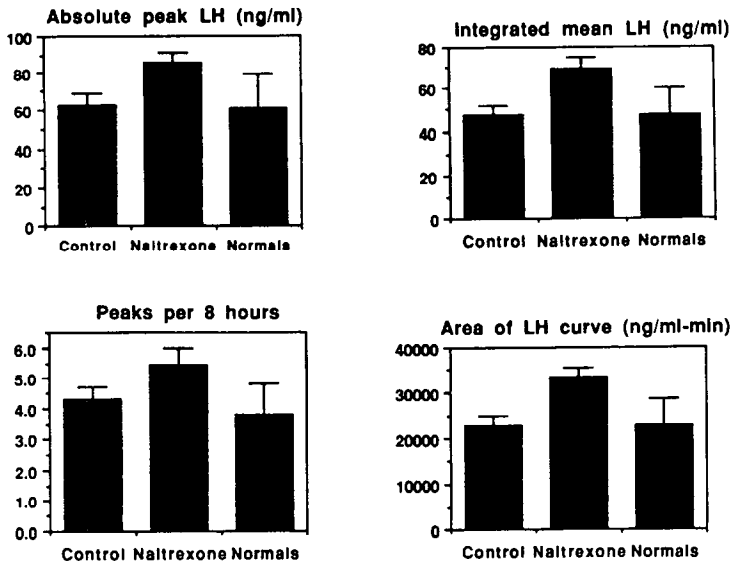
FIGURE 2 from: Ellingboe et al., 1982

163

Naltrexone was associated with highly significant mean increases in the total area under the LH curve (+ 47%; P < 0.0006), the number of secretory pulses (+ 27%; P < 0.0003), integrated mean LH plasma concentrations (+ 47%; P < 0.0005) and absolute peak LH concentrations (+ 36%; P < 0.0001). There was no significant difference between control and naltrexone days in the percent increment of LH per secretory pulse, and no difference was found in log-linear decrements in plasma LH after each secretory spike. Comparisons of the LH values for placebo control days with similar data obtained from 38 normal male subjects of the same age range revealed no signficant differences.

Naltrexone Effects on Plasma LH Levels in Women

Figure 3 shows plasma LH levels for two women studied during the early follicular phase of the menstrual cycle and two women who were studied during the mid and late late phases. Basal LH levels prior to naltrexone administration were normal for the specific menstrual cycle phase. Naltrexone administration induced a significant increase in LH above plasma baseline levels (P < .001). Peak LH values were detected 180 minutes to 210 minutes following naltrexone intake.
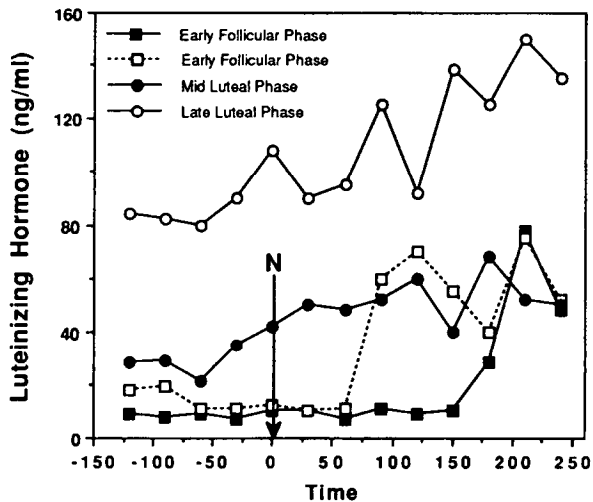
FIGURE 3 from: Mendelson et al., 1986

We have observed that naltrexone administration to women during the early follicular phase of the menstrual cycle induced a significant increase in plasma LH (p = 0.02). Mean plasma LH levels were significantly increased over baseline values prior to naltrexone administration at 1 hour through 90 minutes following administration of 50 mg of naltrexone orally. Figure 4 shows plasma LH values for a representative woman who received 50 mg of naltrexone p.o. A significant increase in LH levels was observed 70 minutes following naltrexone administration and pulsatile surges of LH were detected at 85, 120 and 240 minutes following naltrexone administration. These data indicate that naltrexone increases both pulse frequency and pulse amplitude of LH secretion.

164

Early Follicular Phase
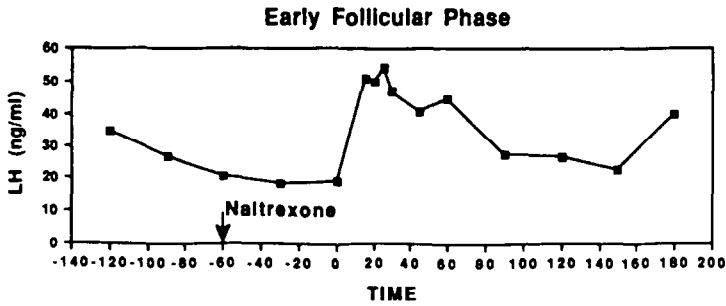
FIGURE 4

DISCUSSION

Findings obtained in our laboratory have been confirmed by other studies which have shown that endogenous opioid systems exert physiologically significant suppression of episodic hypothalamic GnRH secretion (for review see Yen and Jaffe 1986). Taken together these observations also suggest that naltrexone perturbation procedures may be of value for the diagnosis of abnormalities of hypothalamic regulation of gonadotropin release from the pituitary. Currently diagnostic procedures to evaluate such disorders involve administration of the synthetic decapeptide for LHRH (Martin and Reichlin 1987). Use of naltrexone instead of synthetic LHRH for the diagnosis of diseases of gonadotropin regulation may have several advantages including enhanced comfort and safety for patients.

There have been preliminary reports that naltrexone may be effective for the treatment of hypothalamic amenorrhea (Wildt and Lyndecker 1987), for alleviation of late luteal phase dysphoric disorders (Chuong et al., 1988) and for the treatment of male impotence (Fabbri et al., 1989). Naltrexone may be useful for the treatment of a number of gonadotropin secretion disorders in men and women, including hypogonadotropic hypogonadism, delayed puberty and precocious puberty.

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AUTHORS

Jack H. Mendelson, M.D.; Nancy K. Mello, Ph.D.; Siew K. Teoh, M.D.; James Ellingboe, Ph.D. Alcohol and Drug Abuse Research Center, Harvard Medical School/McLean Hospital, 115 Mill Street, Belmont, MA 02178

Neuroendocrine (HPA) and Gastrointestinal Effects of Opiate Antagonists: Possible Therapeutic Application

Mary Jeanne Kreek and Joan Culpepper-Morgan

We have hypothesized that gastrointestinal motility disorders in humans leading to chronic constipation may result, in part, from a relative or absolute excess of one or more of the endogenous opioids or from abnormal binding of these opioids by their specific receptors in the intestinal wall. For over two thousand years, it has been recognized that opium derivatives are effective in the reversal of diarrhea in humans. In the last fifteen years, it has been documented that synthetic opiates which have limited systemic bioavailability after oral administration are effective as anti-diarrheal agents. In early work from our laboratory, prior to the final delineation of specific opiate receptors, but at the time of prediction of the presence and density of specific opiate receptors and after initial attempts to document their existence, we found evidence that there might be specific opiate receptors in the intestinal wall as well as in the brain and spinal cord in humans. (Ingolia and Dole 1970; Dole 1970; Kreek 1973; Dole 1988).

As part of our pharmacological and physiological studies of narcotic addiction and the potential utility of use of the long-acting opioid agonist, methadone, in the maintenance treatment of narcotic dependency, we performed several studies concerning the pharmacokinetics of methadone in humans. In one of these studies, we compared the bioavailability and pharmacokinetics of several different formulations of methadone to be used in the maintenance treatment of addiction, including an experimental formulation in which small amounts of the specific opioid antagonist naloxone had been added (ratio of methadone to naloxone, 10:1; Bristol) to attempt to prevent any illicit parenteral administration of methadone. In our studies, when this combination formulation was administered to well-stabilized, steady dose methadone maintained study subjects, signs and symptoms of narcotic withdrawal at the gastrointestinal level, without systemic signs and symptoms of abstinence, occurred in several subjects; a few subjects developed a full-blown systemic narcotic abstinence syndrome (Kreek 1973). These findings suggested that specific opiate receptors might be present in the intestinal wall in addition to sites within the brain and spinal cord and that reversal of opiate agonist action at these peripheral intestinal wall sites by the antagonist with receptor occupancy had precipitated the observed signs and

symptoms of gastrointestinal narcotic abstinence, including cramps and diarrhea, in our study subjects who were tolerant to and dependent upon narcotics. These observations led to our formulation of the hypothesis as stated above.

After the formal discovery of specific opiate receptors by three groups in 1973 and later the discovery of three classes of specific endogenous opioids which bind to these receptors, beginning with the discovery of the enkephalins by Kosterlitz and Hughes in 1975, several groups have documented the presence of specific opiate receptors and also endogenous opioids of all three classes in the gastrointestinal wall, although significant regional as well as species differences have been documented. For instance, our group has shown that the kappa type opiate receptors are present in the large intestine in the guinea pig in approximately the same abundance as are the mu and delta opioid receptor types, whereas in the rat there is a paucity of kappa receptors in the gastrointestinal tract (Culpepper-Morgan et al 1987). These findings from in vitro receptor binding studies have been further supported by in vivo studies by our laboratory. Administration of the kappa selective opioid agonists U50488-H to guinea pigs resulted in a significant slowing of gastrointestinal transit, similar to the results from administration of a mu selective ligand, such as morphine; in contrast, administration of U50488-H does not cause gastrointestinal transit slowing in the rat (Culpepper-Morgan et al 1988c).

Our group also has shown that the relative density of specific opioid receptor types may change significantly with increased age in both the brain and gastrointestinal tract in guinea pigs, a finding which may have implications for the increased prevalence of gastrointestinal transit disorders (constipation) in elderly persons as well as the apparent increased pain threshold in older subjects (Culpepper-Morgan et al 1988a; Culpepper-Morgan et al 1988b; Unterwald et al 1990a). Recently our group, using the molecular biology technique of solution hybridization protection assays, has shown that the message for the gene preproenkephalin (pENK-mRNA) is expressed throughout the gastrointestinal tract of the guinea pig and that the content of the pENK-mRNA is greater in the colon that any other tissue outside the brain other than the adrenal gland (Zhang et al 1988; Zhang et al 1989). Also we have shown by direct measurement that levels of the terminal active opioid peptide of the proenkephalin peptide, enkephalin-arg-phe, are greater in the guinea pig colon than in any other region of the gastrointestinal tract and comparable to those in regions of the brain known to be high in enkephalin content (Unterwald et al 1990b).

Control of gastrointestinal function is now known to occur at three levels: the brain, the spinal cord, and the enteric nervous system within the intestinal wall. Various studies have shown that the endogenous opioids, and also exogenous opiates, may act at each of these sites to modulate gastrointestinal function, including modulation of secretion and adsorption and also motility and transit. Constipation is a common medical problem which increases in prevalence and severity with age. Over 20% of elderly persons suffer from constipation; oro-cecal transit time has been shown experimentally to be abnormal in such persons

(Piccione et al 1990). Also it has been shown that levels of beta-endorphin, which are normally highest in the morning, are significantly higher in persons over 65 years old as compared with persons under 40 years old (Kreek et al 1986). To address our stated hypothesis, we have asked the following questions:1) would the administration of the specific opioid antagonist naloxone which binds with varying affinities to all three types of opioid receptors ameliorate or reverse chronic constipation in a geriatric population and 2) would naloxone, which has very limited systemic bioavailability in humans after oral administration, because of rapid and efficient metabolism in its "first pass" through the liver (primarily by 3-OH-glucuronidation) be effective in ameliorating chronic spontaneous constipation both in young and middle aged as well as geriatric patients, after oral administration, presumably by action at specific opiate receptor sites in the enteric nervous system.

In our studies of attempting to modulate gastrointestinal motility transit by oral administration of an opioid antagonist, naloxone with its limited systemic bioavailability after oral administration seemed to be most desirable to prevent any additional primary opioid antagonist affects at the brain or spinal cord level which could become side effects or adverse effects during chronic treatment, such as the documented opioid antagonist effects of neuroendocrine function (Ragavan et al 1983). In the case of our studies on narcotic-induced constipation, the use of naloxone administered orally would potentially allow reversal at the gastrointestinal wall level of any opiate effect, without reversing or diminishing any analgesic effect at the brain and spinal cord level and without precipitating narcotic withdrawal in chronic treated tolerant and dependent persons. Thus in our studies regarding modulation of gastrointestinal function, either of a spontaneous type or narcotic induced type of delayed transit, orally administered naloxone has been the opiate antagonist of choice (Kreek et al 1973; Hahn et al 1983; Kreek et al 1983a; Kreek et al 1983b Kreek et al 1983c; Kreek et al 1984; Kreek et al 1985; Kreek et al 1986a, kreek et al 1986b; culpepper-Morgarcal 1988c; Albeck et al 1989; Culpepper-Morgan et al 1989; Albeck et al 1990; Culpepper-Morga et al 1990; Stone et al 1990). Using the guinea pig model, we have shown that oral administration of opioid agonist antagonist naloxone, which is primarily a mu selective opioid antagonist, will reverse the gastrointestinal transit slowing caused by both by the mu preferring opioid antagonist morphine and the kappa preferring opioid agonist U50488H (Culpepper-Morgan et al 1989). In that study we also showed that nalmefene, an antagonist with more kappa receptor type activity than naloxone, had greater efficacy in reversing the kappa agonist U50488H induced gastrointestinal transit slowing than did morphine in a dose response study.

In a study of young and middle aged patients age 18 to 68 with gastrointestinal dysmotility disorders of idiopathic chronic constipation and irritable bowel syndrome with constipation, we have addressed the question of whether or not orally administered naloxone, as contrasted to parenterally administered naloxone, would ameliorate the problem of slow transit and decreased fecal evacuation. Approximately 50% of the study subjects showed a

positive response to the opioid antagonist administration with increased passage of fecal weight as measured objectively; similar findings were made whether naloxone was given by the oral route, thus reaching only the gastrointestinal tract site of opiate antagonist action or when it was given by the parenteral route, reaching the brain and spinal cord sites of intestinal motility control in addition to sites of the enteric nervous system. (Kreek et al 1983a; Kreek et al 1983b; Kreek et al 1985; Kreek et al 1986). In a separate set of two studies performed in a geriatric patient population, with subjects ranging-in age from 72 to 96 years, all mobile and all in stable medical health, naloxone delivered exclusively by the oral route and placebo medication were given to each subject in a double-blinded, random order cross-order design protocol. Sixty percent of patients responded positively as measured objectively by greater than a 25% increase in fecal wet weight and dry weight during the oral naloxone administration. No adverse effects due to naloxone were found in either of these studies (Kreek et al 1983). We have also performed preliminary studies to determine the possible efficacy of reversal of narcotic-induced slowed gastrointestinal transit in chronic pain, as well as in methadone maintained subjects, with very promising results in these preliminary studies (Culpepper-Morgan et al 1990).

Our laboratory has carried out extensive studies of the possible role of the endogenous opioid system in specific aspects of neuroendocrine function, including effects on hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis. This work is related to our basic laboratory and clinical research on the biological basis of addiction. We have confirmed and extended the early findings that acute bolus administration of the specific opioid antagonists will result in an increase in release of cortisol, (which our group went on to show includes an abrupt release in ACTH, beta-endorphin and in turn, cortisol) (Kreek et al 1984). We have studied the differential effects of the specific opioid antagonist nalmefene (which has greater kappa type opioid receptor activity than naloxone) as contrasted to naloxone and have made findings suggesting that kappa as well as mu type opiate receptors may be involved in the tonic inhibition of ACTH and beta endorphin release from the human anterior pituitary (Kreek et al 1987). We have also shown that orally administered naloxone, with its very limited systemic availability, dose not result in any release of cortisol, which could become an adverse side effect in patients with motility disorders who might benefit from chronic opiate antagonist administration (Albecks et al 1989; Albeck et al 1990; Kreek et al 1983; Kreek et al 1984). However we have shown that chronic oral use of the opiate antagonist naltrexone (which has around 35% systemic bioavailability) in former heroin addicts does not allow stabilization of neuroendocrine function, (such as our group has documented to occur during chronic methadone maintenance treatment), because of the sustained bolus-like effect of naltrexone on release of ACTH, beta endorphin and cortisol resulting in increased levels of these neuropeptides and steroids as compared with control levels, even during chronic naltrexone treatment (Kosten et al 1986a; Kosten et al 1986b; Kosten et al 1986c). These findings may or may not be relevant to the failure to reduce drug seeking behavior in over 80% of chronic naltrexone

treated heroin addicts. More studies of the role of the endogenous opioids in neuroendocrine function, gastrointestinal function and also in the biology of the addictive diseases are needed and may be facilitated by development of increasingly selective opioid antagonists which may be used in human and animal studies.

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AFFILIATION:
The Rockefeller University, New York, New York

Cocaine-Induced Changes in Gene Expression in Rat Brain

Bruce M. Cohen, Tuong Van Nguyen, and Steven E. Hyman

Although much is known about cocaine's pharmacologic properties, much remains to be learned about both its acute and long-term effects. In particular, when all of the evidence is weighed from pharmacologic, biochemical, and electrophysiologic studies, it remains unclear what action accounts for cocaine's reinforcing properties in animals and humans and its euphoriant effects and production of dependance in humans (Balster 1988). Thus, additional and novel approaches to the study of cocaine's effects are warranted.

Because regulation of gene expression is likely to be an important mechanism of signal transduction and neural plasticity in the brain (reviewed in Comb et al., 1987). one such approach might be provided by the study of changes in neural gene expression induced by cocaine. Among genes of interest, there is increasing evidence that cellular immediate early genes (IEG's) [Genes that are activated rapidly (within minutes), transiently, and without requiring new protein synthesis] may serve as mediators of neural plasticity, possibly because their protein products induce or repress other genes. A second class of genes, often referred to as late response genes, are induced or repressed more slowly (over hours) and are generally dependent on new protein synthesis. Late response genes include those which encode proteins (e.g. neurotransmitter-synthesizing enzymes, peptide neurotransmitters, receptors, ion channels, and cytoskeletal components) involved in the specific functions of the cell.

We have begun to study cocaine-induced changes in gene expression in order to test the following specific hypothesis: (1) Acute administration of cocaine will regulate expression of cellular IEG's and other genes in a regionally specific and cell specific fashion. (2) Repeated administration of cocaine will result in significant changes in expression of genes involved in neuronal signaling, neuronal growth, and synaptic remodeling. (3) The spatial and temporal pattern of changes in gene expression will suggest neuronal circuits and intracellular signal transduction processes mediating the pharmacologic effects of cocaine.

METHODS

Male Sprague-Dawley rats were treated with vehicle, 3 mg/kg cocaine, or 10 mg/kg of cocaine given slowly by tail vein injection and were sacrificed by decapitation 45 or 75 minutes after drug administration. Brains were rapidly removed and dissected and regions of interest were

frozen on dry ice. For each experimental condition, tissue was pooled in order to yield adequate RNA for analysis.

Frozen brain regions were pulverized under liquid nitrogen and total RNA prepared by homogenization in 4 M guanidinium isothiocyanate followed by centrifugation through a 5.7 M cesium chloride pad (modified from Berger and Chirgwin 1989). RNA was quantitated by spectrophotometry, and equal amounts of RNA (40 mg) were run on each lane of a formaldehyde gel. The RNA was then transferred to a nylon membrane (Gene Screen, DuPont), cross-linked by UV irradiation, and then hybridized with nick translated probes for genes of interest and for the unregulated internal reference gene, cyclophilin (1B15; Danielson et al., 1988). The resulting autoradiograms were then quantitated by densitometry. In cases where the internal reference signal was much stronger than that produced by regulated genes of interest, multiple exposures of the autoradiogram were made in order to ensure that densitometry was in the linear range.

RESULTS

Effects of Cocaine on Immediate Early Gene Expression in Rat Brain:

The first genes studied were three cellular IEG's, the known transcription factors c-fos and c-jun, and the putative transcription factor zif/268 (Christy and Nathans 1989). These genes were studied because each has been shown to respond to trans-synaptic stimulation in other systems (Cole et al., 1989 Wisden et al., 1990). and Fos immunoreactivity has been shown to be increased by cocaine in striatum (Young et al., 1989).

In our experiments, cocaine increased c-fos expression in both dose and time dependent fashion. Forty five minutes after cocaine administration, striking induction of c-fos mRNA was found in two limbic regions, the amygdala and hippocampus (Fig 1), and in the striatum (Fig 2). We did not obtain enough RNA in these preliminary studies to study the nucleus accumbens. In the amygdala, there was a 1.5-fold induction of c-fos mRNA after administration of 3 mg/kg cocaine and a 6.5-fold increase after 10 mg/kg. In the hippocampus there was a 1.7-fold induction after 3 mg/kg and a 7.5-fold induction after 10 mg/kg. In striatum the induction was 9.2-fold after administration of 3 mg/kg cocaine and 10.7-fold after 10 mg/kg. In comparison, inductions in the cerebral cortex and cerebellum were small. This may have partly reflected high levels of c-fos mRNA in the vehicle (control) condition in these regions.

In summary, c-fos was induced in every region examined. However, there was clear regional specificity with modest inductions in some cortical regions and cerebellum and profound inductions in limbic regions and striatum. Based on known patterns of innervation, inductions in cortical regions and cerebellum could be the result of noradrenergic stimulation; inductions in striatum and limbic regions could be the result of cocaine-facilitated neurotransmission in several systems, including dopamine. By 75 min after cocaine administration, c-fos mRNA had returned to basal levels (data not shown), consistent

with the known half-life of c-fos mRNA.
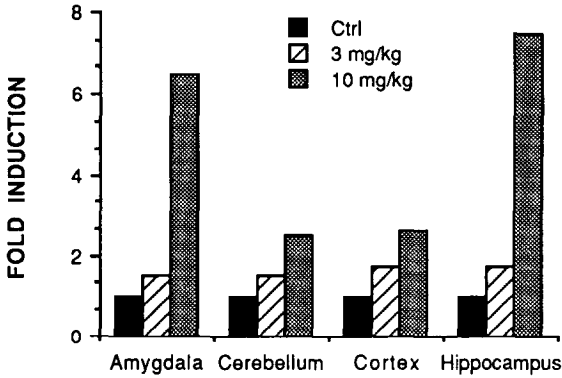
FIGURE 1. Induction of c-fos mRNA by cocaine, 45 minutes after administration by tail vein. Fold induction is determined by densitometry of the autoradiogram. and is derived by dividing the absorbance of the band from the cocaine treated rats by that of the control (vehicle treated) rats which is arbitrarily set at 1. All values are normalized to cyclophilin. Regions shown here are amygdala, cerebellum, frontal cortex, and hippocampus. Experiments were replicated 3 times
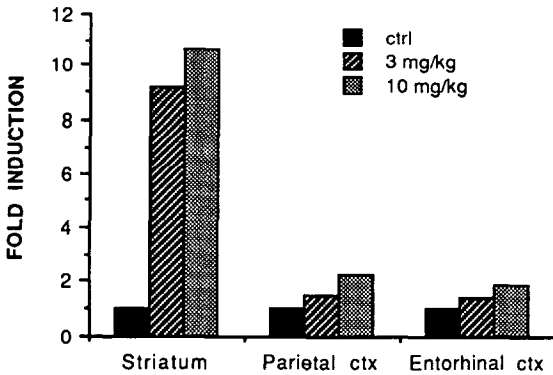
FIGURE 1. Induction of c-fos mRNA by cocaine, 45 minutes after administration. For details, see Fig. 1

Unlike c-fos, the transcription factor c-jun has appreciable basal (unstimulated) levels in many cell types and accordingly shows a less dramatic induction. In response to cocaine, we found a dose dependent increase in c-jun mRNA in amygdala (1.6-fold after 3 mg/kg and

2.3-fold after 10 mg/kg.) There was no significant induction or repression in cerebellum, frontal cortex, or hippocampus (Fig 3) or in other cortical regions or striatum (not shown).
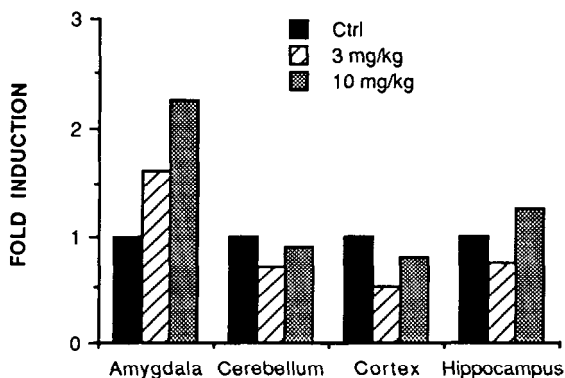
FIGURE 3. **Induction of c-jun mRNA by cocaine, 45 minutes after administration.** For details, see Fig. 1

Regulation of a third IEG, zif/268, was also examined. This gene has been shown to be selectively activated in hippocampal granule cells by high frequency stimulation of the perforant path under conditions known to produce long term potentiation (Cole et al 1989). Forty five minutes after administration of 10 mg/kg cocaine, zif/268 was induced 2.4-fold in amygdala, 1.8 fold in frontal cortex, and 4.8-fold in hippocampus (Fig 4). Consistent with prior studies there is little expression of zif/268 in the cerebellum. Parietal and entorhinal cortex also revealed approximately 2.3-fold and 2-fold inductions respectively (data not shown). In contrast to c-fos, expression remained at high levels at the 7.5 minute time point (data not shown).
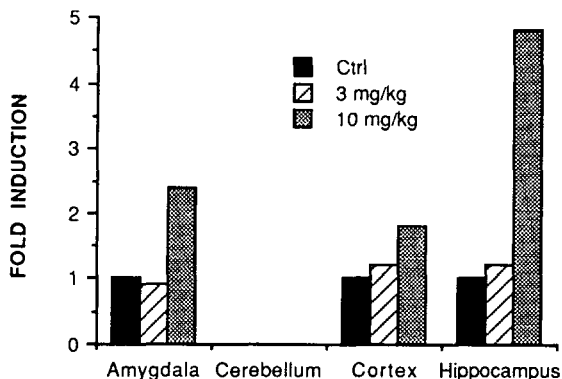
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Effects of Cocaine on Proenkephalin Gene Expression:

It is of particular interest that cocaine co-ordinately induced expression of c-fos and c-jun in amygdala (Figs 1 and 3) because their gene products interact with each other to form heterodimers which bind to genes containing the DNA Sequence TGACTCA (so-called AP-1 sites) to activate or, potentially, repress transcription (Chin et al., 1988). Genes with AP-1 binding sites include those encoding proenkephalin (Hyman et al., 1988 Comb et al., 1988) and vasoactive intestinal polypeptide (Hyman et al., 1988). Sonnenberg et al. (1989) have argued that activation of c-fos and c-jun expression by pentylenetetrazole-induced seizures in dentate gyrus granule cells in the hippocampus is responsible for the subsequent activation of proenkephalin gene expression in those cells.

Seventy five minutes after a single cocaine injection there is a significant increase in proenkephalin gene expression in the amygdala; the induction is 1.3-fold after administration of 3 mg/kg cocaine and 2.5-fold after 10 mg/kg (See Fig. 5).
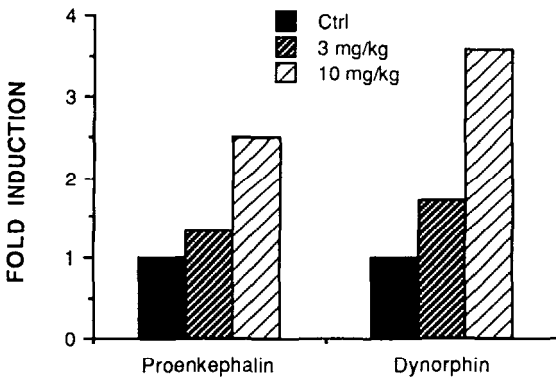
FIGURE 5. Induction of Proenkephalin and Prodynorphin mRNA in Amygdala by Cocaine, 75 minutes after administration.

Prodynorphin is also induced in the amygdala, 1.7 fold by 3 mg/kg and 3.5 fold by 10 mg/kg of cocaine 75 minutes after injection. Additional experiments with an extended time course and repeated dosing may further implicate proenkephalin and prodynorphin as "downstream" target genes for cocaine-induced IEG's. These experiments arc consistent with an interaction between cocaine and opiatergic systems in brain at a molecular level. Such interactions have been suggested at the clinical level by the potential therapeutic effects of buprenorphine inhibiting both opiate and cocaine self-administration (Mello et al.,

1989).

Effects of cocaine on dopamine D2 receptor expression:

In other preliminary experiments we found no reproducible effect of cocaine on dopamine D2 receptor expression after a single dose. Longer time courses and repeat dosing experiments will be performed. It is of interest that we have observed a 2.5-fold increase in D2 receptor mRNA in striatum after 14 days of 1 mg/kg haloperidol delivered by osmotic minipump (manuscript in preparation).

DISCUSSION

Using classical pharmacologic approaches significant progress has been made in characterizing the immediate biochemical properties of cocaine, but a complete understanding of the neural mechanisms that underlie cocaine's reinforcing properties, and development of dependence and withdrawal remain elusive. In particular, classic neuropharmacologic markers (such as receptor number) appear to change only minimally in response to repeated cocaine stimulation. The addition of molecular genetic tools to pharmacology may provide a novel avenue for progress. As briefly detailed above, there is strong evidence that psychotropic drugs can regulate gene expression in the brain, with the implication that such mechanisms can produce long-lasting effects on neuronal functioning. In our preliminary experiments cocaine was found to regulate expression of a variety of genes by 2 to 10-fold in a region specific and dose-dependent manner.

An advantage of the approach proposed here is the ability to focus on post-receptor events occurring within neurons. This is particularly significant because one of the difficulties in analyzing the effects of cocaine is that it affects multiple neurotransmitter systems, including dopamine, norepinephrine, serotonin, and probably their co-released peptides. What may be unique about cocaine's effects (e.g., in comparison with a direct dopamine receptor agonist, such as apomorphine, which has little abuse potential) is this very complexity or simultaneity of actions. By studying the effects of cocaine on gene expression, one is studying the integrated product of all of the synaptic signals converging upon the postsynaptic neuron (see Nguyen et al., in press; for a discussion of the role of synergy in the regulation of proenkephalin gene expression). Thus the study of gene expression provides an integrated measurement of cocaine's effects.

Our preliminary studies have focused on whether cocaine produces effects on gene expression that are of adequate magnitude to suggest biological significance. These initial experiments have utilized single cocaine administrations with a limited dose-response curve and time course. Our conclusions based on these data are that cocaine produces significant dose-dependent, time-dependent, and regionally specific effects on the expression of several genes of interest, that these effects are of a magnitude to suggest that they are pharmacologically relevant, and that more extensive study is warranted.

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AUTHORS AND AFFILIATIONS

Bruce M. Cohen, M.D., Ph.D.[1,3]; Tuong Van Nguyen, M.D., Ph.D.[1,2]; Steven E. Hyman, M.D.[2]

1) Molecular Pharmacology Laboratory, Mailman Research Center, McLean Hospital, Belmont, MA. 02178

2) Molecular Neurobiology Laboratory, Massachusetts General Hospital, Boston, MA

3) To whom correspondence should be sent

Opioid Involvement in the Rewarding Effects of Chronic Cocaine

Ronald P. Hammer, Jr., Daniel W. Clow, and Linda P. Spear

INTRODUCTION

The rewarding properties of cocaine, exemplified by its self-administration potential (Roberts et al. , 1980), have led to the chronic abuse of this drug. The mesocorticolimbic dopamine system is the apparent primary substrate for cocaine effects in the central nervous system. Cocaine treatment increases the extracellular dopamine level in the nucleus accumbens (NAc) (Bradberry and Roth, 1989; Hurd et al., 1989; Pettit and Justice, 1989) and striatum, but this effect is greatest in the NAc (Di Chiara and Imperato, 1988). Dopamine alteration is achieved by selective blockade of the dopamine transporter, which is thought to mediate the reinforcing efficacy of the drug (Ritz et al., 1988). In fact, regional heterogeneity of dopamine transporter sites within the NAc, with greater density in more rostra1 regions (Graybiel and Moratalla, 1989), may underlie selective regional effects of chronic cocaine in the rostra1 NAc (Clow and Hammer, in press; Hammer, 1989). Moreover, chronic cocaine treatment results in greatly enhanced mesocorticolimbic dopamine neurotransmission and supersensitivity of NAc neurons to the inhibitory effect of dopamine (Henry et al., 1989).

A variety of drugs abused by humans, including cocaine and opiates, increase extracellular dopamine content in the NAc (Di Chiara and Imperato, 1988). Both cocaine (Esposito et al., 1978) and opiates (Esposito and Kornetsky, 1977) lower the threshold for intracranial self-stimulation, which itself increases NAc dopamine content (Nakahara et al., 1989). Therefore, the administration of both cocaine and opiates enhances NAc dopaminergic activity. However, naloxone attenuates the effect of cocaine on the threshold for rewarding self-stimulation (Bain and Kornetsky, 1987), suggesting that endogenous opioid systems are involved in cocaine-induced reinforcement. Brain regions receiving NAc projections may function as a common reward pathway during both opiate and cocaine reinforcement (Koob et al., 1987). For example, lesions of the ventral pallidum (VP) disrupt both cocaine and opiate reward (Hubner and Koob, in press).

Although the direct action of cocaine on mesocorticolimbic dopamine systems may initiate cocaine reinforcement, endogenous opioids could interact within reward circuits. Thus, cocaine-induced alteration of opioid systems could affect endogenous opioid function in reward circuits. We have examined the effect of chronic cocaine treatment during development and adulthood on regional opioid receptors, and have observed preferential effects in mesocorticolimbic terminal and reward regions.

CHRONIC COCAINE ALTERS μ-OPIATE RECEPTORS

Endogenous opioid and dopamine systems are functionally related in various brain regions. For example, opioid regulation of dopamine systems in the hypothalamus controls prolactin secretion. Reciprocal feedback is also present, as dopamine denervation produces time-dependent downregulation of NAc μ-receptors located postsynaptically (Unterwald et al., 1989). Cocaine increases NAc dopamine overflow, even with repeated administration (Hurd et al., 1989). Therefore, we suspected that chronic cocaine could alter opioid receptors in critical brain reward regions. Cocaine was administered to male rats by subcutaneously-implanted osmotic minipump at doses of 1 or 10 mg/kg/day in saline vehicle. After 14 days, brains were removed, frozen and sectioned for *in vitro* receptor autoradiography using [^{3}H]naloxone in μ-receptor-preferring conditions. Autoradiographs were analyzed by quantitative densitometry, and the results are shown in Figure 1. Significant, dose-dependent effects were observed in regions containing mesolimbic dopaminergic neurons and their terminal zones, sites along the multisynaptic reward output pathway, as well as in the basolateral amygdalar (ABL) and dorsal raphe (DR) nuclei, but in few of the other 50 brain regions examined (Hammer, 1989).

Chronic cocaine increases μ-receptor labeling in mesolimbic terminal and reward output regions, but this effect is not homogeneous throughout these regions. The rostra1 (r) portion of the NAc shows greater effects than does the caudal (c) portion (Figure 1). This is consistent with the higher level of dopamine transporter sites, hence cocaine effects, in the rostra1 NAc (Graybiel and Moratalla, 1989). Similar cocaine-induced μ-receptor upregulation has recently been observed following repeated daily administration (Kalivas, personal communication) and in *in situ* preparations (Yoburn, personal communication). Furthermore, receptor density is increased in the ventral pallidum (VP) and lateral hypothalamus (LH), while the substantia innominata (SI) and pedunculopontine nucleus (PPN) show no effect. Whereas the VP and LH are directly related to cocaine-induced reward, the SI and the PPN are involved in psychomotor response (Koob et al., 1987). In addition, mesolimbic terminal fields are preferentially affected, since p-receptor density in the striatum (CP) is unchanged. Thus, chronic cocaine increases μ-receptor density along the mesolimbic reward pathway.
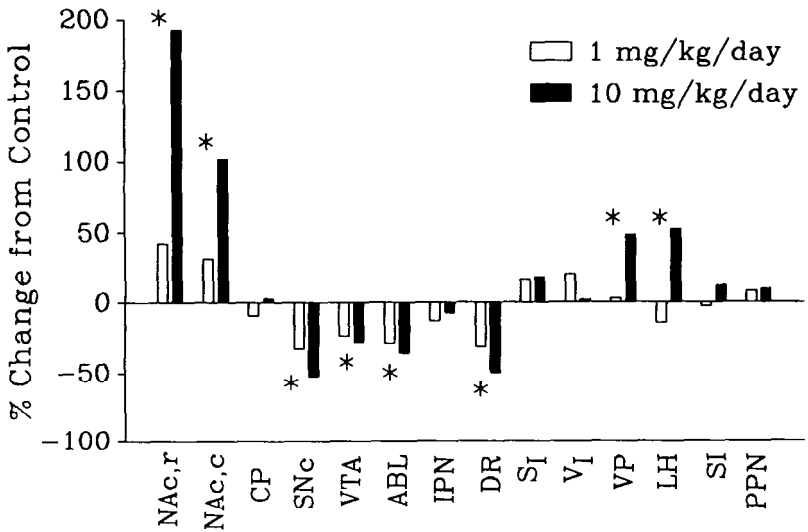
FIGURE 1. Effect of chronic cocaine on regional [^{3}H]naloxone binding, $p \leq 0.05$. See text for abbreviations. Data taken from Hammer, 1989.

The ventral tegmental area (VTA), substantia nigra (SN) and DR show decreased μ-receptor labeling following chronic cocaine treatment. These regions represent the sources of mesolimbic dopaminergic and serotonergic projections. Opioid activity in these regions may be involved in modulation of neuronal activity, hence dopamine and serotonin supply, which are altered by cocaine. The ABL is closely related to the NAc, and reduction of opioid activity herein could alter limbic output to the NAc. In fact, ABL lesions prevent development of cocaine-induced behavioral sensitization (Post et al., 1987). Thus, chronic cocaine decreases μ-receptor density in regions which modulate neurochemical and functional projections to mesolimbic reward regions.

PRENATAL COCAINE EXPOSURE UPREGULATES μ-RECEPTORS

Abundant evidence suggests that cocaine is a neurobehavioral teratogen which produces sustained behavioral effects (Spear et al., 1989a). Dopamine receptor blockade during development by prenatal exposure to haloperidol decreases striatal μ-receptor binding (Moon, 1984). If gestational exposure to cocaine alters brain dopamine content, then the development of μ-receptors may be affected. Cocaine was administered to timed-pregnant rats by daily subcutaneous injection of either 10, 20 or 40 mg/kg cocaine or saline vehicle on embryonic days 8-20. At birth, litters were culled to 10 pups and placed with untreated surrogate foster dams. At 21 days of age, brains were removed from prenatally-treated male pups, frozen and sectioned for *in vitro* autoradiography using [^{3}H]naloxone in μ-

receptor-preferring conditions, and quantitative densitometric analysis was performed. Gestational cocaine exposure produces a sustained, dose-dependent, increase of μ-receptor labeling in dopaminergic terminal fields, limbic and cortical regions (Figure 2). Although this effect is not as selective as that observed following chronic treatment in adults, there are similar trends. For example, cocaine increases μ-receptor density in mesolimbic dopaminergic terminal fields, including the NAc, medial prefrontal cortex (MPF) and olfactory tubercle (OT). μ-Receptor density is also increased in the striatum, but is unaffected in regions of origin of dopaminergic or serotonergic projections (i.e., SN, VTA, and DR). While thalamic and hypothalamic regions [e.g., the laterodorsal thalamic (TLD), preoptic (POA), ventromedial (VMH) and lateral (LH) hypothalamic nuclei] are unaffected, limbic and cortical regions [e.g., the ABL, interpeduncular nucleus (IPN), somatosensory (S_1), visual (V_1) and entorhinal (Ento) cortices] show significant, dose-dependent upregulation.
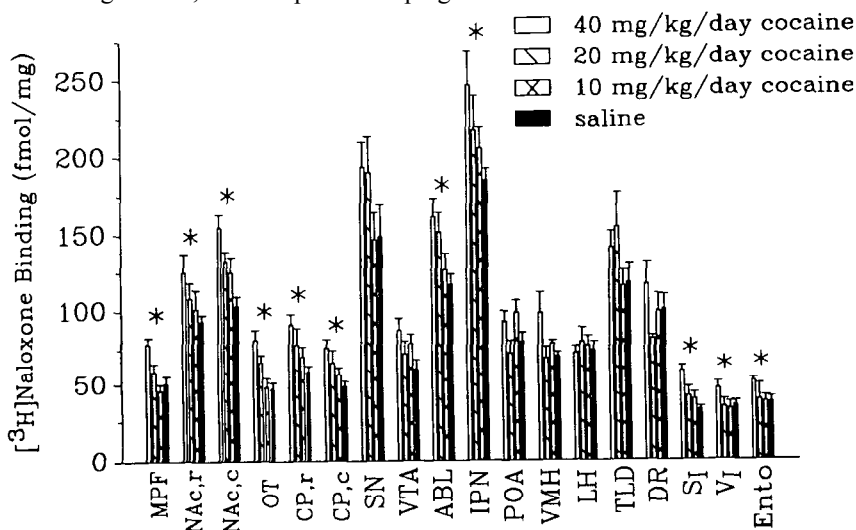
FIGURE 2. Effect of gestational cocaine exposure on regional [^{3}H]naloxone binding. * - $p \leq 0.05$. See text for abbreviations. Data from Clow et al., in prep.

MECHANISMS OF DOPAMINE-OPIOID INTERACTIONS

Dopaminergic lesion or receptor blockade during development or adulthood reduces μ-receptor binding in dopamine terminal fields (Moon, 1984; Unterwald et al., 1989). This effect could occur due to pre- or transsynaptic degeneration (Unterwald et al., 1989), depending on the synaptic location of the opioid receptors involved. However, the level of dopamine could also regulate the expression of opioid receptors in dopaminergic terminal fields, and/or could alter the expression of endogenous opioid peptides, which

subsequently regulate opioid receptor density. Some evidence exists in favor of the latter mechanism. Striatal proenkephalin expression is enhanced following dopaminergic lesion (Gerfen, personal communication). Enhanced expression of enkephalin could act to downregulate the level of its receptor. Although enkephalin demonstrates a greater affinity for δ- than μ-receptors (Paterson et al., 1983), and δ·-receptor level is unaffected by dopaminergic lesion (Unterwald et al., 1989), some post-translational products derived from proenkephalin demonstrate high μ-receptor affinity (Hurlbut et al., 1987). In any case, we might expect the direction of the effect of cocaine on μ-opioid receptors to be opposite to that produced by blockade of dopaminergic activity, and this is precisely what is observed. Cocaine-induced increase of dopamine level upregulates opioid receptors in mesolimbic terminal fields of the adult rat brain (Figure 1). The enhanced dopamine level could act to decrease NAc proenkephalin expression, which might upregulate the level of its receptor in critical reward regions. Alternatively, these processes could be independent, and dopamine could directly affect μ-receptor expression.

The influence of gestational cocaine exposure on dopamine level is unknown, however, dopamine level has been examined in neonatal littermates of the animals exposed to cocaine during gestation as described above. These preliminary data show a trend toward greater whole brain dopamine concentration following gestational cocaine exposure (Spear et al., 1989b). Interestingly, the results of behavioral studies suggest an attenuation of dopamine activity postnatally (Spear et al., 1989a). This subsequent reduction of dopamine activity does not eliminate the prenatal effect on opioid systems. Rather, sustained enhancement of opioid tone occurs in various dopaminergic, limbic and somatosensory systems.

FUNCTIONAL AND CLINICAL IMPLICATIONS

Cocaine-induced upregulation of μ·-receptors in sequential components of a multisynaptic reward pathway may initiate an *opioid cascade,* wherein endogenous or exogenous opiates have greater effects in a system with increased opioid tone. In such a system, opiate compounds may be capable of interfering with cocaine-induced reward, even without directly affecting dopamine. Thus, selective opioid antagonists might be capable of blocking cocaine reward by acting on a substrate containing enhanced opioid tone. This could represent the mechanism by which naloxone acts to attenuate the effects of cocaine on the threshold for rewarding self-stimulation (Bain and Kornetsky, 1987). In addition, buprenorphine, a mixed opiate agonist-antagonist, has been shown to suppress cocaine self-administration in monkeys (Mello et al., 1989; this volume), and may be useful as a pharmacotherapy during cocaine withdrawal. Moreover, the agonist properties of this compound could promote compliance during therapy by enhancing opioid tone in the reward pathway. However, administration of

opiate agonists could further enhance the cycle of cocaine reward, since opiates are known to cause dopamine release (Wood et al., 1980).

The clinical implications of opioid receptor upregulation produced by gestational cocaine exposure are unknown. The magnitude of the effect in a variety of brain regions, and the sustained influence of prenatal exposure suggest that long range effects may exist. Increased opioid tone in neocortical regions might portend cognitive impairment. In fact, cognitive deficits were observed in an appetitive conditioning test following prenatal cocaine exposure (Spear et al., 1989b). Moreover, the combination of enhanced opioid tone and attenuated dopamine activity might be related to hyperactivity and attentional deficits in offspring. These results suggest that sustained neurochemical alterations underlying behavioral deficits in the offspring of cocaine-using mothers might involve endogenous opioid systems.

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ACKNOWLEDGEMENTS

AUTHORS

Ronald P. Hammer, Jr., Ph.D. and Daniel W. Clow, Ph.D.
Laboratory of Cellular & Molecular Neuropharmacology,
Department of Anatomy & Reproductive Biology,
University of Hawaii School of Medicine, Honolulu, HI 96822

Linda P. Spear, Ph.D.
Department of Psychology and Centers for Developmental Psychobiology & Neurobehavioral Sciences, State University of New York,
Binghamton, NY 13901

Preclinical Evaluation of the Effects of Buprenorphine, Naltrexone and Desipramine on Cocaine Self-Administration

Nancy K. Mello

Animal models of drug self-administration can be used to evaluate the potential efficacy of new pharmacotherapies for treatment of drug abuse. Primates will self-administer most drugs that are self-administered by humans and traditionally, this model has proved valuable for the prediction of drug abuse liability (Griffiths and Balster, 1979; Thompson and Unna, 1977; Brady and Lukas, 1984). If medications that significantly suppress drug self-administration in monkey also prove to be. effective in human drug-abusers, this could facilitate rapid identification of promising new pharmacotherapies. Concordant findings from pre-clinical evaluations and clinical studies would significantly reduce the time required to introduce new medications into clinical trials.

There is accumulating evidence that pre-clinical evaluations of new pharmacotherapies for drug abuse treatment are consistent with clinical evaluations. For example, pre-clinical studies of the effects of methadone and of buprenorphine on opiate self-administration have yielded results consistent with clinical studies (Mello et al., 1983; Mello and Mendelson, 1980; Mello et al, 1982; Jones and Prada, 1975, 1977; Martin et al., 1973). This report summarizes our recent studies of three potential pharmacotherapies for the treatment of cocaine abuse using the primate drug self-administration model. Buprenorphine, an opioid mixed agonist-antagonist, naltrexone, an opioid antagonist, and desipramine. a tricyclic anti-depressant, were examined in parallel studies under comparable conditions (Mello et al., 1989, 1990a and b).

METHODS

We compared the effects of treatment with saline, desipramine (0.56 to 10.0 mg/kg/day), buprenorphine (0.237 to 0.70 mg/kg/day) and naltrexone (0.32 to 3.20 mg/kg/day) on cocaine self-administration in rhesus monkey (Mello et al., 1989, 1990a and b). Food self-administration was also studied to ensure that any changes in cocaine self-administration during drug treatment did not reflect a generalized disruption of behavior. Saline treatment and each of 7 doses of desipramine were studied for 20 sessions over 5 consecutive days. Saline treatment and each of 3 doses of buprenorphine were studied for 60 sessions over 15 consecutive days. Saline treatment and two doses of naltrexone also were studied for 60 sessions over 15 consecutive days.

Cocaine (0.05 or 0.10 mg/kg/inj) and food (1 gm banana pellet) self-administration were maintained on an FR 4 (VR 16:S) reinforcement schedule. This second order schedule of reinforcement requires an average of 64 responses for each food pellet or drug injection. The treatment drug or an equal volume of saline control solution was infused over 1 hour at the same time each day through one lumen of a double lumen intravenous catheter. Food and cocaine each were available during 4 one hour sessions each day. Food sessions began at 11 a.m., 3 p.m., 7 p.m. and 7 a.m. Cocaine sessions began at 12 noon, 4 p.m., 8 p.m. and 8 a.m. Drug injections were limited to 20 per one hour session and food pellets to 80 per one hour session. Each pair of food and drug sessions were separated by a time-out period when responses had no programmed consequences.

DESIPRAMINE'S EFFECTS ON COCAINE SELF-ADMINISTRATION

Figure 1 summarizes group data (N = 5) expressed as percent change in cocaine injections from base-line. Desipramine treatment at doses of 0.562 to 1.78 increased cocaine self-administration by 12 to 37 percent. At desipramine doses of 3.2 to 7.86 mg/kg/day, cocaine self-administration was suppressed by an average of 10 to 16 percent. Cocaine-maintained responding was suppressed by 40 percent at the highest dose of desipramine (10 mg/kg/day).

Cocaine self-administration increased (P < .01) or remained equivalent to base-line levels in 4 of 5 subjects during the first 15 days of desipramine treatment (0.56 to 1.78 mg/kg/day). During the second 15 days of desipramine treatment (3.2 to 7.86 mg/kg/day), 3 of 5 monkeys continued to self-administer cocaine at levels equivalent to or significantly above base-line (P < .01). The highest dose of desipramine (10 mg/kg/day) suppressed cocaine self-administration significantly in only one of these 3 monkeys (P < .01) (Mello et al., 1990a).

Suppression of Cocaine Self-Administration by Desipramine

(N = 5)
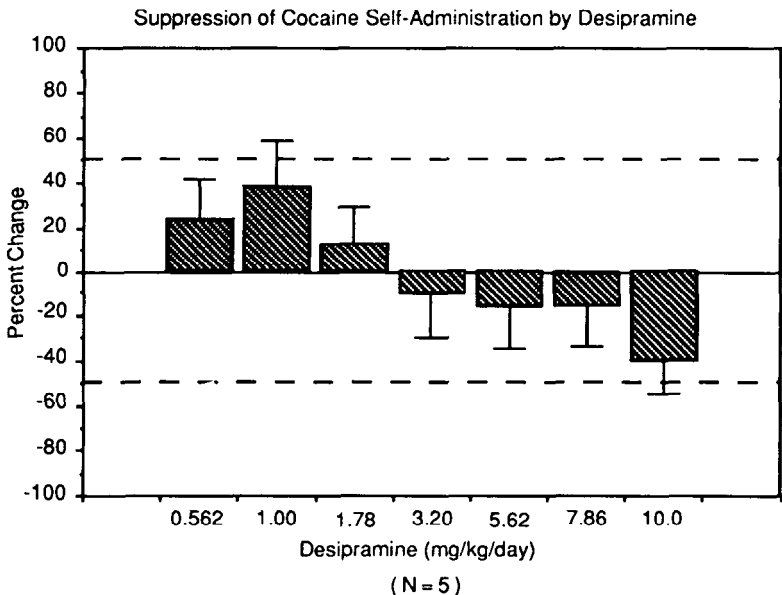
Food-maintained responding remained equivalent to or significantly above (P < .01) base-line levels in 4 of 5 monkeys during desipramine treatment (0.562-10 mg/kg/day). A generalized suppression of both cocaine and food-maintained responding (P < .01) during desipramine treatment occurred in one monkey that self-administered the highest base-line levels of cocaine (6.3 ± 1.03 mg/kg/day).

Clinical studies indicate that at least 10 days of desipramine treatment are required for a positive therapeutic response (Gawin, 1988). Consequently, we re-examined the three highest doses of desipramine (5.62, 7.86 and 10 mg/kg/day) for an additional ten days each. However, 30 consecutive days of high dose desipramine treatment did not significantly suppress cocaine self-administration. Cocaine self-administration averaged 74 to 76 of a maximum of 80 injections per day throughout high dose desipramine treatment. These desipramine doses are considerably above the range usually used in clinical treatment, i.e. 50 to 200 mg/day, which is equivalent to 3.1 to 3.7 mg/kg/day in monkey.

Desipramine's stimulation of cocaine self-administration is consistent with clinical reports of desipramine-related relapse to cocaine abuse (Weiss, 1988). The inconsistent and incomplete attenuation of cocaine self-administration in these primate subjects is also concordant with several clinical reports (McElroy et al., 1989; Baxter, 1983; O'Brien et al., 1988; Arndt et al., 1989). In addition, controlled in-patient clinical studies-indicate that 3 to 4 weeks of desipramine maintenance had no effect on cocaine self-administration, response rates or latency to the first response in comparison to pre-desipramine base-line cocaine self-administration measures (Fischman and Foltin, 1988; Fischman et al, 1990). Consequently, these primate data are in accord with desipramine's inconsistent effects in clinical trials (Mello et al., 1990a).

BUPRENORPHINE'S EFFECTS ON COCAINE SELF-ADMINISTRATION

Buprenorphine, an opioid mixed agonist-antagonist, effectively suppressed heroin self-administration by human heroin abusers in in-patient studies (Mello and Mendelson, 1980; Mello et al, 1982) and by macaque monkeys (Mello et al., 1983). Buprenorphine's effects on cocaine self-administration had not been studied previously, probably because the reinforcing properties of cocaine appear to be controlled by dopaminergic rather than endogenous opioid systems (Ritz et al, 1987; Johanson and Fischman, 1989). However, our endocrine studies of cocaine's effects on anterior pituitary hormones prompted us to explore buprenorphine's effects on cocaine self-administration. Cocaine significantly stimulates pituitary release of luteinizing hormone, a hormone that is under endogenous opioid inhibitory control (Mello et al., 1990c). Moreover, there is accumulating evidence that dopaminergic and opioid systems interact to mediate behavioral and neurobiological effects of drugs (Koob and Bloom, 1988). Co-modulation of neuroendocrine function by endogenous opioid peptides and dopamine (Kuljis and Advis, 1989; Mendelson et al., 1986; Yen, 1986) as well as evidence of cocaine-related increases in opiate receptor density (Hammer, 1989) further encouraged us to examine buprenorphine's effects on cocaine self-administration.

Buprenorphine significantly suppressed cocaine self-administration (P < .001 to .0001) in comparison to saline in all monkeys. On the first day of buprenorphine administration (0.237 and 0.40 mg/kg/day), cocaine self-administration

decreased by 49 to 95 percent in 5 of 6 monkeys. The percent suppression of cocaine self-administration by 15 days of buprenorphine treatment (0.237, 0.40 and 0.70 mg/kg/day) in comparison to the saline treatment base-line is shown in Figure 2 (left) for 6 monkeys. Buprenophine suppressed cocaine-maintained responding by 72 to 93 percent. After abrupt termination of buprenorphine treatment (0.237 and 0.70 mg/kg/day), cocaine self-administration remained suppressed for an average of 16 ± 4.4 and 28 ± 6.6 days, respectively.

Buprenorphine, (0.237 and 0.40 mg/kg/day) initially suppressed food self-administration in some monkeys ($P < .01$), but tolerance developed to buprenorphine's effects on food-maintained responding while cocaine self-administration remained significantly suppressed. Daily patterns of food self-administration across sessions were not disrupted by buprenorphine treatment. Since recovery of food-maintained responding occurred while cocaine-maintained responding remained significantly suppressed, we concluded that buprenorphine's suppressive effects on cocaine self-administration did not reflect a generalized suppression of behavior (Mellog et al., 1989, 1990b).

Suppression of Cocaine Self-Administration by Buprenorphine and Naltrexone
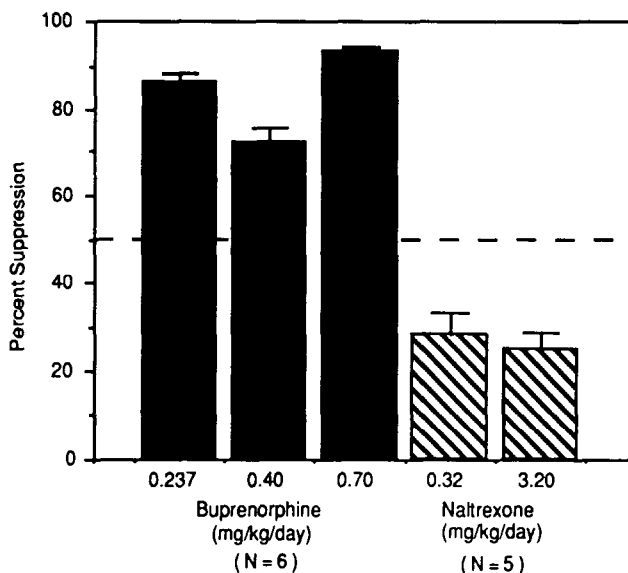
These data suggested that buprenorphine could be a useful pharmacotherapy for the treatment of cocaine abuse (Mello et al., 1989, 1990b). These findings in monkey are consistent with two clinical reports which showed that buprenorphine treatment of heroin abusers who were also polydrug abusers, reduced the number of cocaine-positive urines more effectively than methadone treatment (Kosten et al., 1989 a and b). A second implication of these data is that buprenorphine may also be valuable for the treatment of dual addiction to cocaine and heroin (i.e, the speedball). In-patient and out-patient clinical trials to evaluate the effectiveness of buprenorphine in treating persons with dual dependence on heroin and cocaine are currently underway. A preliminary report

of in-patient trials of buprenorphine from our clinical research center appears in this volume (Mendelson et al., 1990).

The mechanisms by which buprenorphine suppresses cocaine self-administration are unknown. It is likely that buprenorphine's unique combination of opioid mixed agonist-antagonist properties is essential since opioid agonists and antagonists alone have inconsistent effects on cocaine self-administration. Methadone treatment did not reduce cocaine-positive urines in heroin-dependent patients (Kosten et al., 1987a and b). Similarly, opioid antagonists alone are usually ineffective in suppressing cocaine self-administration. In rhesus monkeys, naloxone failed to suppress cocaine-maintained responding (Woods and Schuster, 1971; Killian et al., 1978). In rat, naltrexone pre-treatment resulted in no change in cocaine self-administration (Ettenberg et al., 1982) or increased cocaine self-administration (Carroll et al., 1986). The effects of the long-acting opioid antagonist, naltrexone, on cocaine self-administration in rhesus monkey have not been examined. Naltrexone is a pure mu opioid antagonist with a duration of action equivalent to buprenorphine's antagonist action. We postulated that studies of naltrexone might suggest. the relative importance of buprenorphine's antagonist component in suppressing cocaine self-administration.

NALTREXONE'S EFFECTS ON COCAINE SELF-ADMINISTRATION

In contrast to previous studies of opioid antagonist effects on cocaine self-administration in animal models, one clinical study found that naltrexone treatment of opiate addict polydrug abusers (100 to 150 mg, 3 times per week) reduced cocaine-positive urines significantly in comparison to methadone (Kosten et al., 1989a). Moreover, naltrexone affects a neuroendocrine system that is co-modulated by dopamine and endogenous opioids. Hypothalamic secretion of luteinizing-hormone-releasing hormone (LHRH) is controlled by both dopamine and endogenous opioid peptides (Yen, 1986). Naltrexone stimulates hypothalamic release of LHRH as inferred from increases in luteinizing hormone (LH) (Mendelson et al., 1986; Teoh et al., 1988).

Group data summarized in Figure 2 (right) shows that naltrexone (0.32 mg/kg/day) suppressed cocaine self-administration by an average of 28 (± 3.9) percent over 15 days (P < .001). However, a higher dose of naltrexone (3.2 mg/kg/day) did not suppress cocaine-maintained responding further. Cocaine self-administration was suppressed by 25 (± 4.5) percent over 15 consecutive days. Food self-administration was decreased by 24 percent (P < .05) after 5 days of treatment with the lower dose of naltrexone, then exceeded base-line levels during the higher dose of naltrexone (Mello et al., 1990b). These data are consistent with the clinical report that naltrexone treatment of opioid dependent patients with polydrug abuse problems reduced cocaine-positive urines significantly in comparison to methadone (Kosten et al., 1989a). These data suggest that the antagonist component of buprenorphine may contribute to, but probably does not solely account for buprenorphine's suppressive effects on cocaine self-administration.

COMPARISON OF THE EFFECTS OF BUPRENORPHINE, NALTREXONE, AND DESIPRAMINE ON COCAINE SELF-ADMINISTRATION.

Data shown in Figures 1 and 2 suggest that buprenorphine is likely to be more effective than naltrexone for the treatment of cocaine abuse and that naltrexone and desipramine may be approximately equal in efficacy. The probable mechanisms of action of these three medications are quite different. Desipramine is thought to act as a pharmacologic substitute for cocaine, since the tricyclic anti-depressants and cocaine have parallel effects on neuronal transmitter and receptor mechanisms (Dackis and Gold, 1985). However, desipramine may disrupt the subjective and cardiovascular effects of cocaine without affecting cocaine self-administration (Fischman et al., 1990). Recent controlled clinical studies indicate that cocaine and desipramine interactions may have toxic cardiovascular consequences (Fischman et al., 1990).

The mechanisms by which naltrexone, an opioid antagonist, and buprenorphine, an opioid mixed agonist-antagonist, suppress cocaine self-administration are unclear. The reinforcing properties of cocaine appear to be controlled by dopaminergic systems (Johanson and Fischman, 1989; Ritz et al., 1987). Suppression of cocaine-maintained responding by opioid antagonists and mixed agonist-antagonists support the concept that dopamine and endogenous opiate systems interact to mediate the behavioral and neurobiologic effects of drugs (Koob and Bloom, 1988). This possibility opens up a new domain of potential pharmacotherapies for cocaine abuse, as well as for the combined abuse of heroin and cocaine. As our understanding of co-modulatory interactions between dopaminergic and endogenous opioid systems increases, new approaches and improved pharmacotherapies for cocaine abuse may be developed.

PRE-CLINICAL EVALUATION OF NEW MEDICATIONS FOR DRUG ABUSE TREATMENT: HOW USEFUL IS THIS MODEL?

The primate drug self-administration model has a number of advantages for rapid pre-clinical evaluation of new medications for drug abuse treatment. Unlike out-patient clinical trials, compliance with the treatment drug regimen is ensured and the effects of the treatment drug cannot be confounded by unreported polydrug abuse. Accurate base-line measures of the daily dose and pattern of drug self-administration are available for comparison with drug self-administration during treatment. Moreover, interpretation of the effects of the treatment drug cannot be modulated by factors such as expectancy (i.e., placebo responding). Systematic and targeted pre-clinical evaluation trials are also less costly than extensive clinical trials.

The feasibility and the potential efficacy of using the primate drug self-administration model to evaluate new medications for drug abuse treatment is illustrated by findings from several studies. To date, pre-clinical studies of buprenorphine and methadone for treatment of opiate abuse have yielded results consistent with clinical studies of the same compounds (Mello et al., 1983; Mello and Mendelson, 1980; Mello et al., 1982; Martin et al., 1973). Pre-clinical evaluations of desipramine, buprenorphine, and naltrexone for the treatment of cocaine abuse (Mello et al., 1989, 1990a and b) have also yielded data that are concordant with clinical evaluations (Arndt et al., 1989; Baxter, 1983; Fischman

and Foltin, 1988; Fischman et al., 1990; Kosten et al., 1989a and b; McElroy et al., 1989; O'Briena et al., 1988; Weiss, 1988). We conclude that the primate drug self-administration model should be as effective for the evaluation of new medications as it has been for the prediction of new drug abuse liability. Further studies of a wider range of new medications will be necessary to confirm or refute this hypothesis.

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AUTHOR

Nancy K. Mello, Ph.D.
McLean Hospital-Harvard Medical School
Alcohol and Drug Abuse Research Center
115 Mill Street, Belmont, MA 02178

Buprenorphine Treatment for Concurrent Heroin and Cocaine Dependence: Phase I Study

Jack H. Mendelson, Nancy K. Mello, Siew Koon Teoh, John Kuehnle, Pradit Sintavanarong, and Karen Dooley-Coufos

INTRODUCTION

The prevalence of concurrent cocaine and heroin abuse appears to be increasing in the United States (Kaul and Davidow 1981; Kosten et al., 1986; Kozel and Adams 1986). Enhanced risk for the Acquired Immuno Deficiency Syndrome (AIDS) occurs in persons who intravenously self-administer both cocaine and heroin as a consequence of needle sharing and the immunosuppressive effects of cocaine and heroin (Donahoe and Falek 1988; Klein et al., 1988). In 1980 we observed that buprenorphine suppressed heroin use by heroin addicts and concluded that buprenorphine would be a safe and effective pharmacotherapy for heroin dependence (Mello and Mendelson 1980). More recently, we found that buprenorphine suppresses cocaine self-administration by rhesus monkey (Mello et al., 1989). These observations suggested that buprenorphine may also be a useful pharmacotherapy for cocaine abuse and dependence. The purpose of the current study was to determine the safety and effectiveness of buprenorphine for the treatment of persons with dual intravenous heroin and cocaine dependence.

METHODS

Subjects were adult males between the ages of 31 and 40 who had a DSM-III-R diagnosis of concurrent opiate and cocaine dependence. Their history of opiate dependence ranged from 10 to 19 years, and they currently self-administered four to eight bags of heroin intravenously daily. History of cocaine dependence was 4 to 23 years, and their current use of cocaine ranged from three and a half to one half grams a week intravenously. All were cigarette smokers averaging one to three packs per day, and all consumed alcohol with an average intake of three to six beers each day. The subjects who provided informed consent for participation in these studies had no other DSM-III-R Axis I diagnosis and no significant medical disorders.

The initial portion of the phase 1 open trial consisted of a 30-day inpatient study following methadone detoxification (Table 1). One half of all subjects who entered the program were randomly assigned to receive 4 mg buprenorphine per day and the others received 8 mg per day as the maintenance dose. Buprenorphine was administered sublingually in a solution containing 30 percent

ethanol utilizing procedures similar to those described by Fudala and his associates (1990).

TABLE 1

30-DAY INPATIENT PROGRAM AFTER METHADONE DETOXIFICATION

DAY	CONDITION
1-6	DRUG FREE
7-9	CHALLENGE DOSE (COCAINE, MORPHINE, PLACEBO)
10-14	BUPRENORPHINE INDUCTION (1,2,4/6,8 MG/DAY,SUBLINGUALLY)
15-23	BUPRENORPHINE MAINTENANCE (4 OR 8 MG/DAY, SUBLINGUALLY)
21-23	CHALLENGE DOSE (COCAINE, MORPHINE, PLACEBO)
24-29	BUPRENORPHINE MAINTENANCE (4 OR 8 MG/DAY) OR BUPRENORPHINE DOSE REDUCTION (8,6,4,2,1,0 MG/DAY)
30	DISCHARGE ON BUPRENORPHINE MAINTENANCE (4 OR 8 MG/DAY) FOR OUTPATIENT PROGRAM OR TRANSFER TO NALTREXONE OR DRUG FREEPROGRAM

During the 30-day inpatient program all subjects were asked to complete a drug desire or "craving" questionnaire each day every half hour between 8 a.m. and 12 midnight. Subjects indicated whether they would prefer no drug, heroin, cocaine or speedball. The questionnaires were stamped with a time-clock device and responses were collated at the end of each study day. If subjects provided responses within the appropriate half-hour time interval, they were paid one dollar for each completed questionnaire. During the inpatient program regular daily monitoring of the following indices were also carried out: vital signs, electrocardiogram, blood hemogram, blood chemistries, food intake, sleep/wakefulness behavior, assessment of mood, affect and interpersonal behaviors. Any adverse side effects were also recorded.

Challenge dose assessments were carried out on days 7,8 and 9, prior to induction and maintenance on buprenorphine and on days 21,22 and 23 during buprenorphine maintenance. The purpose of these studies was to assess the effects of buprenorphine on cardiovascular function, vital signs and subjective responses following intravenous administration of 10 mg morphine, 30 mg of cocaine or placebo. Only one drug was administered on a given challenge dose day and the sequence of drug administration during three days was randomized. Prior to intravenous drug challenge, subjects had an indwelling intravenous catheter placed in an arm vein and appropriate chest and arm leads were attached for a continuous monitoring of vital signs and the electrocardiogram.

Following intravenous injection of the challenge drug dose, subjects were instructed to depress a foot pedal immediately upon completion of the i.v. injection. Depression of the foot pedal activated a clock and turned on a discriminative stimulus light. When the subject first detected a drug effect, he was

instructed to depress the foot pedal a second time. This stopped the first clock, activated a second clock and turned on a second discriminitive stimulus light . The subject was instructed to depress the foot pedal the third time when he was <u>certain</u> he felt a drug effect. This foot pedal depression turned off the second clock and activated a third discriminative stimulus light. Utilizing these procedures, it was therefore possible to determine latency of completion of intravenous injection to first <u>detection</u> of a drug effect and latency of completion of intravenous injection to <u>certainty</u> of drug effect.

When the subject reported certainty of drug effect he was instructed to complete a questionnaire containing items describing the intensity of the drug effect and the quality of the effect (pleasant or unpleasant). These questionnaires were administered at 5 to 15 minute intervals for two hours immediately following report of certainty of a drug effect. Thirty minutes after injection of the intravenous challenge dose the subject was also asked to describe whether he had received heroin, cocaine or placebo and to rate the effect of the drug (good or bad) in comparison to his prior experiences with a similar drug.

RESULTS

<u>Drug safety:</u> No significant adverse effects were observed during buprenorphine induction or maintenance. Mild systolic hypotensive episodes were occasionally observed, but no significant systolic or diastolic hypotension occurred. There were minor reports of nausea or constipation, but no significant changes in dietary or bowel habits were noted. During challenge dose studies, buprenorphine did not significantly accentuate or diminish pulse, blood pressure or respiration responses following administration of the challenge drug. No adverse effects on cardiovascular or electrocardiographic function were observed when challenge doses were administered during buprenorphine maintenance.

FIGURE 1

198
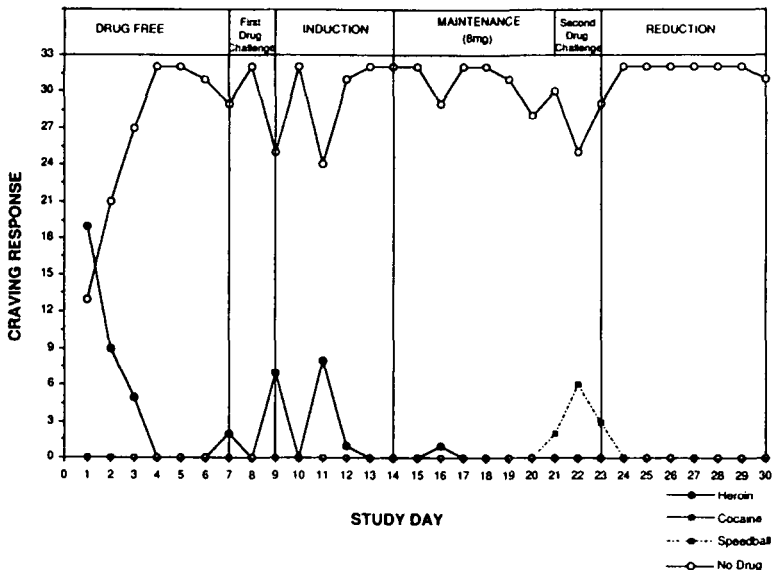
Drug desire and craving: Representative data are presented for two subjects who had different drug desire and craving responses during the 30-day study. Figure 1 shows daily craving responses for one subject whose heroin craving scores decreased from 20 to 0 during the first through fourth drug-free days. When the challenge dose of morphine was administered on day 9 and during early buprenorphine induction on day 11, this subject had an increase in reports of heroin craving with concomitant decrements in reports of desire for no drugs. From day 12 through 30 the subject reported little or no desire for heroin, cocaine or speedball use. Because of his excellent response to a drug-free environment during the initial phases of hospitalization, he decided that a therapeutic trial in a drug-free environment would be of benefit. Therefore he was detoxified from buprenorphine therapy and was transferred to a drug-free residential treatment program.

A second subject (Fig. 2) reported persistent craving for heroin and speedballs throughout his first ten days on the research ward. A progressive decrease in heroin craving occurred following initiation of buprenorphine therapy on the tenth day of the study with a concomitant increase in reports of desire for no drugs. However this subject reported a continuous desire for some use of speedballs during the entire study. On morphine challenge dose days (days 7 and 22) he reported a significant increase in craving for heroin. At the time of discharge from the study, this subject's reports of craving for heroin as opposed to no drug were inversely proportional to those reported when he entered the study. This subject was discharged from the inpatient unit to the outpatient daily buprenorphine (4 mg) maintenance program.

FIGURE 2

199
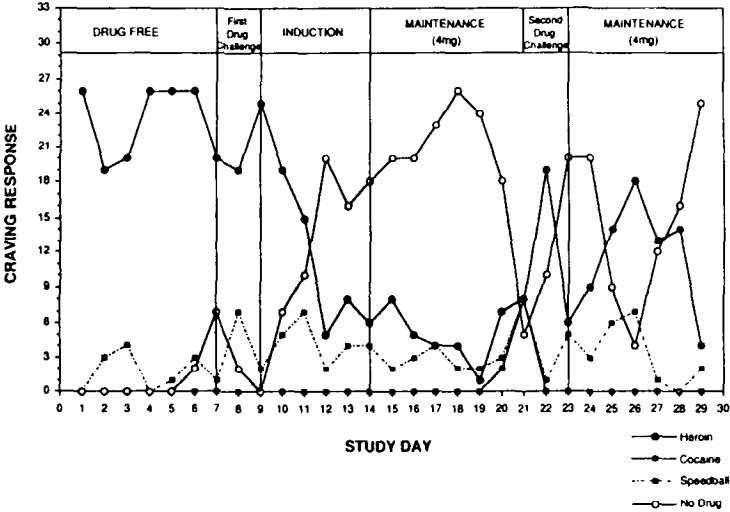
Challenge dose responses; Figures 3 and 4 show time latency data for <u>detection</u> and <u>certainty</u> of cocaine and morphine challenges for two subjects who were maintained on 4 mg of buprenorphine daily. Figure 3 shows that 4 mg of buprenorphine completely blocked a 10 mg morphine challenge dose. The subject reported no detection or certainty of a morphine effect in contrast to his rapid reports of detection and certainty prior to buprenorphine administration. Buprenorphine maintenance also essentially tripled the detection time following cocaine administration although the reported certainty time decreased. Administration of 4 mg of buprenorphine as a maintenance dose also completely suppressed detection of or certainty of a 10 mg intravenous morphine challenge dose for a second subject (Fig. 4). However, 4 mg of buprenorphine shortened the detection time following cocaine challenge.
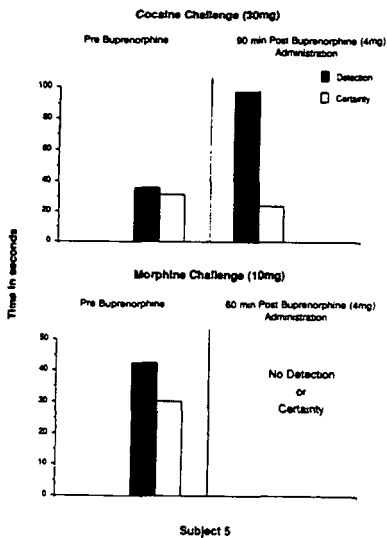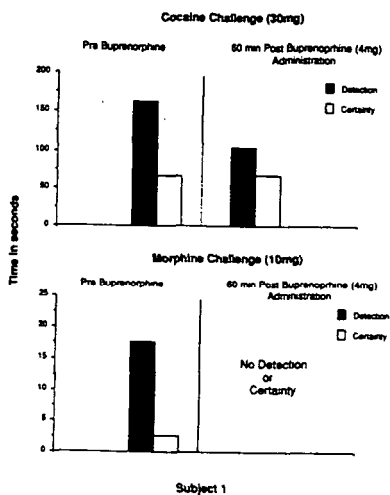
FIGURE 3   FIGURE 4

Figures 5 and 6 show drug intensity and drug quality response for the two subjects described above. Figure 5 shows the reported drug effects for the subject who had an increase in latency for cocaine detection during the cocaine challenge dose when he was maintained on buprenorphine. This subject showed a decrease in drug intensity and a decrement in drug quality during buprenorphine maintenance as compared to his initial challenge dose response. In contrast, figure 6 shows an increase in drug intensity and quality following the cocaine challenge dose when this subject was maintained on buprenorphine.

DRUG EFFECTS OF 30mg COCAINE I.V.

☐ Pre Buprenorphine
■ Post 4mg Buprenorphine
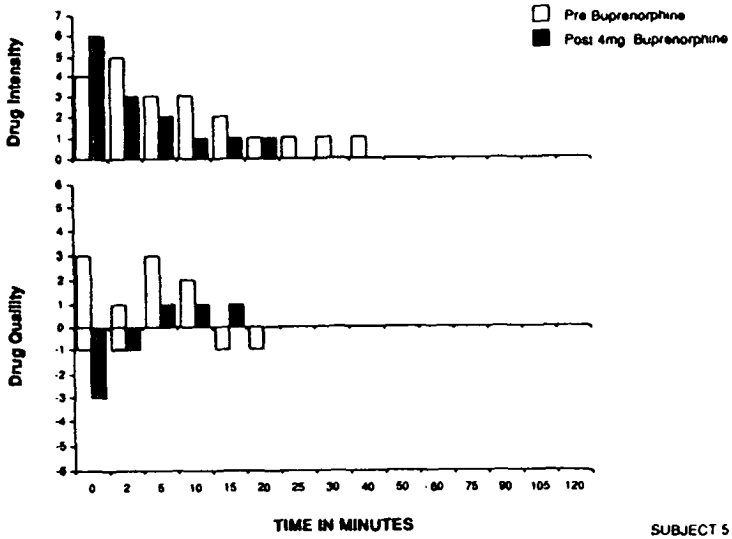
SUBJECT 5

FIGURE 5

DRUG EFFECTS OF 30mg COCAINE I.V.

☐ Pre Buprenorphine
■ Post 4mg Buprenorphine

SUBJECT 1

FIGURE 6

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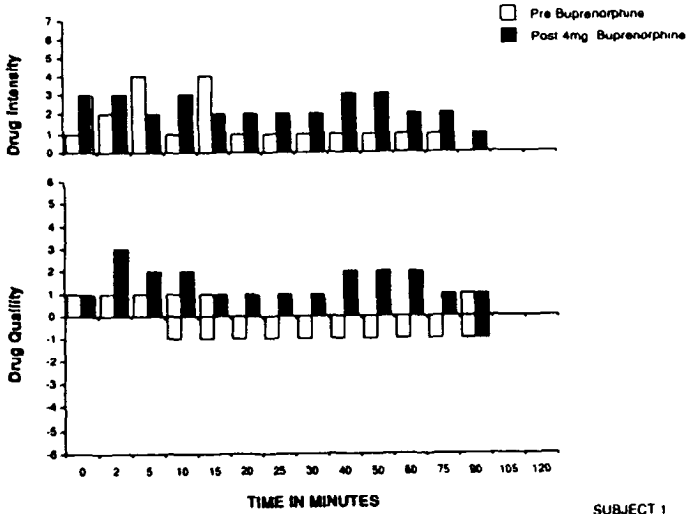
All subjects correctly identified the challenge drug dose prior to buprenorphine maintenance. All subjects correctly identified the placebo challenge dose prior to and following buprenorphine maintenance. However all subjects, whether maintained on 4 or 8 mg buprenorphine per day, identified heroin challenge dose after buprenorphine maintenance as a placebo. Approximately one half of all the subjects believed that buprenorphine maintenance diminished drug intensity quality of cocaine challenge doses whereas others felt that buprenorphine maintenance enhanced both drug intensity and quality of the cocaine challenge dose.

CONCLUSIONS

Buprenorphine maintenance at doses of 4 or 8 mg sublingually per day for ten days completely blocked the subjective effects of 10 mg of morphine administered intravenously. Buprenorphine maintenance also was associated with the significant reduction in subjects' craving reports for heroin. Buprenorphine effects on cocaine craving, as well as speedball craving, were more variable. In some instances buprenorphine appeared to suppress reports of cocaine or speedball craving and also diminished the intensity and quality of the cocaine challenge dose. In other instances, however, buprenorphine maintenance appeared to enhance reports of both intensity and quality of intravenously administered cocaine. A review of demographic data for the subjects indicated that buprenorphine's effects on cocaine challenge doses were not related to the number of years each subject had abused cocaine. However, the suppressive effects of buprenorphine on cocaine challenge dose effects occurred as a function of the dose of cocaine usually self-administered; high cocaine dose users showed a buprenorphine suppressive effect. Further studies to determine the relative effectiveness of buprenorphine maintenance on cocaine desire and the reinforcing properties of cocaine in relationship to severity of cocaine dependence and tolerance are currently in progress.

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Jack H. Mendelson, M.D.; Nancy K. Mello, Ph.D.; Siew Koon Teoh, M.D.; John Kuehnle, M.D.; Pradit Sintavanarong, M.D.; Karen Dooley-Coufos, R.N., M.S. Alcohol and Drug Abuse Research Center, Harvard Medical School-McLean Hospital, 115 Mill Street, Belmont, MA. 02178

Assessing Substance Abuse and Other Psychiatric Disorders: History of Problems, State of Affairs

Lee Robins

DIAGNOSIS AND ASSESSMENT INSTRUMENTS

Designing an instrument to assess substance abuse in clinical and general population subjects requires three steps: selecting the criteria by which substance abusers will be identified, selecting a method for ascertaining whether those criteria are met, and covering those criteria in a way that minimizes errors of both under and overreporting. The other members of this panel will be describing the instruments they have used to assess disorders, and their results. I will concentrate on the criteria, and how instruments can address them most profitably.

It is now well accepted in the medically oriented sectors of the research community that selection of subjects for studies of substance users with problems and the estimation of prevalences of substance abuse in the population should be based on standard nomenclatures, which categorize persons as having or not having specific substance abuse diagnoses. But there are also critics of the diagnostic model.

Criticisms of the Use of Diagnoses in Substance Use Research

Some take the position that the division of the population into cases and non-cases is both arbitrary and irrelevant to understanding levels of substance-related problems in the community. They argue that what is called a disorder is merely the positive tail of a distribution of the community's intake levels, and that the only way to reduce its size is to reduce the average intake of the community as a whole through changing social values, restricting availability of substances, or setting costs so high that the cost-benefit equation of use is altered. No assessment instrument to detect cases could interest people with this view of diagnosis. However, there are other critics of substance abuse diagnoses who take a less extreme position, but make some telling points. While they recognize that categorical diagnoses are a useful simplification for purposes of claiming

reimbursement or clinical communication, they note several major drawbacks to using them in research. First, studies that classify subjects by their diagnoses typically fail to make use of the rich detail on amount of intake and symptoms collected in the course of reaching a diagnosis which would reveal different and meaningful variations in the phenomenology of dependence; second, the claim that research subjects selected on the basis of standard diagnoses of substance dependence are homogeneous is false, because they are composed of subgroups who came to substance abuse by very different routes, some by genetic liability, some by self-treatment of other psychiatric disorders, some by attempts to narcotizing anxiety or pain caused by a major life stress, and some as part of a generally deviant life style. Critics also caution that the diagnostic schema that have been adopted by the official nomenclatures are generally based on psychiatrists' clinical experience in western industrialized countries with their own patients, who typically have such severe disorders that they would meet criteria no matter which diagnostic scheme was adopted, and whose symptoms are similar because they come from the same or similar cultures. The severity of these cases creates the intercorrelation among symptoms that justifies the concept of a syndrome, but these correlations may disappear when the diagnostic scheme is applied to patients of general practitioners or to untreated persons in the community. Critics also note that criteria effective in identifying disorders in one culture may fail in another when the cultures differ in the legality, availability, and costs of substances, in typical patterns of use, and in tolerance of intoxication. Finally, they note that communication via the diagnostic labels of substance use disorders may give only the illusion of shared meaning because of the vagueness of the earlier definitions of substance dependence and the recent rapid shifts in definition in the successive versions of the official nomenclatures. They note, further, that there is every indication that definitions will continue to shift, making results of research using today's nomenclature dated and perhaps unintelligible to future scholars.

We will briefly review the extent to which official systems have changed over time and are likely to continue to do so in the future, and then discuss what to do about that and the other criticisms by those who doubt the value of diagnostic assessment as a research tool.

A HISTORICAL LOOK AT CHANGES IN OFFICIAL NOMENCLATURES

Diagnostic interviews have been written to operationalize substance use disorders as defined by recent versions of the two major diagnostic systems, the American Psychiatric Association's Diagnostic and Statistical Manual (DSM) and the International Classification of Diseases (ICD, Ch. 5). You will be hearing about results from these interviews shortly. These recent versions of the criteria are only the latest in a series of revisions,

which have over time increased the role of substance-related disorders in both standard nomenclatures.

Figure 1.

SPACE ALLOTTED TO SUBSTANCE ABUSE IN OFFICIAL MANUALS
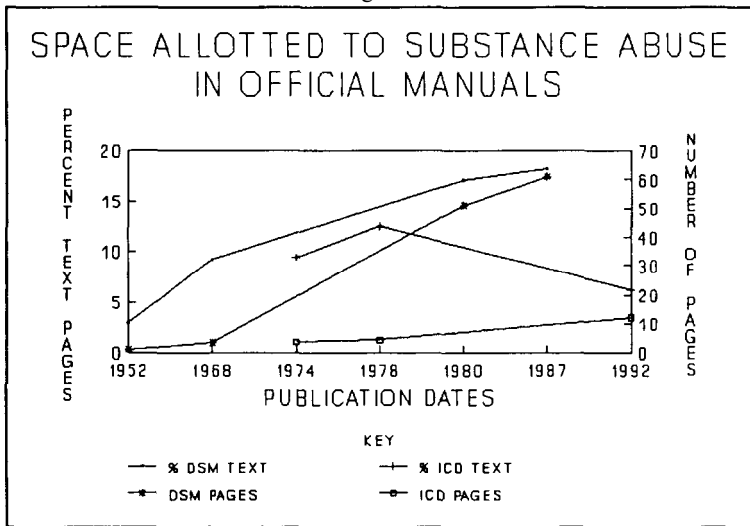
Changes in DSM

American psychiatry's increasing attention to substance-related disorders can be measured by the increasing proportion of the Manual devoted to them in two sections, under the organic mental disorders and as substance abuse disorders (Figure 1) and by their being given increasing prominence as indicated by the level of the diagnostic classification system at which they appear (Figure 2). In the first edition of the DSM (1952), alcohol and drug use disorders together were covered in three-quarters of a page under Acute Brain Syndromes plus 6 lines under Chronic Brain Syndromes Associated with Intoxication and 8 lines under the category of Sociopathic Personality, for a total of 2.9% of the 43 pages of text devoted to "Definition of Terms". In the second edition (1968), two pages were devoted to the alcoholic psychoses, subdivided into delirium tremens, Korsakov's, hallucinosis, paranoid state, acute intoxication, deterioration, pathological intoxication, and other. Drug intoxication appeared only incidentally under psychosis with drug or poison intoxication with the parenthetical "includes psychedelic drugs". Alcoholism and Drug Dependence moved out of the Personality Disorders to a parallel status within the category Personality Disorders and Certain Other Non-psychotic Mental Disorders, and received a total of one and a half pages. Alcoholism was subdivided into four subtypes (episodic excessive drinking, habitual excessive drinking, alcohol addiction, and other), and drug dependence was subdivided by classes of drug: opiates, synthetic opioids, barbiturates, other hypnotics and tranquilizers, cocaine, cannabis, other

Figure 2. Substance Disorders Move Up the Hierarchy
of Manual Headings

	DSM-1 1952	ICD 8 1961	DSM-2 1968	ICD-9 1978	DSM-3 1980	DSM-3-R 1987	ICD-10 1990
HEADING RANK							
SECTION					D	D	D,O
MAJOR HEAD					O	O	
SUBHEAD	O	O,D	O,D	O, D			
SUB-SUB	D	D	O				

O = Organic; D = Dependence

stimulants, hallucinogens, and others. All alcohol and drug-related disorders were covered in 9.2% of the 38 pages of text devoted to Definition of Terms.

DSM-III (1980) was a tome, with 299 pages devoted to the description of diagnostic categories. Substance use organic mental disorders got their own 34-page section within the Organic Mental Disorders. Withdrawal without delirium was added, and organic syndromes were separately described for each class of illicit drug, including a new one, caffeine. Substance use disorders were given their own section of 17 pages, parallel to disorders of childhood, organic syndromes, schizophrenia, mood disorders, anxiety disorders, etc. New disorders here were tobacco dependence, abuse of PCP and hallucinogens, and two combination drug categories, one with an opioid and one without. Further, for alcohol and drugs other than cocaine and hallucinogens, separate diagnoses of abuse and dependence were described. (For cocaine and hallucinogens, an abuse but no dependence syndrome was provided.) This expansion increased substance related disorders' share of the text to 17.1%.

The most recent edition, DSM-III-R (1987), is still longer, with 336 pages devoted to describing disorders. Organic Substance Use disorders now occupy 40 pages, and Psychoactive Substance Use disorders occupy 21, for a total of 18.2% of the total. One new drug class, inhalants, has been added. The continued growth is surprising given that DSM-III-R abandoned listing separate criteria for each of the psychoactive substance use disorders. Each class of substances, except tobacco, can now be said to cause both dependence and abuse. (Tobacco is restricted to dependence.) The uniformity of their symptoms does not apply to the symptoms of withdrawal, listed separately in the mental disorders section for each class of drugs except hallucinogens, with the caution that they "may not apply to cannabis or PCP". In the section on organic mental disorders, there is no effort at uniformity. A table (p. 124) shows 12 organic syndromes

distributed as follows: 7 for alcohol, 5 for PCP, 4 each for amphetamines, cocaine, sedatives, and hallucinogens, 2 each for cannabis and opioids, and 1 each for nicotine, caffeine, and inhalants.

A number of other changes have also occurred in DSM-III-R: Abuse has become a residual disorder, reserved for those who do not meet criteria for dependence. Impairment in social and occupational functioning and emotional or mental problems following substance use are no longer symptoms in themselves. Instead, the symptom requires recognition of the social or emotional problems as substance-caused and continued substance use despite that recognition. Medical problems, absent in DSM-III, can also count as symptoms if use continues after recognition of substance use as their cause. The number of symptoms required has been raised to three (from 2 in DSM-III). The duration criterion has been relaxed by allowing it to be met by symptoms occurring repeatedly over more than a month if they never lasted a whole month.

Have these revisions created the substantive differences that we are warned threaten adequate shorthand communication? Certainly this is the case for the shifts from DSM-I and -II to DSM-III. Indeed, the definitions in those early versions were so sparse that one could almost say that the diagnostic label communicated almost nothing. Whether changes from DSM-III to -III-R are substantive is not self-evident, but you will be hearing empirical evidence about that shortly.

The era of rapid change for DSM are not yet over. There is a Task Force for Substance Use Disorders actively working to redo criteria for DSM-IV.

ICD Changes

Changes over the years in the DSM have been matched by equally massive changes in the international system. In the 40 pages of the ICD-8 Glossary for Chapter V (published in 1974 to accompany the 1967 publication of the classification), there was one and three-quarter pages on alcoholic psychoses, while drugs were combined with poisons, and not treated specifically. Alcoholism and drug dependence got two pages, with alcoholism divided into episodic, habitual, and addiction, and drug dependence divided into 10 classes of drugs. The total pages devoted to substance use disorders were 9.4% of the text. In the edition of the Glossary for ICD-9 (1978), with 36 pages of text, alcoholic and drug psychoses each had a section, together covering 2 pages; the alcohol dependence syndrome got less than half a page, its subtypes having been dropped, drug dependence needed a page to list the same 10 categories of drugs, and nondependent abuse of drugs was added (one-half page). The proportion of space devoted to substance abuse grew to 12.5% of the text.

Because Chapter V of ICD-10 is still in draft form, its final length is uncertain, but it is clearly moving toward textbook size. In the current draft version of 193 pages describing syndromes, organic and behavioral disorders due to alcohol and drugs have been brought together in a single section, which occupies 12 pages. This expansion, however, is not as great as the expansion of other disorders, so substance-related disorders now occupy only 6.2% of the draft text pages.

The criteria for dependence in ICD-10 will resemble those in DSM-III-R. Roth apply common criteria across substances. Both include as criterion symptoms loss of control, withdrawal, substance use to relieve or prevent /withdrawal, tolerance, continued use despite adverse consequences, and neglect of previous interests. However, ICD adds desire or compulsion to use and narrowing of the repertoire of use, criteria suggested by Griffith Edwards, and omits the DSM-III criteria of much time spent on use and intoxication or withdrawal symptoms at inappropriate times and places. Whether this trend toward concordance between the two systems will be abetted or aborted now depends on the DSM-IV Task Force, since ICD-10 is virtually complete.

DESIGNING ASSESSMENT INSTRUMENTS TO COPE WITH THE CRITICISMS.

The historical changes in both DSM and ICD certainly support the view that substance abuse diagnostic criteria are fluid, and that using a diagnostic term like "alcohol dependence" has limited communication value. It is also true that changes are likely to continue, although I, like others who author assessment instruments, wish changes could be called to a halt until there was time to collect data on which differences are substantive and which trivial, and to experiment with proposed variations to choose the most reliable set of indicators applicable to the broadest population groups.

The Task Force for DSM-IV is making an effort to do this sort of evaluation via field trials, but the field trials are not "serious science". They will be carried out in samples of convenience, with minimal supervision across sites to guarantee comparability of data collection. They are certainly better than nothing, but hardly a "state of the art" effort. What can you expect for $10,000 per site? The fact that the field trials are not on a scale sufficient to guarantee that changes will be an improvement is unlikely to cause the Task Forces to leave current criteria unchanged or to make only the changes necessary to make DSM-IV identical with ICD-10; groups empowered to make changes are likely to do so, particularly when it is easy to point to ambiguities and problems with the current criteria in both diagnostic systems.

If we accept the fact that there will continue to be changes, and that there will continue to be differences between DSM and ICD, how should we design assessment tools? The following principles in assessment would be helpful, I believe:

1) Collect and record symptom data in the smallest units possible and 2) put into a single instrument all the elements of the major current diagnostic systems.

These suggestions serve many goals. Because criteria change much more rapidly and radically than does the substance user's experience, the closer we get to recording that experience in detail, the greater the chance that we will be able to reassemble those details into any configuration that future nosologists may suggest. If we attempt to cover all of the symptoms listed by current major diagnostic schemes, items from whichever system "wins out" in the long run will be available.

This "atomic" and multidiagnostic system approach to assessment also permits data collected to be useful to future Task Forces who will make decisions about diagnostic revisions. It provides the raw materials necessary to detect the likely effects of selecting one system over another, dropping criteria, or making eclectic selections from both systems. Diagnostic algorithms can be varied to try different numbers of symptoms and different groupings of symptoms to learn what proportions of the population each potential diagnostic scheme would select as positive and what groups currently found positive it tends to omit or currently negative groups it calls positive, and whether it improves or worsens correlations with genetic liability, impairment, or other non-diagnostic variables that should be correlated with diagnosis.

These suggestions have consequences for interview type. They argue against screening interviews of both the two popular designs, the most discriminating symptoms extracted from a complete survey of symptoms or a decision tree design which terminates the interview as soon as it can be established that the subject must or cannot meet criteria. A screening interview is inexorably tied to a single diagnostic scheme and cannot make diagnoses according to any other system because the relevant questions will not have been asked.

These suggestions also have implications for how questions should be worded. For example, we now evaluate "social" problems by asking whether a family member, friend, employer, or clergyman has complained about the respondent's substance use. If tomorrow there is a proposal to make problems with the employer a separate criterion, we would not be able to separate those whose "yes" answer referred to the employer from those whose "yes" was only in response to one or more of the other

categories of people asked about. For an assessment interview to be flexible enough to be used to evaluate new criteria, questions should contain no "or's" and every item in a string of experiences that are alternative ways of qualifying for a current criterion must be separately scored.

In addition to providing scoring flexible enough to evaluate future diagnostic schemes, this "atomic" interview design meets the needs of those who would prefer a more detailed portrait of the user's patterns and problems than a diagnosis can provide. Its symptoms can be converted into a dimensional scale independent of diagnostic rules through a simple counting of positives. This design also allows applying factor analysis and other psychometric techniques that assess correlations among symptoms to seek alternatives to clinically informed diagnostic criteria.

How can assessments cope with the issue of heterogeneous subtypes? Subtyping based on etiological hypotheses--genetics, secondary effects of other psychiatric disorders, response to life stress, or a general failure to conform to the broader society's standards, requires information <u>external</u> to the symptoms directly attributable to substance use. A stand-alone instrument for evaluating substance abuse will not suffice, although it can identify persons for whom further questions will be needed. Supplemental interview sections are needed to ask for family history and to assess pre-existing psychiatric symptoms and pre-substance abuse life-style and life stresses if respondents are to be divided into some of the suggested etiologically focused subtypes. Onset dates must be obtained both for the substance use disorder and for these possible etiological factors. Because substance abuse can cause depression, can perhaps trigger schizophrenia in a susceptible person, can certainly precipitate life crises, and often embroils the user of illicit drugs in a counter-culture life style, subtyping requires ascertaining that these potential etiological factors predated the substance related problems. For subtyping, the current episode, which is the focus of most assessment tools, has no greater importance than past episodes. The interview must, however, get information about the <u>first</u> episode.

How can assessment instruments respond to the charge that our diagnostic criteria are culture-specific? Our work with the field trials of the Composite International Diagnostic Interview (CIDI), an interview designed for use across nations and cultures, has shown that the substance use disorders are probably the most difficult of all psychiatric disorders to inquire about cross-culturally. Not only are substance-induced behaviors that are a problem in one setting no problem in another, but the very substances used differ and when they are the same, they are measured differently and customarily diluted to different levels, so that, for example, comparable volumes of beer do not provide comparable amounts of absolute alcohol. Further, styles of ingestion vary, so that withdrawal

symptoms may be an early symptom in a culture where there is traditional daily use, but a late symptom indicating a severe disorder in a culture where use is typically sporadic. Opportunities to show some symptoms are greater in industrialized societies: few Chinese alcoholics will have had auto accidents as a result of drinking--bicycle accidents, perhaps. For these problems, I can offer no ready solution. We did learn through the CIDI field trials that local specialists in substance abuse problems are needed to point out problems in translation not only of words but of concepts and units of intake, because psychiatrists have too little information about substance use in the general population to serve as local experts. We are still in early stages of cross-cultural comparisons, and we will need very basic efforts at equilibrating interviews before we are ready to compare prevalence rates or risk factors for substance abuse across cultures.

What can we do to meet the criticism that our interviews, based on clinical experience, miss the problems typical in the general population? Here we have to depend on the ingenuity and ethnographic talents of the interview authors to add missing items, because psychiatrists do not have the necessary experience with substance use in the general population. A few suggestions for items that appear to be missing from current criteria: financial problems because of the cost of the substances, physical danger while buying illicit drugs, poor judgment while intoxicated (perhaps waking up in the wrong bed or engaged to be married to the wrong person). The only advice here is to include a few non-criterion items that seem promising. I don't have much hope that anyone will take this advice. If interview designers follow the earlier suggestions, they will have an interview that everyone will agree is too long, and they will be cutting, not adding questions that are "interesting" but not known to be necessary.

What about length? To do all the things I have suggested would require a very long interview. Perhaps it will be too long to be given in a single session. An alternative to multiple sessions is a collaborative nosological project that would divide the interview into a core of demographic and current diagnostic items, plus modules approaching issues such as the various subtyping hypotheses and new items. Each center would use only some of those modules and so could resolve only some of the issues that critics have raised, but among the centers, all modules would be used, and each would be used in more than one center so that replicability could be assessed. Such a project would require a high degree of coordination and ingenuity in designing analyses, but it might have remarkable payoffs in improving the nosology of the future.

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AUTHOR

Lee N. Robins, Ph.D.
Washington University School of Medicine
St. Louis, MO, 63110

The SCID: A Clinical Instrument for Assessing Psychiatric Disorders

Thomas R. Kosten, K. Bryant, and B. J. Rounsaville

The structured clinical interview for DSM-III-R (SCID) was
developed by Spitzer, Williams and Gibbon to be used by a
trained clinician in order to arrive at diagnoses for the major
axis I disorders included in DSM-III-R. In this paper will we
provide a description of the SCID administration followed by
discussions of its use for developing substance dependence
diagnoses and for assessing comorbid psychiatric disorders in
substance abusers. Substance dependence diagnoses in DSM-III-R
are based on the dependence syndrome and the elements of this
syndrome are assessed by eight items in the SCID interview.
Previous work has shown the internal consistency of these items
in arriving at substance dependence diagnoses for various drugs
of abuse. The SCID can also be used to arrive at rates of
comorbid psychiatric disorders in substance dependent patients,
the so-called "dual diagnosis" patients. This paper will
review the reliability of SCID diagnoses for major psychiatric
disorders in "dual diagnosis" patients compared to SCID
diagnoses in non-substance abusers.

SCID ADMINISTRATION

The SCID is administered by a trained clinician who uses
clinical judgment in assessing responses to structured
questions on a three point scale of yes, no and probably.
Initial probe questions may be clarified by the interviewer's
additional focused questioning, thereby preventing
misunderstanding of items or ambiguous patient responses. This
instrument covers most of the major axis I disorders included
in DSM-III-R, although childhood disorders, organic mental
disorders, and axis II personality disorders are not included
in this particular module. In arriving at substance dependence
diagnoses, eight items are used which reflect the DSM-III-R
criteria, and three of these items need to be endorsed in order
to arrive at a substance dependence diagnoses for each drug.
The interview specifically covers each drug that may have been
abused by the patient and attempts to arrive at a diagnosis for
each of eight classes of abused drugs. In addition, a polydrug

abuse category is included for those patients who have used three or more drugs at any one time without a particular primary drug of abuse during that period.

In both the substance abuse section and in the overall SCID administration, there are lead-in sections which are minimally structured and provide background information on the patient. This background information includes demographics, treatment history for psychiatric and medical disorders, and a brief family and psychosocial history. The structured interview itself functions as a very detailed mental status examination specifically keyed to each of the diagnostic categories, with skip outs provided, if responses to probe questions at the beginning of each diagnostic section indicate no symptoms. This enables the interviewer to rapidly progress through this interview instrument, if the patient has few psychiatric symptoms. The interview usually lasts forty-five to ninety minutes for substance abusers, with longer times among "dual diagnosis" patients. Summary sections are provided at various points in the structured interview in order to consolidate diagnostic information and arrive at a clinically judged diagnosis.

In earlier work we had shown that substance abuse diagnoses made using the SCID had good internal consistency for the items used in making a diagnosis. The internal consistency of the dependence syndrome as assessed by the SCID interview was evaluated among 41 inpatients and 42 outpatients at the Yale Substance Abuse Treatment Unit. The SCID items measuring the dependence syndrome formed a single factorial scale for opioids, cocaine and alcohol. While for sedatives, hallucinogens and cannabis the items fell into two or three factor scales, single scales were done using all of the items, excellent internal consistency. Cronbach's alpha ranged from a low of 0.83 for cannabis to a high of 0.98 for opiates, with values above 0.8 indicating excellent internal consistency. By simply adding the 10 items together, severity measures were determined ranging from 0 to 10. Mean scores ranged from a low of 3.3 for sedatives and cannabis to a high of 8.0 for opiates. Alcoholics had a mean score of 5.6, while cocaine abusers had a mean score of 6.9. The items associated with higher scale scores differed across drug types, particularly in opioids, which were atypical compared to the other drugs of abuse. Patients had mostly high scores for opioid and cocaine dependence, mostly low scores for cannabis and sedative dependence, and scores spread fairly evenly across the range for alcoholism, as shown in figure 1.

SCID RELIABILITY

In the present study, we are assessing retest and interrater reliability of the SCID diagnoses. for this assessment, patients were interviewed by two raters, seven to ten days apart, and their diagnoses were compared using the kappa

Percentage of Patients Reporting 1 to 10
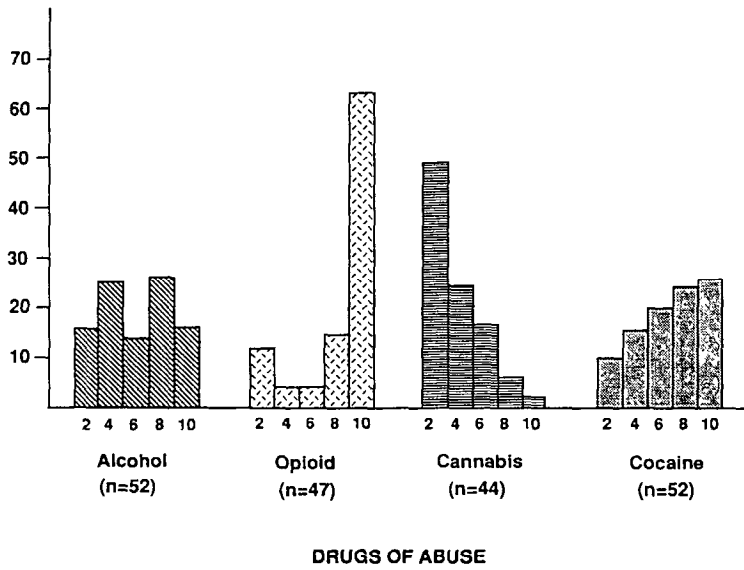## Dependence Syndrome Items by Drug

Figure 1

statistic. The sample included 476 patients drawn from three sites: a general psychiatric setting, both inpatient and outpatient (n = 226), an outpatient drug treatment unit (n = 50), and from the community, including an HMO and a household survey done in New York City (n = 200). Since patients could have multiple lifetime and current diagnoses, the principal diagnoses were used in assessing reliability. The diagnoses were grouped into seven broad categories of disorder: major depression, schizophrenia, bipolar disorder, anxiety disorders, eating disorders, substance abuse, and no psychiatric disorder. For those patients who had multiple lifetime diagnoses, principal diagnoses were assigned hierarchically, ranging from schizophrenia through substance abuse and then no psychiatric disorder. This hierarchical arrangement could be over ridden by the interviewer based on clinical judgment. For example, substance abuse diagnosis could be made the principal diagnosis and an affective disorder made the secondary diagnosis. Reliability was assessed by comparing the principal diagnoses assigned by the first interviewer to the principal diagnoses assigned by the second interviewer.

The 476 patients in this study represented a wide demographic range. The general psychiatric patients had a mean age of 33 years, included 40% males and 87% whites. In this sample 30% had drug dependence and 28% had alcohol dependence. The community sample had a mean age of 33 years, included 35% males and 85% whites. This sample included 23% who had a drug dependence diagnosis and 24% who had an alcohol dependence diagnoses, with 41% of the patients having both drug and alcohol dependence. In the drug abuse sample, the mean age was 30 years, with 61% males and 65% whites. In this sample 100% were drug dependent, and 38% were alcohol dependent. Thus, 100% of the drug abuse sample, 58% of the general psychiatric sample and 47% of the community sample were potential "dual diagnosis" patients. For the three sites, 168 patients were "dual diagnosis" patients and 308 were non-dual diagnosis patients, that is, patients who did not have a substance dependence diagnosis. This "dual diagnosis" was determined by having a lifetime diagnosis of substance dependence in addition to some other psychiatric disorder which might have been either the principal diagnosis or another lifetime diagnosis.

Among the 35% of the patients who met the "dual diagnosis" criteria, rates of non-substance abuse psychiatric disorders were sufficiently high in only 4 of the 7 categories to allow stable estimates of kappa. For anxiety disorders (n = 9) and eating disorders (n = 8) reasonable estimates of kappa could not be made. The most common dual diagnoses were substance abuse and major depression (30%), schizophrenia (18%), and bipolar disorder (14%). Substance abuse was given as the principal diagnosis in 25% of the patients (n = 42), although they all had a secondary psychiatric diagnosis, most often depressive disorder. Among those psychiatric patients without substance abuse diagnoses, depression was the most common

Diagnostic Disagreements - "Dual Diagnosis"
(n = 107)

Rater 2

Rater 1	S	B	D	A
Schizophrenia (S)	(20)	7	3	--
Bipolar(B)	--	(18)	5	--
Depression (D)	1	2	(37)	8
Anxiety (A)	--	--	2	(4)

Figure 2

Diagnostic Disagreements
Psych Alone Patients
(n = 144)

Rater 2

Rater 1	S	B	D	A
Schizophrenia (S)	(33)	--	2	--
Bipolar (B)	1	(32)	3	1
Depression (D)	2	3	(51)	7
Anxiety (A)	--	2	5	(23)

Figure 3

disorder (24%), followed by bipolar disorder (12%), schizophrenia (11%), anxiety disorder (11%) and eating disorders (8%).

Interrater agreement on diagnoses using a test retest design on the principal diagnosis showed kappas ranging from 0.63 to 0.95 among the "dual diagnosis" patients, and ranging from 0.57 to 0.91 for the "psychiatric alone" patients. For depression, kappa estimates were equivalent for dual diagnosis (0.63) and psychiatric alone patients (0.61), but for schizophrenia (kappa = 0.75 vs. 0.91) and for bipolar disorder (kappa = 0.64 vs. 0.85) the dual diagnosis patients demonstrated lower levels of agreement between two raters. On drug dependence itself, the dual diagnosis patients showed excellent interrater agreement with a kappa of 0.95. As shown in the table on the top, diagnostic disagreements among the "dual diagnosis" patients were due to schizophrenia being classified as bipolar disorder by the second rater, bipolar disorder as depression by the second rater, and depression being classified as anxiety disorder by the second rater. As shown in the table on the bottom, diagnostic disagreements among the "psychiatric alone" patients were due to schizophrenia being classified as depression by the second rater, bipolar disorder being classified as depression by the second rater, and depression being classified as anxiety disorder by the second rater. Thus, it was in schizophrenia and bipolar disorder that the diagnostic disagreements contrasted most sharply between the "dual diagnosis" and the "psychiatric alone" patient groups. For schizophrenia, the disagreement on bipolar disorder was 23% in the dual diagnosis patients and 0% in "psychiatric alone" patients. For bipolar disorder, the diagnostic disagreement for depression was 22% in the dual diagnosis patients and only 8% in the "psychiatric alone" patients. Thus, although kappas were not significantly different between these two groups of "dual diagnosis" and "psychiatric alone" patients. the distinction of bipolar disorder from either schizophrenia or major depression disorder may be particularly difficult in substance abusers.

In conclusion, the SCID can be reliably used in substance abusing "dual diagnosis" patients. For the major psychiatric disorders of schizophrenia and bipolar disorder, this reliability may be somewhat lower than in psychiatric patients without substance abuse. Furthermore, the SCID substance abuse items can be added together for each abused drug to form severity measures, thereby providing important information beyond simple presence or absence of drug dependence. Finally, direct comparisons to other research diagnostic interview instruments is needed and future studies using the SCID in substance abusing populations seems well warranted.

Thomas R. Kosten
Kendall Bryant
Bruce Rounsaville

Substance Abuse Treatment Unit
Department of Psychiatry
Yale University School of Medicine
27 Sylvan Avenue
New Haven, Connecticut 06519

The CIDI and CIDI-Substance Abuse Module (SAM): Cross-Cultural Instruments for Assesing DSM-III, DSM-III-R and ICD-10 Criteria

Linda B. Cottler

INTRODUCTION

In all of our research studies involving humans, whether clinical or epidemiological, we need a clear definition of dependence and a way to elicit symptoms of dependence from our subjects.

Although the criteria for dependence have been set by committee and as such are standardized, the techniques used to elicit the criteria are not. Ask a roomful of clinicians or epidemiologists how they elicit the criteria of tolerance from their patients or subjects and you will find a roomful of different operationalizations of the criterion.

Standardized, structured interviews not only allow for consistent coverage of symptom items, which diminishes the possibility for ascertainment bias, but they also offer the possibility to minimize false positives and negatives.

As researchers interested in drug dependence, we are also interested in co-morbid conditions which afflict our patients, in order that appropriate treatment can be assigned. So, we must also be clever about diagnosing other psychiatric disorders as well.

The purpose of this presentation is to familiarize the reader with several recently developed psychiatric diagnostic interviews which standardize the operationalization of criteria.

The essentials of a "perfect" interview are shown in table 1. One may wonder if it isn't impossible to develop such an interview; however, there are several instruments which have many, if not most, of these qualities.

TABLE 1
The Essentials of a "Perfect" Interview

1.   It should provide an accurate operationalization
        of multiple diagnostic systems.
2.   Diagnostic coverage should be broad enough and
        specific enough to capture those who are ill.
3.   It should be highly structured.
4.   It should not allow for individual interpretations
        to be made.
5.   Everyday impairments should not be classified as symptoms.
6.   It should exclude physical origins of illness.
7.   The questions should be easily understood by
        persons from all educational levels.
8.   The language should be nonidiomatic.
9.   The language should be culture-specific.
10. The information obtained should not be dependent
        on record review.
11. The information should be obtainable in one sitting.
12. The interview should be acceptable to everyone.

Although each of the qualities in the table is difficult to achieve, the one which has proven hardest to achieve is cultural specificity, and yet, this is the one which probably gives an instrument its widest appeal. For example, "feeling blue", "going on a binge", "feeling high, tolerant or dependent" are phrases that are not translatable or very difficult to translate into some languages.

WHO/ADAMHA INSTRUMENTS
The instruments which are described in this paper include the CIDI Core, CIDI Full and CIDI SAM. The development of CIDI has relied heavily upon the NIMH Diagnostic Interview Schedule (Robins et al, 1981) the structured standardized psychiatric instrument developed for use in the multisite NIMH Epidemiological Catchment Area (ECA) study. The current version, DIS-III-R, is different from the original version in that it assesses DSM-III-R criteria, in addition to RDC, Feighner, and DSM-III.

The Composite International Diagnostic Interview (CIDI) (Robins et al, 1988) is a fully standardized diagnostic interview which elicits information necessary to assess psychiatric disorders. The collaborative efforts to develop this instrument have been made possible by the World Health Association (WHO) and the US Alcohol, Drug Abuse and Mental Health Administration (ADAMHA) Joint Project on Diagnosis and Classification of Mental Disorders and Alcohol and Drug Related Problems.

The CIDI is a family of epidemiologic instruments that allows non-clinician interviewers to ask questions in a highly standardized fashion to elicit answers which can be combined by computer to produce diagnoses according to

221

published criteria of multiple diagnostic systems. The CIDI Core Version 1.0, the official title, covers two systems-- the prevailing American Psychiatric Association DSM-III-R and the 10th Revision to the International Classification of Diseases (ICD-10)-- for a subset of diagnoses. Specifically, the CIDI Core covers over 45 DSM-III-R disorders.

The substance abuse and dependence sections of the CIDI Core cover DSM-III-R and ICD-10 criteria for the abuse of and dependence on tobacco, alcohol, cannabis, barbiturates and tranquilizers, amphetamines, cocaine, heroin and other opiates, PCP and other hallucinogens and inhalants. A lengthier version, the CIDI Full, exists which covers more diagnoses and diagnostic systems than either the DIS or the CIDI Core. It also elicits onset and recency of each positive symptom.

An even lengthier module was developed to allow for greater diagnostic coverage and broader assessment of the natural history of substance use disorders than is available with the CIDI. The SAM (Substance Abuse Module) is a structured interview which assesses the abuse of and dependence on the substances covered in the DIS and CIDI, yet includes questions to elicit symptom-specific onset and recency, adds specific withdrawal symptoms and physical, social and psychological consequences from each drug, and elicits quantity and frequency information to assess the severity of the dependence syndrome. The SAM can be used as a stand alone instrument or in conjunction with the CIDI. In addition, a treatment module is available for the SAM which covers lifetime treatment utilization patterns.

The reliability of the CIDI and SAM has been established and reported previously (Wittchen et al in press), (Cottler et al, 1989). These results have been found to be good to excellent. In fact, the tests for diagnostic reliability of the CIDI have been conducted in 19 countries around the world. The DIS and CIDI have been translated into at least 40 different languages and dialects which makes these instruments particularly suited for cross-cultural research.

SPECIFIC CHARACTERISTICS
The characteristics of the DIS-III-R, CIDI-Core, CIDI-Full and SAM are shown in figure 1. As indicated, the CIDI-Full is the instrument with the most complete coverage of systems and individual symptoms (i.e., onset and recency information). However, its length will prohibit investigators from administering the entire CIDI-Full, so a modular approach can be taken where only certain sections are selected. These sections can then be covered in depth.

FIGURE 1

Diagnostic Coverage
of WHO/ADAMHA/NIMH Psychiatric Instruments

	DIS-III-R	CIDI-Core	CIDI-Full	SAM
DSM-III	✓		✓	✓
DSM-III-R	✓	✓	✓	✓
ICD-10		✓	✓	✓
PSE			✓	
RDC	✓		✓	
Feighner	✓		✓	
Ind. ONS/REC	✓		✓	✓

The structure of these instruments disallows early diagnostic screening and skip outs and requires every symptom to be asked unless it would be illogical for a symptom to occur, such as a symptom related to panic attack when no panic situation was reported. This structure makes analyses available on a symptom by symptom basis as well as on the diagnostic level.

Examples of DIS and CIDI questions are presented in figure 2.

FIGURE 2

The labels in the left hand margin are unique to these instruments and are informative because they show which criteria the questions are used to assess. For example, the label next to question 17 shows that "a lot of trouble with

223

ASK QUESTION

NO → CODE PRB 1
YES

[1   5] → CODE PRB 5
[1 3 5] → GO TO BOX C

Did you tell a doctor* about (SX)?

YES
NO

(1 2   5) → CODE PRB 5, ASK ONS/REC A

A (NO "2") ———— GO TO BOX B

Did you tell any other professional** about (SX)?
Did you take medication more than once for (SX)?
Did (SX) interfere with your life or activities a lot?

ALL NO → CODE PRB 2
FIRST YES

B (NO "4") ———— GO TO BOX C

Was (SX) ever the result of a physical illness or injury?

YES ——— GO TO ▲ IN BOX D
NO

C (NO "3") ———— CODE PRB 5, ASK ONS/REC A

Was (SX) ever the result of taking medication, drugs or alcohol?

NO → CODE PRB 5, ASK ONS/REC A
YES

Was (SX) always the result of taking medication, drugs or alcohol?

NO → CODE PRB 5, ASK ONS/REC B
YES → [What caused (SX)?]
        RECORD MED/DRUG/ALC
        AND CODE PRB 3

D

When you told the doctor, what was the diagnosis? [What did he say was causing (SX)?]

● NERVES
STRESS
ANXIETY
DEPRESSION
MENTAL ILLNESS

● MEDICATION
DRUGS
ALCOHOL

(NO "3") → CODE
PRB 5, ASK
ONS/REC A

● PHYSICAL
ILLNESS/
INJURY

● NO DEFINITE DIAGNOSIS

Did he find anything abnormal when he examined you or took tests or x-rays?

NO, NO EXAM, DK → RECORD DX
AND CODE
PRB 5, ASK
ONS/REC A

RECORD DX AND
CODE PRB 5, ASK
ONS/REC A

YES

Was (SX) always the result of taking medication, drugs or alcohol?

Was (SX) always the result of a physical illness or injury (such as ———)?

YES → RECORD ILL/INJ AND CODE PRB 4
NO
(NO "3") → CODE PRB 5, ASK ONS/REC C

YES → RECORD MED/DRUG/
        ALC AND CODE PRB 3
NO

When (SX) was not due to taking medication, drugs or alcohol, was it always the result of a physical illness or injury?

When (SX) was not due to a physical illness or injury, was it always the result of taking medication, drugs or alcohol?

NO → CODE PRB 5, ASK
        ONS/REC D
YES → [What caused (SX)?]
        RECORD MED/DRUG/
        ALC AND ILL/INJ
        AND CODE PRB 4

NO → CODE PRB 5, ASK ONS/REC D
YES → [What caused (SX)?] RECORD MED/
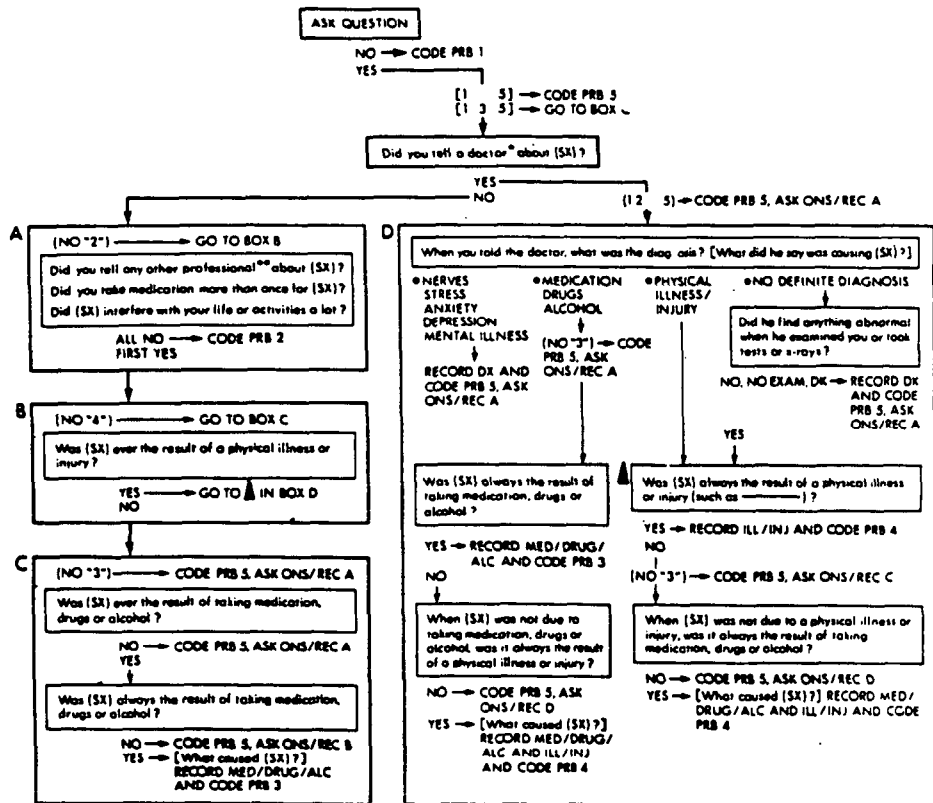   DRUG/ALC AND ILL/INJ AND CODE
        PRB 4

FIGURE 3

Somatization disorder criterion B8, ICD 10 Somatization disorder, Persistent pain disorder in (ICD-10) and DSM-III-R Somatic Pain disorder.

A strong feature of the DIS and CIDI instruments is the unique infrastructure which determines whether a symptom is clinically relevant and of psychiatric origin. This infrastructure, known as the Probe Flow Chart (Figure 3) assesses whether the symptom was severe enough for the respondent to tell a physician, tell any other professional, take medications, or interfere with his life or activities a lot. If a doctor was told about the symptom, the cause is elicited and recorded on the MD line. The Probe Flow Chart also determines the proper questioning to elicit etiology of symptoms not discussed with a physician.

When a symptom is determined to be possibly psychiatric in origin (CODE 5), the onset and recency is elicited. The onset and recency questions and their codes are shown in figure 4.

A severity probe which corresponds to PSE items is included in the CIDI-Full to cover those symptoms which have occurred in the previous month. The severity question is also shown in figure 4.

The questions on drug abuse and dependence are as structured as the questions for ascertaining other psychiatric symptoms. Problems which may have arisen as a consequence of drug use are elicited for drugs used 6 or more times. (on a lifetime basis)

FIGURE 4
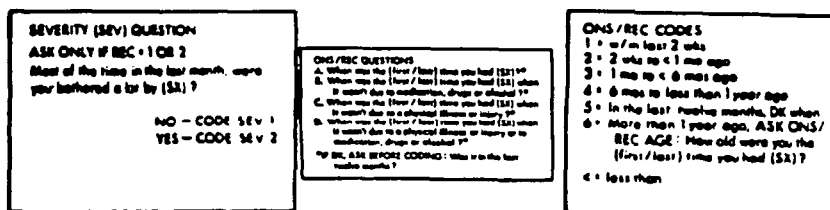
CONCLUSION

The questions in these interviews have developed as the criteria have developed; therefore, many have undergone extensive revision. These instruments have been shown to be reliable, and the CIDI has been shown to be acceptable among persons from many different cultures. Used together, they can provide a useful mechanism for obtaining valuable data for the study of comorbidity of psychiatric illnesses.

However, the interview is only one part of epidemiological research. Another major task is the development of the algorithms used to score the diagnoses, and the checking and rechecking of these programs. For each of these instruments described, computer input and training materials are available.

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AUTHOR
Linda B. Cottler, Ph.D.
Assistant Professor of Epidemiology in Psychiatry
Washington University School of Medicine
Department of Psychiatry
4940 Audubon
St. Louis, MO 63110

Categorizing Substance Abusers Using the ASI: Implications for Evaluation and Treatment

A. Thomas McLellan, Mary Randall, Natalie Joseph, and Arthur I. Alterman

INTRODUCTION - Although the current patterns of drug use and abuse have been described, including the background and demographic characteristics of substance abuse patients (1-3), there still has been little information regarding the pattern of treatment-relevant problems seen among these patients; either compared among various "drug pattern" subgroups within the present population, or compared with populations from earlier years. This is potentially important information in that a great deal of prior work has indicated that the severity of treatment problems such as psychiatric illness, unemployment and poor family relations have been more predictive of outcome from substance abuse rehabilitation than simple demographic or drug pattern variables (4-6).

At the Substance Abuse Treatment and Research Center of the Philadelphia VA hospital we have been collecting admission information on the treatment problems of substance abuse patients since 1980, using the Addiction Severity Index (ASI) (7). This structured interview provides reliable and valid information on the nature and severity of treatment problems in seven areas of functioning commonly affected in substance abusers: medical condition, employment, alcohol use, drug use, legal status, family and social relations and psychiatric condition (8&9). Thus we had access to data that were pertinent to treatment issues associated with the observed changes in the substance abuse patient population.

It could be that the treatment problems among our current admission cohort are more severe across all drug-pattern subgroups than the problems of patients admitted prior to the current cocaine epidemic. A second possibility is that while the number and types of drugs used have changed, the treatment problems may have remained similar to those of past cohorts. Still a third possibility is that the treatment problems of the "newer" subgroups of substance abusers (eg. cocaine and multiple substance abusers) are more serious, or at least different, than those of the "traditional" subgroups (eg. alcohol and opiate abusers) and that the changes observed in the total population of substance abuse patients have been due to the emergence of these "newer" subgroups of substance abusers. These possibilities are of course, not exhaustive but they do suggest significant considerations related to evaluating and treating the current population of substance abusers.

Part I of this paper establishes criteria for categorizing patients into single (alcohol, opiate and cocaine) and multiple substance use groups and then compares the prevalence of these groups in each of the admission cohorts. Part II of the paper compares the admission problems of subgroups of 1987 patients with corresponding groups of 1980 patients. Part III examines the admission problems

of four drug-pattern subgroups from the 1987 population. The results are discussed in terms of evaluation and treatment issues associated with rehabilitation of the current group of substance abuse patients.

PART I - Categorization of Substance Abuse Admissions by Drug-Pattern and Prevalence

Admission Interview Procedure - Since 1980, admission to all treatment programs at the Center has been nerformed bv the Central Intake Unit. At admission each patient receives a complete medical examination (including laboratory testing and physical), the Beck Depression Inventory and a personal interview using the Addiction Severity Index (ASI). The ASI has received extensive testing and has been shown to be reliable and valid in a wide range of patient populations. **Please** Note: All ASI's used in the present study were performed by trained clinical evaluators (psychology technicians) having years of clinical research experience with the instrument. The patient is then referred to the most appropriate treatment program in the Center. The total time required is approximately two hours. These admission data serve a clinical need as the basis for each patient's initial treatment plan; and a program evaluation need as a "baseline" status for subsequent comparison with a repeated ASI evaluation at post-treatment follow up.

Subjects. Subjects were male veterans admitted to the Treatment Center during the calendar years 1980 and 1987. They were divided into groups based upon their major drug of choice, their frequency of use of that drug and their histories of other substance abuse, as discussed below.

Rationale for Grouping Decisions - It should be recognized at the start that many drug-pattern groupings are possible given the range of substances available and the various definitions of a "drug problem." In dividing the population of substance abuse patients into groups for this study we wanted to employ criteria that would be comparable to current DSM Ill or DSM III-R diagnostic criteria and would provide comparable groups from both the 1980 and the 1987 cohorts. A "drug problem" in this study was defined as the regular use of a substance for a period of at least five years (three for cocaine - see below). "Regular use" in turn, was defined as use of the substance at a frequency of at least three times per week and at an intoxicating dosage sufficient to prevent or seriously hinder adequate performance of normal duties. Our experience has indicated that very few users are able to avoid negative, "problematic" consequences when a substance is used at intoxicating doses three times per week or more. We purposely required this significant frequency and duration of drug use, as well as a failure to maintain abstinence (indicated by at least one previous treatment attempt) to define a substance use pattern as a "problem."

We are aware that most patients have used several drugs by the time of treatment admission and that most patients have had additional, irregular use of non-preferred substances when the regular or preferred agent is unavailable. However, we were not interested in these irregular use patterns but instead chose to base our patient categorization scheme on those substances which were used regularly and in a "problematic" fashion. Finally, we wanted to insure that drug "problems" as defined in this study would meet current diagnostic standards as a problem. Thus, while it should be clear that a diagnostic interview was not a formal part of the standard admission, we feel certain that the use of these criteria insure that all study patients in these groups would meet minimum criteria for a Substance Dependence diagnosis using the DSM III or DSM III-R criteria.

Marijuana use was not considered in the inclusion/exclusion criteria for essentially pragmatic reasons. No patient in either cohort reported marijuana as a treatment problem but a majority of subjects form all groups reported frequent use of

marijuana. Excluding users of this drug would have eliminated the majority of subjects. Similarly, the Cocaine subjects and the subjects from the multiple substance group who reported cocaine use, were required to have only <u>three years of regular use</u> as compared with all other groups who required five years of regular use. This decision was based on the finding that no patient admitted during 1987 reported more than four years regular use of cocaine, since there was comparatively little cocaine available in the Philadelphia area and virtually no freebase or "crack" cocaine available prior to 1983. Given the recent nature of this cocaine phenomenon we felt it would be non-representative to require five years of regular use for patients in this group. However, we were concerned that comparisons among the drug groups might be influenced not only by the drug choice but also by the <u>severity</u> of the drug problem. For this reason we have required <u>both</u> a minimum of three years regular (three times per week or more) use <u>and</u> at least one prior treatment attempt as inclusion criteria. We do not claim that this is the best way to categorize these patients, only that it satisfies the major points of interest to us and that the groupings are at least clinically meaningful.

PART I RESULTS - Using the criteria outlined, four drug-pattern subgroups of patients were extracted from the two admission cohorts. The descriptions and prevalence of these subgroups are provided in the text below.

<u>Alcohol</u> - Subjects in this group reported a minimum of five years of regular (three times per week or more) alcohol use, a history of alcohol tolerance/withdrawal, at least one prior treatment attempt, but less than one year of regular use of any other drug except marijuana. These alcohol-only patients made up 34% of all 1980 admissions; but only 17% of 1987 admissions to this Center. The difference between these two proportions was statistically significant (p<.04).

<u>Opiate</u> - Subjects in the opiate group reported a minimum of five years of regular (three times per week or more) opiate use, a history of opiate withdrawal and/or tolerance, at least one prior treatment attempt but less than one year of regular use of any other drug except marijuana and less than one year of problematic alcohol use (intoxication three times per week or more). These Opiate patients comprised 40% of all 1980 admissions; but only 6% of 1987 admissions to this Center. The difference between these two proportions was statistically significant (p<.01).

<u>Cocaine</u> - Subjects in this group reported a minimum of three years of regular (three times per week or more) cocaine use, at least one prior treatment attempt but less than one year of regular use of any other drug (except marijuana) or alcohol. Using this definition of a cocaine drug problem, no patient admitted during 1980 could be included in this group. However, these patients comprised 26% of 1987 admissions to this Center. The difference between these two proportions was statistically significant (p<.001).

<u>Multiple Substance</u> - Subjects in the Multiple Substance group reported regular use (three times per week or more) of two or more substances (including alcohol but excluding marijuana) of at least five years duration (three years for cocaine) and had at least one prior treatment. These Multiple Substance patients comprised 11% of all 1980 admissions; but 39% of 1987 admissions to this Center. The difference between these two proportions was statistically significant (p<.03). The 1980 Multiple Substance group could be subdivided into 36% who reported joint use of opiates (usually heroin) and alcohol; 31% who reported combined use of opiates and benzodiazepines (usually diazepam -Valium®); 26% who reported combined use of opiates and amphetamine; and the remaining 7% of these multiple use patients who reported regular use of three or more substances sequentially and in combination. The 1987 Multiple Substance group could be subdivided into 41%

who reported joint use of cocaine and alcohol; 26% who reported combined use of cocaine and opiates (usually heroin); 16% who reported combined use of cocaine, alcohol and opiates; 10% who reported use of benzodiazepines plus opiates; and the remaining 7% of these multiple use patients who reported regular use of three or more different types of drugs sequentially and in combination.

PART II - A BETWEEN-YEARS COMPARISON OF SINGLE SUBSTANCE ABUSERS 1980 - 1987

Given the changes in the prevalence of the various drug-pattern subgroups from 1980 to 1987, it became important to address the question of whether these recent admissions had more severe problems than those from 1980, prior to the recent cocaine epidemic. Since none of the 1980 admissions reported regular use of cocaine, it was not possible to compare the 1987 cocaine abusers with a comparable group from 1980. Similarly, since the majority of the multiple substance group from 1987 reported combinations of substances that included cocaine, it was not possible to find a truly comparable multiple substance group from the 1980 cohort. For this reason, Part II of this paper focuses only on the alcohol and opiate groups admitted to this Center during 1980 and 1987.

Subjects - were selected from the Alcohol and Opiate subgroups of the 1980 and 1987 admission cohorts (See Part I). One hundred and three subjects were selected from the 1980 Alcohol group while 100 consecutive admissions to the 1987 Alcohol group were included for this comparison. Similarly, 139 Opiate subjects were selected from the 1980 cohort while 50 consecutive Opiate admissions were selected from the 1987 cohort. The low proportion of 1987 admissions in this group reduced the number of available subjects. As described above (See Part I) all subjects were male veterans and all were interviewed using the ASI by independent members of the Central Intake Unit of the Center.

PART II RESULTS - Sociodemographic Comparisons - There were significant changes in the demographic characteristics of the alcohol admissions to this Center over the seven year period. There were significant reductions in the average age (from 46 to 40, p<.02), in the proportion of white patients (from 35% to 24%, p<.03) and in the proportion of married patients (from 37% to 21%, p<.05). While there were no significant changes in the educational backgrounds of these groups, there was a lower proportion of full-time employed and a higher proportion of unemployed patients in the alcohol admissions from 1987 than from 1980. Finally, there was a significant increase in the average number of prior treatments for psychiatric illness in the 1987 alcohol sample (from 1 to 3, p<.02). A very different picture of changes was seen among the opiate groups with significant increases in the average age of these patients (from 31 to 39, p<.05) and in the proportion of white patients (from 36% to 54%, p<.05). There was an increase in the years of education reported by the 1987 patients and fewer of these admissions were unemployed (from 40% to 28%, p<.02) but there was no significant change in the marital status patterns reported. Finally, there were significant increases in the average number of prior treatments for both drug dependence and psychiatric illness among the 1987 sample.

Treatment Problem Status at Admission - Data from the ASI on the nature and severity of treatment problems were analyzed for the 1980 and 1987 cohorts. In the case of the Alcohol groups only 7 of 23 between-year comparisons were significantly different at the .05 probability level and of these, 5 indicated better admission status for the 1987 group while 2 indicated worse status for this group. Similarly, only 9 of 23 between-year comparisons of the opiate groups were significantly different using the .05 criterion and of these, 5 indicated worse status for the 1987 group while 4 indicated better status. These few significant

differences across all the ASI variables showed no consistent trend for better or worse status in either admission year or for either substance abuse group.

PART III - ADMISSION STATUS OF ALCOHOL, OPIATE, COCAINE AND MULTIPLE SUBSTANCE ABUSERS TREATED DURING 1987.

The results from Part II suggested very few significant differences in the treatment problems of Alcohol or Opiate patients admitted to this Center during the years 1980 and 1987. It therefore became all the more important to examine the 1987 cohort of admissions to contrast the nature and severity of treatment problems presented by the cocaine and multiple substance groups with those from the alcohol and opiate groups. We felt this type of comparison could be potentially important for the development of evaluation and treatment strategies for the current population of substance abuse patients at the Center.

Subjects - were chosen from the 1987 cohort of male veteran patients admitted to the Center. The subjects were divided into the four drug-pattern groups based on the criteria described in Part I.

PART III - RESULTS Sociodemographic Comparisons. There were significant demographic differences among the four groups on virtually all variables examined. The cocaine group was significantly younger (average= 31±6) than all other groups which did not differ significantly from each other. With regard to racial distribution there were again differences among the groups. Perhaps surprisingly, the cocaine group was almost entirely black while the opiate group was much more evenly distributed between blacks and whites.

The alcohol group had significantly fewer years of formal education than all other groups (p<.05), were less likely to have been employed prior to treatment (p<.01) and were more likely to have been widowed (p<.01) than all other groups. The cocaine group was more likely to be employed full time (p<.01) and (with the multiple substance group) more likely to be married (p<.05) than the other groups. The opiate group was not significantly different from all other groups on any of the demographic characteristics (p>.10). The multiple drug use group was significantly more likely to have graduated from college (p<.05) and less likely to be single (never married) (p<.05) than all other groups.

Problem Status at Admission - We performed one-way analyses of variance for each of the ASI measures shown in Table 2. As can be seen, with the exception of the psychiatric problem area the results of the analyses for almost all of the specific comparisons were highly significant (p<.05 or less). All significant main effects from the ANOVAs were followed by post-hoc comparisons using Bonferroni-corrected t-tests (two-tailed probabilities). The results of the comparisons are discussed below by problem area.

Medical Problems - There were significant (p<.02) group differences in the severity of the medical problems reported by the four groups at treatment admission with the alcohol and opiate groups reporting similar levels of problems and similar durations (approximately 10 days each). The cocaine and multiple drug groups reported significantly fewer medical problems from the other two groups but did not differ from each other.

Employment/Support Problems - Again, the alcohol and opiate groups reported the highest (and approximate equal) levels of problems with the fewest days employed, the lowest wages earned and the greatest amounts of welfare income in the thirty days prior to admission. In addition, the cocaine and the multiple drug groups were

similar, showing lower levels of problem severity with more days employed, higher wages and lower levels of welfare income than the alcohol and opiate groups.

Alcohol - Not surprisingly the alcohol group had the highest levels of alcohol use among all groups as measured by both the number of days where any drinking occurred as well as the number of days where the drinking led to intoxication. However, the cocaine and the mixed drug groups also had high scores; significantly less than the alcohol-only group but significantly higher than the opiate only group.

Drug Use - The opiate group had significantly higher scores (p<.03) on the drug composite. measure than the cocaine and multiple substance groups (which did not differ from each other). All three of these groups differed significantly (p<.05) from the alcohol group. With regard to the use of specific drugs during the month prior to treatment admission, all groups reported approximately equal rates of marijuana use (average 12 days per month). The opiate group naturally reported a higher frequency (p<.01) of opiate use than all other groups, all of which reported very low frequencies of use and were not significantly different from each other.

Legal Status - The opiate group had significantly more legal problems than all other groups. In the case of the composite score and the crime days (days committing a crime for profit in the past 30 days) measures, the alcohol, cocaine and multiple substance groups were not significantly different from each other (p>.10). The opiate group also reported substantially higher amounts of illegal income generated during the month prior to admission ($808.00) than all others. The cocaine and multiple drug use groups reported approximately equal and intermediate levels of illegal income while the alcohol group reported significantly lower illegal income than all others.

Family and Social Relations - The opiate and the alcohol groups were significantly different from the two other groups (p<.05) on the ASI family/social relations measure. However, it was surprising to note that, in this case, the opiate and alcohol groups reported lower problem levels than the other two groups. The ASI family composite score is basically a measure of conflicts among family, friends and associates during the past 30 days and in this regard, the cocaine-only and the multiple substance groups reported significantly (p<.05) more days of conflict with family members than the other two groups (which did not differ from each other). We speculate that the alcohol and opiate groups may have estranged their families and social contacts to the point where interaction was negligible and then appeared to have low problem levels in this area for artificial reasons.

Psychiatric Status - There were no group differences on any of the psychiatric status measures including the composite score, the days of problems reported or the Beck Depression Inventory scores (p>.10) It should be noted that the overall frequency of problems reported and the admission Beck scores arc indicative of clinically significant levels of psychiatric distress among all groups.

DISCUSSION - In an attempt to study the changes in the prevalence of drug use patterns and in the treatment problems of male veterans admitted to the Philadelphia VA Substance Abuse Treatment Research Center, we evaluated patients with the same drug patterns admitted during different years; and patients with different drug patterns admitted to treatment during the same year. In the between-years comparison we examined alcohol and opiate patients admitted during 1980 and 1987. In the between groups comparison we examined three types of single substance abusers (alcohol, opiate and cocaine) as well as a group of multiple substance abusers entering rehabilitation during 1987. The use of a standard

evaluation instrument (ASI) also enabled us to assess the treatment problems of these four groups.

The comparison of drug-pattern prevalence in the two admission cohorts revealed two notable findings. Perhaps the major finding was that cocaine, alone or in combination with other drugs, was the major drug of abuse among these male veteran patients admitted to this Center in 1987. Eighty-eight percent of the 1987 admissions reported cocaine use alone or in combination, as contrasted with virtually no mention of the drug by this same population in 1980. While this has not been an unnoticed change here or elsewhere (See 2&3) it is remarkable that in this relatively short period of time, cocaine abuse has grown to the point where it rivals alcohol abuse in terms of prevalence. This is even more striking in that until only recently cocaine was considered a "white collar" or "high society" drug. Yet it has become a major problem in this predominantly black, lower socioeconomic group of male veterans. In fact, cocaine plus opiates has become the modal drug problem even among our methadone maintenance patients, the majority of whom had been classified as opiate-only prior to the recent cocaine epidemic.

A second, related finding was that these drug problems (even cocaine) rarely occur alone. The modal patterns of drug use among these male veterans during 1987 were cocaine and alcohol, or cocaine, opiates and alcohol. In fact 5 1% of the 1987 admissions were classified as multiple substance abusers (usually alcohol plus cocaine or cocaine plus opiates) while only 26% of patients from the 1980 cohort were similarly classified (usually opiates plus alcohol). Of particular interest was that the recent (past 30 days) alcohol problems of the "cocaine" group were not significantly different from the recent alcohol use in the "alcohol" group. Similarly, the number of days of depressant use (barbiturates or benzodiazepines) was equal across all groups, further suggesting the prevalence of multiple drug use.

The between-years comparison of treatment admissions to this Center evaluated the demographic and associated treatment problems of alcohol and opiate patients admitted to this Center during the years 1980 and 1987. Despite the changes in the demographic characteristics of these drug-pattern groups over time, there were no overall statistically significant or clinically remarkable changes in the severity of the treatment problems in either of these single substance abuse groups compared between years. Of particular interest in this regard is the work of Hendricks (9) in Holland who used a Dutch translation of the ASI to examine groups of opiate addicts entering an abstinence oriented treatment setting in that country. The patterns and severity of problems in that sample were essentially identical to those reported by various groups working with opiate addicts in this country (See 8), again suggesting the similarity of treatment problems among opiate abuse patients.

The between-groups comparison of the treatment problems presented by all four drug-pattern groups (alcohol, opiates, cocaine, or multiple substance) indicated, perhaps surprisingly, that the alcohol and particularly the opiate groups, had generally more problems and at greater severity than either the cocaine or the multiple substance groups. Clearly the most severely affected patients were the opiate group with the worst levels of problems in medical status, employment, drug use and crime. The alcohol group was next in severity with very serious medical and employment problems. However, the admission data indicated severe but generally similar levels of psychiatric symptoms (particularly depression) across all groups.

CONCLUSION - In conclusion, we have found again as we did in 1979 (See 1) that the nature of the patient population is changing. The substance abuse patterns of our current patients are becoming more complex and the former distinctions

among drug and alcohol abusers or between opiate and cocaine abusers are blurring. While there is as yet no indication that the treatment problems of the current population of substance abusers are more severe, or even different than the problems of former populations, it remains for clinicians and clinical researchers to develop and deliver appropriate patient evaluation strategies and treatment interventions to address the full range of addiction related problems in this population.

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Affiliation: The Penn/VA Center for Study of Addiction - Department of Psychiatry, Philadelphia VA Medical Center and University of Pennsylvania

Reprint Requests to Dr. McLellan
Bld #7 Phila VA Medical Center
University Avenue, Phila. PA 19104

TABLE 1 - ADMISSION STATUS COMPARISONS: 1980 AND 1967

	1980 ALCOHOL		1987 ALCOHOL	1980 OPIATE		1987 OPIATE
NUMBER OF PATIENTS	103	P<	100	139	P<	50
	mean (s.d.)		mean (s.d.)	mean (s.d.)		mean (s.d.)
MEDICAL COMPOSITE (#)	.403 (.314)		.373 (.357)	.234 (.213)	*	.364(.248)
days of med. probs.	11 (6)		10 (12)	7 (4)		10 (3)
EMPLOYMENT COMPOSITE	.804 (.313)	*	.702 (.257)	.681 (.313)		.733 (.236)
days worked	7 (2)		8 (9)	9 (4)		6 (2)
money earned($)	186 (223)		293 (140)	327 (113)		328 (261)
welfare income($)	52 (77)		36 (88)	68 (57)		50 (37)
ALCOHOL USE COMPOSITE	.621 (.210)	*	.540 (.239)	.206 (.136)	*	.149 (.129)
days drinking	18 (6)		14 (9)	9 (3)	*	6 (2)
days intoxicated	18 (4)	*	12 (9)	3 (2)		3 (3)
DRUG USE COMPOSITE	.021 (.018)		.034 (.061)	.315 (.177)		.368 (.212)
days opiate use	0 (0)		0 (0)	28 (4)		24 (10)
days cocaine use	0 (0)	*	<1 (1)	1 (3)	* *	6 (3)
days depressant use	2 (3)		0 (0)	7 (3)		6 (2)
days marijuana use	6 (3)	*	3 (3)	6 (2)		6 (3)
LEGAL COMPOSITE	.027 (.031)		.042 (.127)	.185 (.211)		.158 (.129)
crime days	0 (1)		1 (4)	12 (8)		10 (6)
illegal income($)	0 (0)		3 (18)	630 (313)		808 (856)
FAMILY/SOCIAL COMPOSITE	.237 (.214)		.215(.217)	.248 (.212)		.232(.243)
conflicts w/family	3 (3)		2 (1)	4 (4)		2 (1)
conflicts w/ others	5 (3)	*	1 (1)	5 (3)	*	2 (1)
PSYCHIATRIC COMPOSITE	.221 (.177)		.245(.227)	.246 (.183)		.241 (.212)
days psych. probs.	9 (3)		11 (2)	10 (4)		10 (3)
Beck Depression Inv.	14 (3)	*	11 (2)	18 (13)		15 (6)

#All measures reflect the 30 days prior to admission. Factor scores range from 0 to 1.0; and higher scores mean greater problem severity. *=p<.05; **=p<.01 by two tailed t-tests. HOWEVER, MANOVAs between years showed NO SIGNIFICANT OVERALL GROUP DIFFERENCES.

TABLE 2 - ADMISSION ON STATUS OF THE FOUR DRUG PATTERN GROUPS

	ALCOHOL ONLY	OPIATE ONLY	COCAINE ONLY	MULTIPLE DRUGS	BETWEEN GROUPS ANOVA
NUMBER OF PATIENTS	119	50	106	100	
	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	
MEDICAL COMPOSITE (#)	.373 (.357)	.364(.248)	.265 (.185)	.226 (.188)	*
days of med. probs.	10 (12)	10 (3)	7 (3)	5 (5)	*
EMPLOYMENT COMPOSITE	.702 (.257)	.733 (.236)	.560 (.410)	.608 (.311)	* *
days worked	8 (9)	6 (2)	13 (5)	11 (4)	*
money earned($)	293 (140)	328 (261)	715 (401)	605 (314)	*
welfare income($)	36 (88)	50 (37)	4 (8)	11 (5)	*
ALCOHOL USE COMPOSITE	.540 (.239)	.149 (.129)	.264(.237)	.289 (.169)	*
days drinking	14 (9)	6 (2)	8 (3)	9 (2)	*
days intoxicated	12 (9)	3 (3)	4 (2)	5 (3)	*
DRUG USE COMPOSITE	.034 (.061)	.368 (.212)	.223 (.200)	.284 (.211)	* *
days opiate use	0 (0)	24 (10)	1 (4)	4 (2)	* *
days cocaine use	<1 (1)	6 (3)	10 (4)	8 (3)	* *
days depressant use	0 (0)	6 (2)	2 (1)	3 (1)	*
days marijuana use	3 (3)	6 (3)	3 (2)	6 (2)	NS
LEGAL COMPOSITE	.042 (.127)	.158 (.129)	.071 (.033)	.046 (.069)	*
crime days	1 (4)	10 (6)	2 (1)	1 (1)	* *
illegal income($)	3 (18)	808 (856)	58 (30)	47 (31)	* *
FAMILY/SOCIAL COMPOSITE	.215(.217)	.232(.243)	.473 (.296)	.502 (.329)	*
conflicts w/family	2 (1)	2 (1)	3 (2)	5 (3)	*
conflicts w/ others	1 (1)	2 (1)	3 (1)	2 (2)	NS
PSYCHIATRIC COMPOSITE	.245(.227)	.241 (.212)	.214 (.191)	.243 (.217)	NS
days psych. probs.	11 (2)	10 (3)	8 (3)	11 (4)	NS
Beck Depression Inv.	11 (2)	15 (6)	14 (6)	12 (6)	NS

All measures reflect the 30 days prior to admission. Factor scores range from 0 (less) to 1.0 (more) seve
* = p<.05; ** = p<.01 by ANOVA (See text for individual comparisons)

235

Diagnosing Personality Disorders in Substance Abusers

Helen   Pettinati

The focus of this paper is twofold. First, I will briefly
delineate some of the problems that confront the addiction
field in our efforts to diagnose and make sense of person-
ality disorders in substance dependent populations. Second,
I will provide some preliminary data on personality disor-
ders from a large treatment outcome project ongoing at
Carrier Foundation.

PROBLEMS IN DIAGNOSING PERSONALITY DISORDER

The presence of a personality disorder during treatment for
substance dependence may influence the management of the
patient and his/her compliance with treatment regimens. For
some personality disorders, treatment interventions are
being proposed (Liebowitz et al., 1988), and finally,
prognosis may be affected by the presence of a personality
disorder.

Five major issues in evaluating personality disorder will be
addressed: comorbidity with Axis-I disorders; comorbidity
with other Axis-II disorders; choosing the instrument to be
used; choosing the diagnostic system to be used; and ensur-
ing the validity of the patient's self-report about his/her
personality with regard to deception, poor memory, or the
inability to discriminate behaviors which are independent of
alcohol or drug use.

Issue #1. Comorbidity with Axis-I Disorders. We have been
taught that personality traits are pervasive, enduring, and
not subject to major changes. In this way, Axis-II psycho-
pathology is meant to be distinct from Axis-I disorders.
However, the interface between Axis-I and Axis-II psycho-
pathology is unclear when successful treatment for the
Axis-I condition eliminates Axis-II psychopathology. Nace
(1990, p. 65) has suggested that a change in personality
traits can be demonstrated in alcohol dependent patients
following treatment of a substance use disorder because such
treatment programs exert a "favorable impact on co-occurring
character pathology." Reports such as these, however, raise

the question of whether diagnosed character pathology is actually symptom expression or, perhaps, represents bad habits which have been acquired as part of the Axis-I condition that will get extinguished over time with recovery. Schuckit (1989) reports that careful history taking, e.g., sequencing of ASPD with regard to substance abuse behaviors, can minimize this problem. Blume (1989) urges the interviewer to continually remind subjects that their descriptions of behaviors should be independent of substance use. Typically, however, clear timelines and unbiased reports are often not possible due to the subject's poor memory or long-term substance use which began at an early age.

Issue #2: Comorbidity with Other Axis-II Disorders. Much discussion has focused on the merits of a categorical vs. dimensional approach to evaluating personality-(e.g., Frances and Widiger 1986). For example, traits, like impulsiveness, can intersect with several categorically-determined personality disorders, and data implicating dysregulation of the serotonergic system in impulsive behavior, that could have implications for several diagnoses, underscores the value of a dimensional approach to personality classification (Depue and Spoont 1986). Also, when using a categorical approach, patients may meet criteria for several personality disorders, raising suspicion that a single trait might be involved. However, while dimensional approaches are conceptually more satisfying, prior attempts to use constellation of traits to predict outcome in substance abuse has been relatively discouraging (Pettinati et al., 1982).

Issue #3: Instruments Used for Evaluation. The construction of personality assessment instruments well reflects the categorical vs. dimensional division in the field. In addition, we also struggle with comparing studies using structured interviews vs. self-report questionnaires. While space does not allow me to discuss the assets and liabilities of each of these instruments, it is important to know that little work has been done to psychometrically compare instruments, and the little that has been reported has not been encouraging (see, e.g., Hogg et al., 1990). Therefore, until more investigation into instrumentation takes place, comparisons across studies that have used different instruments will be difficult.

Issue #4: Diagonstic Criteria Used for Evaluation. Similar issues have been raised regarding use of different diagnostic systems (e.g., Gerstley et al., 1990). Woody et al. (1985) reported that 45% of opiate addicts met DSM-III criteria for ASPD; while only 19% of these Ss met RDC criteria for ASPD. Marin et al. (1989, p. 509) reported that there were "848 different ways one can meet criteria for ASPD."

<u>Issue #5. Validity of Patient's Self-Report.</u> Finally, there is the issue of ensuring valid patient self-reports. Usually, deception can be overcome, if interviews are conducted by the same, experienced interviewer. Difficulty occurs, however, if a patient has a poor memory or is unable to discriminate which behaviors are related to drug and alcohol use. Two useful strategies that have been used are delaying the evaluation to allow as much of an abstinent period as possible, and continually reminding the patient that their descriptions of their behavior should be independent of substance use. Family informants also may be helpful.

CARRIER RESEARCH STUDY SAMPLE

<u>Description of Ss</u>. Table 1 shows some of the sociodemographic characteristics of the Carrier study sample of 140 patients who have been entered thus far into a one-year treatment outcome project, with 72 of them having a DSM-III-R diagnosis of cocaine abuse or dependence, 12 females and 60 males, and 68 of them having a DSM-III-R diagnosis of alcohol abuse or dependence, 19 females and 49 males. Of note, is that 75% of the cocaine dependent subjects have a concomitant DSM-III-R diagnosis of alcohol abuse or dependence. Also, marijuana use is prevalent in this subgroup, although none of the cocaine deDendent Ss met DSM-III-R criteria for marijuana dependence.

Table 1

SUBJECT DEMOGRAPHICS: SUBSTANCE DEPENDENCE BY GENDER						
	COCAINE			ALCOHOL		
PATIENTS:	TOTAL	FEMALE	MALE	TOTAL	FEMALE	MALE
$\underline{n}$	72	12	60	68	19	49
$\bar{X}$ Age	29.9	26.7	30.5	38.1	40.9	36.9
% Black	31.0	54.5	26.7	4.7	0.0	6.7
$\bar{X}$ Yrs Educat	12.7	12.6	12.7	14.1	14.2	14.0
Hollingshead:						
1-2 (%)	12	0	14	33	27	36
3-5 (%)	60	66	59	41	46	38
6-7 (%)	28	34	27	26	27	26
Marital Stat:						
% Married	29.6	9.1	33.3	45.3	47.4	44.4
% Single	56.3	81.8	51.7	26.6	21.1	28.9
% Div/Sep	14.1	9.1	15.0	28.1	31.5	26.7
$\bar{X}$ SCL-90-R	99	133	93	81	84	80
$\bar{X}$ ASI Rating:						
$\bar{X}$ Alcohol	4.0	4.3	3.9	6.2	5.8	6.4
$\underline{X}$ Drug	6.7	6.9	6.7	0.6	0.6	0.6
$\bar{X}$ Psych	3.0	4.2	2.8	3.2	3.6	3.0

Procedure. Carrier Foundation is a private, nonprofit psychiatric hospital in a middle to upper income area in a rural setting north of Princeton. Specialized addiction

238

inpatient treatment is 28 days and outpatient treatment is 6
weeks. Both are followed by an outpatient aftercare program
for 12 weeks. Diagnoses of Axis-I and Axis-II psychopatho-
logy are made using the SCID-I and SCID-II during the last
half of intensive treatment, typically after 4 weeks of
abstinence. A follow-up SCID-II is given at 3 months post-
intensive treatment. The SCID-II interview questions
included a qualitative statement to exclude any behaviors
that have occurred due to alcohol or drug use. In deter-
mining diagnoses, specific questions that are directly
related to drinking and drug behaviors are excluded, e.g.,
"...did you ever drive a car when you were drunk?"

Results. Of the total sample of 140 Ss, 40% met criteria
for an Axis-II disorder. Significantly more cocaine
abuse/dependent Ss compared to alcohol abuse/dependent Ss
met Axis-II criteria: 50% vs. 29% (p = .001). There was a
nonsignificant
trend for fewer
males than females
to meet Axis-II
criteria: 38% vs.
48% (Q = .19).
Figure 1 reveals
this gender differ-
ence within the
cocaine dependent
group. Prevalence
of Axis-II disor-
ders is highest in
the cocaine depen-
dent females at 75%
compared to the
alcohol dependent
females at 32%
(p = .02) and com-
pared to both cocaine
dependent males
at 45% (p = .11),
and alcohol
dependent males
at 29% (p = .0004).
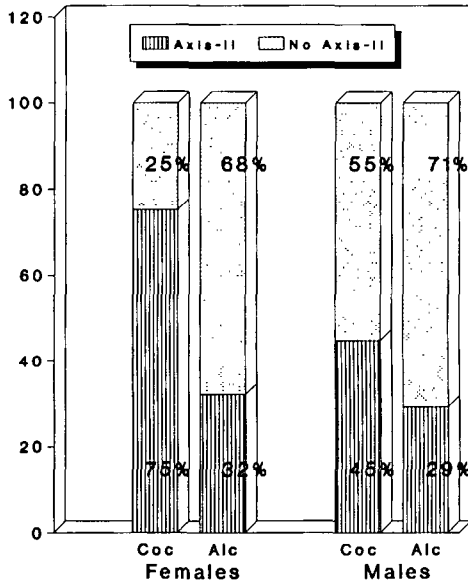
Figure 1: Percent DSM-III-R
Axis-II for Female & Male
Substance Dependent Ss at
the End of Intensive Treatment.

Type of Axis-II. The six most common personality disorders
in our sample, in order of prevalence based on the total of
140 Ss, were: Avoidant (15.7%), Paranoid (12.1%), ASPD
(9.3%), Self-Defeating (8.6%), Borderline (7.9%), and
Obsessive-Compulsive Disorder (5.7%). We found that type of
personality disorder differed depending upon the preferred
substance (cocaine or alcohol), and depending upon gender.

239

For example, the cocaine dependent females showed a higher prevalence of Histrionic, Borderline, Schizoid, and Paranoid Personality Disorder compared to alcohol dependent females, who, in our sample, typically met criteria for Self-Defeating Personality Disorder. Cocaine dependent males also had a high prevalence of Borderline, Schizoid and Paranoid. However, in contrast to the cocaine dependent females, they showed a high prevalence of ASPD and Obsessive-Compulsive Disorder. The alcohol dependent males had some features common to alcohol dependent females, but also some that were common to cocaine dependent males. In summary, our data support reports of gender differences (e.g., Griffin et al., 1989; Hesselbrock et al., 1985) and substance differences (Mirin and Weiss 1988) in personality profiles of substance dependent Ss.

DSM-III-R Cluster Analyses. As mentioned previously, a number of patients in this sample met criteria for more than one personality disorder. We determined which diagnosis received the most severe rating, and proceeded to group Ss by the DSM-III-R cluster that represented their most severe Axis-II diagnosis.

Cluster A represents diagnoses characterized by emotional withdrawal or odd behavior: Paranoid, Schizoid, Schizotypal. Cluster B represents dramatic, emotionality, and suicide potential: Antisocial, Borderline, Histrionic, Narcissistic. Cluster C represents resistive submissiveness and anxiety: Avoidant, Dependent, Obsessive-Compulsive, Passive-Aggressive. We also included Self-Defeating in Cluster C. Clusters A, B, and C occurred in the alcohol dependent subgroup at a rate of 15%. 30%, and 55%, respectively. Clusters A, B, and C occurred in the cocaine dependent subgroup at a rate of 22%, 44%, and 33%. respectively. Not surprisingly, cocaine dependent subjects had significantly more severe diagnoses from Clusters A and B in contrast to alcohol dependent Ss who had diagnoses from Cluster C (p = .009). Of note, there was a significant relationship between cocaine route of administration and Personality Disorder Cluster. Freebasing or using cocaine I.V. was related to having diagnoses within Clusters A or B, compared to using cocaine intranasal which was associated with Cluster C diagnoses (p = ,002).

Outcome. The relationship between Axis-II psychopathology and relapse was not straightforward. Relapse was defined as re-meeting dependence criteria for a duration of 1 week, which could be shorter if a major intervention occurred that prevented further substance use, e.g., jail or admission to inpatient treatment. Figure 2 graphs the proportion of cocaine dependent Ss who had an Axis-II diagnosis at the end of intensive treatment, divided into those who relapsed vs. no relapse by 6 months. There was no relationship between

having a personality disorder and relapse. However, we redid this analysis, except instead of generically defining personality disorder, we selected Ss who had the most severe Axis-II psychopathology.

The requirement for this subgroup was that a S would have a Cluster A diagnosis and a Cluster B diagnosis. Figure 3 shows the significant relationship (p = .03) between relapse by 6 months and having this severe personality profile (Cluster A plus Cluster B diagnoses). (This analysis was not possible for alcohol dependent Ss due to so few of the Ss having this severe personality profile.) Of interest, the cocaine dependent Ss who relapsed by 6 months were predominantly, but not exclusively, Ss who met criteria for ASPD ti for Paranoid Personality Disorder. Thus far, it was not predictive of relapse if only ASPD or Paranoid Personality Disorder had been present. In trying to interpret this relationship, it occurred to us that meeting criteria for Paranoid Personality Disorder in a cocaine population might index a lingering neurotoxic effect of intense cocaine use, rather than inherent maladaptive paranoid character structure.

Figure 2: Percent Cocaine Dependent Ss with DSM-III-R Axis-II Disorders Who Relapsed by 6 Months Post-Treatment.
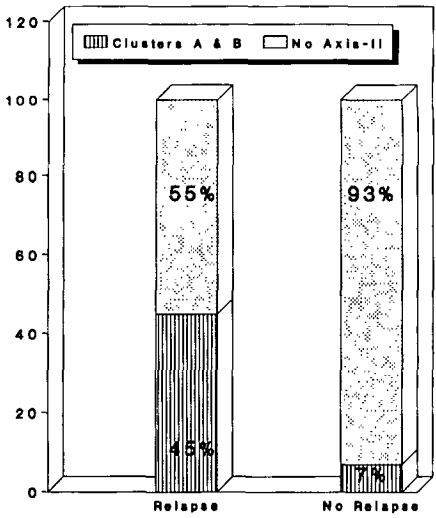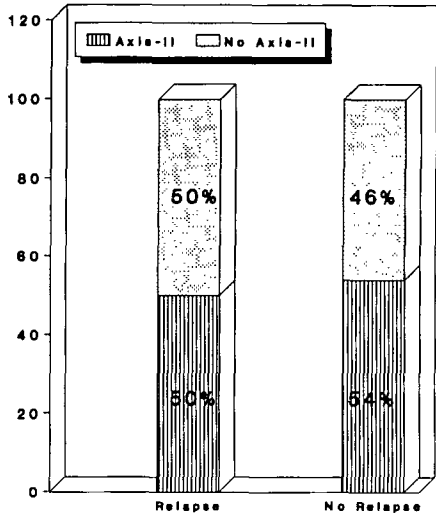
Figure 3: Percent Cocaine Dependent Ss with Personality Disorders from DSM-III-R Clusters A & B Who Relapsed by 6 Months Post-Treatment.

241

<u>Change In Axis-II Psychopathology.</u> We could not look at changes in personality disorder 3 months post-treatment in the ASPD plus Paranoid Personality Disorder group because essentially most had relapsed by then (also, 20% were un-locatable). We have only one <u>S</u> thus far who has agreed to repeat the SCID-II and has been successful in maintaining abstinence for 3 months. Of interest, he continued to meet criteria for ASPD, but no longer met criteria for Paranoid Personality Disorder. Obviously, these data need to be examined in larger samples before we know the extent and potential reversibility of paranoid psychopathology.

Of the follow-up SCID-11s we did have available, we found approximately 70% of <u>S</u>s no longer met criteria for any personality disorder. However, generalizations from our data were limited to patients with personality disorders, from Cluster C, because those were the <u>S</u>s who had not yet relapsed, or were cooperative to sit for the interview.

In conclusion, further investigation is needed on a number of fronts if we are to fully understand the relationship between personality disorder and substance dependence. The intent of this presentation was to provide a framework for organizing the issues and controversies relevant to person-ality assessment in the substance abuse population, fully realizing that empirically-based answers are simply not yet available.

Helen M. Pettinati, Ph.D., Carrier Foundation, Belle Mead, NJ 08502

The Actions of FMRF-NH$_2$ and FMRF-NH$_2$ Related Peptides on Mammals

Robert B. Raffa

ABSTRACT

Since the initial isolation and characterization of FMRFamide (Phe-Met-Arg-Phe-NH$_2$) from a molluscan source, the potential for the interaction of this neuropeptide with opioid systems has been suspected. Immunoreactive FMRFamide-like material is found in mammals (particularly in brain, spinal cord and GI tract) and mammalian-derived FMRFamide-related peptides (FaRPs) have been identified. A considerable amount of data supports the hypothesis that FMRFamide or mammalian FaRPs function as endogenous antiopiates, particularly with regard to opioid-induced antinociception and other opioid-induced behaviors. They have also been reported to be capable of altering the rate of morphine tolerance development and precipitating withdrawal in morphine-dependent animals. These data imply that FMRFamide or FaRPs are competitive antagonists at opiate receptors. The relatively low affinity for opiate receptors also suggests other possibilities, including mammalian FMRFamide (or FaRP) receptors equivalent to those in invertebrates, partial agonism at opiate receptors or, an indirect (modulatory) role. Whatever the actual mechanism, FMRFamide-like peptides and other 'anti-opiate' peptides might have critical roles in the development of opioid tolerance and dependence or in the pharmacologic study or clinical treatment of these phenomena.

INTRODUCTION

In 1984 Tang and coworkers (Tang et al., 1984) proposed that a peptide with FMRFamide (Phe-Met-Arg-Phe-NH$_2$)-like properties might be an endogenous opioid antagonist or, more broadly, an endogenous 'antiopiate' (Galina and Kastin 1986). Since that time, two FMRFamide-like peptides (FaRPs) have been isolated from bovine brain and have been shown to have antiopiate properties similar to FMRFamide (Yang et al 1985). There is considerable data to support the hypothesis that FaRPs function as endogenous antiopiates in mammals as presented, by other authors in this volume. It is the purpose of this presentation to give a broad overview of the general pharmacology of FMRFamide and related

peptides in non-mammalian, as well as mammalian, species as a background for the antiopiate action of FMRFamide and FaRPs and to place the antiopiate action of these neuropeptides in the broader contex of a rather wide spectrum of pharmacologic activity (for a more detailed review see Raffa 1988).

MOLLUSCAN NEUROPEPTIDES

FMRFamide is a neuropeptide that was originally isolated from ganglia of the clam <u>Macrocallista</u> <u>nimbosa</u> and identified by Price and Greenberg in 1977. FMRFamide is known as 'molluscan cardioexcitatory neuropeptide' because of its effects on molluscan heart, such as the ability to augment and prolong contractions of cardiac muscle, to correct arrhythmias and to initiate beating of quiescent hearts. FMRFamide or FMRFamide-related peptides are widely distributed throughout molluscan species (Price <u>et</u> <u>al</u>., 1987), where they have generally the same type of cardioactive actions (excitatory or inhibitory). The mechanism of these and other actions of FMRFamide and FaRPs in molluscs appears to be one of modulation of neurotransmitter action (e.g., Walther <u>et al</u>., 1984; Muneoka and Matsuura 1985; Evans and Myers 1986; Raffa and Bianchi 1986). Modulation of neurotransmitter action can also be shown in mammals (acetylcholine action on isolated guinea pig ileum) (Raffa and Jacoby 1989a).

In addition to their multiple actions on the cardiovascular system of molluscs, FMRFamide and FaRPs have actions on other types of excitable tissues, such as noncardiac muscle and nerve (Greenberg <u>et al</u>., 1988). As in the case of cardiac tissue, the actions of FMRFamide and FaRPs on these other tissues are varied and complex. Thus, despite the fact that FMRFamide and FaRPs may play a role in cardioregulation in molluscs (as a neurohormone), that is apparently not their only role. It may be just one part of a more general pharmacology.

In molluscs, the C-terminal amide and the Arg^3 residue are critical for activity with conservative substitutions for Phe^1 or Met^2, or N-terminal extension not so crucial (Painter <u>et</u> <u>al</u>., 1982). A similar SAR appears to be true for activity in mammals (Raffa and Jacoby 1990).

THE FAMILY OF FaRPs

Numerous 'FMRFamide-like' peptides have now been identified in molluscs, including FLRFamide and several with the generic sequence X-FLRFamide (Ebberink <u>et</u> <u>al</u>., 1987; Price et al., 1987a,b). In addition, at least three N-terminal extended analogs of FLRFamide have been found in cockroach heads (Holman et al., 1986) and in lobster (Trimmer <u>et</u> <u>al</u>., 1987).

Immunoreactive FMRFamide-like material (irFMRFamide) has been demonstrated in mammals, with particularly high levels found in the CNS and GI tract (see TABLE 1 next page).

TABLE 1: irFMRFamide-like Activity in Mammals

Dockray et al.	1981	rat, cow, dog	Brain, GI tract, pancreas
Weber et al.	1981	rat	Brain, spinal cord, post. pituitary
Dockray and Williams	1983	rat	Spinal cord, hypothalamus, low in cerebellum and the striatum
O'Donohue et al.	1984	rat	GI tract and pancreas
Chronwell et al.	1984	rat	Cell bodies, n. fibers in brain, laminae I & II of sp. cord
Kubben et al.	1986	rat, human	Gastric antrum cells, pancreatic cells
Chen et al.	1989	monkey CNS	Similar distribution to rats

TABLE 2. Actions in Mammals

Cardiovascular

Mues et al.	1982	rat	iv, B.p.(+), heart rate(+),
Wong et al.	1985		icv resp. rate(+)
Barnard & Dockray	1984		
Chai et al.	1986	rat	icv Block of [D-Ala^2,Leu^5]-enkephalin decrease in bp and heart rate

Feeding

Kavaliers & Hirst	**1985**	**mouse**	**icv Attenuation of morphine-, kappa-,**
Kavaliers et al.	**1985**		**deprivation & defeat-induced feeding**

Endocrinology

Sorenson et al.	1984	rat	Inhibition of glucose-stimulated insulin release in isolated pancreas

Behavior

Raffa et al.	1986	mouse	it Increased grooming

Amnesia

Telegdy & Bollok	1987	rat	icv Interference with 'consolidation of learning' and memory 'retrieval'

Yang and coworkers have now isolated, sequenced and characterized two peptides from bovine medullae oblongatae and midbrains that cross-react with FMRFamide antiserum and have properties similar to FMRFamide and FaRPs (Yang et al., 1985; Majane and Yang 1987).

ACTIONS OF FaRPs IN MAMMALS

FMRFamide and FaRPs have a multiplicity of actions in mammals including, not unexpectedly, cardiovascular (see TABLE 2). Some of these appear to be clearly related to an antiopiate action, but with others such a mechanism is not as obvious.

FaRPS as ANTIOPIATES

The partial homology between FMRFamide and Met-enkephalin-Arg[6],Phe[7]-NH$_2$ (Tyr-Gly-Gly-FMRF-NH2) has been used to suggest a possible relationship between FaRPs and the opioid peptides Greenberg et al., 1983). In fact, the antinociception (Tang et al., 1984; Yang et al., 1985) and feeding (Kavaliers and Hirst 1985; Kavaliers et al., 1985; Kavaliers 1987) produced in rodents by opioid agonists is antagonized by FMRFamide and related peptides (see summary of the antiopiate actions of FMRFamide in TABLE 3). In addition, the peptides inhibit the development of, and increase in CSF in parallel with, morphine tolerance and precipitate withdrawal in morphine-tolerant animals (Tang et al., 1984; Lake et al., 1989; Malin et al., 1989). Based on these and considerable additional supportive data, FaRPs have been postulated to be endogenous opioid antagonists (Tang et al., 1984). The affinity for opioid receptors, however, appears to be relatively low (Zhu and Raffa 1986; Zadina and Kastin 1986).

TABLE 3. Antiopiate Actions of FMRFamide

Greenberg et al.	1983	molluscs	Reviewed comparative pharmacology and structural similarity to the endogenous opioids.
Tang et al.	1984	rat icv/it	Blocked increased TF latency produced by MS and YGGFM-RF; IgG from FMRFa antiserum produced Nx-reveirsible increased latency; Anti-FMRFa IgG attenuated the tolerance development to MS; MS added to sp. cord perfusion fluid released FMRFa-like peptides.
Kavaliers and Hirst	1985	rat icv	Blocked defeat-induced analgesia.
Raffa	1989	mouse icv	Block of MS(icv)-induced increase in horizontal locomotor activity.

MS = morphine; Nx = naloxone; TF = tail-flick test.

In contrast to the antinociceptive endpoint, however, we have found that central administration (i.c.v) of FMRFamide mimics the action of morphine and enkephalin analogs on mouse colon, namely, inhibition of

propulsive motility (Jacoby <u>et</u> <u>al</u>., 1987). This action is reversed by the opiate antagonist naloxone. Hence, FMRFamide appears to act in this preparation as an opioid agonist, not antagonist. We subsequently demonstrated similar results using two mammalian-derived FaRPs F-8-Famide and A-18-Famide (Raffa and Jacoby 1989b) and a series of FaRPs with C-terminal sequence Arg-Phe-NH$_2$. [D-Met^2]-FMRFamide was identified as particularly active (Raffa and Jacoby 1990).

CONCLUSION

There is now substantial data demonstrating that FMRFamide and FaRPs interact with mammalian opiate systems. Of course, endogenous FaRPs might act differently than exogenously administered peptide and there may be families of interacting (competing) peptides. Nevertheless, the proposal that FaRPs might be endogenous opiate antagonists is intriguing and has important implications for the study and treatment of the development of tolerance and dependence. Whether the mechanism of their action is via competitive interaction with opiate receptors or as functional antagonists remains to be elucidated.

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AUTHOR

Robert B. Raffa, Ph.D.
Central Nervous System Research, R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776

Animal Conflict, Analgesia and the Modulatory Effects of FMRFamide-Related Peptides

Martin Kavaliers

INTRODUCTION

The neuropeptide Phe-Met-Arg-Phe-NH$_2$, (FMRFamide) was the first member of a growing interphyletic family of peptides to be isolated and sequenced. It was originally isolated from ganglia of the clam <u>Macrocallista nimbosa</u> (Price and Greenberg 1977). FMRFamide related peptides (FaRPs) have subsequently been shown to have a broad phylogenetic distribution, with extensive FMRFamide-like immunoreactivity being reported in the mammalian central nervous system. Two endogenous FaRPs peptides, an octapeptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH$_2$ (F8Fa) and an octadecapeptide, Ala-Glyn-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH$_2$ (A18Fa), were isolated from the bovine central nervous system, with highest concentrations present in the dorsal spinal cord and periaqueductal grey of the hypothalamus (Yang et al., 1985). Subsequently, these, or closely related FaRPs, were immunohistochemically described and biochemically characterized from equivalent areas of the central nervous system of the laboratory rat (Kivipetto & <u>et</u> <u>al</u>., 1989).

FMRFamide and the FaRPs have been implicated in the mediation of a variety of behavioral and physiological functions in invertebrates and vertebrates. In molluscs, FaRPs are involved in the regulation of cardiac and other muscular activities (Greenberg <u>et</u> <u>al</u>., 1983). In mammals, FMRFamide was found to produce a variety of effects, including alterations in blood pressure and microvasculature, as well as direct effects on neuronal tissue (reviewed in Raffa 1988). To date the most substantive evidence, however, suggests that in mammals the FaRPs may function as endogenous modulators (antagonists) of opioid mediated analgesia and nociception.

FMRFamide AND FaRP MODULATION OF OPIOID ANALGESIA

Evidence for a possible relationship between FMRFamide and endogenous opioid peptides was described by Greenberg et al., in 1983. Subsequently, there has accumulated substantial data suggesting that FaRPs have a modulatory effect on opioid mediated responses in mammals. The first direct evidence for a specific role for FMRFamide, or a FaRP, in the regulation of opioid activity was made by Tang et al., in 1984. They showed that both FMRFamide and an immunoreactive FMRFamide-like peptide purified from bovine brain could antagonize morphine-induced analgesia in male rats. In addition, they showed that intrathecal or intracerebroventricular (icv) injections of antibody (IgG) directed against FMRFamide produced a naloxone-reversible, low level analgesia, as measured by increased tail-flick latency, and decreased tolerance to morphine-induced analgesia. In 1985 Yang et al., demonstrated that icv administrations of F8Fa and A18Fa produced a hyperalgesia in rats and attenuated the increased tail-flick latency produced by peripheral administration of morphine. In both cases, F8Fa had significantly more potent effects than A18Fa. Recently, it was shown that peripheral administration of a dansyl derivative of FMRFamide (Dansyl-Arg-Phe-NH,), which readily penetrates the blood-brain barrier, also blocked morphine-induced analgesia in rats, without having any evident effects on the control level of nociception (Brussaard et al., 1989).

In laboratory mice icv injections of FMRFamide were also shown to block morphine and restraint stress-induced opioid analgesia (Kavaliers and Hirst 1986). FMRFamide by itself had no significant effects on day-time basal nociceptive responses. Recently, it was demonstrated that icv injections of IgG from antiserum against these FaRPs augmented morphine- and restraint-induced analgesia in mice (Kavaliers 1990, Kavaliers and Yang 1989). As in rats, the octapeptide, F8Fa, had significantly greater inhibitory effects on analgesia than did the octadecapeptide A18Fa. The IgGs by themselves also potentiated, in a naloxone reversible manner, basal day-time nociceptive sensitivity in male mice, as measured by the latency of a foot-lifting response to a warmed surface (Kavaliers and Yang 1989). The FaRPs, F8Fa and to a lesser extend A18Fa, also influenced basal nociceptive sensitivity, significantly reducing the elevated night-time response latency while not affecting the day-time response (Kavaliers 1990). Peripheral administrations of naloxone had similar inhibitory effects on the day-night rhythm of nociceptive sensitivity. In nocturnal rodents increased dark period thermal response latencies have been associated with higher night-time levels of central opioid peptides and opioid binding sites (Naber et al., 1981).

These observations with rats and mice, along with the central distribution of the FaRPs, suggest that these peptides may function as endogenous modulators of opioid analgesia and possibly related opioid mediated behavioral functions (see also a number of apparent pro-opioid effects of FMRFamide described in Raffa, 1988). These observations with rats and mice raise the possibility that the FaRPs may have more general roles in the modulation of opioid-mediated analgesic responses associated with natural aversive events in biologically realistic situations.

SOCIAL CONFLICT AND THE MODULATORY EFFECT'S OF FaRPs

Social conflict and aggression are key facets of animal behavior (Wittenberger 1975). Animals use aggression to control space, compete for resources and acquire mates. They use it in parental interactions and in sibling rivalries and on occasion to thwart predators or members of other species. Exposure to threatening environmental stimuli, including those associated with aggression and predation, has been found to induce analgesia in animals from diverse phyla, and it is now considered that such pain inhibition is a highly adaptive component of the organism's defense repertoire (Kavaliers 1990).

Intraspecific Aggression

Intraspecific aggression has been used as a biologically relevant means of examining central opioid activation and its behavioral and physiological consequences (Miczeck et al., 1982). In the typical laboratory 'resident-intruder' paradigm a small male intruder mouse is introduced into the home cage of a larger dominant, isolated resident animal and the ensuing agonistic encounter is monitored. In both wild and laboratory-bred mice, intraspecific aggressive interactions, which are made up of a number of components including threats, attacks and fighting, can result in the display of a specific posture by the vanquished individual. This defeat behavior is considered to represent a generalized natural biological response to the stress of social confrontation. Both the aggressive encounter and subsequent defeat experience obtained in a 'resident-intruder' interaction have been shown to induce opioid-mediated analgesic responses in the subordinate, intruder mice. These behaviors, which are analogous to the responses obtained after central administration of either opioid peptides or morphine, are reduced by various opiate antagonists including naloxone (Teskey and Kavaliers 1988).

Results of initial studies showed the icv administrations of FMRFamide

reduced, in a dose-dependent manner, the analgesic consequences of defeat in intruder male CF-1 mice in a 'resident-intruder' paradigm (Kavaliers and Hirst 1988). FMRFamide also affected the aggressive interactions, reducing the number of bites required to obtain defeat in the subordinate mice during the aggressive encounters: Naloxone had similar effects on defeat-induced analgesia and the propensity for defeat. Recently, F8Fa and A18Fa were also found to reduce in a dose-dependent manner, and IgG prepared from F8Fa and A18Fa antiserum to enhance, defeat-induced analgesia, with F8Fa having greater effects than A18Fa (in preparation). Likewise, the FaRPs decreased, while the IgGs increased, the number of bites to defeat and the intensity of the aggressive interaction. These observations indicate that FaRPs have a role in the modulation of endogenous opioid activation and/or expression of opioid-mediated analgesia that is associated with social conflict. These findings also suggest that the FaRps may have inhibitory influences on the expression of aggression. Defeat-induced analgesia has been shown to be independent of the number of bites received by the vanquished mouse (Teskey and Kavaliers 1988). Therefore, the reduced number of bites obtained after treatment with either FMRFamide, FaRPs, or naloxone does not by itself account for the lowered nociceptive thresholds that are observed in the defeated mice. Mice which receive a low number of bites can still display a high nociceptive level. This suggests that FaRP sensitive, and possibly opioid mediated, mechanisms are associated with the expression of the aggressive interactions.

Predator Exposure

Predation in its broadest sense is a basic behavior that affects all individuals. All organisms may be exposed at some time to the threat of predation. The first and most important step in the initiation and avoidance of predation is prey and predator recognition, respectively (Endler 1986). There is substantial evidence for natural predator recognition by many prey animals. It has been shown that exposure of deer mice or white-footed mice to a natural predator, the short-tailed weasel (also called ermine or stoat), elicits significant analgesic responses. Brief ecologically relevant, non-visual exposure to a weasel elicited non-opioid mediated (benzodiazepine sensitive) analgesic responses, while longer exposures led to more prolonged opioid-analgesia in these species (Kavaliers 1988, 1990). Similar patterns of analgesic responses were also observed in deer mice that were exposed to just the scent of a weasel (in preparation).

Short-tailed weasels are also major predators of meadow voles. Meadow voles are small (30-60 gm) herbivorus rodents that under long

days are active in the daytime. They display a marked daytime morphine-induced analgesia that can be attenuated by icv treatment with either F8Fa, A18Fa or naloxone (in preparation). Results of initial studies revealed that a brief (30 sec) exposure of the voles to the scent of a weasel elicited a significant analgesic response that was unaffected by naloxone and benzodiazepine agonists and antagonists, but was sensitive to serotonergic (5-HT) manipulations (in preparation). Unexpectedly, this 5-HT sensitive analgesia was also reduced by icv administrations of either F8Fa or A18Fa. More prolonged exposure (5 min) to the weasel scent also elicited a naloxone-insensitive analgesia. This analgesia was reduced by benzodiazepine antagonists, but was unaffected by the FaRPs and the 5-HT manipulations. A longer (15 min) exposure to the scent of a weasel led to a naloxone-sensitive, analgesia that was also attenuated by the FaRPs. This opioid sensitive analgesia was unaffected by either benzodiazepine or 5-HT manipulations. These findings suggest the involvement of FaRP sensitive anxiety in the initiation of non-opioid analgesia associated with brief exposures to the predator odor. These responses are consistent with the non-opioid analgesia evident in certain strains of mice after social conflict (Rodgers and Shepherd 1989) and maternal aggression-induced analgesia in male voles (in preparation).

These observations with meadow voles show that the FaRPs, F8Fa and A18Fa, can attenuate opioid-mediated analgesia elicited by exposure to a natural, ecological relevant, stressor associated with a predator. They also raise the intriguing possibility that FaRPs may affect naloxone insensitive, 5-HT mediated analgesia. This is consistent with evidence from invertebrates for interactions between FMRFamide and 5-HT systems (Greenberg et al. 1983), as well as with preliminary data from rats for 5-HT and vasopressin related effects of FMRFamide (Robert et al. 1989, Telegdy and Bollok 1987).

CONCLUSIONS

Results of investigations with ecologically relevant stimuli associated with aggression and predation have revealed the FaRPs have significant modulatory (antagonistic) effects on opioid mediated analgesia in several species of rodents. These investigations have, however, also raised the possibility that the FaRPs may also have modulatory effects on non-opioid (naloxone insensitive) analgesia whose expression involves serotonergic systems.

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AUTHOR

Martin Kavaliers, Ph.D.
Division of Oral Biology
Faculty of Dentistry
The University of Western Ontario
London, Ontario Canada N6A 5B7

FMRF-NH$_2$-Like Peptides Antiopiates

Hsiu-Ying T. Yang, Elizabeth A. Majane, and Jinmin Zhu

The cardioactive peptide, Phe-Met-Arg-Phe-NH$_2$ (PRRF-NH$_2$), was isolated from the Macrocallista nimbosa clam by Price and Greenberg (1977). Subsequently, a widespread occurrence of FMRF-NH$_2$-like peptides was demonstrated in brain, intestine and pancreas of several vertebrate species (Weber et al., 1981; Dockray et al., 1981); furthermore, this clam peptide was found to display many biological actions in rats and mice (Raffa 1988). Interestingly, in molluscs, a possible relationship between FMRF-NH$_2$ and enkephalins was suggested and supported by the finding that Tyr-Gly-Gly-Phe-Met-Arg-Phe-Nil$_2$, an amidated form of the opioid peptide Met[5]-Enkephalin-Arg[6]-Pfie[7], can display both opioid and FMRF-NRr-like activities (Greenberg et al., 1983). This hypothesis prompted us to study the effect of FMRF-NH$_2$ on opiate analgesia in the mammalian system and FMRF-NH$_2$ was found to be capable of attenuating opiate analgesia. However, many studies have indicated that the FMRF-NH$_2$ immunoreactivity (IR) detected in vertebrates is not due to FMRF-NH$_2$ (Dockray and Williams 1983; O'Donohue et al., 1984). In order to study the physiological role of FMRF-NH$_2$-IR in the mammalian system we have characterized FMRF-NH$_2$-IR of mammalian origin including the isolation of two peptides from bovine brain. In addition the possible relation between mammalian FMRP-NR$_2$-IR and the opiate system was explored.

EFFECT OF FMRF-NH$_2$ ON OPIATE ANALGESIA

The effect of FMRF-NH$_2$ on analgesia induced by opiates was studied in rats using tail flick latency as the measurement of antinociception (Tang et al., 1984; Yang et al., 1985). FMRF-NH$_2$, when injected intraventricularly or intrathecally prior to opiate, can decrease the prolongation of tail flick latencies induced by opiates (Fig.1, left). This opiate modulating activity is dose dependent and the effect is more potent when given intrathecally than if given intraventricularly. This result is in good agreement with the finding of high levels of FMRF-NR$_2$-IR in the rat spinal cord (Majane et al., 1989).

FMRF-NR$_2$-LIKE PEPTIDES OF MAMMALIAN ORIGIN

FMRF-NH Inunoreactive peptides of Bovine Brain: Structure and Biological Activity

Chromatographic studies have revealed that the mammalian FMRF-NH-like peptides are composed of multiple molecular forms; none of them can be identified as authentic FMRF-NH_2 (Dockray and Williams 1983; O'Donohue et al., 1984). Two FMRF-NH_2-like peptides were isolated from bovine brain extract and biochemically characterized as Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro Gln-Arg-Phe-NH_2 (A-18-F-NH_2) (Yang et al., 1985).
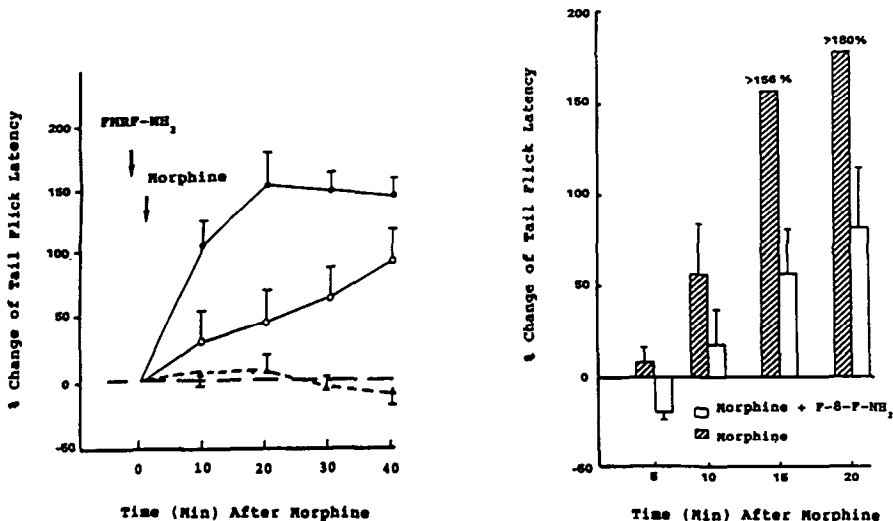
Figure 1. Effect of FHRF-NH (left) and F-8-F-NH_2 (right) on morphine analgesia. Left: rats were injected intrathecally with morphine sulfate at 10 µg/20 µl (●); FMRF-NH_2 at 1.5 µg/10 µl (▲); and FMRF-NH_2 followed 5 min later by morphine sulfate (o). Right: rats were injected intraventricularly with morphine sulfate at 20 µg/5 µl (▨); and F-8-F-NH_2 at 5 µg/5 µl followed 2 min later by morphine sulfate (▢).

Similary to inverterbrates, an interaction between FMRF-NH_2-IR and opioid peptides has been implied in vertebrates. In rats, intrathecal or intraventricular administration of IgG prepared from FMRF-NH antiserum was found to induce a long lasting moderate analgesia (Tang et al., 1984). This analgesia was not observed if rats were pretreated with naloxone. Because of these observations, the effects of F-8-F-NH_2 and A-18-F-NH_2 on nociception and the analgesic effect of morihine were studied (Yang et al., 1985). F-8-F-NH_2 and A-18-F-NH_2, when injected intraventricularly, were found to decrease basal tail flick latencies. The

effect was short lasting and F-8-F-NH$_2$ was more potent than A-18-F-NH$_2$. F-8-F-NH, when injected prior to morphine, was found to markedly reduce tie morphine induced analgesia (Fig.1, right). This effect of F-8-F-NH$_2$ appears to last longer than the effect on the basal tail flick latency. Allard et al. (1989) have demonstrated the presence of high affinity F-8-F-NH$_2$ binding sites in rat spinal cord membranes and these specific binding sites are not affected by μ, δ and κ receptor agonists or naloxone. Though the molecular mechanism underlying the morphine modulating action of F-8-F-NH$_2$ still remains to be established, it is clear that F-8-F-NH$_2$ does not exert the morphine modulating activity by interacting directly with opiate receptors.

F-8-F-NH$_2$, which shares only the c-terminal Arg-Phe-NH$_2$ with FMRF-NH$_2$, was found to have a similar morphine modulating activity to FMRF-NH$_2$. In studying the F-8-F-NH$_2$ receptors, Allard et al. (1989) have found that FMRF-NH$_2$ can displace, though with a low affinity, the ^{125}I-YLFQPQRF-NH$_2$ (the radiolabelled ligand of F-8-F-NH$_2$) bound to the rat spinal cord membrane. Brussaard et al. (1990) have found that intraperitoneal injections of 1-dimethylaminonaphthalene-5-sulfonyl-Arg-Phe-NH$_2$ (a derivative likely to penetrate the blood brain barrier) can effectively inhibit morphine induced analgesia in rats. These observations taken together seem to suggest that the Arg-Phe-NH$_I$ sequence may be essential for the morphine modulating activity.
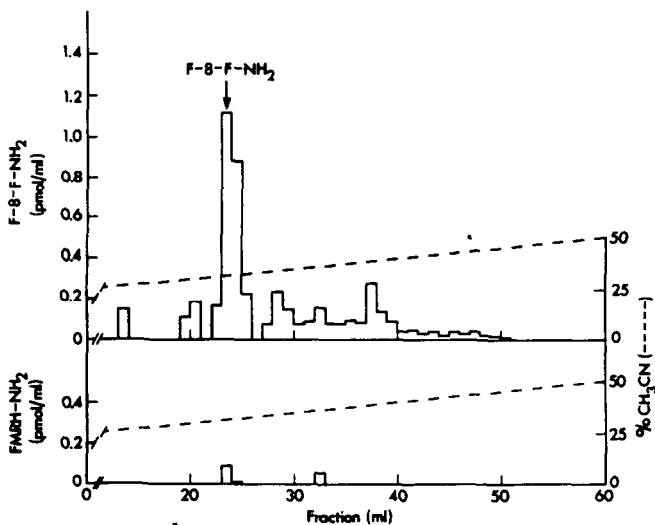
Figure 2. HPLC characterization of F-8-F-NH$_2$-IR and FMRF-NE$_2$-IR in human cervical spinal cord. Extract from 0.55 g tissue was applied to an Altex ODS-5 column. The column was eluted with a linear gradient of 20-50 % CH$_3$CN in 0.1 % trifluoroacetic acid over 60 min at a flow rate of 1 ml/min. Fractions were analyzed by F-8-F-NH$_2$ (top) and FMRF-NH$_2$ (bottom) RIAs.

Characterization of FMRF-NH$_2$-IR in Various Mammalian Species.

Using HPLC coupled with radioimmunoassays (RIA) developed for F-8-F-NH$_2$ and FMRF-NH$_2$, FMRF-NR$_2$-IR in spinal cords of bovine, rat, mouse, guinea pig and human were studied (Majane et al., 1988). One major immunoreactive peak eluting in the position of synthetic F-8-F-NH$_2$ was detected using F-8-F-NH$_2$ RIA in the extract from bovine spinal cord. One major immunoreactive peak was also observed in all other species by the F-8-F-NH$_2$ RIA, however, the retention time of this immunoreactivity was found to vary according to species except in human. In the carefully collected spinal cord of human, the F-8-F-NH$_2$ immuroreactive peak was found to have a retention time very similar to that of the bovine (Fig. 2). Whether F-8-F-NH$_2$-IR of human spinal cord is identical to bovine F-8-F-NH$_2$ awaits further verification. The F-8-F-NH$_2$ immunoreactive peak was also detected by the FMRF-NR$_2$ RIA (Fig. 2) but the amount of immunoreactivity can be grossly un there is a novel system of FMRF-NH$_2$-like peptides in mammalian CNS and furthermore that FMRF-NH$_2$-immunoreactive peptides of mammalian sources may have structures substantially different from FMRF-NH$_2$. Further characterization of F-8-F-NH$_2$-IR in various species may aid in understanding the structure-activity relationship of the F-8-F-NH$_2$ or FMRF-NH$_2$-like peptides because the biologically active portion of the molecule may be conserved throughout the species.

F-8-F-NH$_2$-IR in Ret Nervous System: Distribution and Possible Relationship with Opiates

The distribution studies have revealed that F-8-F-NH$_2$-IR is unevenly distributed in the rat CNS with the highest concentration found in the spinal cord and the pituitary (Majane et al., 1989).

In the spinal cord, F-I-F-NR$_2$-IR is highly concentrated in the dorsal horn. Immunohistochemically, this immunoreactivity was localized in nerve terminals in the substantia gelatinosa (Panula et al., 1987). Dorsal rhizotomy did not change F-8-F-NE$_2$-IR in the affected regions of the spinal cord while spinal cord transection resulted in 30-50 % lovering of F-8-F-NH$_2$-IR caudal to the transection. The lesion study suggests that at least part of the spinal F-8-F-NH$_2$-IR is contained in a descending neuronal pathway and does not originate from sensory ganglia.

Using an in vitro superfusion technique, the release of F-8-F-NH$_2$-IR from rat spinal cords was studied (Zhu and Yang 1990). F-8-F-NH$_2$-IR was released from intact spinal cord when the superfusion medium was adjusted to contain 56 mM KCl. This release was abolished by eliminating calcium from the depolarizing solution. The released immunoreactivity was analyzed by HPLC coupled with the F-8-F-NH$_2$ RIA and the main immunoreactivity released was identical to the main immunoreactivity of rat spinal cord. Addition of morphine to the perfusion medium was found to inhibit the 56 mM KCl induced release of F-8-F-NH$_2$ in a dose

dependent manner (Zhu and Yang 1990).

Accumulating evidence suggests that FMRF-NH$_2$-like peptides including F-8-F-NH$_2$ may have a role in neuronal function in the spinal cord. F-8-F-NH$_2$ was found to produce depolarizing and hyperpolarizing responses in cultured mouse spinal neurones (Guzman et al., 1990); furthermore, F-8-F-NH$_2$ specific binding sites were demonstrated in the rat spinal cord (Allard et al., 1989). These observations, along with the high level of F-8-F-NH$_2$-IR in the substantia gelatinosa, the inhibitory action of morphine on the secretion of F-8-F-NH$_2$ immunoreactivity and the morphine modulating activity of F-8-F-NH$_2$ suggest that spinal F-8-F-NH$_2$-IR may have a role in opioid-mediated antinociception.
In rat pituitaries, F-8-F-NH$_2$-IR is present in very high concentrations and furthermore is stored exclusively in nerve terminals in neural lobe (Majane et al., 1990). The posterior pituitary also contains high levels of dynorphin and Arg^8-vasopressin (AVP). Dynorphin is co-stored with AVP within magnocellular neurons in the supraoptic and paraventricular nuclei (Watson et al., 1982; Whitnall et al., 1983) and is co-released from the neurohypophysis during the antidiuretic response (Hollt et al., 1981). Because of this, the effects of opiates on the secretion of AVP were widely investigated and many studies have implied a modulatory role for the endogenous opioid peptides (dynorphin system) on AVP secretion from the pituitary (Bicknell et al., 1985; Brady et al., 1988; Yamada et al., 1989), although the results have been somewhat inconsistent. Because of these reports along with the morphine modulating activity of F-8-F-NR$_2$, F-8-F-NH$_2$-IR in pituitaries of Brattleboro and salt loaded rats, animals known to have modified biosynthesis of pituitary AVP and dynorphin (Sherman et al., 1986), was studied. In the pituitary, F-8-F-NH$_2$-IR was found be below the level of detection in the homozygous Brattleboro rat while a normal level of F-8-F-NH$_2$-IR is detected in control Long Evans rats. Furthermore, in the Brattleboro rat, levels of F-8-F-NH$_2$-IR in hypothalami and spinal cords were not decreased. Thus, in the Brattleboro rat, it appears that the absence of F-8-F-NH$_2$ is not due to a genetic defect in F-8-F-NH$_2$ biosynthesis but probably due to an enhanced release of this peptide (Majane and Yang 1990). In pituitaries of rats given 2% NaCl solution instead of of H$_2$0, F-8-F-NH$_2$-IR was found to decrease rapidly and reach a minimum level by the 4th day (Rajane and Yang 1989). As expected a parallel decrease of pituitary AVP was also observed in the salt-loaded rat. In the salt-loaded rat, this co-depletion of AVP and F-8-F-NH$_2$ resembles that of AVP and dynorphin. However, it should be noted that F-8-F-NH$_2$-IR is not co-localized with AVP in the same nerve terminals in the neurohypophysis because F-8-F-NH$_2$ has not been detected in the hypothalamic magnocellular neurons (Panula et al., 1990). These results taken together suggest that F-8-F-H$_2$-IR is released from pituitaries of hyperosmotic rats and that there may be an interaction between F-8-F-NH$_2$ and AVP. Though the physiological significance of F-8-F-NH$_2$-IR in the pituitary function is still unclear, the pituitary may be an interesting site to further explore the possible role of FMRF-NH$_2$-like peptides as

an endogenous antiopiate.

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AUTHORS

Hsiu-Ying T. Yang, Ph.D.; Elizabeth A. Majane, M.S. and Jinmin Zhu, Ph.D., Lab. of Biochem. Genetics, NIMH, Neuroscience Center at St. Elizabeths, Washington D.C. 20032

Upregulation of Rat Brain Opioid Receptors by the Chronic Administration of Morphine: Possible Evidence for an Anti-Opiate Model of Tolerance and Dependence

Richard B. Rothman, Hsiu-Ying T. Yang, and Joseph B. Long

INTRODUCTION

The mechanism(s) underlying the development of tolerance and dependence to opiates has been the subject of intensive investigation for several decades (1). The experimental systems used include clonal cell lines (2,3), smooth muscle bioassay systems (4) and whole animal studies (5). These various investigations have identified several potentially interactive mechanisms which include acute desensitization (6), receptor down-regulation (7), altered receptor interactions with G proteins (8) and the generation of spare receptors (4).

In a thorough review of this area of research, Smith et al (9) divided models of tolerance and dependence into two groups: "receptor models" which postulate that changes in the opioid receptors are of primary importance, and "homeostatic models" which postulate that factors extrinsic to the opioid receptors functionally antagonize the actions of morphine. They concluded that although no one mechanism could fully account for the phenomena which describe tolerance and dependence, the participation of endogenous substances which bind to opioid receptors, and block the effects of morphine, was likely on theoretical grounds. They further identified opioid peptides such as ß-endorphin(1-27) and dynorphin as candidate endogenous antagonists.

The hypothesis that chronic administration of morphine to animals triggers the secretion and/or activation of endogenous opioid antagonists was experimentally examined by Malin et al. (10) who demonstrated that CSF from donor tolerant/dependent rats, but not naive rats, was capable of precipitating withdrawal in recilpient tolerant/dependent rats. Unfortunately, the chemical identity of this substance, or substances, was never established.

The development of the opiate binding assay in 1973 (11-13) made it possible to examine the hypothesis of Collier (14) that chronic morphine altered opioid receptors. However, careful investigation in many

laboratories failed to confirm this hypothesis (15). In retrospect, it seems clear that these early studies, which were conducted before the recognition of opioid receptor subtypes, used [3H]ligands which we now know label more than one binding site. Thus it is possible that if chronic morphine altered only one of the binding sites labeled by the [3H]ligand, the change might not have been detected.

These considerations, and subsequent observations that chronic morphine produced an increase in the Bmax of opioid receptors labeled by [3H][D-ala2,D-leu5]enkephalin ([3H]DADL) (16) led us to reexamine the hypothesis of Collier using this [3H]ligand. Previous studies had demonstrated that [3H]DADL labels two binding sites: a higher affinity (δ_{ncx}) site at which mu ligands are weak competitive inhibitors, and a lower affinity (δ_{cx}) site, at which mu ligands are potent, noncompetitive inhibitors (17). Rats were made highly tolerant and dependent by the subcutaneous implantation of morphine pellets. The results demonstrated that the chronic administration of morphine produced a highly significant increase (45% to 65%) in the Bmax of the lower affinity [3H]DADL binding site without increasing the Bmax of the higher affinity [3H]DADL binding site (18). The total number of binding sites increased by 17%, confirming the original observations of Holaday et al. (16). Subsequent studies by Danks et al (19) further demonstrated that chronic morphine not only increased the Bmax of the δ_{cx} site, but also increased its Kd as well. Similar findings were obtained using [3H]DAGO to label mu opioid receptors (20).

The consistent finding of a chronic morphine-induced increase in the Bmax of the δ_{cx} binding site, an effect similar to that observed in rats chronically administered antagonists such as naltrexone (21) prompted speculation that the administration of morphine resulted in a secretion of endogenous antagonists, which like naltrexone, produce an increase in the Bmax of the opioid receptors. Consistent with this hypothesis, the CNS synthesizes and secretes peptides which are known to attenuate the actions of morphine. Some of these peptides, such as ß-endorphin(1-27) (22), dynorphin (23,24) and met5-enkephalin (25), presumably produce this attenuation via direct interactions at opioid receptors. Other peptides, such as α·-MSH (26), tyr-MIF (27) MMP-8 (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH2) (28) CCK-8 (29) and glucagon (30) presumably functionally attenuate actions of morphine via interactions at their own specific receptor.

These peptides, which we generically referred to as "anti-opiate" peptides, a term describing the ability of the peptides to attenuate the actions of opiates, were suggested to participate in the development of tolerance and dependence (18,27,28). According to the anti-opiate model of tolerance and dependence, the continuing administration of morphine results in increasing levels of anti-opiates, which attenuate the

effects of morphine, thereby maintaining homeostasis. Administration of an exogenous antagonist, such as naloxone, abruptly disturbs this homeostasis; resulting in the withdrawal syndrome. Synergism between exogenous and endogenous antagonists accounts for the observation that the more tolerant the animal, the less naloxone is required to trigger withdrawal. Finally, the anti-opiates upregulate the δ_{CX} binding site, much as administration of an exogenous antagonist would.

An attractive aspect of the anti-opiate model of tolerance and dependence, is that it generates several testable predictions. 1) Administration of morphine should increase CSF levels of anti-opiates; 2) I.c.v. administration of anti-opiates to a tolerant/dependent animal should trigger withdrawal. 3) I.c.v. administration of anti-bodies directed against anti-opiates should prevent the development of tolerance and dependence. 4) I.c.v. administration of anti-bodies directed against anti-opiates should prevent the morphine-induced increase in the Bmax of opioid receptors. 5) Chronic administration of anti-opiates should increase the Bmax of the same opioid receptor subtypes upregulated by chronic morphine.

A discouraging aspect of the anti-opiate model of tolerance and dependence is that there are so many peptides which potentially act as anti-opiates. Clearly, criteria must be established for deciding which anti-opiate peptides might play pivotal roles in tolerance and dependence, and therefore be appropriate candidates for additional research. It was felt that a peptide satisfying predictions 1-3 would be a suitable candidate for further study. In view of recent data demonstrating that 1) CSF levels of MMP-8 increase during chronic administration of morphine; 2) administration of MMP8 to tolerant/dependent rats triggers withdrawal; 3) that administration of anti-MMP-8 IgG to tolerant/dependent rats prevents naloxone-induced withdrawal and 4) that MMP-8 has its own CNS binding site, it was felt that MMP-8 more than satisfied the criteria mentioned above. In the present paper, we report on our experiments which begin to examine predictions 4 and 5.

METHODS

 Chroic Drug Studies. For the chronic MMP8 studies, stainless steel cannulae were surgically implanted in the lateral ventricle of male Sprague-Dawley rats (250-300 gm). These were connected to ALZET 2001 minipumps, filled with MMP-8 (5 µg/µl), which were implanted subcutaneously between the scapulae in the back of the neck. Brain membranes were prepared after 4 or 6 days of infusion. In the chronic morphine experiments, ALZET minipumps were filled with either control IgG or anti-MMP-8 IgG (1 µg/µl), which were prepared as previously described (31). These rats were then subcutaneously implanted with either placebo or morphine pellets, two on day #1, and four on day #2 as previously described (18). Brain membranes were prepared on day #5.

Preparation of Membranes. Each rat brain, minus cerebellum, was homogenized in 10 ml ice-cold .32 M sucrose containing 10 mM TRIS-HCl, pH 7.4, using a glass homogenizer and a teflon pestle. The homogenate was centrifuged at 1,000 x g for 10 min, and the supernatant centrifuged at 20,000 x g for 15 min. The P2 pellet was resuspended with 5 ml 10 mM TRIS-HCl, pH 7.4, and incubated for 15 min at 25° C. The lysed membranes were then centrifuged at 20,000 x g for 15 min, washed once by centrifugation, and the pellets kept at -70° C.

Ligand binding assay. Each pellet was resuspended into 10 ml of buffer (50 mM TRIS-HCl, pH 7.4). One ml was retained for analysis of protein using the Lowry method (32). The rest was added to a 50 ml tube containing 40 ml of buffer. Aliquots of the membranes (750 µl) were then added to 12 x 75 mm polystyrene test tubes prefilled with 50 µl of buffer, 100 µl of either drug or buffer, and 100 µl of [^{3}H][D-ala^2-MePhe^4,gly-ol^5]enkephalin ([^{3}H]DAGO) in a protease inhibitor cocktail (bacitracin [1.0 mg/ml], bestatin [0.1 mg/ml], leupeptin [0.04 mg/ml] and chymostatin [0.02 mg/ml]). Incubations, which proceeded for 4 to 6 hr at 25° C were terminated by rapid filtration over Whatman GF/B filters, using a Brandell MR24 cell harvester.

Experimental Design. The method of binding surface analysis, which has been described in detail elsewhere (33) was used. Briefly, two concentrations of [^{3}H]DAGO were each displaced by eight concentrations of DAGO (0.25 nM to 64 nM), generating 18 data points per membrane preparation. The binding surfaces of each experimental group (n=4 or 5) were pooled, and fit to the one site binding model for the best-fit estimates of the Kd and Bmax. Statistical differences between experimental groups were determined as described by Munson and Rodbard (34). The data of group 1 (parameters Kd_1 and $Bmax_1$) were fit simultaneously with the data of group 2 (parameters Kd_2 and $Bmax_2$) without any constraints. The data were then refit with the constraint that $Kd_1=Kd_2$, and again with the constraint that $Bmax_1=Bmax_2$. An increase in the sum-of-squares was tested for statistical significance using the F-test (34).

Chemicals. [^{3}H]DAGO (SA=47.8 Ci/mmol) was purchased from DuPont New England Nuclear Corp. Control IgG and anti-MMP IgG were prepared as previously described (31). MMP-8 was purchased from Peninsula Laboratories. The sources of other reagents have been published elsewhere (18).

RESULTS

As reported in Table 1, chronic i.c.v. infusion of MMP-8 (5 µg/hr) for 4 days produced a highly significant increase in the [^{3}H]DAGO Bmax, without altering the Kd. However, a 6 day infusion resulted in a small, but statistically significant decrease in the Bmax, without a significant change in the Kd.

TABLE 1

EFFECT OF CHRONIC I.C.V. INFUSION OF MMP-8 ON [^{3}H]DAGO
BINDING SITES

	Bmax (percent of control)	Kd (nM)
A. 4 day infusion		
VEHICLE	100±3	0.74±0.04
MMP-8	153±7.1*	0.66±0.06
B. 6 day infusion.		
VEHICLE	1 00±3	0.69 ± 0.04
MMP8	82.7±2.9*	0.75±0.05

Each parameter value is the mean±SD (n=4 or 5). *p<0.01 when compared to the vehicle group (F-test).

In the next experiment (Table 2), rats implanted with either placebo (PBO) or morphine (MOR) pellets received chronic i.c.v. infusions of either control IgG or anti-MMP IgG. The administration of control IgG did not alter the usual response to chronic morphine, which increased both the Bmax and Kd. Administration of anti-MMP IgG to the PBO rats also increased the Bmax, but did not significantly change the Kd. Concurrent administration of anti-MMP to MOR animals also substantially increased the Bmax, and attenuated the increase in the Kd produced by MOR.

DISCUSSION

In the present study we began to systematically examine several testable hypotheses of an anti-opiate model of tolerance and dependence, focusing on the phenomenon of morphine-induced upregulation of a specifically defined set of opioid receptors.

Consistent with the prediction that chronic i.c.v. infusion of MMP-8 will increase the Bmax of opioid receptors, we observed that a 4 day infusion of MMP-8 increased the Bmax to 153 percent of control, without altering the Kd. However after 6 days of infusion, the Bmax was slightly lower than control. One interpretation of these observations is that the CNS initially responds to an increased level of anti-opiate by increasing the Bmax of opioid receptors, but that later on other homeostatic mechanism(s) come into play, permitting the density of opioid receptors to return to normal.

Whereas chronic naltrexone increases the Bmax of [^{3}H]DAGO binding sites without altering the Kd, chronic morphine increases both the Kd and the Bmax. Thus, the effect of MMP-8 to upregulate [^{3}H]DAGO binding

sites resembles the effect of chronic naltrexone more than it resembles the

TABLE 2

EFFECT OF CHRONIC I.C.V. INFUSION OF ANTI-MMP8 IgG AND MORPHINE PELLETS ON [³H]DAGO BINDING SITES

	Bmax (fmol/mg protein)	Kd (nM)
CONTROL-IgG/PBO	176±7	1.09±008
CONTROL-lgG/MOR	272±11	3.09±0.10*
ANTI-MMP-8-lgG/PBO	228±6.5*	1.02±0.05
ANTI-MMP-8-IgG/MOR	288±8*	2.14±0.07*5

Each parameter value is the mean±SD (n=4 or 5). *p<0.01 when compared to the CONTROL-IgG/PBO group (F-test). §p<0.01 when compared to the CONTROL-lgG/MOR group (F-test).

effect of chronic morphine. It will be of interest to see if administration of MMP-8 produces a sensitization to morphine like that caused by the chronic administration of naltrexone (21).

Perhaps the most interesting finding of the present study is that chronic administration of anti-MMP IgG produces a substantial increase in the Bmax of [³H]DAGO binding sites without altering the Kd. Since the IgG probably does not readily penetrate into the brain from the ventricular space, these data support the hypothesis that MMP-8 in the CSF exerts a tonic negative control over the the density of [³H]DAGO binding sites. This interpretation is at odds with the results obtained from chronic i.c.v. infusion of MMP-8. Clearly, further experiments, including direct measurement of the MMP-8 receptor (35), will be required to address this paradox.

Unlike the prediction of the anti-opiate model that administration of anti-MMP IgG would prevent the morphine induced increase in the Bmax, both chronic morphine and chronic anti-MMP IgG increased the [³H]DAGO Bmax, but the effects were not additive. It seems possible, however, that perhaps another anti-opiate peptide might be responsible for mediating this effect. The observation that anti-MMP-8 IgG partially attenuated the ability of chronic morphine to increase the Kd suggests that MMP-8 may partially contribute to this particular effect of chronic morphine.

The data of the present study collectively indicate that there are important interactions between CSF MMP-8 and the mu opioid receptor. Considerably more work, including correlative behavioral tests, will be required to further explore the predictions of the anti-opiate model of

tolerance and dependence as well as the interactions between the MMP-8 and opioid systems.

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AFFILIATIONS

[1]Unit on Receptor Studies, LMC, NIDDK, Bethesda, MD 20892.
[2]Laboratory of Biochemical Genetics, NIMH Neuroscience Center at St. Elizabeths Hospital, Washington, DC 20032
[3]Neuropharmawlogy Branch, Walter Reed Army Institute of Research, Washington, DC 20307-5100

FMRF-NH$_2$ Like Mammalian Octapeptide in Opiate Dependence and Withdrawal

**David H. Malin, J. Ronald Lake, Maria V. Hammond,
David E. Fowler, Juan E. Leyva, Robert B. Rogillio,
Joy B. Sloan, Thomas M. Dougherty and Karen Ludgate**

There were early reports of a morphine-tolerance-inducing peptide factor in brain homogenate of morphine-tolerant rats (Ungar and Cohen 1966). Later studies (Ungar, Ungar, Malin and Sarantakis 1977; Malin and Radcliffe 1975) suggested that extracts of such homogenates, when injected i.p. into recipient animals, induced accelerated dependence formation and tolerance to morphine analgesia (tail pinch test). The extracts also had a naloxone-like morphine antagonist effect on the in vitro vas deferens assay. Han et al. (1980) found that intracerebral injection of brain extract from morphine-tolerant rats or from acupuncture-tolerant rats induced immediate tolerance to morphine analgesia. They characterized the active factor as a peptide. Lu et al. (1982) reported that intrathecal injection of cerebrospinalfluid (CSF) from tolerant rats induced tolerance to opiate analgesia. Wahlstrom and Terenius (1980) found that CSF from morphine-tolerant rats and human heroin addicts contained a factor capable of inducing morphine tolerance in recipient rats.

CONCEPT OF AN ANTI-OPIATE PEPTIDE

An anti-opiate peptide is one that, at least in some locations in the nervous system, counteracts the effects of endogenous and exogenous opioids. This could be accomplished through a number of different mechanisms. 1. The peptide might be a competitive antagonist at opiate receptors. 2. The peptide might stimulate the same cells that opioids inhibit via different receptors on the same target neurons. 3. The different receptors for opioids and anti-opiate peptides might form a complex in the cell membrane, such that the peptides would serve as negative modulators for the opioids, diminishing the cell's responsiveness to them. 4. The receptors for the anti-opiate peptides might even reside on neurons without opiate receptors that interact synaptically with other neurons that do have opiate receptors. In any of these cases, activation of anti-opiate peptides by chronic opiate exposure might hypothetically contribute to tolerance by, counteracting effects of exogenous opiates and to dependence by counteracting effects of endogenous opioid peptides.

CHRONIC EFFECT OF EXOGENOUS OPIATE ANTAGONIST

If altering the balance between endogenous opioids and endogenous

anti-opioid substances is responsible for a dependent/abstinentstate, one might expect that exogenous antagonists such as naloxone might also upset this balance and produce a similar state. However, naloxone administered acutely to a non-dependent organism does not induce a withdrawal syndrome (Malin et al. 1985). On the other hand, 7 days of twice daily naloxone injections (0.6 mg/kg s.c.) in a non-dependent rat resulted in a syndrome of wet shakes, scratches and aggression resembling moderate opiate abstinence syndrome. The signs were totally reversible by a small dose of morphine, but not by a renewed dose of naloxone. The same signs were seen after only a day of continuous naloxone infusion (0.7 mg/kg/hr) via subcutaneous Alzet osmotic minipump (Malin et al. 1985). In addition, there was a 53% increase in oxygen consumption, reminiscent of the respiratory hyperactivity routinely seen during opiate abstinence. Interestingly, the abstinence-like behavioral and respiratory signs receded a day later, despite continued naloxone infusion. Malin et al. (1986) found that this "endorphin blockade syndrome" was, like actual opiate abstinence syndrome, potently reversible by clonidine, suggesting both syndromes may share an underlying mechanism of noradrenergic hyperactivity.

ANTI-OPIATE PEPTIDE IN CSF OF DEPENDENT PATS

Perhaps the most striking action of exogenous anti-opiates such as naloxone is their precipitation of an abstinence syndrome in opiate dependent organisms. Perhaps a similar action might be used as an assay for the presence of endogenous anti-opiate peptides in the nervous system of opiate dependent rats.

This question led to the design of an assay procedure for abstinence-precipitating bioactivity in CSF (Malin et al. 1987). Recipient rats were rendered dependent since acute naloxone administration precipitates withdrawal only in dependent organisms. CSF was withdrawn from dependent donor rats after 6 hours of abstinence in order to allow time for clearance of morphine from the CSF; otherwise, the presence of morphine might mask the presence of anti-opiate substances in the CSF. The donor rats were exposed to a higher chronic morphine infusion rate than the recipients, since pilot experiments suggested that this combination was the most effective for precipitating withdrawal. The aim was to induce a large amount of endogenous opiate antagonist in the donor CSF relative to the amount of morphine in the recipient CSF. An i.c.v. injection was employed, since anti-opiate peptides or proteins might not readily cross the blood/brain barrier. CSF seemed to be the most natural, non-toxic material to inject into the ventricles. Finally, the third ventricle was chosen as the injection site. It was desired to use a site producing the most immediate abstinence signs. In a preliminary study, naloxone was injected at various ventricular sites inmorphine dependent rats, and the third ventricle resulted in the shortest latency symptoms (see Fig. 1).

CSF withdrawn from morphine-dependent rats 6 hours into abstinence precipitated an abstinence syndrome when infused into the third ventricle of morphine-dependent recipient rats. The effect was similar to that of infusing the opiate antagonist naloxone, suggesting that opiate dependent organisms might secrete an endogenous opiate antagonist substance. CSF withdrawn from non-dependent donors failed
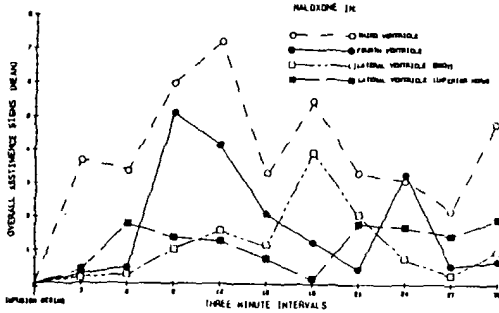
FIGURE 1

*Time course of abstinence
signs precipitated by
10 ug NX in various ven-
tricular sites.*
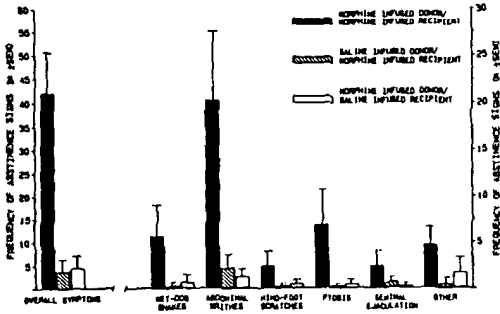
FIGURE 2

*Abstinence signs precipi-
tated by CSF from morphine
dependent and non-depen-
dent rats in dependent and
non-dependent recipients.*
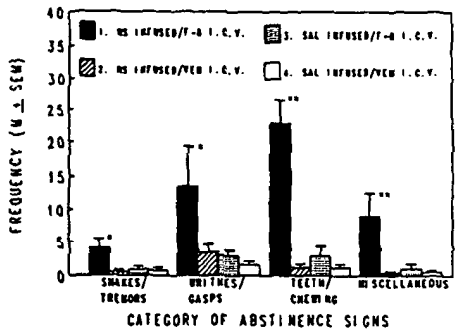
FIGURE 3

*Abstinence signs precipi-
tated by 2 ug $F-8-F-NH_2$
or saline i.c.v. in de-
pendent and non-dependent
rats.*
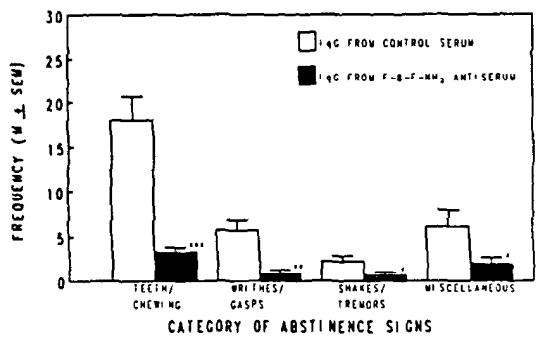
FIGURE 4

*Abstinence signs precipi-
tated by 10 ug NX i.c.v.
in rats pretreated with
IgG from $F-8-F-NH_2$ enti-
serum or control IgG.*

273

to precipitate an abstinence syndrome in morphine-dependent recipients, and CSF from morphine-dependent donors did not precipitate an abstinence syndrome in non-dependent recipients (see Fig. 2).

Additional experiments were carried out to provide a preliminary characterization of the active substance in the CSF from dependent rats (Malin et al. 1987). The abstinence-precipitating bioactivity of CSF from morphine dependent donors was destroyed by incubation with 3 different proteolytic enzymes, but it survived heating in a 100°C water bath. Bioactivity appeared in the filtrate, but not the retentate following ultrafiltration through a collodion filter with a 10,000 M.W. cut-off. All of these results are consistent with the hypothesis that the active factor in the CSF is a peptide or peptides. This substance remains to be further characterized.

F-8-F-NH$_2$, A PUTATIVE ENDOGENOUS ANTI-OPIATE PEPTIDE

One likely candidate for the role of an endogenous anti-opiate peptide is the octapeptide FLFQPQRF-NH$_2$ (F-8-F-NH$_2$), also known as FMRF-NH$_2$-like mammalian octapeptide or morphine-modulating octapeptide. Evidence supporting this conclusion is discussed in this volume in chapters by Yang, Kavaliers, Rothman and Raffa.

Criteria For Selective Involvement In Dependence

Undoubtedly, multiple transmitters, modulators and neurohormones are involved in narcotic dependence to some extent, if only to permit the expression of a particular abstinence sign or symptom. But what, in operational terms, does it mean to say that a particular neurohumoral substance is "selectively" or "specifically" involved in dependence? It seems reasonable that the substance meet at least the following 3 criteria in order to be considered as one of the specific or selective causes of dependence and subsequent withdrawal syndrome.

1. Selective presence. Elevated levels or activated release in dependent as opposed to non-dependent organisms.

2. Sufficient causation. The introduction or activation of this substance causes or precipitates abstinence signs, the only means of detecting and quantifying a state of dependence.

3. Necessary causation. Selective inactivation of the substance alleviates or reverses dependence as determined by absence of subsequent abstinence syndrome.

The following experiments evaluate a possible role for F-8-F-NH$_2$ in morphine dependence with respect to these 3 criteria.

ELEVATED LEVELS OF F-8-F-NH$_2$ IMMUNOREACTIVITY IN DEPENDENT PATS

A collaborative experiment with Dr. H.-Y.-T. Yang, NIMH, examined the effect of chronic morphine infusion on F-8-F-NH$_2$ immunoreactivity in CSF. Twenty-eight rats were rendered dependent by 7 days of continuous, s.c. morphine sulfate infusion at a rate of 1.5 mg/kg/hr (Alzet osmotic minipump). Twenty eight control rats were infused in the same manner with saline alone. CSF (approximately 180 μl) was

then withdrawn from the cisterna magna and stabilized by the addition of the aminopeptidase inhibitor bestatin and HCl, followed by heating in a 100°C water bath (Malin et al. In Press).

The F-8-F-NH$_2$-like immunoreactivity in CSF from morphine-dependent and control rats was characterized by HPLC coupled with F-8-F-NH$_2$ radioimmunoassay. The main immunoreactive peak of CSF eluted at the same position as the F-8-F-NH$_2$ immunoreactive peak of rat spinal cord. The CSF samples from dependent rats had 100% greater immunoreactivity than samples from saline-infused controls. This difference was significant, $t(40) = 1.89$, $p < .05$.

Chronic opiate exposure may be necessary to produce this elevation, since 4 hours of morphine infusion did not result in elevated F-8-F-NH$_2$ immunoreactivity in CSF. F-8-F-NH$_2$ levels may return to normal during abstinence, since F-8-F-NH$_2$ immunoreactivity did not differ in CSF drawn 6 hours after termination of morphine or saline infusion.

F-8-F-NH$_2$ PRECIPITATES OPIATE ABSTINENCE SYNDROME

An experiment was performed to determine whether F-8-F-NH$_2$ could, like CSF from morphine dependent/abstinent rats, precipitate an abstinence syndrome in morphine dependent rats (Malin et al. 1990). Recipient rats were cannulated in the third ventricle and continuously infused s.c. for 7 days with morphine sulfate (0.3 mq/kg/hr) or with saline vehicle alone. Half of each group were then gradually injected in the third ventricle with 2 µg F-8-F-NH$_2$ in 20 µl saline with 0.06 µg bestatin, while the other half were injected with saline and bestatinvehicle. Thus, there were 4 groups: morphine dependent rats injected with peptide, morphine dependent rats injected with vehicle, non-dependent rats injected with peptide and non-dependent rats injected with vehicle. Each rat was observed under "blind" conditions for 20 minutes. As Fig. 3 shows, only dependent rats injected with F-8-F-NH$_2$ displayed an appreciable number of abstinence signs. According to Dunnett's Test, this group differed significantly from all others in overall abstinence signs, as well as in each individual category of abstinence sign.

A higher dose of F-8-F-NH$_2$ can induce an abstinence-like syndrome or "quasi-morphine abstinence syndrome" (Collier et al. 1974) in opiate naive rats (Malin et al. 1990). Thirty-one rats were cannulated in the third ventricle and 7 days later injected through this cannula with 15 µg F-8-F-NH$_2$ in 30 µl saline with 0.06 µg bestatin, or with saline/bestatin vehicle, or with saline alone. In order to test whether the effects of F-8-F-NH$_2$ were morphine-reversible, a fourth group received the intraventricular F-8-F-NH$_2$ injection preceded by 3.5 mg/kg morphine sulfate s.c. Only the group receiving F-8-F-NH$_2$ without morphine displayed an appreciable number of abstinence-like signs. This group had significantly more overall abstinence signs, as well as significantly more signs in each major category.

PREVENTION OF NALOXONE-PRECIPITATED ABSTINENCE SYNDROME BY IgG FROM F-8-F-NH$_2$ ANTISERUM

This collaborative experiment with Dr. H.-Y.T. Yang, NIMH, determined

whether IgG from F-8-F-NH$_2$ antiserum could reverse the state of dependence induced by chronic morphine infusion, as evidenced by lack of subsequent naloxone precipitated abstinence syndrome (Malin et al. In Press). Fourteen rats were cannulated in the third ventricle and rendered dependent by infusion of 0.3 mg/kg/hr morphine sulfate s.c. for 7 days. Each rat was then gradually injected through the cannula with 12 µg IgG in 20 µl saline. Seven rats received IgG from F-8-F-NH$_2$ antiserum, while the other seven received control IgG from pre-immunized rats. Forty minutes later, each rat was injected through the same cannula with 10 µg naloxone.

Subjects pretreated with IgG from F-8-F-NH$_2$ antiserum had only 23.0% as many overall abstinence signs as rats pretreated with control IgG. This is a highly significant difference, $t(12) = 5.28$, $p < .001$. As shown in Fig. 4, rats pretreated with IgG from F-8-F-NH$_2$ antiserum had significantly lower frequencies of teeth chatter/chewing, writhes/gasps, shakes/tremors, and miscellaneous less frequent signs (seminal ejaculation, ptosis, scratches, diarrhea and hopping).

The guiding assumption of this experimental methodology was that the IgG and naloxone had to be present at the same brain sites at the same time for one to prevent the effects of the other. That is why both were 'introduced through the same cannula. Pilot experiments revealed that the time interval between injections was a critical variable, presumably because of the contrasting molecular size and diffusion rates of the IgG and naloxone.

DISCUSSION

There is at least preliminary evidence that F-8-F-NH$_2$ meets three criteria for selective involvement in opiate-dependence and abstinence: selective presence (in CSF from dependent rats), ability to cause abstinence-like symptoms, and reversal of symptoms through inactivation of F-8-F-NH$_2$. Antibody against F-8-F-NH$_2$ would not seem to be a promising treatment modality for opiate dependence, since IgG does not ordinarily cross the blood/brain barrier. However, it might be possible to synthesize F-8-F-NH$_2$ antagonists, perhaps F-8-F-NH2 analogs that bind to, but do not stimulate, F-8-F-NH$_2$ receptors. Such peptide analogs might conceivably be modified to cross the blood/brain barrier, making them potentially useful for reducing tolerance to opiate analgesics, for alleviating the state of dependence prior to detoxification or for managing abstinence syndrome.

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AUTHORS

David H. Malin, Ph.D.
J. Ronald Lake,
Maria V. Hammond,
David E. Fowler,
Juan E. Leyva,
Robert B. Rogillio,
Joy B. Sloan,
Thomas M. Dougherty,
Karen Ludgate
University of Houston - Clear Lake
Houston, Texas 77058

Illicit Drug Use During Pregnancy: Effects of Opiates and Cocaine on Human Placenta

M.S. Ahmed, D. Zhou, T. Schoof, C. Spong, K. Sagduyu, A. Agbas, and D. Maulik

Introduction

Human placental villus tissue contains components of the opiate system namely kappa receptors and their endogenous ligand Dynorphin 1-8. The purified receptor has a molecular weight of 63 kd and retains high affinity binding to its ligands (1). In spite of the lack of innervation in the placental tissue, opiate cholinergic interactions were demonstrated in an in vitro system (2,3). Agonists inhibit acetylcholine release and antagonists alone potentiate it. Antagonists reverse the inhibitory effect of agonists. A correlation exists between route of delivery and number of receptor binding sites. Abdominal deliveries have higher receptor sites than vaginal (4). Placentas obtained from patients abusing a mixture of pentazocine and pyribenzamine ("T's and blues") during pregnancy did not have any detectable opiate receptor binding sites (4). These results could be explained by receptor down regulation. We investigated this assumption and report here on the effect of chronic opiate administration during pregnancy (methadone program) on the number of opiate receptors and their function in placentas obtained from these patients.

Recently we identified a cocaine binding protein in human placental villus tissue membranes (48,000 xg pellet). The protein has an apparent molecular weight of 76 kd and an $S_{20,w}$ of 5.1. The protein has a high affinity (kd 16.7 nM) and low affinity (107.4 nM) binding sites for ^{3}H-cocaine. The binding of this protein to norcocaine, pseudococaine, nomifensine, imipramine, desipramine, amphetamine and dopamine indicate that it share some but not all the properties of the brain cocaine receptor (5). We report here on the effect of documented cocaine use prior to delivery on the placental ^{3}H-cocaine binding protein.

Results and Discussion

Placentas (n=4) were obtained from patients in a methadone program had no detectable opiate binding sites in a villus tissue membrane preparation. The ligands used in a radioreceptor assay were ^{3}H-Bremazocine, ^{3}H-EKC and ^{3}H-Dynorphin. The villus tissue released an amount of acetylcholine in vitro comparable to normal placentas (300±50 p.mole/g wet wt. tissue) (2,3), however the tissue did not respond to the inhibitory or stimulatory effects of agonists and antagonists. These results provide evidence for opiate receptor down regulation following chronic opiate administration in human placentas.

Placentas were obtained from patients admitting to cocaine use during pregnancy with a positive urine analysis for benzoylecgonine at delivery (n=12). The binding of ^{3}H-cocaine to its protein in this placenta indicated a shift in its affinity to the drug (Kd 45 & 150nM). The physiological function of the protein is unclear. However, the change in its affinity may result in an impairment of a placental function necessary for fetal development.

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Acknowledgements

Affiliation

University of Missouri-Kansas City Schools of
Basic Life Sciences and Medicine/Truman Medical Center

Substance Abuse Patterns in Pregnant Women

Janet S. Knisely, Evelyn R. Spear, Diane J. Green, James T. Christmas, and Sidney H. Schnoll

Recent evidence of the deleterious effects of substance abuse on pregnancy has focused attention on the early detection of abuse patterns in pregnant women. Random urine toxicology studies have demonstrated the prevalence of illicit drug use in various populations to be 10-25 percent. Even in patient populations previously thought to be at low risk for substance abuse in pregnancy, the prevalence of use is as high as 8 percent.

Pregnancy complicated by substance abuse in general, and cocaine in particular, is associated with increased perinatal morbidity and mortality. Pregnancy complications include increased incidence of spontaneous abortion, fetal death in utero, premature labor and delivery, small for gestational age infants, behavioral abnormalities in childhood, and possibly sudden infant death syndrome. The overall cost to society of substance abuse in pregnancy is difficult to calculate, but underscores the need for early identification, treatment, and prenatal monitoring of these high risk pregnancies.

Recent studies in the greater Richmond area have suggested increased substance abuse in the general population. Of particular concern is the marked increase in cocaine use, especially among women of childbearing age. In an attempt to identify patients at risk for substance abuse, as well as identify pattern of use, a modified version of the Lincoln Council on Alcoholism and Drugs Health Questionnaire is administered to all patients at the time of their first prenatal visit. The current study was undertaken to evaluate the accuracy of this questionnaire in an indigent, predominantly inner-city, pregnant population, as well as to further identify abuse patterns of this population.

The patient population consisted of 305 consecutive patients presenting to our routine obstetric clinic for their first prenatal visit. All patients were administered a questionnaire by a trained registered nurse. Urine samples *were* obtained for routine prenatal labs and a portion of each sample was screened for amphetamines, barbiturates, benzodiazepines, cannabinoid, benzoylecgonine, opiates, methagualone, phencyclidine, methadone, propoxyphene, and ethanol.

The overall prevalence of self-reported substance abuse in this population is similar to that previously documented in other major urban populations. Of 305 patients screened, 11% admitted to current drug use (within past 30 days) and 23% reported past drug use. The majority of those patients identified as current substance abusers reported using two or more substances. This has an obvious potential impact on information derived from studies that have evaluated the effects of one drug without regard to other substances. Also, those drug users identified by urine toxicology, self-report more use of drugs (primarily multiple past use) than the total sample population. Questionnaires such as the one evaluated in this study may help to identify patients at risk for current substance abuse by eliciting a history of past substance abuse.

Urine toxicology screening identified an additional 10% of this population as current users. Twice as many cocaine users and three times as many marijuana users were identified with urine toxicology than with the questionnaire. Thus, a more accurate reflection of the number of abusers is obtained using both screening measures.

AFFILIATION:

Substance Abuse Medicine
Virginia Commonwealth University
Medical College of Virginia
Richmond, Virginia 23298

Specificity of Conditional Responding to Naturalistic Stimuli in Humans With Different Drug-Use Histories

Ronald N. Ehrman, Steven J. Robbins, James R. MacRae, and Anna Rose Childress

There has been much interest in recent years in the possibility that environmental stimuli which reliably signal the administration of a drug acquire conditioned properties and motivate relapse. Evidence for this idea has come from studies demonstrating that drug-related cues (e.g., the presence of paraphernalia1 evoke drug-related responses in the laboratory (e.g., Childress, et. al. 1988).

To establish a conditioning basis for such responses, one must demonstrate both that addicts show greater responsivity to drug-related than to non-drug-related cues and that subjects lacking a drug history (drug-naive subjects) fail to show this difference. However, such results might only reflect that drug users are more responsive to unconditionally arousing stimuli than are non-users. Therefore, to conclude that drug cue responding is conditioned, one needs to demonstrate a cross-over pattern of responding in two drug-use populations. In other words, opiate addicts should respond more to opiate-related stimuli than to cocaine-related cues; cocaine addicts should show the reverse pattern. Such results would demonstrate that drug-cue responding results from a specific set of past conditioning experiences.

Individuals with a history of cocaine use but not opiate use and subjects with no history of cocaine or opiate use were tested in three laboratory sessions. No subjects with a history of opiate use alone were included due to their scarcity in our clinical population. Physiological and self-report measures of drug-related responding were collected while subjects were exposed to either cocaine stimuli, opiate stimuli, or non-drug-related stimuli. The stimuli consisted of a 10 minute audio tape, a 10 minute video tape, and a manual task.

Subjects in the cocaine-only group showed a strong tendency to

respond <u>only</u> to the cocaine stimuli. Following the cocaine video, these subjects showed significant increases from baseline in heart rate and craving for cocaine, and significant decreases in skin temperature and GSR. By contrast, the opiate video caused a significant decrease in skin temperature but no reliable change on the other measures. The decrease in skin temperature to the opiate video was significantly smaller than the decrease caused by the cocaine video. The only reliable change caused by the neutral video was a <u>decrease</u> in heart rate.

By contrast, drug-naive subjects were unresponsive to all three sets of stimuli. The only reliable change from baseline in this group is a <u>decrease</u> in heart rate during the opiate video.

These results are particularly striking given that the opiate video used here has been found to evoke strong responding in opiate addicts in earlier studies.   In other words, the opiate cues which failed to evoke responding in cocaine-only patients were nevertheless effective at producing arousal in subjects with the appropriate drug-use history.   To date, these results constitute the best evidence that responding to drug-related cues in the laboratory reflects a specific history of past conditioning.

AUTHORS

Ronald Ehrman, Ph.D., Steven Robbins, Ph.D., James MacRae, & Anna Rose Childress, Ph.D. are affiliated with the Veterans Administration Medical Center, Philadelphia, PA and the Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

A Comparison of Drug Use and Adjustment in Urban Adolescent Children of Substance Abusers

Janet Gross and Mary E. McCaul

Children of alcoholics and other drug abusers are at elevated risk for substance abuse and related psychosocial problems. Adolescents with a positive family history for drug abuse or alcoholism (FHP) were compared to a similar group of low SES, urban youth who were at risk for school failure but did not report any family history of substance abuse (FHN). A survey of depression, self-esteem, behavioral competence and dysfunction, and drug/alcohol use found that, overall, FHP adolescents exhibited more use of illicit drugs compared to FHN youth. A greater number of FHP cases fell into the clinical range on the psychosocial measures of behavioral dysfunction and depression compared to FHN youth. There were differential effects for boys versus girls, with FHP girls exhibiting a greater number of problem cases. Implications for substance abuse prevention with urban youth are discussed.

Johns Hopkins University School of Medicine

College Ungergraduate Drug Use and Life-Styles, 1969, 1978 and 1989

**Martin Ionescu-Pioggia, Deep Varma,
Harrison Pope, and Jonathan Cole**

We examined long-term trends in college drug use and life-style by conducting anonymous questionnaire studies of college seniors at the same institution on three separate occasions over 20 years: 1969, 1978, and 1989. The methodology employed in the three studies was virtually identical; a copy of the original questionnaire is given in Walters, Goethals, & Pope, Arch Gen Psychiat, 1972. Identical comparisons were performed in the three studies. Non-users (those who never have used an illicit drug) were compared with all users (those who used any illicit drug $\geq$ once). Next, non-users were compared with "hallucinogen users" (those who used LSD or another hallucinogen $\geq$ once). Non-users were also compared with "old users" (those who first used an illicit drug $\geq$ 3 years ago).

Between 1969 and 1978, the most striking difference observed in incidence of drug use was the dramatic surge in cocaine use reported in 1978 (Pope, Ionescu-Pioggia, & Cole, Arch Gen Psychiat, 1981). Whereas in 1969 not one respondent reported having used cocaine >10 times, 8.5% of the respondents in 1978 claimed to have used the drug >10 times, while a striking 30.4% reported having used it $\geq$ once. In addition, although incidence of most drug use remained relatively unchanged between 1969 and 1978, prevalence of both weekly use of alcohol and marijuana increased significantly. By 1978, weekly alcohol use had risen from 38% to 44% and, more strikingly, weekly marijuana use had risen from 16% to 26%, suggesting the drug's increasing social acceptability.

Nevertheless, by 1989, aside from incidence of alcohol use, all other forms of drug use had decreased significantly from their levels in both 1969 and 1978, especially cocaine use, which had peaked so markedly in 1978 (Pope, Ionescu-Pioggia, Aizley, & Varma, in

press). Of special note, weekly marijuana use dropped off significantly from 26% of the respondents in 1978 to 5.7% in 1989.

In all study years, only a small number of significant differences were observed between the non-user group and the three comparison groups on life-style indices. In none of the years was any statistically significant difference found between non-users and drug users in relation to grades, athletics, or other undergraduate activities. In 1969, however, the "alienation" question elicited significantly more positive responses from users as compared to non-users. By 1978, only two parameters continued to distinguish non-users from drug users: visits to a psychiatrist and heterosexual activity. As in 1969, users were more likely to have seen a psychiatrist than non-users. Heterosexual activity was again more prevalent among drug users than non-users; whereas only half of the non-users reported having had sexual intercourse with at least one partner, roughly 90% of users, had engaged in similar activity. It is interesting to note that these frequencies were virtually identical to those reported in 1969. No differences in homosexual activity emerged between non-users and drug users in any of the three study years.

In 1989, drug users continued to report having made more psychiatric visits than non-users. As in 1969 and 1978, approximately 90% of drug users reported having had intercourse with at least one partner, whereas only 52.1% of non-users reported the same extent of heterosexual activity. Perhaps more strikingly, the observation that respondents in 1989 reported almost identical frequencies of heterosexual activity as in the previous years, along with the consistency of weekly alcohol use (roughly 40%) over the course of the three studies, suggests that although illicit drug use has decreased significantly, the pursuit of "pleasurable activity" by undergraduates has remained unchanged over 20 years.

AFFILIATIONS: McLean Hospital-Harvard Medical School, Burroughs Wellcome Co. and Duke University Medical School.

Impulsivity and Substance Abusers: Changes With Treatment and Recovery

J.M. Jensen, H.M. Pettinati, B.D. Evans, K. Meyers, and V.N. Valliere

This study evaluated impulsive characteristics in two groups
of substance dependent subjects to further investigate the
relationship of impulsivity as an enduring personality
trait, versus when it changes with treatment and recovery.
Patients (N=160) in a 28-day residential treatment program
for cocaine or alcohol use disorders were given a self-
report measure on impulsivity derived from the California
Psychological Inventory and the Current and Past
Psychopathology Scales S's were asked to complete this at
admission and at 1, 3, and 6 months post-treatment. DSM-
III-R Axis I and II diagnoses were made using the Structured
Clinical Interview for DSM-III-R (SCID) and Structured
Clinical Interview for DSM-III-R Personality Disorders
(SCID-II) during the last half of intensive treatment
(approximately 4-6 weeks of treatment). Axis II assessment
excluded behaviors due to alcohol or drug use.

The mean pre-treatment impulsivity score for the total
sample (N=160) was 6.8 (range 0-14, $\underline{sd}$=3.4). $\underline{S}$'s with dual
diagnoses were significantly more impulsive than $\underline{S}$'s who
were not psychiatrically complicated (Axis II only vs. No
Psych. Dx.: X=7.7 vs. 6.0, $\underline{t}$=2.4, $\underline{df}$=96,_ $\underline{p}$ <.02, two-
tailed; Axis I and II vs. No Psych. Dx.: X=8.8 vs. 6.0,
$\underline{t}$=2.8, $\underline{df}$=78, $\underline{p}$ <.01, two-tailed). S's with an Axis II
personality disorder in DSM-III-R Cluster B or Cluster C
were significantly more impulsive at admission than S's
with no Axis II diagnoses (B vs. No Axis II Dx.: X=9.0 vs.
6.1, $\underline{t}$=3.64, $\underline{df}$=100, $\underline{p}$ <.001, two-tailed; C vs. No Axis II
Dx.: X=8.3 vs. 6.1, $\underline{t}$=2.64, $\underline{df}$=99, $\underline{p}$ <.02, two-tailed). No
significant differences were noted between S's in Cluster A
compared to S's with No Axis II diagnoses. Impulsivity
decreased significantly for the $\underline{S}$'s (N=87) still-in the
study at 1 month post-treatment (pre vs. post: X=6.8 vs.
4.5, $\underline{t}$=7.22, $\underline{df}$=85, $\underline{p}$ <.001, two-tailed). By clusters,
significant decreases in impulsivity from pre- to one month
post-treatment were noted for Clusters B and C and the $\underline{S}$'s
without an Axis II diagnosis (pre vs. post: Cluster B,

287

X=8.9 vs. 5.4, t=3.3, df=18, p <.01, two-tailed; Cluster C, X=6.7 vs. 3.3, t=3.2, df=24, p <.01, two-tailed; no Axis II, X=6.3 vs. 4.3, t=3.1, df=102, p <.01, two-tailed).

An item analysis revealed differences by gender and substance in areas of decision making, thrill seeking and temperament. Although Cluster A S's did not differ from S's without an Axis II personality disorder, Clusters B subjects and C subjects did. Overall, both cocaine and alcohol subjects decreased in impulsivity by one month post-treatment.

CARRIER FOUNDATION, NEW JERSEY

Cathinone, A Phenylpropylamine Alkaolid from Khat Leaves that Has Amphetamine Effects in Humans

P. Kalix, S. Geisshusler, R. Brenneisen, U. Koelbing, and H.-U. Fisch

The leaves of the khat* bush, which is grown in certain regions of East Africa and of the Arab Peninsula, have a stimulating effect, and they are therefore chewed habitually by many people living in the that region. Since khat use may be compulsive, the habit has been seen as a phenomenon of drug addiction; indeed, khat is known to induce moderate but persistent psychic dependence (Eddy et al., 1965).

Khat has been almost unknown in other parts of the world because only fresh leaves are active, but due to the availability of air transport, the drug has now made its appearance in London (Gough and Cookson, 1984), Rome (Nencini et al., 1989) and New York (Browne, 1990). This is worrying because khat consumption may induce toxic psychosis; a number of such cases have recently been reviewed in the literature (Pantelis, et. al., 1989).

A reinvestigation of the constituents of the khat plant by the United Nations Narcotics Laboratory (Szendrei, 1980) has led to the identification of the alkaloid cathinone in the leaves, which is now considered to be the main psychoactive constituent of khat. Indeed, results of experiments in animals and in isolated tissues indicate that this alkaloid is a potent amphetamine-like substance (WHO Advisory Group, 1980; Kalix and Braenden, 1985). We decided therefore to evaluate the effects of cathinone in humans under controlled conditions, and thus to determine the role of this alkaloid in the syndrome that is observed after khat consumption.

We administered cathinone in individual sessions to six healthy male volunteers using placebo-controlled balanced experimental design. The drug was given per os in gelatine capsules as hydrochloride in an amount corresponding to 0.5 mg base per kg body weight; this dose corresponds to the consumption of 100 g khat having average cathinone content (Geisshtisler and Brenneisen, 1987). Blood pressure and heart rate were automatically recorded with a monitor, and

*Catha edulis, Celastraceae.
 Common names: khat, qat, gad, tschat, miraa etc.

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
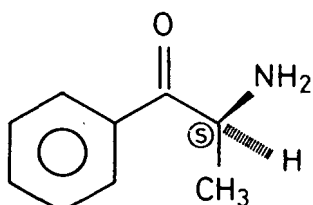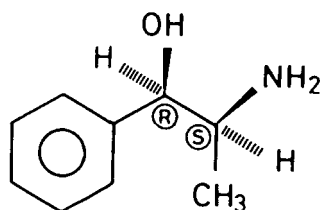
(-) CATHINONE

(-) NOREPHEDRINE

blood samples were drawn for the determination of the plasma concentrations of cathinone and of its metabolite norephedrine by HPLC. The subjective and psychic effects of cathinone were evaluated by submitting to the subjects sets of questions developed for recognizing amphetamine effects in humans (Martin et. al., 1971).

Cathinone produced clearcut increases in blood pressure and heart rate, effects which can be seen as reflecting the sympathomimetic syndrome that may appear after khat consumption; these changes coincided with the presence of cathinone in blood plasma. Cathinone underwent rapid metabolic reduction to norephedrine, this metabolite did not sustain the effects of cathinone. The subjective effects reported by the test persons showed that cathinone has in humans marked psychostimulant and euphorigenic effects; these can be expected to reinforce the habit of chewing khat.

Our results implicate cathinone as the dependence-producing constituents of khat, and they agree with the concept that cathinone is mainly responsible for the syndrome observed after khat consumption. The objective and subjective effects of cathinone in humans that we have observed may lead, together with epidemiological data, to a reconsideration of the use of khat as a stimulant and social drug.

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AFFILIATION

Department of Pharmacology[1], University of Geneva, Switzerland
Department of Phytochemistry[2], and Department of Psychiatry[3]
University of Berne, Switzerland

A Substance Abuse Consultation Service: Characteristics of Patients and Pedagogical Potential

F.R. Levin, W.W. Weddington, C.A. Haertzen, A. Cohen, and D.A. McDuff

Substance use disorders occur commonly among persons who use medical, surgical, or psychiatric clinical services. In spite of high prevalence rates, oftentimes physicians fail to detect substance use disorders, particularly alcoholism. A method to teach medical students and residents as well as to offer intervention services to substance abusing patients in a teaching hospital is to offer a substance abuse consultation service (SACS). To learn if a SACS had clinical or teaching potential, we conducted a pilot study to determine characteristics of referred patients of SACS in a 700-bed university teaching hospital.

Two hundred eighty-nine inpatients were referred over a three month study period. A majority of patients were on the medical service, although one-third were psychiatric inpatients. Alcohol was the most frequently ever-abused substance (68% of patients), followed by cocaine (53%), opiates (46%), and marijuana (22%). Excluding nicotine dependence, polysubstance abuse was frequent; over 60% of patients reported ever abusing two or more substances concurrently. Interestingly, although phencyclidine (PCP) was infrequently reported by patients on all services, significantly more patients on psychiatry reported abuse of PCP compared to patients on medical or other services. We found that there was a greater percentage of black drug users on the medical and surgical services compared to the psychiatric service whereas patients on the psychiatric and surgical services tended to be younger than the medically ill patients. Drug-abusing patients on the medical and surgical services were significantly more likely to have received prior drug abuse treatment than the drug users on psychiatry. Over two-thirds of the patients reported being jailed or incarcerated. Surgical patients reported the longest periods of incarceration.

Generally, all evaluations performed by the consultation team included patient education. Whenever necessary, pharmacologic management was provided. Patients were also given treatment referrals to inpatient rehabilitation, methadone maintenance, and drug-free programs. There were significantly more referrals to outpatient drug-free programs on the psychiatric service whereas there were significantly more referrals for methadone treatment on

the medical and surgical services. This is not surprising given that there were more IV drug users on these two services. We did not find a significant difference for treatment acceptance or refusal among the various services.

The SACS was utilized by the medical staff and provided patients for a variety of services, including psychiatry, to trainees for interviewing, diagnosing, interventions, recommendations regarding pharmacological and behavioral treatment, and referral. Results of our study point out that liaison activities would complement consultation services. Although not addressed by our study, it is important that learning and behavioral outcome by trainees on a SACS be compared to student who receive training on more traditional psychiatric clerkships.

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Francis Rudnick-Levin, M.D., Department of Psychiatry, University of Maryland and the Addiction Research Center of the National Institute on Drug Abuse.

William W. Weddington, M.D., Department of Psychiatry, University of Maryland and the Addiction Research Center of the National Institute on Drug Abuse.

Charles A. Haertzen, Ph.D., The Addiction Research Center of the National Institute on Drug Abuse.

Arthur J. Cohen, B.A., Department of Psychiatry, University of Maryland.

David A. McDuff, M.D., Department of Psychiatry, University of Maryland

Preliminary Report on Psychiatric Comorbidity in Adolescent Substance Abusers

Spyros J. Monopolis, Robert K. Brooner, Regina M. Jadwisiak, Emily Marsh, and Chester W. Schmidt, Jr.

INTRODUCTION

Substance abuse in adolescence has been related to high rates of concurrent psychopathology, including disorders of conduct, mood, and anxiety. This is a preliminary report on the prevalence of psychopathology among adolescents admitted to an outpatient substance abuse treatment program and its relationship to previous psychiatric or substance abuse treatment, a history of arrests and physical violence, HIV high risk sexual behaviors (multiple sexual partners, sex without use of condoms), self-harming behaviors (suicidal thoughts/threats and attempts/gestures), and family history of psychiatric or substance abuse problems.

METHODS AND SUBJFCTS

Our data were collected through a detailed chart review of all consecutive admissions from July 1, 1988 - June 30, 1989. Diagnoses were made according to DSM III-R. The sample consisted of 141 subjects, 86 (61%) males, 55 (30%) females, 112 (79%) white, 24 (17%) black and 5 (4%) other. Their age range was 11-19 years (mean 15.88 years). Statistical analysis of the obtained results was performed through the use of X^2 (chi square) to calculate differences in percentages between groups.

RESULTS

Sixty-one percent of the subjects had a substance use disorder. The most prevalent diagnoses were for alcohol (37%) and cannabis (24%). Sixty percent had a non-substance use psychiatric disorder. Conduct disorders (40%) and adjustment disorders (13%) were the most common diagnoses made. Males had a significantly higher prevalence of any substance use disorder (p < .001), cannabis use disorder (p < .005) and conduct disorder (p < .001). Females had a significantly higher prevalence of adjustment (p < .05) and mood disorders (p < .005). Youngsters with any psychiatric disorder showed significantly higher prevalence

293

of past psychiatric treatment (p < .025), family history of
psychiatric problem (p < .025), arrests in last 24 months (p <
.05), and a history of assaulting others (p < .001). Adoles-
cents without a psychiatric disorder had a significantly higher
prevalence of substance use disorder in general (p < .05), and
alcohol use disorder (p < .05) specifically. Subjects with a
substance use disorder had a significantly higher prevalence of
a family history of substance abuse (p < .05), and HIV high-risk
sexual behaviors (p < .005). Youngsters without a substance use
disorder evidenced a significantly higher prevalence of psychia-
tric disorder in general (p < .005), and adjustment disorder (p
< .05) specifically.

DISCUSSION

Concomitant psychiatric disorder was fairly common in this
cohort of adolescent substance abusers. As expected, males
tended to present with conduct disorder and females with adjust-
ment and mood disorders. The presence of any psychiatric disor-
der was related to significantly more illegal and aggressive
behaviors, past psychiatric treatment, and a family history of
psychiatric problems. Surprisingly, the presence of a psychia-
tric disorder was not significantly related to self-harming
behaviors or HIV high-risk sexual behaviors. The presence of a
substance use disorder was, however, related to higher rates of
HIV high-risk sexual behaviors, as well as a family history of
substance abuse. These findings are generally consistent with
our studies of adult substance abusers. These preliminary data
support the need for further systematic studies in this area.

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AFFILIATIONS

Departments of Psychiatry, Francis Scott Key Medical Center
Johns Hopkins University School of Medicine, Baltimore, MD 21224

Putative Aminoalkylindoles (AAI) Antagonists

F.M. Casiano, R. Arnold, D. Haycock, J. Kuster, and S.J. Ward

INTRODUCTION

Aminoalkylindoles (AAIs) are a new class of antinociceptive molecules proposed to interact with a cannabinoid receptor (see Ward et. al., these proceedings).

One essential criterion to define the AAI receptor as a cannabinoid receptor is the demonstration of the existence of competitive antagonists with similar potency against both AAI-mediated and cannabinoid-mediated effects. Thus, the purpose of the present study was to identify AAI antagonists and determine their effects against cannabinoid-mediated events.

METHODS

Putative AAI antagonists were identified as AAI molecules with affinity for the AAI binding site radiolabelled with [^{3}H]-Win 55212-2 yet devoid of significant inhibitory activity in the electrically stimulated mouse vas deferens (MVD) preparation. Putative cannabinoid antagonist activity was identified using delta-9 tetrahydrocannabinol (THC) as a prototypic cannabinoid agonist.

RESULTS AND DISCUSSION

Several AAI analogs had affinity for the AAI binding site, did not demonstrate inhibitory activity in the HVD preparation, yet did attenuate the agonist actions of AAIs and THC in this assay. AAI receptor binding affinity determined by competition anaylsis in the Win 55212-2 binding assay (see Haycock et al., these proceedings) was highly correlated (r = 0.96) with antagonist potency determined from Schild anaylsis in the MVD preparation (n = 5 analogs). The in vitro pharmacological profile of these AAI antagonists is illustrated by an AAI analog:
[5-Bromo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]
(4-methoxyphenyl)methanone

The AAI analog in Figure 1 produced rightward shifts in the
concentration-effect curves for pravadoline and Win 55212-2 in
the MVD preparation. While the AAI antagonist produced a
reduction in the Emax for the concentration-effect curve of
pravadoline, the rightward shifts in the concentration-effect
curve for Win 55212-2 were parallel. A similar rightward
parallel shift in the concentration-effect curve for the
cannabinoid THC was seen over the same AAI antagonist
concentration range. The potency of the AAI antagonist in the
MVD assay is summarized in Table 1; the IC_{50} for the antagonist
analog to inhibit [^{3}H]-Win 55212-2 binding was 515 nM.

Table 1
Potency of MI Antagonist in The MVD Preparation

agonist	pA2	slope
pravadoline	6.6 (6.3 - 6.9)	-1.3 (-1.7 - -0.9)
Win 55212-2	7.3 (6.7 - 7.9)	-0.7 (-0.9 - -0.5)
T H C	6.5 (6.1 - 6.9)	-1.06 (-1.4 --0.7)

It may be seen from Table 1 that the potency of the AAI
antagonist against both AAIs and THC is similar, suggesting
that AAIs and THC may interact with the same receptor. This
conclusion is consistent with that drawn from the actions of
AAI and cannabinoid agonists in AAI and cannabinoid binding
studies (see Ward et al. and Haycock et al., these
proceedings).

The data also suggest that the antagonism of AAIs and THC by
the molecule in Figure 1 is competitive in nature. Thus, the
AAI antagonist analog produced an increase in the Kd for the
binding of [^{3}H]-Win 55212-2 to rat brain membranes, without
increasing Bmax (data not shown). In addition, the Schild
slopes for the interaction of the AAI antagonist analogs with
both AAIs and THC did not differ from -1.

The antagonism of AAI and cannabinoid agonist actions is
selective since the AAI antagonist analog. at concentrations up
to 10 uM, did not inhibit the MVD-inhibitory actions of
normorphine (data not shown).

In summary, AAI analogs that antagonize both AAIs and a
cannabinoid in a competitive manner can be identified using the
[^{3}H]-AAI binding and MVD assays. These molecules represent the
first cannabinoid antagonists in vitro.

AFFILIATION

Department of Neurosciences. Sterling Research Group, Sterling
Drug, Rensselaer, NT 12144

Evaluation of Ether and Free Hydroxyl Analogs of Delta-8, Delta-9, 11-THC as Cannabinoid Antagonists

D.R. Compton, W.R. Prescott, C.S. Siegel, P.M. Gordon, R.K. Razdan, and B.R. Martin

Although one mechanism of action of Δ^{t9}-tetrahydrocannabinol (Δ^{t9}-THC) is presumed to be a THC receptor, there currently is no strong evidence for the existence of a specific THC antagonist. The existence of such a cornpound may be crucial in determining whether the ligand binding site described[1,2] in fact a receptor via which cannabimimetic responses are produced. There has been one report[3] that the acid metabolite of Δ^9-THC will attenuate the cataleptic effects of the cannabinoids, which suggests a specific antagonist may exist. One qualified success in the quest for an antagonist is the fact that $\Delta^{t9,11}$-THC was found to significantly reduce the effect of Δ^{t9}-THC in the monkey.[4,5] Thus, it is reasonable to continue evaluating weakly active cannabinoids as potential antagonists. Oddly, though both the 1-methyl ether of Δ^{t8}- and Δ^{t9}-THC have been found to be inactive in the monkey,[6] neither of these compounds have been evaluated for possible antagonist properties.

The primary objective of this research effort was to design novel ether (and selected non-ether) analogs of Δ^8-, Δ^9-, and $\Delta^{t9,11}$-THC, and to evaluate these cannabinoids and parent ether congeners for agonist and antagonist properties. The compounds evaluated besides Δ^8-, Δ^{t9}-, and $\Delta^{t9,11}$-THC include: **1**) 8ß-hydroxy-$\Delta^{9,11}$-THC; **2**) 1-O-methyl-$\Delta^{t9,11}$-THC; **3**) 1-O-methyl-Δ^{t8}-THC; **4**) 1-O-methyl-Δ^9-THC; **5**) 1-O-biphenylmethyl-Δ^9-THC; **6**) 1-O-biphenylmethyl-$\Delta^{t9,11}$-THC; **7**) 1-O-(Cphthalimidobutyl)-Δ^{t8}-THC, 8) 1-O-(4-aminobutyl)-Δ^{t8}-THC; **9**) 1-O-(3aminopropyl)-Δ^{t8}-THC; 10) 1-O-(aminohexyl)-Δ^{t8}-THC 11) 1-O-(2-morpholinoethyl)-Δ^{t8}-THC, 12) 3-norpentyl-3-propyl-$\Delta^{9,11}$-THC; 13) 1-O-methyl-3-norpentyl-3-propyl-$\Delta^{9,11}$-THC; and 14) 8-(N-morpholino)-amino-$\Delta^{;9,11}$-THC. A complete description of the synthesis of these compounds is to be published elsewhere.

Activity of these 17 compounds was evaluated in mice using a multiple-evaluation procedure (locomotor activity, tail-flick latency, hypothermia, ring-immobility) and activity in rats determined in a discriminative stimulus paradigm. Cannabinoid methyl ethers previously considered inactive have been found to produce limited activity in the mouse, though the effects observed with the 1-

methyl ether of Δ^{t8}-THC (3) was different from that observed with the 1-methyl ether of Δ^{9}-THC (4). Compound 3 produced minimal effect ($ED_{50} > 100$ mg/kg) except in the tail-flick procedure ($ED_{50} = 33$ mg/kg), while compound 4 was largely inactive ($ED_{50} > 100$ mg/kg) except in the ring-immobility procedure ($ED_{50} = 17$ mg/kg). Additionally, though a large dose might be required, these data suggest that $\Delta^{59,11}$-THC might produce activity in humans (in contrast to previously reported contentions of inactivity), since the ED_{50} values for this analog varied between 5.4 and 56 mg/kg. In general, a correlation exists between activity in the mouse multiple-evaluation procedure and production of activity in the rat. None of the cannabinoid analogs were capable of attenuating the effects of Δ^{9}-THC (3 mg/kg) in either the rat (doses up to 10 mg/kg) or in the mouse (doses up to 30 mg/kg). Thus, the inactivity of these cannabinoids seems to be due to a failure to bind to or "recognize" molecular mechanism(s) of action, rather than a failure to "activate" those presumed mechanisms. It is also clear that those compounds with minimal activity are not mixed agonist-antagonists.

AFFILIATLONS
Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, and [†]Organix, Inc., Woburn, MA 01801

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Delta-9-THC Interactions With PCP and Ethanol in Rats Responding Under a Fixed Consecutive Number Schedule

Pamela Doty, Mitchell J. Picker, and Linda A. Dykstra

The effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) in combination with either phencyclidine (PCP) or ethanol were examined in two separate groups of rats responding under a fixed consecutive number schedule without an added external discriminative stimulus. Under this schedule, a minimum of 13 consecutive responses on the left lever followed by one response on the right lever produced food reinforcement. When administered alone, PCP (0.3-10.0 mg/kg), Δ^9-THC (0.3-5.6 mg/kg) and ethanol (0.3-1.0 g/kg) produced dose-dependent decreases in rate of responding. PCP and Δ^9-THC increased the number of runs to obtain one reinforcer (R/Rf) at doses that decreased rate of responding. Ethanol did not alter R/Rf. In each respective group of rats, a complete dose-effect curve for PCP or ethanol was redetermined in combination with six doses of Δ^9-THC. Δ^9-THC produced dose-dependent leftward shifts in the PCP dose-effect curves for rate of responding and R/Rf and in the ethanol dose-effect curve for rate of responding. The interactive effects of Δ^9-THC and PCP or Δ^9-THC and ethanol on rate of responding were dependent on the dose and behavioral end point evaluated (ED_{50} or ED_{75}). In some instances the nature of the interaction (additive, supra-additive or infra-additive) for rate of responding differed according to the model used to assess the interaction (dose addition or effect addition). The interactive effects of Δ^9-THC and PCP or Δ^9-THC and ethanol on R/Rf were additive when evaluated according to the effect addition model. (Supported by NIDA contract 271-87-8126).

AFFLIATION

Departments of Psychology and Pharmacology, University of North Carolina, Chapel Hill, N.C. 27599-3270.

Conformationally Restrained Aminoalkylindoles: Potent, Stereoselective Ligands at the Cannabinoid Binding Site

K.G. Estep, T.E. D'Ambra, E.M. Olefirowicz, M.R. Bell, M.A. Eissenstat, D.A. Haycock, and S.J. Ward

Analogs of pravadoline, an aminoalkylindole (AAI) analgesic, have recently been shown to interact with a cannabinoid binding site. The established SAR for AAIs indicates a morpholinylethyl side chain and a bicyclic aroyl sustituent at the indole 3-position are optimal for potency. A number of structurally diverse conformationally rigid AAIs were synthesized to explore the relationship between AAI activity and the spatial orientation of the amine side chain. By restricting the amine substituent to lie in the southeast quadrant of the molecule, the *in vitro* potency was increased by as much as 40-fold over the analogous acyclic analogs. A selected set of such compounds was resolved to probe the stereoselective requirements of the receptor. In each case, all of the AAI agonist activity was found to be mediated by the R enantiomer, which further restricts the amine substituent to lie below the indole plane.   An optically pure benzoxazine was tritiated and used to develop an AAI receptor binding assay. Activity in this assay has been shown to correlate well with functional agonist activity in mouse vas deferens isolated tissue preparations.   Nine diverse AAI structures were examined using CAMD techniques and a three-point pharmacophore model for *in vitro* AAI activity was generated. This model consists of the mutual positions in space of two aromatic moieties and the nitrogen atom of the AAI side chain, and is markedly different from the published cannabinoid pharmacophore.

Pravadoline
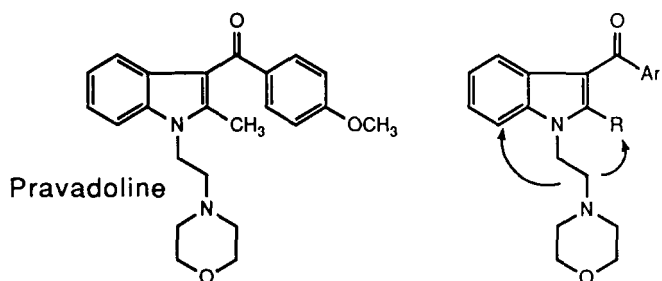
AFFILIATION

Departments of Medicinal Chemistry and Neuroscience,
Sterling Research Group, Rensselaer, NY 12144

Cannabinoid-Like Effects of Aminoalkylindole Compounds on the Physical Properties of Brain Membranes: Fluorescence Polarization Studies

J.C. Moldovan, S.J. Ward, and A.S. Bloom

A recently studied group of aminoalkylindole(AAI) analgesic compounds, of which Pravadoline is the prototype seem to exhibit a "novel" mechanism of action suggestive of binding to a receptor. These compounds are active in the mouse vas deferens test and also inhibit the activity of rat brain adenylate cyclase. Similar properties were also seen with some cannabinoids. Considering that the AAI group can show some other cannabinoid-like effects, we studied the influence of these drugs on artificial and biomembranes. It was previously reported that cannabinoids reduced the order within membrane bilayers, an effect considered to be consistent with fluidization of membranes. In these studies fluidity was determined using the fluorescence polarization technique. Low pharmacologically relevant concentrations of delta-9-tetrahydrocannabinol (THC) decreased the polarization of the fluorescence emission of DPH in synaptic plasma membranes(SPM). High concentrations (30 µM) of THC, increased DPH polarization (indicating that this drug decreased DPH mobility) in SPM. The purpose of this study was first, to compare the effects of the AAI compounds and cannabinoids on the fluidity of brain SPM, and artificially prepared membranes, utilizing three different probes. Secondly, we compared the effects of four AAI compounds, in order to determine their potency, within the AAI group of drugs.

METHODS

Male Sprague-Dawley derived rats weighing 275 to 300 g were used in all experiments. THC and AAI compounds were administered in vitro using DMSO as a vehicle. SPM were prepared from whole rat brain homogenates using Ficoll and sucrose density gradients. To make the vesicles, aliquots of lipid stock were placed into glass culture tubes and were dried under a stream of nitrogen. PBS was added to each tube (total lipid concentration was 90 nmol/ml) and the tubes were vortexed, then sonicated for 30 sec in a bath sonicator. This method produces large, multilamellar vesicles. The fluorescence polarization of DPH, TMA-DPH and 12-AS in membranes was determined using a SLM - 8000C T-format fluorometer.

 RESULTS AND CONCLUSIONS

I.	In SPM, compounds (+)-I and II, behaved similarly to THC when studied using the DPH probe (Fig 1). Polarization was decreased at low doses and

increased at high doses. This indicates that it had a biphasic effect on brain membrane fluidity.

II.   All the compounds increased the polarization (decreased fluidity) in brain measured using the 12-AS probe, as did THC.

III. With the TMA-DPH probe, a decrease in polarization was seen in SPM with all four drugs.

IV.   In vesicles prepared from egg-yolk PC, all four drugs increased polarization using either DPH or 12-AS. This effect was also seen with THC. With the TMA-DPH probe, the effect was biphasic, with a small initial decrease in polarization, followed by a larger increase.

V.   In vesicles prepared from EYPC and cholesterol (2:1), the DPH and 12-AS probes showed an increase in polarization, with all four compounds. In general, fluidity was increased measured using the TMA-DPH probe.

VI.   The rank-order potencies of the AAI compounds for fluidizing SPM, as measured, using DPH, correlated well with their relative potencies for inhibiting the mouse vas deferens contraction and inhibiting the activity of adenylate cyclase in brain. Furthermore, stereoselectivity was observed in both kinds of vesicles only when DPH was used. These data offer further support for the hypothesis that alterations in membrane properties are involved in the actions of cannabinoids and synthetic cannabinoids, such as the aminoalkylindoles.

EFFECTS OF AAI DRUGS ON BRAIN MEMBRANES DPH PROBE
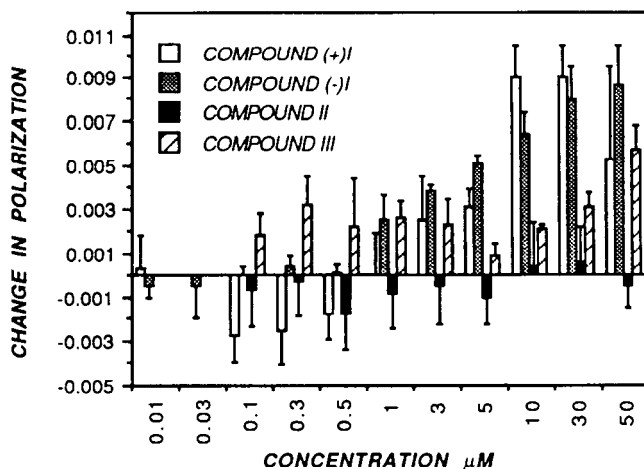
AFFILIATIONS

Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226 and Sterling Research Group, Rensselaer, NY 12144.

Characterization of Aminoalkylindole Binding: Selective Displacement by Cannabinoids

D.A. Haycock, J.E. Kuster, J.I. Stevenson, S.J. Ward, and T. D'Ambra

Aminoalkylindole (AAI) compounds are potent and efficacious antinociceptive agents. This antinociceptive activity is unrelated to the opioid system and, for many AAIs, to inhibition of cyclooxygenase.

Studies using the isolated mouse vas deferens preparation suggest a receptor mediated mechanism. There appears to be no link, however, between AAIs and purinergic, adrenergic, opioid, dopaminergic, GABA, serotonergic, neurokinin, bradykinin prostaglandin or cholinergic receptors studied in the mouse vas deferens assay (MVD) or in radioligand binding assays.

The purpose of this study was to characterize the binding properties of a potent AAI to further elucidate the mechanism of action of these compounds.

Radioligand binding studies were performed using male Sprague-Dawley rat cerebellar membranes from a 48,000 x g pellet which was washed twice by suspension in 20 mM HEPES buffer, pH 7. The final assay volume was 1 ml and included: 20 mM HEPES, pH 7; 0.5 nM [3H]-(R)-(+)-WIN 55212-2 (specific activity 59 Ci/mmOl, Dupont NEN); 1 mg/ml BSA; 100-120 ug cerebellar membrane protein and varying concentrations of competing compounds. Nonspecific binding was determined in the presence of 1 uM unlabeled WIN 55212-2. Compounds were solubilized in (1) a mixture of methane sulfonic acid-ethanol, (2) ethanol or (3) DMSO. The experiments were controlled for vehicle effects. Further dilutions of compounds or radioligand were in buffer containing 5 mg/ml BSA to prevent absorption to glass.

Incubation was initiated with the addition of tissue homogenate, carried out at 30 degrees C. for 90 min. and stopped by rapid filtration and rinsing over Whatman CF/B filters (presoaked in 5 mg/ml BSA) on a 48-channel cell harvester. Radioactivity on the filters was measured by liquid scintillation spectrometry.

The ligand appeared to bind to a single class of sites (Kd = 2 nM: Bmax = 1.2 pmoles/mg protein) and was saturable, heat-labile, reversible and stereospecific. The amount of binding varied with brain region and was reduced in the presence of GTP-analogs. Other AAIs in the series completely inhibited binding with potencies extending over a 1000 fold range. IC50 values correlated with ability to inhibit neuronally-induced contractions in the MVD (r = 0.98, P < 0.01). Over 60 compounds representing known neurotransmitter systems failed to inhibit binding. Cannabinoids, however, completely inhibited binding (IC50 0.5 - 3000 nM) in an apparently competitive manner.

These data suggest that the binding properties of [3H]-WIN-55212-2 satisfy the major criteria for describing a functional receptor, that the receptor recognizes cannabinoid compounds and that AAIs may exert their antinociceptive effects through the same mechanism as cannabinoids.

AFFILIATION:

Departments of Neuroscience and Chemistry
Sterling Research Group
81 Columbia Turnpike
Rensselaer, New York 12144

Marijuana Use by Methadone Maintenance Patients

Andrew J. Saxon, Donald A. Calsyn, Paul A. Blaes, Virginia M. **Haver, and Diane M. Greenberg**

Patients maintained on methadone commonly smoke marijuana, yet the impact of cannabis use on treatment outcome has received limited attention. Fewer than 30% of methadone programs nationwide screen urines for tetrahydrocannabinol (THC). However, a recent examination of THC in urine specimens of methadone patients found THC-positive specimens also frequently positive for other illicit drugs. To assess the effect of marijuana use in this population, weekly random urine specimens were obtained from 117 patients receiving methadone treatment at the Seattle VA Medical Center. Specimens were screened for opiates, cocaine, and benzodiazepines by enzyme immunoassay (EIA) with thin layer chromatography (TLC) confirmation. Monthly screening by TLC with EIA confirmation also detected miscellaneous drugs of abuse. Over 24 months specimens were also tested periodically for THC by EIA with gas chromatography/mass spectrometry confirm. The number of tests for THC varied from 1 to 17 with a median of 4. Patients also completed the Addiction Severity Index (ASI), the Millon Clinical Multiaxial Inventory (MCMI), the Rey Auditory Verbal Learning Test, the Shipley Institute of Living Scale, and the Trail Making Test.

Fifty-one percent of patients tested positive for THC at least once, and 20% tested positive every time. Whites (24.7%) were more likely than blacks (0%) to test consistently THC+ (χ^2 = 7.9, p < .01). THC-positive and THC-negative patients did not differ on numbers of UA's positive for cocaine, opiates, or benzodiazepines. However, patients consistently THC+ had a smaller percentage of positive UA's for other drugs of abuse ($\bar{X}$ = 8.0, σ = 12.6) than clients intermittently THC+ ($\bar{X}$ = 19.9, σ =4.4, U=253.5, p<.06). Only 8% of patients were consistently negative for both THC and other drugs during the testing period. Another 10% were consistently THC+ but negative for other drugs. Marijuana users were younger ($\bar{X}$ =42.4, σ = 6.5) than non users ($\bar{X}$ =45.9, σ =9.4, t=2.27, p<.05), but did not differ in race, employment, education, marital status, months in treatment, or methadone dose level.

THC+ patients reported more months of marijuana use lifetime and in the 30 days prior to admission on the ASI. THC+ patients obtained significantly higher scores on the MCMI scales of schizoid, avoidant, passive-agressive, schizotypal, and psychotic thinking. Marijuana users and non-users did not differ on any of the interviewer severity ratings from the ASI, including psychiatric severity. There were no differences between THC-positive and THC-negative patients on neuropsychological measures assessing abstraction, cognitive flexibility, or memory.

These findings suggest that methadone patients who use marijuana are no more likely to use other illicit drugs than those who do not use marijuana, and that past and current histories of marijuana use at time of entrance into treatment are good predictors of ongoing use in treatment. No evidence from this study supports the notion that users of marijuana perform more poorly in treatment or demonstrate more cognitive impairment than non-users. The MCMI results do imply that THC+ patients are more socially withdrawn and isolated and have more tangential thought patterns, but, overall, the routine use of THC urine screening in methadone treatment does not appear warranted at this time.

Affiliations
1. Seattle Veterans Affairs Medical Center
2. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle
3. Department of Laboratory Medicine, University of Washington, Seattle

Response to Marijuana as a Function of Potency and Breathhold Duration

James P. Zacny and L.D. Chait

Both anecdotal reports and laboratory studies indicate that marijuana smokers often hold the smoke in their lungs for prolonged periods (10-15 s), apparently in the belief that prolonged breathholding intensifies the effects of the drug. The present study examined the effects of systematic manipulation of breathhold duration (0 and 20 s) on the physiological and subjective response to active (M; 2.3% delta-9-THC) and placebo (P; 0.0% delta-9-THC) marijuana in a group of ten regular marijuana smokers.

During the 8-session experiment, subjects were exposed twice to each of four experimental conditions [P0; P20; M0; M20], scheduled according to a randomized block design. A controlled smoking procedure was used during each session in which the number of puffs (4) and puff volume (50 cc) were held constant. Dependent measures were assessed immediately before smoking and shortly after smoking (either 5 or 15 min afterwards). Expired-air carbon monoxide (CO) levels were used to monitor smoke absorption. Heart rate was used to measure the pharmacological effects of marijuana. Subjective effects were measured with three questionnaires: the Addiction Research Center Inventory (ARCI), a Visual Analogue Scale (VAS) and an End-of-Session (E-O-S) questionnaire. Breathhold duration most strongly affected CO absorption [Breathhold Duration: $F(1,9)=51.7$, $p<0.001$]; significantly more CO was absorbed from both P and M smoke after 20 s of breathholding (mean CO boost=6.9 ppm) than after no breathholding (mean=4.4 ppm). Smoking M increased heart rate [Drug: $F(1,9)=79.2$, $p<0.001$]; the mean increase in heart rate 5 min after smoking M was slightly greater after the 20-s breathhold (25 bpm) than after no breathholding (20 bpm), and this difference approached significance (paired t-test, $T=2.11$, $p<0.06$). Most subjective effects of marijuana, including scores from the Marijuana scale of the ARCI, "high" ratings from the VAS, and "marijuana peak effect" ratings from the E-O-S questionnaire, were unaffected by the breathhold manipulation.

The results confirm previous findings (Zacny and Chait, 1989) that prolonged breathholding does not substantially enhance the effects of inhaled marijuana smoke. It is possible that prolonged breathholding of marijuana smoke is largely a superstitious behavior. Alternatively, breathholding may have some adaptive value which was not apparent in the present study (e.g., reduction of the irritancy of the smoke when inhaled). Our results suggest that marijuana smokers could achieve the same level of intoxication while lowering their exposure to CO and other possibly toxic smoke constitutents simply by modifying a single aspect of their smoking behavior. (Supported by NIDA Grant DA-03517)

Affiliation: Department of Psychiatry, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois, 60637

Human Avoidance Responding With Added Point Loss During Acute Tobacco Abstinence

Don R. Cherek, Robert H. Bennett, and Ralph Spiga

Free-operant avoidance responding of male tobacco smokers during conditions of ad libitum smoking of their preferred brands was compared to responding during three tobacco abstinence conditions. The abstinence conditions were (1) placebo gum, (2) nicotine gum, or (3) no gum. During placebo and nicotine gum conditions, subjects were given two pieces of gum to chew thirty minutes prior to each of five sessions conducted at 0900, 1000, 1200, 1400, and 1600 hrs. Subject's responding was maintained by tandem schedule of point loss postponement. During one component a variable number of added, unavoidable point losses were presented. Overall response rates increased during nicotine abstinence conditions (placebo and no gum). The largest increases in response rate were typically observed during the ten seconds immediately following an unavoidable point loss. Results were discussed in terms of factors maintaining tobacco use.

Affiliation: Substance Abuse Research Center
                   Department of Psychiatry & Behavioral Sciences
                   University of Texas Health Science Center
                   1300 Moursund - UTMSI
                   Houston, Texas 77030

The Role of Nicotine Titration in the Smoking Behavior

C. Cohen, J. Le Houezec, C. Martin, and R. Molimard

The purpose of the study was to test the effects of smoked nicotine on smoking behavior and related subjective measures in smokers. Three cigarettes with varying nicotine delivery were used: A (0.6mg). B (subjects' usual brand, 0.72mg). C (1.76 mg). They delivered similar amounts of CO and tar in standard machine tests.

The subjects were tested after 9 h smoke deprivation. Each session included a fixed-rate smoking procedure of two cigarettes of one type and a 2 hours <u>ad</u> <u>libitum</u> smoking period.

Cigarettes C were stronger, less satisfying and caused more dizziness and palpitation than A and B. C decreased various measures of cigarette smoking including expired CO, amount of tobacco burned, and smoking rate during the subsequent 2h <u>ad</u> <u>libitum</u> smoking period. There were no differences in strength and smoking satisfaction between A and B. However, the first cigarette A was less potent in alleviating the self-reported need to smoke than B and subjects smoked a greater amount of tobacco from the second than from the first cigarette A.

The results show that the subjects regulated their nicotine intake and suggest that compensatory reaction to a decrease in nicotine delivery may not be immediate. These findings suggest that nicotine as well as sensory factors determine regulation of nicotine intake.

AFFILIATION

U.F.R. Biomédicale, Université Paris V
45, Rue des Saints-Pères, 75006 Paris, France

Smoking Cessation, Depression and Dysphoria

Sharon M. Hall, Ricardo Munoz, and Victor Reus

DEPRESSION AND SMOKING TREATMENT
Dysphoria has long been implicated in smoking treatment
failure (e.g. Guilford, 1966). Glassman et al. (1988)
found that subjects with a history of Major Depressive
Disorder were over-represented in smoking treatment. We
completed a study of the effects of history of Major
Depressive Disorder on quitting smoking. We
hypothesized that: (1) depressive history subjects
would show greater dysphoria at baseline than no
history subjects; (2) immediately after quitting,
subjects with a history of Major Depressive Disorder
would report greater dysphoria than subjects without
such a history: and (3) at 12, 26, and 52 weeks post-
treatment, depressive history subjects would be more
likely to relapse than no history subjects.

Subjects were men and women who smoked at least 10
cigarettes per day. All subjects (N=65) received
aversive smoking treatment plus relapse prevention
skill training. Assessments were held before
treatment, one and two weeks after the quit date, and
at 12, 26 and 52 weeks after quitting. Major
Depressive Disorder and Dysthymia sections of the
Diagnostic Interview Scale (DIS) were administered at
pretreatment. Smoking, moods, withdrawal symptoms, and
depressive symptoms were measured at each assessment.
Subjects also completed mood and withdrawal symptom
scales daily for the first two weeks after quitting
smoking. Carbon monoxide levels were obtained.
Outcome and psychometric data up to Week 26 were
available for analysis.

At intake, 46% of the 65 subjects had a history of
Major Depressive Disorder. Also, t-tests indicated
depressive history subjects scored higher than no
history subjects on the Profile of Mood States anger-
irritability scale (POMS; p<.03), the trait scale of
the State-Trait Anxiety Index (p<.03), and the Beck

Depression Inventory (Total Score, p<.04 and Cognitive Score p<.05). These subjects reported lower scores on the POMS vigor (p<.06) and higher scores on the PCMS fatigue (p<.06) scales. Pretreatment cigarette intake, Tolerance scale scores, and self-efficacy did not correlate with depression history, or with baseline moods. Thus, the correlations between depression and poor mood reflected more than greater nicotine dependence or negative reporting bias. We completed a series of hierarchical regressions with pretreatment POMS scale scores and history of major depressive disorder as independent variables, and scores during the first week after the quit date as dependent variables. Depression history subjects reported greater increases in POMS anger (p<.05) and depression (p.<.09) during the first quit week than no history subjects. We did not find comparable changes in physical withdrawal symptoms and craving.

We combined the DIS diagnoses from these 65 subjects with diagnoses from 33 subjects who participated in an earlier study of weight gain prevention following smoking cessation. In this combined sample, the frequency of a history of Major Depressive Disorder was 44%. At six months, in this combined sample (N=98), CMH $X^2(1)$=3.51, p<.06 for the association of abstinence status, presence of an intimate partner, and history of Major Depressive Disorder. Abstinent subjects with a history of Major Depressive Disorder were less likely to have a partner (Fisher's Exact Test, p=.004). Among subjects who relapsed at 26 weeks, there was no relationship between Major Depressive Disorder and partner status.

Considered together, these data suggest 1) a path by which a history of depression may lead to smoking relapse. It is through heightened dysphoria during quitting. 2) A history of Major Depressive Disorder may increase the probability of smoking relapse. This relationship, however, may be mediated by social factors.

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Effect of Repeated Nicotine Administration in Nonsmokers

Stephen J. Heishman, Frederick R. Snyder, and Jack E. Henningfield

Among the most well documented effects of nicotine on behavior is its potential to reverse the deficits in performance that may accompany tobacco abstinence (US DHHS, 1988). Such deficits have been reversed by either resumption of cigarette smoking (Revell, 1988; Snyder *et al.,* 1989) or administration of nicotine polacrilex (Snyder & Henningfield, 1989). The majority of evidence suggests that nicotine deprivation is a necessary condition for nicotine to produce reliable enhancement of performance; however, others have suggested that the potential of nicotine to enhance performance is a more robust effect, not limited to conditions of nicotine deprivation (Warburton, 1988; Wesnes & Warburton, 1983). Preliminary data from our lab indicated that nicotine did not enhance cognitive performance in nonsmokers (Snyder *et al.,* 1987). The purpose of this study was to examine further the effects of repeated nicotine administration in nonsmokers to determine if there are conditions under which performance enhancement occurs and the possible course of development of tolerance to nicotine's pharmacological effects.

METHOD

Participants were seven males 29-36 years old, who reported never smoking more than five tobacco cigarettes and never abusing any drug. Subjects lived on a residential research unit while participating in nine consecutive experimental days in which they were administered various doses of nicotine polacrilex for 15 min four times each day (0900, 1030, 1300, and 1430). Nicotine in the form of polacrilex gum was chosen because it provided a means to control dose and is of low abuse liability and toxicity. Before and after each dose of nicotine, subjects performed a battery of cognitive tasks and completed various subjective forms. Physiological measures were made before, during, and after each dose. Blood samples were taken 30 min after drug administration ended. Placebo was given on day 1. On days 2-9, four doses were administered each day in this order: 0, 2, 4, and 8 mg.

RESULTS

Performance on four cognitive tasks was either not affected or was impaired by nicotine. On a digit recall task, nicotine 4 and 8 mg increased the number of attempted trials and decreased mean response time to complete each problem. However, this increase in task speed was accompanied by a decrease in accuracy, which showed evidence of recovery to placebo levels with repeated dosing. Nicotine also decreased accuracy on a logical reasoning task; there was no evidence

of tolerance development. Performance on a letter searching task and rapid arithmetic was not affected by nicotine.

Nicotine produced dose-related increases in several subjective measures, including ratings of dose strength, LSD and MBG scales from the Addiction Research Center Inventory, and Total Mood Disturbance and Tension-Anxiety scales from the Profile of Mood States. Some measures showed evidence of tolerance development (LSD and Tension-Anxiety scores), whereas other did not (dose strength). Systolic blood pressure was increased only with the 8 mg dose of nicotine on days 2 and 3. In contrast, diastolic blood pressure and heart rate were increased by lower nicotine doses across all dosing days and revealed no evidence of tolerance development. Skin temperature was not consistently affected by nicotine. Plasma nicotine levels were increased dose-dependently across all dosing days.

DISCUSSION

This study demonstrated that nicotine polacrilex produced dose-related effects on several subjective and physiological measures. Over the course of 8 days of nicotine administration with an escalating dose sequence each day, tolerance was observed to develop to some, but not all measures. One of the main purposes of this study was to clarify the controversy concerning nicotine's purported enhancing effect on human performance. Much of the past reseach on this question has used smokers who were either tobacco deprived or not before being given a test dose nicotine. In both situations, the effect of the test dose of nicotine is confounded with pre-existing plasma nicotine levels and potential withdrawal effects. To avoid such confounding, this study tested nonsmokers. Our results indicated that nicotine did not enhance performance on any of four cognitive tasks and that accuracy was impaired on two of the tasks. These results agree with recent studies reporting no enhancement of cognitive performance by nicotine polacrilex in nonsmokers (Hindmarch *et al.,* 1990; Snyder *et al.,* 1987). However, these findings and those of the present study are not consistent with previous reports that nicotine enhances cognitive performance (Warburton, 1988; Wesnes & Warburton, 1983, 1984).

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AFFILIATION

Clinical Pharmacology Branch, Addiction Research Center, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224

Smoking Cessation for Adolescent Substance Users: Preliminary Considerations

J.L. Jaeger, K. Meyers, and P. Sargiotto

In addition to being a major public health problem, cigarette smoking, in particular, nicotine dependence, has finally gained recognition as a substance use disorder. Despite ample documentation regarding concurrent tobacco and substance consumption and a synergistic smoking-substance interaction leading to elevated health risks, smoking cessation efforts among substance users have received little research attention to date. Specifically, a paucity of data exists among adolescents and adults which examines the feasibility of urging substance users to curtail <u>both</u> smoking <u>and</u> substance use behaviors. Issues regarding the timing as well as technique of smoking cessation efforts and the impact of smoking cessation efforts on the maintenance of sobriety have also not been extensively investigated. To begin to address these issues among adolescents, prevalence of cigarette smoking was examined in a sample of adolescents who reported use of other substances in the past year (N=81; 74% regular users, 26% experiment only) and who were receiving either inpatient substance abuse or psychiatric treatment at a private, non-profit psychiatric hospital. A subsample of current smokers (N=31) and their parents (N=8) were selected to preliminarily examine indices of interest in smoking cessation.

Preliminary analyses document high prevalence rates of current smoking among this population (total sample: 77% smokers, 74% smoke daily; among regular substance users, 80% smokers, 79% smoke daily; among experimental users, 67% smokers, 57% smoke daily), and hence, a correspondingly high need for smoking cessation. Unfortunately, few smokers felt smoking needed to be addressed (22%), wanted to quit (40%), were considering quitting (29%). or would actually try to quit smoking (47%). Furthermore, demand for a smoking cessation program was lacking (less than 20% of adolescents were willing to participate). Interestingly, 87.5% of parents were supportive of proposed smoking cessation efforts although most (75%) felt it should be contingent upon their son/daughter's progress in treatment.

In an attempt to identify potential mediating factors of smoking cessation, adolescent subjects were grouped according to their intention to quit smoking. The two groups (do not intend vs. intend to quit smoking) did not significantly differ in terms of their knowledge of health consequences, personal relevance of smoking, and value and capability for stopping. They did significantly differ by their reasons for smoking; those not intending to quit smoking reported significantly more pleasure from smoking (X=13.5, s.d.=1.77 not intend vs. X=11.2, s.d.=1.20 intend; t=3.13, df=15, p=.007), more craving for cigarettes (X=13.6, s.d.=1.30 not intend vs. X=11.1, s.d.=2.52 intend, t=2.53; df= 15, p=.023) and more smoking out of habit (X=9.3, s.d.=1.67 not intend vs. X=5.9, s.d.=2.42 intend: t=3.29, df=15, p=.005) than those intending to quit. Since these variables suggest physical dependence though the groups did not significantly differ in their X cigarettes per day (X=18.3, s.d.=11.33 intend vs. X=25.8, s.d.=10.92 not intend; t=1.67, df=23, p=.ll), future work is needed to determine whether intent to quit is related to an attributional or physical state of dependence given their diverse implications for intervention.

Although additional work is clearly needed and an increase in the sample size critical, it appears that most adolescent substance users who smoke are not interested in smoking cessation. Of those who are, self-directed smoking cessation versus a formal program seems to be the preferred technique. Future work is needed among adolescent substance users who smoke to assess whether intention to quit will actually result in a curtailment of smoking, and to identify the appropriate timing of smoking intervention both in terms of client acceptability and intervention effectiveness.

CARRIER FOUNDATION, NEW JERSEY

317

Behavioral and Subjective Effects of Buspirone and Lorazepam in Sedative Abusers: Supra-therapeutic Doses

T.S. Critchfield and R. Griffiths

Two previous studies with substance abusers concluded that buspirone hydrochloride (Buspar®), a non-benzodiazepine anxiolytic, has little abuse liability at doses up to 40 mg. To date no study has evaluated higher doses for abuse liability. A double-blind acute dose run-up was conducted comparing buspirone (10, 20, 40, 60, 80, 100, and 120 mg) with the benzodiazepine anxiolytic lorazepam (1, 2, 4, and 8 mg) and placebo on subjective ratings and psychomotor performance. In a residential research unit, four male subjects (ages 27-36) with histories of recreational sedative abuse received active drug approximately every other day. Within subjects, buspirone doses generally occurred in an ascending sequence; lorazepam doses occurred in mixed order, interspersed among the buspirone doses. Psychomotor performance data and subject ratings were collected across 12 hours following drug ingestion. Mean subject ratings of drug strength increased with dose for both drugs, and peak strength ratings generally were similar for the highest doses. However, lorazepam elevated staff ratings of sedation more than did buspirone. In addition, on a drug identification questionnaire, high doses of lorazepam were most often identified as a benzodiazepine or a barbiturate, while high doses of buspirone were most often identified as an antidepressant, a phenothiazine, or other drugs. Lorazepam tended to produce dose-related impairment on circular lights, digit-symbol substitution, digit recall, and a picture memory task, while buspirone produced relatively little impairment. In contrast to subject ratings of drug strength (which were comparable for the two drugs), ratings of drug liking were of substantially lower magnitude and of shorter duration for buspirone than for lorazepam. The present data extend the abuse-liability characterization of buspirone by suggesting that even at supratherapeutic doses it produces less sedation, psychomotor impairment, and liking than a commonly prescribed benzodiazepine anxiolytic. Supported by NIDA grant DA03389.

AFFILIATION:
Johns Hopkins University School of Medicine, Baltimore, MD 21224.

Diazepam Preference in Males With and Without a Family History of Alcoholism

Harriett de Witt

Several lines of research, including both epidemiological and experimental data, suggest that there is an association between alcoholism and use or abuse of benzodiazepines. There is also growing evidence that alcoholism is influenced by genetic factors, raising the possibility that the tendency to use or abuse benzodiazepines may also be affected by the same genetic mechanism. The present study sought to determine whether a family history of alcoholism would affect the subjective and behavioral effects of diazepam. Fourteen light-drinking males with alcoholic first-degree relatives (family history positive; FHP) and no personal history of alcohol problems were compared to 13 controls with no known alcoholic relatives (FHN). Both groups reported drinking on average less than five drinks per week (FHN 4.23 and FHP 4.53). Subjects participated in a seven-session, double-blind laboratory choice procedure comparing diazepam (DZ; 20 mg) to placebo (PL). Four-hour sessions were conducted one or two evenings per week in a laboratory-based "recreational environment". On the first four sessions subjects sampled capsules containing either DZ or PL, and on the last three sessions they ingested whichever capsules they preferred. Dependent measures included: i) the number of times DZ was chosen over PL, ii) the dose of DZ ingested on choice sessions, and iii) subjective effects measures including drug liking ratings, drug identification and current mood states (using the Profile of Mood States; POMS)

The FHP group chose DZ slightly, but not significantly, more often than the FHN group (47% versus 38%), and they also
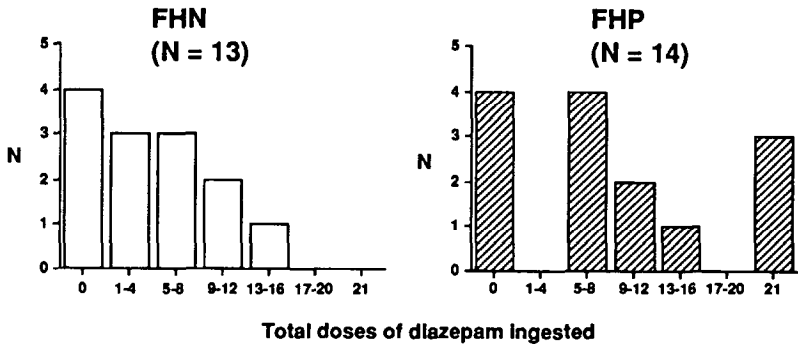
Fig 1 Total number of diazepam doses (4 mg each) ingested over three sessions (maximum 21), by subjects without (FHN) and with (FHP) first-degree alcoholic relatives.

ingested somewhat higher doses of DZ (24.4 mg versus 19.3 mg per- session for the FHP and FHN groups, respectively). Fig 1 shows the total diazepam ingested for the two groups. The two groups did not differ in their liking of the drug or in their drug identifications. Diazepam produced typical tranquilizer-like subjective effects in both the FHN and FHP groups, and did not produce differential effects on measures of "euphoria" in the two groups. The only group differences obtained were in subjects' overall ratings of mood states (regardless of drug): The FHP group scored higher on Arousal and Positive Mood scales and lower on Fatigue than the FHN group.

These results indicate that a family history of alcoholism does not necessarily influence subjective or behavioral responses to acute administration of diazepam, suggesting that it also does not infer a greater risk for developing problems of excessive use or abuse with this class of drugs. Whether these conclusions would apply to individuals with multi-generational family histories of alcoholism, or to individuals exposed to higher doses of benzodiazepines over longer periods of time, is not known.

AFFILIATION

Department of Psychiatry, The University of Chicago, Chicago, IL 60637

Divided Attention Task Performance in Women With and Without Familial Alcoholism

B.W. Lex, E.M. Rhoades, S.K. Teoh, and J.H. Mendelson

Our earlier reports showed FHP women to have less sensitivity to alcohol. We now report greater sensitivity to alcohol for FHP women on a divided attention task (DAT) that combines a compensatory tracking task with a visual search task. Subjects had similar results on the 6 baseline trials. The DAT was presented at 15, 30, 60, 90, 120, 150, and 180 min after alcohol (0.56 g/kg) or placebo. Results were analyzed for 35 healthy female volunteers (15 FHP and 20 FHN women) with comparable drinking histories, background characteristics, height/weight ratios, and X age of 23.2 years. Subjects received either alcohol (17) or placebo (18) but not both, under randomized, double-blind conditions. Blood alcohol levels peaked at about 80 mg/dl from 45 to 60 min after alcohol administration, and declined to about 50 mg/dl at 180 min. BALs for FHP and FHN women were similar on the ascending limb of the BAL curve, but differences in mean BALs for FHN and FHP women occurred on the descending limb of the BAL curve. Differences may stem from measurement error. After 0.56 g/kg alcohol, 7 FHP women had greater performance decrements (0.8 sec) for visual search response time than 10 FHN women on the ascending limb of the BAL curve (30 and 60 min). FHP and FHN women received comparable 95% ethanol doses as calculated by body weight, but FHN women had significantly lower BALs at 90, 120, 150, and 180 min. However, scores (cm) on the tracking task showed no significant differences for FHP and FHN women after either alcohol or placebo. Thus, reaction times were greater for FHP women after alcohol, but shorter after placebo. We will continue this study with women as their own controls. (Supported by NIAAA Grant Numbers 06794 and 09616).

AFFILIATION:

Alcohol and Drug Abuse Research Center, McLean Hospital, Department of Psychiatry, Harvard Medical School.

Differential Craving Between Recovering Abstinent Alcoholic-Dependent Subjects and Therapeutic Users of Benzodiazepines

Irwin Lucki, Joseph R. Volpicelll, and Edward Schweizer

Treatment of alcohol dependence is characterized by physical withdrawal symptoms and strong craving for alcohol during the early stages of abstinence. The withdrawal syndrome is thought to be followed by a subjective state marked by persistent preoccupation with thoughts of alcohol and frequent or intense urges to take alcohol that may contribute to relapse, that has been called "craving". Chronic use of other sedative hypnotics, such as benzodiazepines (BZs), also result in a withdrawal syndrome when they are discontinued. The development of physical dependence to BZs may cause certain patients to continue taking their medication after it is no longer needed. However, no studies have yet examined whether discontinuation from BZs is followed by persistent urges to take BZs or mental preoccupation with thoughts of BZs after withdrawal symptoms have abated. The presence study measured periods of craving in abstinent alcohol-dependent subjects and former chronic users of BZ tranquilizers 3 months after they stopped using their drugs. A questionaire matched for alcohol and tranquilizers was administered to former users of these drugs to measure self-reports of the intensity and frequency of craving, and estimates of its effect on continuing abstinence by drug abusers.

The long-term craving for alcohol or tranquilizers was examined after detoxification treatment in two groups of patients. One group consisted of 25 recovering male alcohol-dependent subjects (Age = 41 ± 1.6 years) treated at the Veterans Administration Hospital for a substance abuse disorder. Before treatment, alcohol use averaged 21.1 ± 1.9 oz (range = 12 - 40) and the duration of use was 192.6 ± 20.2 months (range = 36 - 348). All subjects were known to be physically dependent on alcohol before treatment because of the presence of withdrawal symptoms shortly following abstinence. The second group consisted of 43 patients (24 females, 19 males; Age = 49 ± 2.2 years) treated at the Psychopharmacology Research and Treatment Clinic for chronic use of therapeutic doses of BZ medications. Before treatment, patients were using either: diazepam (N=9, Mean dose = 9.5 mg/day (2-40)), lorazepam (N=16, Mean dose = 2.3 mg/day (0.5-4.0)), or alprazolam (N=18, Mean dose = 1.8 mg/day (0.25-4.0)). The average

duration of BZ use by chronic users was 67 (13 - 216) months. All subjects (alcohol-dependent and chronic BZ users) were asked to complete a craving questionaire that asked about their desire or urge for alcohol or tranquilizers during the previous week. The wording of the questions on each survey was matched but referred only to the use of alcohol or tranquilizers. All patients were drug-free for 3 months at the time that the questionaire was administered.

The frequency of the desire, urge, or craving for a drink was significantly greater for abstinent alcohol-dependent subjects (1.80 ± .14, mean ± 1 SEM) than the craving of abstinent chronic users of BZs for a tranquilizer pill (0.91 ± .14, Chi-square = 16.89, P < 0.001). The periods of craving were rated as more intense by alcohol-dependent subjects (2.24 ± .27) than tranquilizer users (1.09 ± .18, Chi-square = 8.82, P < 0.01). Abstinent alcohol-dependent subjects (1.52 ± .17) reported more frequent occasions of thinking about having a drink than chronic BZ users thought about taking tranquilizers (0.70 ± .13, Chi-square = 9.56, P < 0.01). Alcohol-dependent subjects (1.92 ± .20) also missed drinking significantly more than tranquilizer users missed taking BZs (0.55 ± .14, Chi-square = 18.22, P < 0.001). There were no differences in the response to these questions from subjects that used different tranquilizers.

Both alcoholic-dependent subjects (0.72 ± .21) and BZ users (0.42 ± .13) reported that periods of craving rarely lead to them taking a drink or resuming BZ medication (Chi-square = 1.57, P > 0.05). Also, alcohol-dependent subjects reported it would be somewhat difficult to resist taking a drink if they knew a bottle was in the house (0.96 ± .24), but this did not differ significantly from chronic BZ users who generally reported it was not difficult at all (0.42 ± .13) to resist an analogous situation (Chi-square = 1.90, P > 0.05). The low estimated contribution of craving to relapse by these patients may be related to their successful abstinence.

In conclusion, differences were found between the craving for alcohol of abstinent alcohol-dependent subjects and the craving for tranquilizers of chronic users of BZs after abstinence from their drug use for a period of 3 months. Alcohol-dependent subjects reported a more frequent and intense desire to drink than BZ users to take a tranquilizer, more frequent occasions of thinking about alcohol than BZ users, and that they missed alcohol more than BZ users missed tranquilizers. These differences may be related to: 1) differences in the reinforcing effects of alcohol and BZs in these subjects during the period when they were used, 2) differences in exposure to stimuli associated with each of the drugs during the abstinence period, and 3) differences in the after-effects between chronic alcohol and BZ use.

Departments of Psychiatry and Psychology, University of Pennsylvania, 207 Piersol Bldg., Philadelphia, PA 19104-4283.

Human Aggressive Responding: Effects of Response-Cost and Ethanol

Ralph Spiga, Don R. Cherek, and Robert H. Bennett

Two experiments examined the effects of response-cost (FR requirement)
and ethanol on human aggressive responding. Four male research
subjects were provided non-aggressive and aggressive response
options. Non-aggressive responses were button presses maintained by
presentation of points exchangeable for money on a fixed ratio (FR)
100 schedule. Aggressive responses were defined as button presses
which ostensibly subtracted points from a fictitious partner. Point
loss engendered aggressive responding. These point losses were
attributed to a fictitious partner. Aggressive responding was
maintained by escape from scheduled point losses for 125s. Subjects
were exposed to FR schedules that required 10, 20, 40 or 80 button
presses before a point was ostensibly subtracted from a fictitious
partner. For one subject in the first experiment the number of
aggressive-response ratios completed increased as a function of
response requirement. For the other subject aggressive response
ratios completed decreased as the FR was increased. During the second
experiment ethanol doses of 0.125, 0.25 and 0.375 g/kg were
administered prior to each of the first three sessions of the day.
For both subjects the number of aggressive response ratios completed
decreased as a function of increasing FR value. For both subjects
ethanol administration increased the number of aggressive response
ratios completed under FR 20 response-cost conditions but not under FR
40 or 80 conditions. However, under all FR conditions subjects
retaliated by subtracting a point from the other fictitious subject
for every point ostensibly subtracted by that subject. Aggressive
responding in both experiments appeared to correlate with paper and
pencil measures of hostility.

Affiliation: Substance Abuse Research Center
               Department of Psychiatry & Behavioral Sciences
               The University of Texas Health Science Center at Houston
               1300 Moursund - UTMSI
               Houston, Texas 77030

The Discriminative Stimulus Effects of Abecarnil

Nancy A. Ator and Roland R. Griffiths

Baboons (N=4) were trained to discriminate lorazepam (1.8 mg/kg p.o.) under a two-lever drug versus no drug dis-crimination procedure. Previous work with the lorazepam training condition in baboons and rats showed that drug-lever responding occurred in tests with benzodiazepines (BZ) and with the BZ-receptor ligands zopiclone and CL 218,872, but did not reliably occur after barbiturates, other sedative/anxiolytics, or the BZ-receptor ligands PK 9084 or CGS 9896. Abecarnil (ZK 112,119, Schering), is a ß-carboline which binds the BZ receptor and has shown BZ agonist-like effects in other paradigms. The discriminative stimulus effects of abecarnil (0.32-32 mg/kg p.o.) were studied at 1 hr after administration and for 15 hr using a time-course procedure in which 10-min test sessions were conducted at 2-hr intervals. Generalization from the lorazepam stimulus to abecarnil was an increasing function of dose, with the peak stimulus effects at 3-5 hrs after drug in all baboons (i.e., at 1 hr, mean drug lever responding was <50%: at 3 hrs, drug lever responding was >80% in all baboons at the 32 mg/kg dose; at 5 hours, drug lever responding was >80% in all baboons at the 18 mg/kg dose also). Drug lever responding generally decreased 9-13 hrs after administration. When flumazenil (0.32 mg/kg i.m.) was given 4 hrs after abecarnil (18 and 32 mg/kg), complete antagonism of the abecarnil stimulus occurred in a test session 1 hr later, indicating that the discriminative stimulus effects of abecarnil are mediated through the BZ receptor.

AUTHORS AND AFFILIATIONS:

Nancy A. Ator, Ph.D.[1] and Roland R. Griffiths, Ph.D.[1,2]
Dept. of Psychiatry and Behavioral Sciences[1] and Department of Neuroscience[1,2] The Johns Hopkins University School of Medicine. Baltimore, Maryland 21205

Reduced Amplitudes of Somatosensory Evoked Potentials Observed After Chronic Ethanol Exposure

Cathy J. Bogart and Larry P. Gonzalez

INTRODUCTION

Few studies have examined the effects of ethanol exposure on somatosensory evoked potentials (SEPs), despite neuroanatomical evidence of chronic ethanol-induced abnormalities. Excessive alcohol consumption is known to cause peripheral neuropathy in humans and laboratory animals. The sciatic nerves of rat pups with fetal alcohol syndrome showed shrunken and retracted axons in about 30-40 percent of myelinated fibers, in addition to relatively thin myelin sheaths (Baruah & Kinder, 1989). In another study utilizing the fetal alcohol syndrome model, the number of myelinated fibers in the ventral funiculi of the spinal cord was significantly reduced in rat pups exposed to ethanol (McNeil1 *et. al.,* unpublished manuscript). Exposure to ethanol has also been reported to result in a significant reduction of myelinated axons and a lower rate of glucose metabolism in layer V of the somatosensory cortex in rats (Al-Rabiai & Miller, 1989).

The purpose of this study was to examine SEPs recorded from rats which received chronic exposure to ethanol versus ethanol-naive animals and to determine the effect of an acute dose of ethanol on SEPs. Such a study is needed to provide a functional measure of the neuroanatomical abnormalities already observed in the somatosensory pathways following ethanol exposure.

METHODS

Six male, Sprague-Dawley rats, weighing 315-350 g, received chronic exposure to ethanol in vapor inhalation chambers for six weeks. The average blood ethanol level at the time of withdrawal from the chambers was 170 mg/dl (S.E.=63.3). Twenty-four hours after withdrawal, the animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and chloral hydrate (100 mg/kg, i.p.) for chronic electrode implantation. A stainless steel screw electrode was implanted stereotaxically over the right somatosensory cortex, and a reference electrode was placed 2 mm behind.

Immediately after the surgery, which lasted approximately one hour, baseline SEPs were recorded. Recordings were time-locked to 3-milliamp electrical pulses of .5 msec duration. The pulses were delivered to the left hindpaw at a rate of .5 pulses/sec. A ground strap, soaked in a saline solution, was attached to the right hindpaw. Thirty-two sweeps were averaged for each recording.

After six baseline recordings were completed, the animals were given an injection of ethanol (1.5 g/kg, i.p.). Subsequent SEPs were recorded 5, 30 and 60 minutes post-injection (six trials each). The same procedures were carried out on seven ethanol-naive rats and were repeated in the ethanol-exposed animals 10 days later.

CONCLUSION

Figure 1 represents the effects of acute and chronic ethanol exposure on SEPs. The results indicated that the baseline SEPs of ethanol-exposed rats, 24 h after withdrawal, had significantly lower amplitudes than controls (F = 10.75, p>.007). The amplitudes of pre-injection SEPs in ethanol-exposed animals were an average of 340 uv smaller than those of controls. Both groups showed a significant amplitude reduction from baseline SEPs within 5 minutes of the ethanol injection (F = 4.97, p > .025). The SEP response to

the acute ethanol dose was similar in both groups.

When the SEP recording procedures were repeated 10 days later, the ethanol exposed animals did not differ from controls in their baseline SEPs nor in their response to an acute dose of ethanol.

These results indicate that chronic exposure to ethanol produces a large, but temporary, reduction in electrophysiological responsiveness.

University of Oklahoma Health Sciences Center, Department of *Psychiatry & Behavioral Sciences, Oklahoma City, OK 73190*
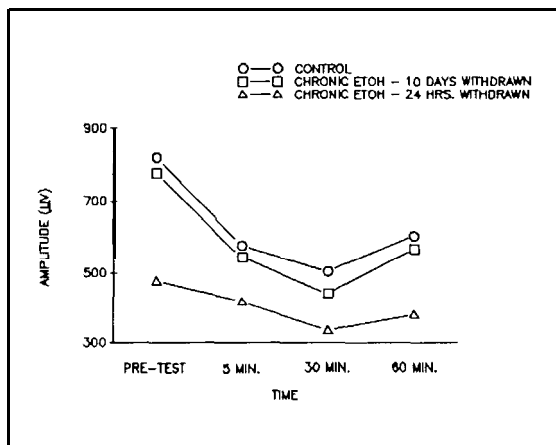
Behavioral Effects of Chronic Abecarnil Administration in Baboons

Christine A. Sannerud, and Roland R. Griffiths

Abecarnil (ZK 112,119, Schering) is a ß-carboline which exerts pharmacological effects at the benzodiazepine (BZ) receptor complex, and is being developed as an anxiolytic (Stephens et al., J. Pharmacol. Exp. Ther., 253:334-348. 1990). The effects of chronic administration of and spontaneous withdrawal from abecarnil were studied in 4 male baboons, surgically implanted with i.g. catheters. Baboons were maintained on vehicle during a 2 week baseline phase of the study. Baboons then received 100 mg/kg/day abecarnil via continuous i.g. infusion for 6-8 consecutive weeks. Observational sessions, conducted before, during, and after chronic administration permitted the scoring of a variety of behaviors and postures, movement and position in cage, and the assessment of coordination, level of activity, sedation and tremor. Chronic administration of 100 mg/kg/day abecamil produced few behavioral signs of sedation in the 4 baboons; lip droop and slight limb tremor were seen in only 2 of 4 baboons. Flumazenil (5.0 mg/kg, i.m.) administered on day 8 of chronic abecarnil administration produced signs of a mild precipitated withdrawal syndrome that was less severe than diazepam or lorazepam. During spontaneous withdrawal from abecarnil, there were transitory increases in signs of limb tremor, twitch/jerk, and abnormal postures, and decreases in food intake. No signs of vomiting, myoclonic jerks, or seizures (withdrawal signs observed during precipitated or spontaneous withdrawal from chronic high doses of BZ) were observed in any baboon. These patterns of behavioral changes indicated a mild BZ-like withdrawal syndrome from abecarnil that was less severe than from diazepam or lorazepam.

AUTHORS AND AFFILIATIONS:

Roland R. Griffiths, Ph.D.[1,2], Christine A. Sannerud, Ph.D.[1]
Department of Psychiatry and Behavioral Sciences[1] and Department of Neuroscience[2], The Johns Hopkins University School of Medicine. Baltimore, Maryland 21205

Self-Injection of Barbiturates, Benzodiazepines and Other Sedative-Anxiolytics in Baboons

Roland R. Giffiths, Christine A. Sannerud, Richard J. Lamb, Nancy A. Ator, and Joseph V. Brady

Self-injection of twelve sedative-anxiolytics was examined in baboons. Intravenous injections and initiation of a 3-h time-out were dependent upon completion of a fixed-ratio schedule requirement (FR 80, 160 or 320), permitting eight injections/day. Before testing each dose of drug, self-injection performance was established with cocaine. Subsequently, a test dose was substituted for cocaine. At some doses, all five of the benzodiazepines examined (alprazolam, bromazepam, chlordiazepoxide, lorazepam, triazolam) maintained levels of drug self-injection above vehicle control in each of the baboons tested. Peak levels of benzodiazepine self-injection were generally submaximal. Of the benzodiazepines examined, triazolam maintained the highest levels of self-injection. Among the three barbiturates tested, methohexital generally maintained high rates of self-injection in contrast to hexobarbital and phenobarbital which only maintained low levels. The low level of self-injection maintained by hexo-barbital was unexpected. Of the four non-benzodiazepine non-barbiturate sedatives examined, both chloral hydrate and methyprylon occasionally maintained high levels of self-injection. Although there were differences within and across animals, baclofen maintained intermediate levels of self-injection. The novel anxiolytic buspirone maintained only low levels of self-injection that were not different than vehicle. This study further validates the self-injection methodology for assessing sedative-anxiolytic abuse liability and provides new information about drug elimination rate as a determinant of drug self-administration.

AUTHORS AND AFFILIATIONS:

Roland R. Griffiths, Ph.D.[1,2], Christine A. Sannerud, Ph.D.[1], Richard J. Lamb, Ph.D.[1], Nancy A. Ator, Ph.D.[1], and Joseph V. Brady, Ph.D.[1,2]
Department of Psychiatry and Behavioral Sciences[1] and Department of Neuroscience[2], The Johns Hopkins University School of Medicine. Baltimore, Maryland 21205

Effect of (-) and (+) Nicotine of [^{3}H]DA Uptake and Release in Rat Striatum

S. Izenwasser, H.M. Jacocks, and B.M. Cox

The effects of both (-) and (+)nicotine isomers were examined on *in vitro* uptake and release of [^{3}H]dopamine from rat striatum. Both isomers inhibited [^{3}H]dopamine uptake in chopped tissue at concentrations well below those necessary for promoting release of preloaded [^{3}H]dopamine. (-)Nicotine was more potent than (+)nicotine both at inhibiting uptake and at promoting release. Unlike other dopamine uptake inhibitors such as cocaine or methylphenidate, however, (-)nicotine only inhibited approximately 50% of the total uptake and did not compete against binding of [^{3}H]GBR 12935, a selective dopamine uptake inhibitor. The nicotinic receptor agonists carbachol and DMPP also inhibited uptake whereas the nicotinic antagonists chlorisondamine and mecamylamine blocked nicotine's effect. These findings suggest that nicotine may inhibit [^{3}H]dopamine uptake via a different mechanism than classical uptake inhibitors and that nicotine's effect on dopamine uptake may be mediated by a receptor similar to the nicotinic acetylcholine receptor. These receptors seem to be located on different terminals than those which are accumulating dopamine, however, since tetrodotoxin prevented the effect of nicotine on [^{3}H]dopamine uptake and nicotine had no effect on uptake in a synaptosomal preparation. (Supported by a grant from NIDA).

AFFILIATION
Department of Pharmacology, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

Studies of the Effect of Nicotine on Synaptosomal Calcium Accumulation Using FURA-2

Cecelia J. Hillard and Wendy K. Graf

It is becoming clear that there are several subtypes of the nicotinic receptor in the CNS and that these subtypes may subserve diverse actions of nicotine. There is good evidence that there are a population of nicotinic receptors located presynaptically at catecholaminergic synapses that, when stimulated, enhance the release of dopamine (Rapier et al., 1990, Westfall, 1974) and norepinephrine (Andersson et al., 1982). Recent studies of Wonnacott and coworkers have demonstrated that nicotinic receptor mediated dopamine release from striatal synaptosomes is calcium dependent but tetrodotoxin insensitive (Rapier et al., 1988). The studies reported in this abstract demonstrate, using direct methods, that nicotinic receptor activation results in an increase in intrasynaptosomal calcium concentration.

METHODS

Synaptosomes were prepared from rat forebrain using discontinuous Ficoll density gradients (Cotman and Matthews, 1971). Synaptosomes were loaded with FURA-2-AM, a membrane permeant ester of FURA-2 for 45 min. After washing, 85% of the FURA-2 was found to be within the synaptosomes. An SLM 8000 spectrofluorometer was used in these studies, the excitation wavelength was 340 nm and a 418 cutoff filter was used in the emission light path. A SFA-II Rapid Kinetics Accessory (Hi-Tech Scientific, Wiltshire, England) was used for the time course studies. This attachment consists of two drive syringes, one containing the synaptosomes and the other containing drug solutions; a drive plate that pushes both syringes, and a thermostatted cuvette. When the drive plate was pushed, exactly 200 µl of both the synaptosomal preparation and the drug entered the cuvette and mixed. After approximately 20 msec dead time, the intensity of FURA-2 excitation was recorded. Intrasynaptosomal calcium concentrations were typically 400-500 nM.

RESULTS AND DISCUSSION

Nicotine produced a concentration-related increase in 340 nm fluorescence at concentrations between 10 µM and 1 mM (Fig 1). Rapid (subsecond) time based studies indicated that the increase occurred within 50 msec of nicotine addition. The effect of nicotine was blocked by mecamylamine. Suberyldicholine, a nicotinic agonist with greater affinity for CNS nicotinic receptors, also increased

synaptosomal FURA-2 fluorescence at concentrations between 0.1 μM and 10 μM. These studies support the hypothesis that an increase in the concentration of intrasynaptosomal calcium is a functional consequence of nicotinic receptor activation.
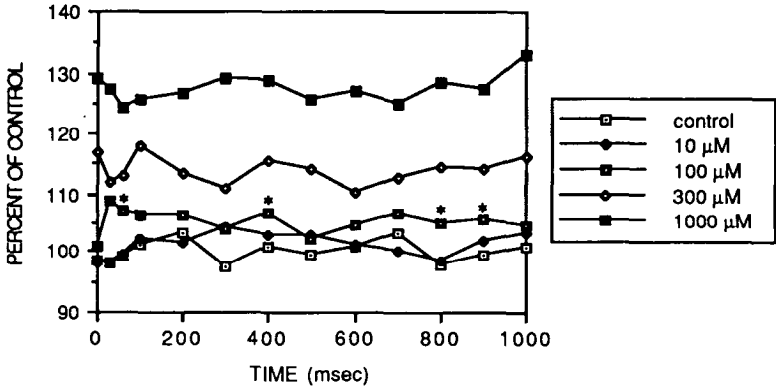
FIGURE 1. EFFECTS OF NICOTINE ON INTRASYNAPTOSOMAL FURA-2. Fluorescence intensity of FURA-2 at 340 nm was determined using the rapid kinetics attachment. Final protein concentration was 0.5 mg/ml. Each point is the mean of 3 separate experiments. Concentrations of 300 and 1000 μM nicotine produced significant changes at all time points. Asterisks indicate points at which 100 μM nicotine produced significant increases (p<0.05).

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ACKNOWLEDGMENTS

AFFILIATION

Department of Pharmacology and Toxicology; Medical College of Wisconsin; 8701 Watertown Plank Road; Milwaukee, WI 53226

Lack of Neurotoxicity After Intra-Raphe Micro-Injections of MDMA ("Ecstasy")

Joseph M. Paris and Kathy A. Cunningham

INTRODUCTION

The current upswing in the abuse of methamphetamine, 3,4-methyl-enedioxymethamphetamine (MDMA; "ecstacy") and related compounds follows closely on the heels of an epidemic of cocaine abuse in the United States. Repeated systemic administration of MDMA produces depletions of serotonin (5-hydroxytryptamine; 5-HT) and its primary metabolite 5-hydroxyindoleacetic acid (5-HIAA), decreases 5-HT reuptake sites and diminishes tryptophan hydroxylase activity in various forebrain regions (Stone et al., 1986; Commins et al., 1987). The spontaneous firing rate of dorsal raphe (DR) 5-HT neurons is depressed by intravenous MDMA presumably as a result of increased 5-HT release (Sprouse et al., 1989). MDMA has been shown to be neurotoxic to the fine fibers originating from the DR 5-HT neurons but not the beaded fibers from the median raphe (MR) nucleus (O'Hearn et al., 1988). We investigated whether direct microinjections of MDMA into the DR or MR resulted in neurotoxicity as measured by 5-HT and 5-HIAA levels and 5-HT immunocytochemistry.

METHODS

Male Sprague-Dawley rats (N=43) were anesthetized and stereotaxically injected with (+)MDMA (50 μg base in 2 μl of 0.1% ascorbic acid in saline; NIDA) into the DR (N=15) or MR (N=15) or 2 μl of the ascorbic acid vehicle (DR, N=6; MR, N=7). Injections were made using a 10 μl syringe over 10 min. Two weeks later, the rats were sacrificed by decapitation and hippocampi and striata were dissected out. Thirteen rats were perfused and prepared for 5-HT immunocytochemistry.

RESULTS

HPLC analysis (shown in table) did not reveal any significant changes in the concentrations of 5-HT and 5-HIAA in the hippocampus and striatum of rats administered MDMA into the DR or MR.

CATECHOLAMINES AND INDOLEAMINES IN INTRA-RAPHE MDMA TREATED RATS

	5-HT	5-HIAA	mg/fmol protein DA	DOPAC	NE	HVA
CAUDATE						
DR	39.8	36.8	516.6	54.1	28.6	30.9
	±1.5	*±1.6*	*±46.2*	*±11.2*	*±1.7*	*±3.6*
MR	42.5	37.9	450.7	41.8	24.3	26.3
	±2.8	*±3.0*	*±19.9*	*±2.3*	*±2.8*	*±3.5*
VEH	41.0	40.3	421.8	44.3	31.8	27.2
	±1.2	*±1.5*	*±27.2*	*±2.7*	*±6.6*	*±1.9*
HIPPOCAMPUS						
DR	24.1	21.1	ND	ND	17.4	11.6
	±1.5	*±0.7*			*±1.3*	*±0.6*
MR	23.5	22.0	ND	ND	14.9	11.2
	±1.4	*±1.8*			*±1.2*	*±1.4*
VEH	20.0	19.5	*NO*	ND	15.4	12.4
	±1.2	*±1.3*			*±1.3*	*±1.6*

Analysis of the raphe sections stained for 5-HT also did not re-
veal any apparent neurotoxicity. Although inclusion bodies have
been observed in DR 5-HT perikarya of MDMA-treated animals
(Ricaurte et al., 1988), no such pathologies *were* observed in our
tissue sections.

CONCLUSIONS

The results suggest that a direct cerebral injection of (+)MDMA
does not result in neurotoxicity to 5-HT neuronal systems origi-
nating in the DR or MR and support the hypothesis that metabolism
of MDMA to an active neurotoxic substance is required. Alterna-
tively, we cannot rule out the possibility that multiple injec-
tions of MDMA into these raphe nuclei might prove to be neurotox-
ic.

ACKNOWLEDGEMENTS

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*Department of Pharmacology and Toxicology, University of Texas
Medical Branch, Galveston, Texas, 77550, USA.*

[3H]1,3-D1(2-Tolyl)Guanidine Labels Two High Affinity Binding Sites in Guinea Pig Brain: Evidence for Allosteric Regulation by Calcium Channel Blockers and Sigma Ligands

Richard B. Rothman, Artin Mahboubi, Audrey A. Reid, Chong-Ho Kim, Brian R. deCosta, Arthur E. Jacobson, and Kenner C. Rice

INTRODUCTION

Previous studies of sigma receptor binding have reported displacement curves characterized by low Hill coefficients (1), which can be evidence for multiple binding sites. In a previous communication (2), we reported that [3H]DTG labeled two binding sites in guinea pig brain termed DTG site 1 and DTG site 2. In the present study, we confirm and extend these observations, and provide evidence for allosteric binding sites associated with both DTG site 1 and DTG site 2.

METHODS

Guinea pig brain membranes were prepared, and equilibrium binding assays were conducted as previously described (3). Dissociation experiments, which monitored the dissociation of [3H]DTG from site 1 and site 2, used a dilution method, as described elsewhere (4).

RESULTS

Quantitative ligand binding studies resolved two high affinity [3H]DTG binding sites with Bmax vlaues of 1045 and 1423 fmol/mg protein. The Ki values of selected agents for DTG site 1 and 2 are reported in Table 1. Dissociation experiments (Figure 1) demonstrated that low concentrations of the inorganic calcium blockers, Ni^{2+} (1 mM), La^{3+} (0.1 mM), and Cd^{2+} (0.1 mM) selectivley increased the dissociation of [3H]DTG from site 2. DTG and haloperidol increased the dissociation of [3H]DTG from both site 1 and site 2, although they were more potent at site 1.

DISCUSSION

Equilibrium binding studies readily resolved two binding sites with different ligand selectivity patterns. Whereas site 1 has the characteristics of the sigma receptor as it is currently defined (5) a binding site with the

characteristics of site 2 has not to our knowledge been previously reported in guinea pig brain. The dissociation experiments provide additional independent data for the existence of two high affinity [^{3}H]DTG binding sites.

TABLE 1
Ki (nM) VALUES OF DRUGS FOR DTG SITE 1 AND SITE 2

	Site 1	Site 2
Haloperidol	0.36	36.1
(+)-Pentazocine	2.0	456
R-(+)-PPP	5.1	442
Carbepentane	5.2	1523
BD738	6.4	188
8D737	8.0	502
DTG	11.9	37.6
S-(-)-PPP	30.5	1544
Dextromethorphan	121	53503

The selective modulation of [^{3}H]DTG site 2 by calcium channel antagonists suggests that this binding site may be associated with a calcium channel. Moreover, the increase in the dissociation of [^{3}H]DTG

FIGURE 1: DISSOCIATION EXPERIMENT
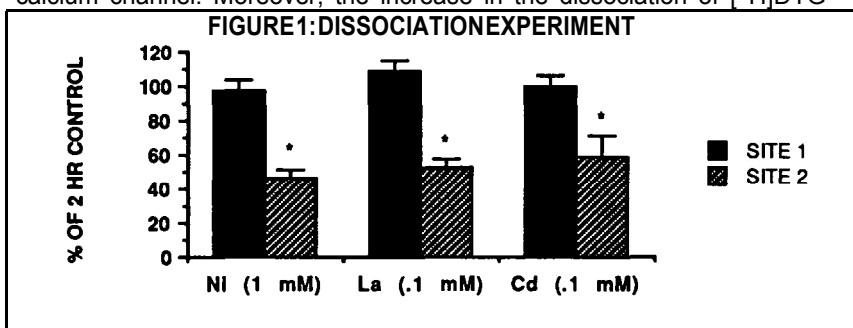
produced by haloperidol and DTG suggest that both DTG site 1 and 2 possess binding sites which allosterically modulate the sites labeled by [^{3}H]DTG. Bowen et al. (6) have similarly reported the presence of an allosteric binding site associated with sigma binding sites.

References will be supplied by the author upon request.

AFFILIATIONS

[1]Unit on Receptor Studies, LCS, NIMH, Bldg. 10-3D41, Bethesda, MD 20892.
[2]Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

Synthesis and Anticonvulsant Activity of Dextromethorphan Analogs

F.C. Tortella and A.H. Newman

Dextromethorphan (DM, (+)-3-methoxy-N-methylmorphinan) is a widely used cough suppressant that does not possess the addiction liability or analgesic properties of classical opiates. [3H]DM binds to a high affinity site (Kd 57 nM) and a low affinity site (Kd 27 µM) in the guinea pig brain. Recently, several sigma ligands including (+)-3-PPP, (+)-SKF-10047 and haloperidol have been shown to inhibit [3H]DM binding with a rank order of potency very similar to that for sites labeled with [3H](+)-3-PPP and allosteric modulation of both these sites occurs with ropizine and phenytoin. These studies suggest that DM and (+)-3-PPP bind to at least one common site.

The anticonvulsant activity of DM was first described as a dose-related protection against maximal electroshock seizures (MES) in rats. Not only was DM anticonvulsant but it potentiated the anticonvulsant activity of the prototypic anticonvulsant drug diphenylhydantoin. Subsequently, DM and its primary metabolite dextrorphan (DX) have been shown to inhibit N-methyl-D-aspartate (NMDA)-induced convulsions. DX does not interact with DM binding sites but does bind with high affinity to the PCP receptors associated with the NMDA receptor complex. DX is also a potent anticonvulsant (2.5 times more potent than DM) in the rat MES assay. Since DM is rapidly and completely metabolized to DX, DX could possibly be responsible for, or at least contribute to, the seizure protection associated with the parent drug.

We have prepared a series of DM analogs in which the 3-methoxy group is replaced with bioisosteric functions that are

not expected to be metabolized to 3-hydroxy, the functional group on the DM metabolite DX. These agents were prepared in search of novel anticonvulsants and in hopes of correlating anticonvulsant action of these agents with binding affinities to the receptor systems above. Preliminary testing in the supramaximal electroshock test in rats showed the 3-amino-analog was anticonvulsant with an ED_{50} value (24.9 mg/kg [16.2-38.41) equivalent to DM (24.1 [19.7-29.51).

TABLE 1: ANTICONVULSANT ACTIVITY OF DM ANALOGS.
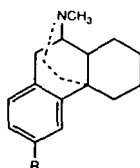
COMPOUND	R	MES Anticonvulsant ED_{50} (mg/kg. s.c.)
DM	$-OCH_3$	24-38
AHN 649	$-NH2$	25
AHN 668	$-N(CH_3)_2$	2 5
AHN 667	$-NHCH_3$	1A*
AHN 664	$-Cl$	1A**
AHN 644		1A**
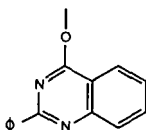
* Inactive at doses up to 100 mg/kg
* Inactive at doses up to 50 mg/kg

AUTHORS

Frank C. Tortella, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington D. C.

Amy Hauck Newman, Department of Applied Biochemistry, Walter Reed Army Institute of Research, Washington D. C.

The Competitive NMDA Receptor Antagonist, CPP, Allosterically Modulates NMDA Receptor Associated PCP Binding Sites in the Absence of Steric Hinderance

A.A. Reid, W.D. Bowen, C. Setterlund, and R.B. Rothman

INTRODUCTION

Phencyclidine (PCP) binding sites have been hypothesized to be located within ion channels associated with glutamate receptors specifically activated by N-methyl-D-aspartate (NMDA) (Fagg, 1987; Jarvis et al., 1987). Previous reports have proposed a steric hinderance model for binding of compounds to these PCP receptors in which NMDA receptor agonists such as glutamate act to open the ion channel and allow access to PCP binding sites; this effect is reversed by competitive NMDA receptor antagonists such as CPP, AP5 and AP7 (Kloog et al., 1988). In order to further evaluate the mechanism whereby NMDA receptor antagonists effect binding of PCP ligands and examine the plausibility of alternative hypotheses such as allosteric modulation, we conducted experiments which defined the association kinetics of [^{3}H]TCP and [^{3}H]MK801 binding to NMDA-associated PCP receptors in the absence and presence of CPP in two different membrane preparations containing high and low concentrations of nonsequestered glutamate.

METHODS

Guinea pig membranes were prepared as previously described (Rothman et al., 1989). For Prep 1, membrane pellets were resuspended with 5 mM Tris-HCl pH 8.2 buffer (protein~0.8 mg/ml; nonsequestered glutamate = 13.4±5.9 µM). For Prep 2, membrane pellets were prepared as described for Prep 1 and then centrifuged for 10 min at 37000 x g. These pellets were resuspended with the same volume of buffer (protein~0.7 mg/ml; nonsequestered

glutamate = 0.7±1.0 µM) for use in the binding assays. [³H]TCP and [³H]MK801 binding assays were conducted as previously described (Rothman et al., 1989).

RESULTS

Association time course studies indicated that equilibrium binding conditions were achieved within 2 h for [³H]TCP with both prep 1 and prep 2. CPP inhibited the binding of [³H]TCP with both membrane preparations; this inhibition was observed at equilibrium. The inhibition of binding resulted in a lenghtening in the time required to reach equilibrium such that 6 h was required for [³H]TCP in the presence of CPP (250 µM-prep 1; 50 µM-prep 2). The inhibition produced by CPP resulted from an ~1.5-fold increase in the Kd of TCP for PCP receptors (p<0.01, F-test); the inhibition of binding by CPP did not effect the Bmax. The inhibition of [³H]TCP binding produced by CPP was completely reversed by glutamate (50 µM). Glutamate (50 µM), itself, had no effect on [³H]TCP binding. The initial rate of association of [³H]TCP increased 7.0-fold (prep 1) and 7.4-fold (prep 2) in the absence of CPP and 11.1-fold (prep 1) and 8.1-fold (prep 2) in the presence of CPP with a 10 fold increase in the TCP concentration. Similar results were obtained with the [³H]MK801 binding assays, although quantitative differences were observed.

CONCLUSIONS

The association time course studies suggest that CPP allosterically modulates NMDA-associated PCP binding sites in the absence of steric hinderance since the inhibition of binding produced by CPP persisted at equilibrium and was reversed by glutamate and the initial rate of association of PCP ligands in the presence of CPP increased proportionally to their concentration.

REFERENCES available upon request from author.

AFFILIATIONS
Laboratory of Medicinal Chemistry, NIDDK and Unit on Receptor Studies, LCS, NIMH, NIH, Bethesda, MD 20892 and Section of Biochemistry, Brown University, Providence, RI 02912.

Dextromethorphan Inhibits But Dextrorphan Potentiates Behavior Induced By PCP and Ketamine in Rats

J.I. Székely, L.G. Sharpe and J.H. Jaffe

INTRODUCTION

Dextromethorphan (DM) is a non-opioid cough suppressant which is rapidly broken down to its demethylated metabolite dextrorphan (DO) (Ramachander *et al.,* 1977). Many data indicate that their pharmacological profiles are similar. In receptor binding assays, both displace PCP (phencyclidine) (Murray and Reid, 1984) and N-allylnormetazocine (Su, 1981) i.e. the prototypic ligands of PCP- and sigma-receptors, respectively. Furthermore, DM also binds to a third type of binding site for which other non-opioid antitussive agents like caramiphen and carbetapentane display high affinity (Craviso and Musacchio, 1983). Therefore, this group (Musacchio *et al.,* 1989) postulated that the DM binding sites might have selective physiological functions beyond the inhibition of cough reflex. If it is true, in appropriate in vivo models DM and DO should elicit differential effects since DO hardly displaced DM from its own binding sites in the above mentioned studies. Since DM and DO do not induce striking behavioral effects when administered alone (Benson *et al.,* 1954) we approached the issue indirectly. In this preliminary study we compared the eventual modulation of a number easily recordable PCP-induced changes in spontaneous activity by DM and DO, respectively. Ketamine (K), as a PCP-like agent was used for comparison.

METHODS AND MATERIALS

The PCP (10 mg/kg) and K (60 mg/kg) elicited hyperactivity was measured in activity cages in 12 successive 15-min periods. Animals were pretreated either with saline (SAL), DM or DO, the latter two in a dose of 30 mg/kg which is close to their anticonvulsant ED, (Ferkany *et al.,* 1988). All injections were given i.p. In the subsequent 16 sessions the various treatment combinations were applied in counterbalanced order. In addition to the automatically recorded total motor activity counts, every 15 min, eight

Modulation by Dextromethorphan (DM) of (Drug) Induced
Changes in Various Parameters

Modulation by Dextrorphan (DO) of (Drug) Induced
Changes in Various Parameters

A Phencyclidine (PCP)

Total Motor
Activity

ACTIVITY COUNT / 15 MIN
(Mean + S.E.)

15 30 45 60 75 90 105 120 135 150 165 180

D Ketamine (K)

15 30 45 60

B

NUMBER OF
SYMPTOMS RECORDED
(Mean + S.E.)

15 30 45 60 75 90 105 120 135 150 165 180

E Stereotypy

15 30 45 60

C

ATAXIA SCORE
(Mean + S.E.)

□ SAL + SAL
▫ DM + SAL
▨ SAL + PCP
■ DM + PCP

(SAL = SALINE)

Ataxia

15 30 45 60 75 90 105 120 135 150 165 180

F

□ SAL + SAL
▫ DM + SAL
▨ SAL + K
■ DM + K

(SAL = SALINE)

15 30 45 60

TIME AFTER THE SECOND TREATMENT

G Phencyclidine (PCP)

Total Motor
Activity

ACTIVITY COUNT / 15 MIN
(Mean + S.E.)

15 30 45 60 75 90 105 120 135 150 165 180

J Ketamine (K)

15 30 45 60

H

NUMBER OF
SYMPTOMS RECORDED
(Mean + S.E.)

15 30 45 60 75 90 105 120 135 150 165 180

K Stereotypy

15 30 45 60

I

ATAXIA SCORE
(Mean + S.E.)

□ SAL + SAL
▫ DO + SAL
▨ SAL + PCP
■ DO + PCP

(SAL = SALINE)

Ataxia

15 30 45 60 75 90 105 120 135 150 165 180

L

□ SAL + SAL
▨ DO + SAL
▨ SAL + K
■ DO + K

(SAL = SALINE)

15 30 45 60

TIME AFTER THE SECOND TREATMENT

342

signs of drug-induced "serotonergic" stereotypy were checked for their presence or absence (Martin *et al.,* 1982) and the degree of ataxia was scored on a scale from 0 to 5 (Sturgeon *et al.,* 1982). MANOVA followed by paired comparisons was used for statistical analysis. Boxed parts of the figure indicate the duration of significant ($p < 0.05$) differences between groups pretreated with DM or DO vs. the SAL pretreated ones.

RESULTS

Pretreatment with DM inhibited the PCP induced hyperactivity mainly in the first 15 min. Subsequently facilitation was seen which was not statistically significant (Panel A). The PCP elicited stereotypy was also inhibited by DM (Panel B) but the ataxia was not (Panel C). DM inhibited the K-induced motor activity as well (Panel D). However, the stereotypy and ataxia induced by K were not modified by DM (Panels E, F). Contrary to DM, DO did not inhibit the PCP induced hyperactivity (Panel G). The PCP elicited stereotypy and ataxia were even potentiated by DO (Panels H, I). On the other hand, the K elicited symptoms were not modified in any direction by pretreatment with DO (Panels J, K, L).

DISCUSSION

The main finding of this preliminary study is that DM and DO modulate the behavioral effects of PCP and K in opposite directions. This finding is surprising since in many biological assays DM and DO exert qualitatively similar actions. There are several possible explanations. DM may act as an antagonist, or weak agonist, whereas DO as a stronger agonist, at the sigma and/or PCP receptors. (Actually, in our ongoing experiments DO, in higher doses, seems to elicit PCP-like effects.) On the other hand DM might act at the receptors that bind DM but not DO. Thus, activation of these DM-receptors (Musacchio *et al.,* 1989) might bring about the suppression of some PCP-receptor mediated events. If so, other selective ligands of these receptors like caramiphen and carbetapentane should elicit similar inhibition. In ongoing experiments our first experience with the former one are in line with this hypothesis. Collectively, these data suggest that the DM binding sites, discovered by Musacchio and his coworkers, might have selective physiological functions beyond those associated with cough suppression.

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AFFILIATION: NIDA Addiction Research Center, POBox 5180, Baltimore, MD 21224.

Effects of Subject Expectancy on the Disposition of Tetrahydrocannabinol (THC) from Smoked Hashish Cigarettes

J. Camí D. Guerra, B. Ugena, J. Segura, and R. de la Terré

The specific effect of expectancy on cannabis consumption has been studied as part of a wider balanced-placebo clinical trail designed to assess possible interaction between alcohol and hashish. Data presented refers to subjects who did not receive the alcohol beverage but smoked cigarettes containing hashish (1 g tobacco cigarette containing 200 mg of hashish with 11.5% of THC) (n = 24) or placebo (n =24).

The hashish high (AUC_{0-330}) reported by subjects who received and expected the drug was higher but not statistically different, from what experienced by subjects who also received the drug but received placebo rated a hashish high lower than, but not statistically different from, those who received the drug. In subjects who received hashish, the sum AUC_{0-240}) of tetrahydrocannabinol (THC) and COOH-THC of those who expected the drug was greater than in those who did not expect it ($p < 0.05$). The ratio THC/COOH-THC AUC_{0-240} was lower in the group with positive drug consumption than in the group with negative expectancy ($p < 0.02$). An increased metabolism of THC was shown in subjects with positive expectancy. This could be explained, in part, by an increase in liver extraction induced by a higher heart rate in these subjects as compared with those with negative expectancy ($p < 0.007$). Positive expectancy can induce similar subjective effects to those observed when the drug is administered. In subjects with positive expectancy who ingested the drug, the bioavailability of THC from the smoked cigarette was higher.

AFFILIATION:

Department of Pharmacology and Toxicology. Institut Municipal d'Investigació Médica. Barcelona, Spain.

HIV Infection in Female Intravenous Drug Abusers (IVDAs) of Childbearing Age in NYC Drug Clinics and Their Demographic Profile

David C. Ajuluchukwu, Lawrence S. Brown, Jr., Janet Mitchell, Rosalind Thompson, and April M. Harris

INTRODUCTION:

The epidemiology of the acquired immune deficiency syndrome (ADDS) has changed, with the proportion of women with AIDS steadily increasing. While women represent 9% of all reported AIDS cases in the U.S., in New York they account for 13% of the total cases. AIDS has become the leading cause of death in New York City, among women 25 to 34 years of old. Intravenous drug use continues to be the second most common risk behavior associated with the prevalence of ADDS, for women, it is the number one risk behavior associated with AIDS. Intravenous drug use is reported as the node of transmission for 62% of all females with AIDS in New York City. There are an estimated 50,000 female intravenous drug users (IVDUs) in New York City, rendering women at increasing risk of infection with the human immuno-deficiency virus (HIV). Women of color have been disproportionately affected by AIDS. In New York City, as of March 30, 1990, 52% of all women with AIDS were Black and 32% were Hispanic.

METHOD:

In this retrospective study 59 (fifty-nine) females were recruited from a methadone maintenance program which maintains about 2,100 patients a year in six clinics located in Brooklyn and Manhattan areas of New York City. Subjects ware recruited from three Manhattan and one Brooklyn clinics. All subjects were contacted by a i-raineH HnfirviTTnh Assistant and were asked to participate voluntarily in the study.fn extensive and well-sructured questionnaire was administered to these subjects. Be questionnaire was designed to obtain, retrospective information on iimojt^hir-,, family planning, perinatal and post-partum information. Data obtained from the questionnaire were ceded and entered in the computer. Statistical associations were determine using univariate and multivariate methods of analysis.t

RESULTS:

The findings show that 96% of these women have been pregnant at least once. This strongly suggests that family planning and safer sex education are needed to help prevent perinatal HIV infection. Only 18% of the total sample used sane farm of birth control. Over 56% of the total sample have had at least one abortion. 4% of the sample population who knew their HIV status were positive.

DISCUSSION:

Due to the sample snail size, the variables did not show statistical significance However, the investigators strongly believe that future research should have an increased focus on holistic intervention for this patient population so that their basic needs can be addressed as part of the intervention strategy. Health education strategies that are culturally sensitive should be designed and effectively carried out for this patient population in carder to prevail, perinatal transmission of HIV. This study also demonstrates the need and the importance of providing onsight services (Gynecological) for this population. There is an on-going prospective study which will examine in depth the needs and the strategies prevailing perinatal HIV transmission at ARK.

AFFILIATIONS:
Addiction Research and Treatment Corporation, Brooklyn, New York
Harlem Hospital, Manhattan, New York
Centers for Disease Control, Atlanta, Georgia

Factors Associated with Elevated Risk of HIV Among Hispanic IVDAs

Mark E. Barrett and Robert J. Battjes

INTRODUCTION

Intravenous drug abusers (IVDAs) currently have the fastest increasing HIV infection rates of all major risk groups. They potentially pose a great risk of spreading HIV infection into the heterosexual population. Understanding seroconversion in this group is vital for designing prevention strategies.

Among the IVDA population, minorities have been found to have higher rates of infection than White IVDAs. Studies of IVDAs in Chicago, Illinois over the past few years, indicate that Hispanics have infection rates that are appreciably higher than those of Blacks and Whites. This finding raises important questions about the specific risk factors responsible for higher HIV infection rates in Hispanics. Possible hypotheses might be that Hispanics are more likely to engage in AIDS risk behaviors, or that they are more likely to travel to high prevalence areas--e.g., Puerto Ricans traveling to Puerto Rico or New York--increasing their exposure to risk.

This paper attempts to account for higher infection rates in Hispanic IVDAs by using a set of risk behaviors as predictors in a multiple regression model. To the extent that the variance in HIV infection rates can be accounted for by these predictors, Hispanic ethnicity should drop from significance in the model.

METHOD

Sample. The present study uses data from a national study of HIV seroprevalence among IVDAs, which is being conducted by NIDA. It was gathered in Chicago from January 1988 through June 1989. The sample consisted of 733 subjects, 18 year and older, who were intravenous heroin addicts seeking admission to methadone treatment programs. Therefore, it must be noted that these subjects may not be representative of all IVDAs in the area but are a select group who are seeking treatment for heroin addiction. They were paid $10 for participation in the study.

Measures. Questionnaires were administered that solicited information on a number of drug use and sex behaviors and attitudes related to HIV transmission, as well as basic socio-demographic information. HIV antibody tests were used to determine HIV seropositivity. Samples that were repeatedly reactive by ELISA were confirmed with Western blot. Blots were considered positive if antibodies to gp41 and/or p24 plus gp120 or gp160 were detected.

Research design. The basic research aim was to determine which specific risk behaviors were responsible for the higher HIV infection rates found in Hispanic IVDAs in the sample. Therefore, we looked at the association of selected demographic and risk behaviors with 1) Hispanic ethnicity, and 2) HIV serostatus. The specific risk factors were: needle sharing/cleaning behaviors; use of shooting galleries; frequency of injection of cocaine, heroin, and speedball; risks from exposure to the virus during travel in high prevalence areas; and sociodemographic variables. Chi-Square tests were used to determine the significance of all univariate associations.

Only those independent variables which were significant predictors of HIV status in the univariate tests were included in the regression model. A logistic regression procedure was used to do the analyses.

RESULTS AND DISCUSSION

Univariate tests. Many of the significant HIV risk factors were variables on which Hispanics were significantly different than non-Hispanics: use of shooting galleries, high frequency of needle sharing; and IV drug use in high HIV prevalence geographical areas. In addition to these, Hispanics were less educated, more likely to be male, younger, married or living as married, to not have had STDs, to have a higher frequency of sexual intercourse in high HIV prevalence areas, and to more often use shooting galleries and share needles.

Logistic regression models. Two logistic regression models were tested, a full model and a stepwise model. The full model, presented in Table 1, included each of the predictors that were significant in the univariate analyses. In the full model, only three variables were significant at the .05 level. These were Hispanic ethnicity and the two categories of "less than daily" and "daily" speedball use, which were both highly significant. (IV drug use in high HIV infection geographical areas was only marginally significant, p = .06). The stepwise model was used to select the best set of significant predictors (p < .05 level). The stepwise model is also presented in Table 1. In addition to both levels of speedball use and Hispanic ethnicity, "daily" needle sharing and IV use in high HIV prevalence areas achieved significance in the reduced model.

This study was successful in identifying many of the variables associated with HIV infection among IVDAs. If all of the relevant risk factors had been included, membership in the Hispanic group should have dropped from significance in the model. However, it remained signficant even in the presence of several risk measures, such as needle sharing, frequent intravenous drug use, and IV drug use in high HIV prevelance areas. This suggests that additional explanatory factors that were not included in this model may be needed to fully explain the dramatically higher prevalence of HIV infection among Hispanic IVDAs in the sample. It should be noted that more sensitive and reliable measures may have been better able to explain HIV infection. This study underscores the need for outreach programs to target speedball users because of the high risk of this practice.

TABLE 1
LOGISTIC REGRESSION MODELS

PREDICTOR VARIABLES	FULL MODEL	STEPWISE
Hispanic		
No	*	
Yes	.15 (.01)#	.14 (.01) [2.18]
STD		
None	*	
Yes	.09	
Sex in high HIV area	*	
No		
Yes	.03	
IV in high HIV area	*	
No		
Yes	.13 (.061)	.11 (.042)[1.82]
Cocaine injection		
None	*	
LT Daily	.08	
Daily or more	.10	
Speedball Injection	*	
None		
LT Daily	.32 (.015)	.29 (.001)[2.87]
Daily or more	.37 (.005)	.35 (.001)[4.14]
Shooting Gallery		
None	*	
1/wk or less	.01	
Sev/wk to daily	.06	
Shared needles		
None	*	
1/wk or less	.04	
several times/wk	.00	
daily or more	.11	.12 (.048)[1.93]

* omitted category

NOTE: p values given in parentheses; odds ratios for predictors in stepwise model are given in brackets.

AUTHORS
Mark E. Barrett, Ph.D., Department of Alcoholism and Substance Abuse
Robert J. Battjes, D.S.W., National Institute on Drug Abuse.

HIV-Risk Behaviors Among a Middle Class Population: A Preliminary Investigation Among Adolescent vs. Adult Substance Users

K. Meyers, J.L. Jaeger, V.N. Valliere, J.M. Jensen, H.M. Pettinati, B.D. Evans, and P. Sargiotto

AIDS research, prevention, and intervention efforts have been targeted to at-risk populations, primarily gay men and IV drug users (IVDU). However, non-IVDU substance abusers have recently been recognized as an additional population at risk for HIV. It has been suggested that certain substances may be a co-factor in the development of HIV, either by immunosuppressive influences or the strong association with unsafe sexual practices. However, little data exists on the prevalence of high-risk behaviors among this population. Hence, this study preliminarily examined behavioral risk factors among adolescent (N=49), young adult (N=13). adult (N=31) and older adult (N=2l) subjects receiving substance abuse treatment at a private, non-profit psychiatric hospital. (Age groups are defined as follows: 12-17 yrs., adolescents; 18-25 yrs., young adults; 26-35 yrs., adults; 36+ yrs., older adults.) Subsamples of each age group were selected to assess knowledge of HIV-related issues.

Preliminary analyses show that substance abusers in a middle income, private psychiatric setting do engage in HIV-risk behaviors. Although these behaviors occurred in both the sexual-and drug-related domains, high-risk sexual behavior (some superimposed upon an intoxicated state) appears to be predominant, particularly within the adolescent and young adult subgroups. Unprotected sex was the predominant sexual behavior among all age groups although it was more prevalent among adolescents and young adults (87.2% adolescents; 84.6% young adults; 51.6% adults: 33.3% older adults). Promis-cuous sexual activity (3 or more partners) was relatively common for both adolescents (66.7%) and young adults (50.0%). although uncommon for adults (5.6%) and older adults (5.9%). Having a partner at risk for HIV was reported by 17.9% of adolescents and 30.8% of young adults, although this was more pronounced among the adult (41.9%) and older adult (28.6%) population. Given that 80% of adolescents, 40% of young adults, 10% of adults and 20% of older adults were unaware of their partner's risk status of

at least one HIV-risk behavior, reported rates of having a partner at risk for HIV may be an underestimate.

Drug-related HIV-risk behaviors were not as prevalent as those that were sexually-related. Sex for drugs, and the use of crack cocaine or IV drugs were relatively uncommon for adolescents (22.4%, 22.4%, 8.2% respectively), adults (16.1%, 9.7%, 12.9% respectively) and older adults (9.5%, 4.7%. 9.5% respectively), but somewhat common among young adults with the use of crack cocaine predominate (23.1% sex for drug; 30.8% crack use, 15.4% IV drug use).

Given the knowledge level of this population (at least 89% of each group were aware of modes of transmission), interventions which will actually modify behavior is needed. Furthermore, it may be more beneficial to target interventions to HIV-risk behaviors of specific age groups. Since for some individuals, high-risk sexual behaviors were superimposed upon an intoxicated state, the role of substance use to sexual behavior and whether it continues during sobriety is being explored.

CARRIER FOUNDATION, NEW JERSEY

Chronic Cocaine-Induced Supersensitivity id Blocked by Co-treatment With the Peptide Cycle-(Leu-Gly)

Larry P. Gonzalez, Janet F. Czachura, and Kevin W. Brewer

INTRODUCTION

Chronic exposure to cocaine is sometimes accompanied by an increased responsiveness to acute cocaine administration. Much of the available data suggests that changes in central dopaminergic systems may be important in the mediation of this "reverse tolerance." The study presented here investigates the effects of cycle-(Leu-Gly) (CLG) on responses to acute cocaine after chronic drug exposure. This peptide is reported to modulate dopamine receptor sensitivity under various treatment conditions. We have reported, for example, that dopamine receptor supersensitivity following chronic haloperidol exposure is blocked by co-treatment with CLG (Fields et al., 1986). We report here the effects of chronic cocaine, with and without CLG, on acute responses to cocaine and haloperidol.

METHODS

All animals were implanted with biotelemetry temperature/activity transmitters (Mini-mitter Co.). Of the three groups examined, one group (Saline/Saline) received daily injections of saline (1 ml/kg, i.p.) for 21 days (n = 6) and two groups received daily injections of cocaine HCl (10 mg/kg, i.p.) for the same number of days (n= 7/group). Animals in the saline group, and in one of the cocaine groups (Cocaine/Saline) also received subcutaneous injections of saline (1 ml/kg) on Day 1 and every third day of chronic treatment; the second cocaine group (Cocaine/CLG) received injections of CLG (8 mg/kg, s.c.) on Day 1 and during every third day of chronic treatment. Chronic cocaine animals were tested for their responses (temperature and gross locomotion) to an acute cocaine injection on days 1 and 10 of chronic administration; saline animals were tested for their responses to acute cocaine (10 mg/kg, i.p.) for the first time on day 19. On the last day of chronic treatment (day 21) all animals received an acute injection of cocaine and were tested for cocaine-induced changes in motor behavior in motility monitors that permit quantification

of fine, repetitive motor movements. Seven days after the termination of chronic treatment all animals were tested for their response to haloperidol (0-5 mg/kg).

RESULTS and CONCLUSIONS

Groups receiving cocaine and cocaine plus CLG did not differ in any of their drug-induced responses to the initial cocaine injections on Day 1, suggesting that CLG is not a direct antagonist of the acute effects of cocaine. Nor did these groups differ from the response of the Saline/Saline group to their first cocaine dose on Day 19. Animals receiving chronic cocaine with saline exhibited significantly more cocaine-induced locomotion on day 10 than on day 1 ($p<0.001$) and showed a greater hyperthermic response on day 10 ($p<0.001$). Treatment with CLG, along with chronic cocaine, reduced the locomotor (Figure 1) and temperature responses to cocaine on day 10 of chronic treatment as compared to animals receiving chronic cocaine and saline ($p < 0.0001$). The cocaine/CLG group also exhibited significantly less cocaine-induced 8 Hz motility (representing stereotyped sniffing) when tested on day 21 ($p< 0.05$), as compared with animals receiving chronic cocaine but no CLG (Figure 2). Chronic cocaine, with or without CLG, did not significantly alter haloperidol-induced catalepsy.
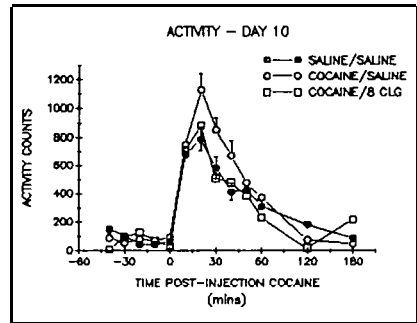
Figure 1
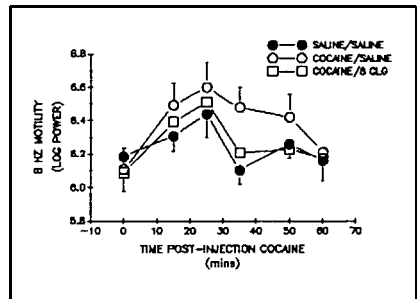
These data indicate that although CLG does not block the acute response to cocaine in control animals, it does block or reduce the development of supersensitivity to some cocaine-induced responses after chronic cocaine treatment. Neuronal adaptation to the chronic presence of cocaine may be an important factor leading to physical dependence and may be an impediment to abstinence. CLG, by reducing this adaptation, may be useful in the treatment of human cocaine abusers.

Figure 2

University of Oklahoma Health Sciences Center, Department of Psychiatry & Behavioral Sciences, Oklahoma City, OK 73190

Differential Dopaminergic Potency of Cocaine, Procaine and Lidocaine Infused Locally in the Nucleus Accumbens <u>In Vivo</u> with Calibration by Capillary Electrophoresis <u>In Vitro</u>

L. Hernandez, N.A. Guzman, and B.G. Hoebel

Cocaine, procaine and lidocaine (each at 7.3 mM) were infused via a microdialysis probe in the nucleus accumbens for 20 min in freely moving rats. Cocaine was about twice as potent as procaine; both increased extracellular dopamine significantly. Lidocaine had no detectable effect (Figure 1). Calibration of drug efflux from the probe *in vitro* using capillary electrophoresis to measure the relative concentration of drug inside and outside the probe (281100) suggested that about 40 nmoles of cocaine reached the accumbens. These results (1) confirm an earlier report that local injections of cocaine increase extracellular dopamine (Hemandez & Hoebel, 1988), and (2) show that the effect is independent of local anesthesia. (3) Procaine may enhance mood like cocaine by a dopaminergic effect. (4) Cocaine levels in small samples can be assayed by capillary electrophoresis. This work will be reviewed elsewhere (Hemandez <u>et al.,</u> submitted; Hoebel <u>et al.,</u> in press).
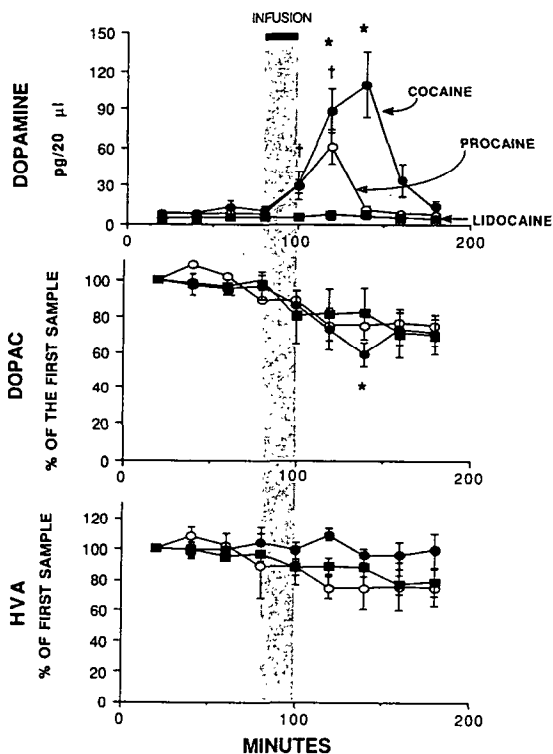
Figure 1. Cocaine (7.3 mM) applied locally in the accumbens by microdialysis infusion instead of bolus injection confirmed the increase in dopamine (*p < 0.01). Equimolar procaine infused by this same technique also had a significant effect, but significantly less than cocaine. Lidocaine had no detectable effect.

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AFFILIATION: Department of Psychology, Princeton University, Princeton, New Jersey 08544-1010

Genetic Differences in the Development of Cocaine-Kindled Seizures

R.J. Marley, J.M. Witkin, and S.R. Goldberg

The repeated administration of cocaine can result in an enhanced responsiveness to the effects of cocaine. There are strong genetic differences in acute sensitivity to the stimulant properties of cocaine and in the rate of development of increased sensitivity to its stimulant properties. Increased sensitivity to the convulsant effects of cocaine (kindling) also develops following the repeated exposure of rats to subconvulsant doses of cocaine. There have been no systematic studies of genetic factors influencing this kindling process. We have compared differences in the cocaine-kindling process with differences in susceptibility to seizures induced by acute administration of cocaine to BALB/cByJ, C57/6J, DBA/2J and SJL/J mouse strains. Mice were injected with cocaine HCl and observed for signs of clonic seizure activity. Evaluation of the cocaine-kindling process employed the highest dose of cocaine at which no seizure activity was observed in drug naive animals (50 mg/kg). Cocaine HCl, or saline, was administered daily to animals from each strain for 5 or 10 days. 72 hrs after cessation of treatment, both cocaine- and saline-treated mice were challenged with 70 mg/kg cocaine.
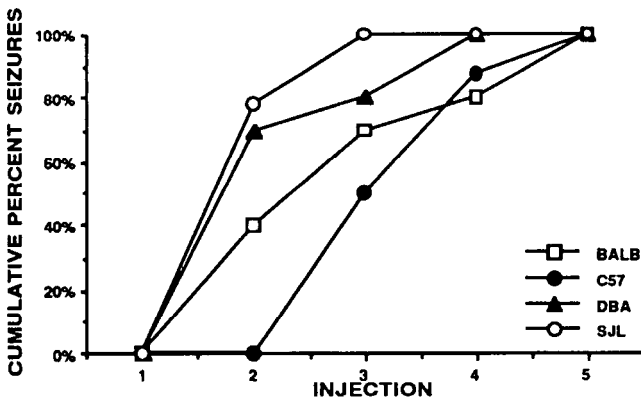
Fig. 1. Development of cocaine-kindled seizures.

The ranking for susceptibility to acute cocaine-induced seizures was C57 > BALB = DBA > SJL. The sensitivity of these strains to the development of cocaine-kindled seizures was opposite to that observed for acute seizure sensitivity (Fig. 1). While an increase in susceptibility to cocaine-induced seizures following repeated administration was initially observed in all the strains, this sensitization did not always persist. Only the SJL strain maintained an increased level of sensitivity to the convulsant effects of cocaine over the entire testing period. Among the other 3 strains, the repeated administration of cocaine after the appearance of a "kindled" seizure resulted in a loss of the apparent sensitization to the convulsant properties of cocaine. This "desensitization" was most readily apparent among C57 mice. The differences in response to repeated cocaine administration were further substantiated when the animals were subjected to an acute challenge with a convulsant dose of cocaine 72 hrs after the cessation of chronic treatment.
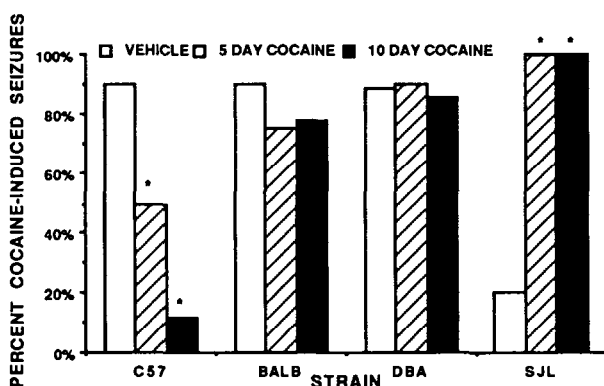
Fig. 2. Susceptibility to seizures induced by a 70 mg/kg challenge dose of cocaine 72 hrs after the end of the treatment period.

C57 mice chronically treated with cocaine were more resistant to cocaine-induced seizures than their saline controls, suggesting that for C57 mice, chronic cocaine exposure results in the development of tolerance to the convulsant properties of the drug. In contrast, chronically-treated SJL mice were much more sensitive to a challenge dose of cocaine. Based on these results it is concluded that (1) genetic factors mediate both acute sensitivity to the convulsant properties of cocaine and the effects of repeated administration of cocaine; (2) the mechanisms underlying sensitivity to acute cocaine-induced seizures may not be the same as those mediating sensitization to the convulsant effects of cocaine; and (3) the repeated administration of cocaine results in the development of sensitization or tolerance to the convulsant effects of cocaine depending on the genetic background of the individual being examined.

AFFILIATION:
NIDA-Addiction Research Center, Baltimore, Maryland 21224

Preliminary Evidence that the High Affinity Dopamine Reuptake Inhibitor, GBR12909, Antagonizes the Ability of Cocaine to Elevate Extracellular Levels of Dopamine

**Richard B. Rothman, Andrea Mele, Audrey A. Reid,
Hyacinth Akunne, Nigel Grieg, Andrew Thurkauf,
Brian R. deCosta, Kenner C. Rice, and A. Pert**

INTRODUCTION. GBR12909 (GBR) is one of the most potent inhibitors of dopamine (DA) reuptake known (1). In a previous paper (2) we presented evidence that following its systemic administration to rats, GBR binds persistently to the DA transporter, resulting in a modest increase in the extracellular levels of DA (ECDA) in the caudate nucleus, as well as an attenuation of the ability of cocaine to elevate ECDA levels. The present report expands on that preliminary study (2).

METHODS. Caudate membranes were prepared as previously described (2). [^{3}H]Cocaine ([^{3}H]COC) and [^{3}H]GBR12935 binding assays and in vivo microdialysis studies were also conducted as described (2). For ex vivo experiments, GBR was administered (i.p.) to rats, and the caudates were dissected at various times after drug administration. Caudates were stored at -70° C until assayed.

RESULTS. Administration of GBR (20 mg/kg) produced a decrease in [^{3}H]GBR12935 binding measured in vitro that was still present 4 hr after drug administration. In subsequent studies, rats were sacrificed 1 hr after drug administration. Administration of GBR (0, 10, 40, 100 mg/kg) produced a dose-dependent decrease in [^{3}H]COC binding (ED50 of about 40 mg/kg). Administration of GBR (0, 1, 10, 20, 40 and 100 mg/kg) also produced a dose-dependent decrease in [^{3}H]GBR12935 binding (ED50= about 10 mg/kg), resulting from a decrease in the Bmax, as well as an increase in the Kd. Control studies indicated that the increase in the Kd was due to residual GBR. The maximum decrease in the Bmax was about 50%. Control studies demonstrated that [^{3}H]GBR12935 labeled a single class of binding sites with low affinity for cis-flupentixol (Ki=1612 nM), which has high affinity for the "piperazine acceptor site" (1).

Administration of GBR (25 mg/kg) produced a modest increase in ECDA, quantitated as a percent of baseline levels, which was stable for at least 4 hr. Although the increase was not statistically significant at any one time point, analysis of variance demonstrated a significant difference

between the saline and GBR groups (p<0.01). Administration of COC (0.1, 1.0 and 10 mM) through the microdialysis probe produced substantial increases in ECDA which were significantly attenuated by administration of GBR. Calculating the increase in ECDA produced by COC as the ECDA in the presence of COC minus the ECDA in the absence of COC, demonstrated that GBR inhibited the ability of COC to elevate ECDA levels by over 50% at all doses of COC (Figure 1).
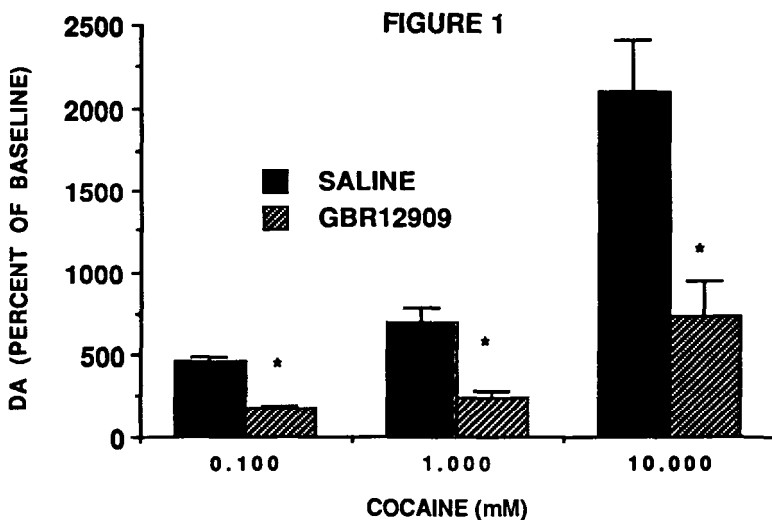
DISCUSSION. The ex vivo binding studies demonstrate that systemic administration of GBR results in a persistent occupation of the DA transporter. Administration of GBR at a dose which produces a substantial inhibition of [^{3}H]COC and [^{3}H]GBR12935 binding (25 mg/kg), produced only a modest increase in the levels of ECDA, yet substantially attenuated the increase in ECDA levels produced by COC. Defining ECDA as the dependent variable, these data are consistent with the hypothesis that GBR acts as a partial agonist. The clinical significance of these findings awaits clinical trials of GBR.

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AFFILIATIONS

Unit on Receptor Studies, LCS, NIMH
Biological Psychiatry Branch, NIMH
Laboratory of Medicinal Chemistry, NIDDK
Laboratory of Neurosciences, NIA; Bethesda, MD 20892.

Lack of a Genetic Correlation Between the Convulsant and Epileptogenic Effects of Cocaine and Lidocaine

J.M. Witkin, S.R. Goldberg, and R.J. Marley

The acute administration of cocaine produces clonic convulsions in rats, mice and humans. Furthermore, the repeated administration of cocaine can result in an increased susceptibility to cocaine-induced seizures (kindling). While many of the effects of cocaine have been attributed to its actions on monoamine systems, the convulsant and epileptogenic properties of cocaine are thought to be related to its local anesthetic effects. The local anesthetic properties of cocaine are similar to those of lidocaine, however, lidocaine has no direct effects on monoamine systems. Thus, lidocaine provides a means for investigating the local anesthetic properties of cocaine. We have assessed the convulsant effects of lidocaine in 4 isogenic strains of mice (BALB/cByJ, C57/6J, DBA/2J and SJL/J) and compared these results with similar experiments evaluating cocaine-induced seizures. The rank order for susceptibility to acute lidocaine-induced seizure (SJL > DBA > BALB > C57) was not the same as for cocaine (C57 > BALB = DBA > SJL).
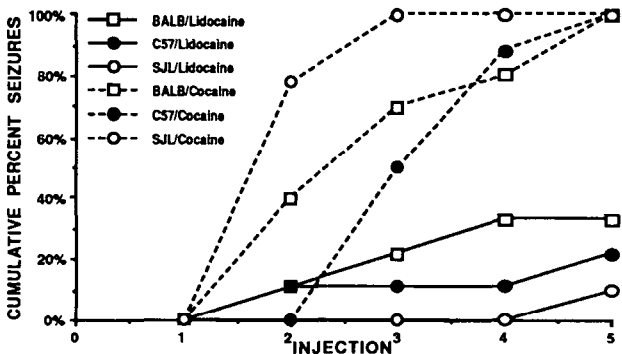
Fig. 1. Development of lidocaine- vs. cocaine-kindled seizures.

The epileptogenic properties of these 2 drugs were also compared in 3 of the 4 strains. To equate the different strains for differences in acute sensitivity to lidocaine-induced seizures, the highest doses of lidocaine at which no seizure activity was observed in drug naive animals (40 mg/kg for the SJL mice; 60 mg/kg for the BALB and C57 mice) were used for the investigations of the lidocaine kindling process. Lidocaine, or saline, was administered daily via i.p. injection for 5 days. The rank order of sensitivity for lidocaine kindling was BALB > C57 > SJL as opposed to SJL > BALB > C57 for cocaine kindling using 50 mg/kg (Fig. 1). Three days after the last injection, the lidocaine- and saline-treated mice were challenged with a convulsant dose of lidocaine that was 10 mg/kg higher than the treatment dose. To assess the effects of chronic cocaine administration on susceptibility to lidocaine-induced seizures, animals were injected with 50 mg/kg cocaine or saline for 5 consecutive days and challenged with the same convulsant dose of lidocaine used in the chronic lidocaine studies. On this measure of chronic treatment, the 3 strains did respond in a similar manner to lidocaine and cocaine (Fig. 2). Chronic treatment with lidocaine or cocaine resulted in sensitization to lidocaine-induced seizures among SJL mice, but gave rise to tolerance to these seizures among BALB and C57 mice.
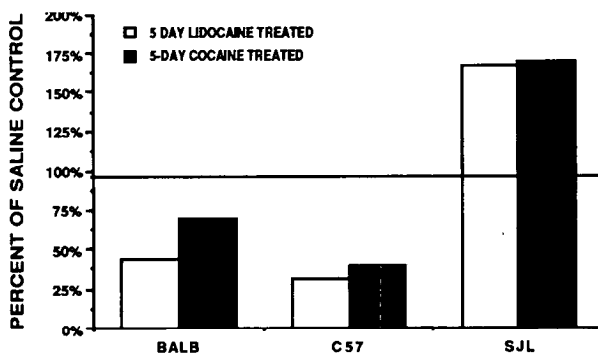
Fig. 2. Susceptibility to lidocaine-induced seizures following 5 daily injections of either lidocaine or cocaine.

Based on these results, the following conclusions were made: (1) genetic factors mediate acute sensitivity to the convulsant and epileptogenic properties of lidocaine; (2) the convulsant and epileptogenic effects of cocaine do not completely parallel those of lidocaine; (3) the repeated administration of lidocaine or cocaine results in the development of sensitization or tolerance to the convulsant effects of lidocaine depending on the genotype being examined; and (4) the local anesthetic properties of cocaine may account for individual differences in the ultimate effect of chronic cocaine administration on seizure susceptibility.

AFFILIATION: NIDA-Addiction Research Center, Baltimore, MD

Day Hospital Versus Inpatient Cocaine Dependence Rehabilitation: An Interim Report

Arthur I. Alterman

INTRODUCTION. This poster is an interim report which describes baseline, 4 and 7 month post-treatment entry outcome findings for **a** random assignment comparison study of Day Hospital and inpatient rehabilitation treatment for cocaine abusing men.

METHODS
Subjects. The research subjects were 80 men seeking treatment for cocaine abuse/dependence at a VA Medical Center. To qualify for the study, the individual had to be 50 years of age or under, have **a** stable residence and provide several informants for followup contact purposes. He could have no history of a psychotic disorder or dementia and no major medical problems. He could qualify for no current substance abuse diagnosis other than alcoholism or marijuana.

METHODS
Procedure. Informed consent was first obtained. Subjects were randomized to one month of our Day Hospital (n=41) program or inpatient (n=39) treatment at another VAMC 30 miles removed.

A 3 hour baseline battery was administered during week 1 which included the Addiction Severity Index (ASI) and the NIMH Diagnostic Interview Schedule (DIS) for DSM-III. Drug urine screens were taken twice weekly and at followup, and the ASI was done 4 and 7 months following entry into treatment. Nominal compensation was provided for completing the assessments.

RESULTS

Baseline. Few differences were revealed between the two groups. The subjects were about 33 years of age, had almost 12 years of education, and were almost entirely black. They averaged under 3 years of cocaine use, but had been drinking alcohol to intoxication for over 7 years.

Treatment completion. Inpatients (34 of 39, or 87%) were significantly more likely to complete treatment (p=.0013) than Day Hospital patients (21 of 41, or 51%).

Treatment outcome. The ASI data revealed considerable self-reported reduction 'of drug and alcohol-related problems for both groups at followups. The groups did not differ significantly on any of these variables. The reductions reported at 4 months generally were maintained at 7 months. About 60% of the subjects reported being abstinent at 4 months. At the same time, 11 of 20 (55%) urine screens obtained for Day Hospital patients were negative for cocaine, as contrasted with eight of 17 (47%) of those obtained from the inpatient subjects. 9 of 16 urine drug screens (56%) were negative for each of the groups at the 7 month follow. Thus, the urine screen data were consistent with the ASI findings.

The ASI data indicated significant improvements for both groups at 4 and 7 months with respect to legal, family/social and psychological problem levels. Group differences were not found in these areas.

CONCLUSIONS

While inpatients were more likely to complete the initial one month of rehabilitation treatment, both groups showed significant reductions in substance-related problems at both followup evaluations. No group differences were revealed. Improvements shown at 4 months were generally maintained at 7 months. The urine drug screen data supported these findings.

A Treatment Crisis: Cocaine Use by Clients in Methadone Maintenance Programs

A.F. Kolar, B.S. Brown, W.W. Weddington, and J.C. Ball

Cocaine use by opiate addicts being treated with methadone poses particular problems for methadone maintenance treatment programs (MMP) because of their physical deterioration, diminution in social functioning, as well as increased criminal activities. Many opioid addicts use cocaine intravenously, further risking the spread of HIV through needle and paraphernalia sharing. Given the prevalence of cocaine use and the treatment challenges posed by cocaine users in MMP, we undertook a study to examine the methods used in one metropolitan area to detect and respond to cocaine abuse by their clients.

METHODS

Eleven MMPs in the Baltimore area participated in the study. The 11 programs had a total census of 2,414 clients and ranged from a current census of less than 100 clients to a census of almost 400 clients. At each program one interviewer (AFK) interviewed either the program or clinical director with the medical director, staff counselors and/or nurses using a semi-structured questionnaire. Data came from face-to-face confidential staff interviews as well as record reviews. Urine toxicology reports were examined at the programs to determine the incidence of cocaine use the month prior to the survey. In addition we counted the number of clients who tested positive for cocaine in two or more urine samples during two consecutive months between July, 1988 and January, 1989.

RESULTS

Based on at least one positive urine for cocaine, the rate of cocaine use during the previous month was 15.7% (379/2414). Cocaine use by clients per program ranged from 5.9% to 33.0%. During the two consecutive months prior to our survey, 9.2% (222/2414) of the clients submitted 2 or more urines positive for cocaine. Urine screening methods differed and urine collection frequency also varied. The staff of the programs in urban Baltimore reported that clients' cocaine use as almost exclusively intravenous. Client use of crack or freebase cocaine was more common at suburban programs.

Urine screening for cocaine was obtained (1.7 ± 1.0) (mean ± SD) times per month. Increased urine testing is contained in the program policy statements of 8 of 11 MMPs. The remaining 3 programs relied on counselor's discretion in association with program guidelines. One program charged the cost of more frequent urine testing to the client.

The 11 program directors reported that after one urine sample is positive for cocaine the client is confronted by the counselor. Three programs mandated increased individual therapy after one positive urine. By the second positive urine 6 of the 11 programs report adding a group therapy experience, typically lasting 8 to 12 weeks.

Four of 11 programs discontinue take home privileges for methadone doses if a client submits one cocaine positive urine sample. Six of the 11 programs stop one take home dose for each positive urine sample.

Two programs implement contingency contracts with their cocaine abusing clients, by decreasing the individual's methadone dose by 5 to 10 mg. each time the client's urine is positive for cocaine.

All 11 programs hospitalize clients showing medical or psychiatric sequelae of cocaine abuse. They differ, however, in their criteria for referral for inpatient detoxification. Hospital detoxification is usually for 7 days. After 3 positive urines for cocaine, all 11 MMPs offer or mandate inpatient cocaine detoxification.

Policies for detoxification from methadone and discharge from the treatment programs consequent to cocaine abuse differ. One MMP specified discharge from the clinic after the user submitted 3 urine samples positive for cocaine. In some programs a client is discharged only when "everything else failed."

The mean (SD) number of discharges specific to cocaine use per program for the prior month was $4.0 \pm (4.6)$. This represented a discharge rate, (number of clients discharged in the prior month because of cocaine over the total census in the prior month of each of the 11 programs) from 0.0% to 5.4%. The estimated number of clients discharged in the prior year at individual programs ranged from 0 to 200.

DISCUSSION

The treatment response to cocaine-abusing clients at the 11 MMPs revealed a pattern of responses which combine intensified treatment with additional surveillance. Administrative detoxification and discharge were only utilized as a last resort.

The relatively low prevalence of cocaine use (15.1%) evidenced by the positive urine screens may appear surprising. While one can assume some diminution in cocaine use consequent to treatment entry, it also seems likely that the infrequent urine screens employed in most MMPs do not detect a large number of cocaine users.

A.F. Kolar, M.D.; B.S. Brown, Ph.D.; W.W. Weddington, M.D., and J.C. Ball, Ph.D.
Addiction Research Center, National Institute on Drug Abuse, Baltimore, RD.

An Open Pilot Study of Bupropion and Psychotherapy for the Treatment of Cocaine Abuse in Methadone-Maintained Patients

**Arthur Margolin, Thomas Kosten, Ismene Petrakis,
S. Kelly Avants, and Therese Kosten**

Bupropion hydrochloride (Wellbutrin) is a "second generation" anti-depressant whose mechanism of action, while essentially unknown, is apparently fundamentally different from either tri-cyclic or MAO-inhibitor anti-depressants--it has mild dopaminergic activity and little or no effect on serotonin and norepinephrine systems. In light of the hypothesized connection between cocaine "withdrawal," depletion of brain dopamine, and post-cocaine use depression, the fact that bupropion has been shown to be an effective anti-depressant which has few anti-cholinergic side-effects, decreases dopamine turnover, and is well-tolerated by most patients suggested its use for the treatment of cocaine abuse. We tested bupropion in an open pilot study with six cocaine-abusing methadone patients. For eight weeks subjects took bupropion 100 mgs three times daily and participated in twice weekly psychotherapy sessions. Outcome measures included pre- and post-treatment psychophysiology sessions measuring physiological reactivity (skin temperature and skin conductance level) and self-report of "craving" to neutral and cocaine-related cues; during the study, and during a four-week follow-up period, urine samples were taken twice weekly and tested for cocaine use; in addition, subjects completed a pre- and post-treatment "self-schemata" questionnaire, in which they were asked to list traits that described different aspects of themselves, such as their addict sense of self and their ideal sense of self.

Results showed that bupropion had few side-effects in five out of six subjects. One subject was terminated from the

study because of episodes of hypomania. Four subjects experienced mild side-effects of dry mouth and jitteriness. Dosage of bupropion was decreased to 100 mg twice daily and these symptoms disappeared. Self-report of patients who used cocaine during the study gave no evidence of clinical interactions between bupropion and methadone or bupropion, methadone and cocaine. At the end of eight weeks, cocaine use as determined by number of patients having positive urines for cocaine dropped sharply, from a pre-treatment mean of 4.5 subjects/week to 1 subject/week. Subjects reported an increase in intensity and duration of non-drug related positive affect, and a decrease in negative affect. They also reported fewer episodes of cocaine craving consequent to dysphoric states. Levels of cocaine, craving were also reduced to cocaine-related cues in post-treatment psychophysiological sessions. No subjects showed a marked response to neutral or cocaine cues in skin temperature. Two subjects were reactive to cocaine cues as measured by skin conductance levels, both pre- and post-treatment. Cocaine craving was reduced to zero post-treatment in these two subjects, while two low-reactive subjects continued to self-report mild craving. This lack of concordance between self-report and skin conductance levels warrants further investigation with greater numbers of subjects. Subjects also showed a shift in their sense of self: at the end of eight weeks they felt less like their addict sense of self and more like their ideal sense of self. These results suggest that bupropion and psychotherapy may be a useful psychopharmacotherapy for the treatment of cocaine abuse. We are planning to conduct a double-blind placebo controlled trial using bupropion and psychotherapy in both methadone-maintained patients and in "pure" cocaine abusers.

ARTHUR MARGOLIN, PH.D.
THOMAS KOSTEN, M.D.
ISMENE PETRAKIS, M.D.
S. KELLY AVANTS M.S.
THERESE KOSTEN, PH.D.

YALE UNIVERSITY, DEPARTMENTS OF PSYCHIATRY AND PSYCHOLOGY

Incidence of Personality Disorders in Cocaine vs. Alcohol-Dependent Females

H.M. Pettinati, R.A. Cabezas, J. Jensen, B.D. Evans, and K. Meyers

Evaluating substance dependent patients for Axis-II diagnoses, i.e., personality disorders, is important in treatment planning and, potentially, in determining prognosis. Preliminary reports have found differences in the prevalence of personality disorders in this population, based on the kind of chemical addiction (Mirin & Weiss, 1988). and gender (Griffin, 1989). Currently, there is minimal information on personality disorders with regard to prevalence and type of disorder in female cocaine dependent patients from various tx settings. In particular, comparisons between these women and other female substance abusers, e.g., female alcohol dependent Ss, are lacking. The present study included 140 patients (80% inpatients; 51% cocaine, 20% female) who had come for short-term, intensive treatment for (DSM-III-R) cocaine and/or alcohol use disorders at a specialized addiction unit in a private, nonprofit, psychiatric hospital. They were assessed 4-6 weeks into intensive tx using the Structured Clinical Interview for DSM-III-R Personality Disorders (SCID-II). Interviewers were careful to ask Ss to not include drug/alcohol related behaviors when responding to questions. Significantly more cocaine compared to alcohol dependent Ss met DSM-III-R, Axis-II criteria for a personality disorder (p=.02), regardless of gender. Preliminary analyses showed that significantly more cocaine dependent females (75%) met DSM-III-R criteria for a personality disorder compared to alcohol dependent females (32%; p=.02). This approached significance when compared to cocaine dependent males (45%; p =.ll).   Thus, the type of personality disorder differed with the type of substance abused and gender. This was particularly apparent when Ss were grouped by their primary severe personality diagnosis, according to DSM-III-R cluster. Specifically, while self-defeating personality disorder was most prevalent for alcohol dependent females (50%), paranoid, borderline and histrionic were most prevalent for cocaine dependent females (33.3% for each).

Interestingly, Avoidant personality disorder was prominent in all four subject groups. Also, the difference approached significance between the frequency of female cocaine dependent $\underline{S}$s with personality disorders in Cluster B vs female alcohol dependent $\underline{S}$s with personality disorders in Cluster C ($\underline{p}$=.12).

CARRIER FOUNDATION, NEW JERSEY

Clinical Features of Cocaine Induced Paranoia

Sally L. Satel, Steven M. Southwick, and Frank H. Gawin

In a sample of 50 cocaine dependent males, 68% reported highly distressing transient paranoid states. Paranoid (34/50) and non-paranoid (16/50) groups did not differ by demographic features or by usage parameters. For those reporting paranoia, the mean duration and amount of cocaine use prior to the development of paranoia was not significantly different from the mean lifetime duration and amount used by the non-paranoid group. This suggests that development of binge-limited paranoia in heavy user (min .5g/wk) is not a simple result of exceeding a threshold of usage and that affected individuals likely possess a predisposition to this drug induced state. Paranoia became more severe and developed more rapidly with continued use; this is consistent with a sensitization model of cocaine induced paranoia.

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AFFILIATION

Yale University School of Medicine
Substance Abuse Treatment Unit
New Havená CT 06519

Liver Function Tests In Non-Parenteral Cocaine Users

Javier Tabasco-Minguillan, David M. Novick, and Mary Jeanne Kreek

Cocaine is associated with experimental hepatotoxicity in the mouse, but its effects on the liver in man have not been systematically studied. Recently, several cases of hepatic injury following acute cocaine intoxication have been reported. We studied 46 cocaine users with no history of parenteral drug use or homosexuality. Liver function tests were similar in 21 users of cocaine only (Group A) and 25 users of cocaine and alcohol (Group B). The mean alcohol consumption of Group B was 64 ± 12 g/day. All mean values of liver function tests for both groups were within the normal range. Only three patients, two of whom were hepatitis B carriers, had an alanine aminotransferase level more than five units above normal limits. Group B patients were significantly more likely to complain of headaches (p=0.009), irritability (p=0.03), and loss of memory (p=0.02) than Group A patients. We conclude that 1) non-parenteral cocaine use is rarely associated with significant liver function test abnormalities, and 2) alcohol may potentiate some adverse effects of cocaine.

AFFILIATIONS

Department of Medicine, Beth Israel Medical Center, New York, NY 10003, and The Rockefeller University, New York, NY 10021.

Evidence for Kinetic Disparity Among Pertussis Toxin Substrate G-Proteins Coupled to Opiate Receptors

Donna J. Carty

Opiate dependence is a multiple receptor phenomenon suggesting post-receptor loci as targets for changes during the development of dependence. A common feature of all opiate receptors is their ability to modulate effector function using heterotrimeric GTP-binding proteins that are pertussis toxin substrates. Therefore, to understand and perhaps interfere with the mechanisms of drug dependence, it is important to understand what unique functional characteristics each G-protein has. A single cell may contain several members of each of the pertussis toxin substrate G-protein subfamilies, G_i's and G_o's, Using native G-proteins, we have found that within G-protein subfamilies there may not be stringent receptor or effector specificity. All three G_i's open K^+ channels with similar potencies and both forms of G_o stimulate phospholipase C activity. Hence the intrinsic properties of G-proteins were analyzed for possible differences.

We find that there are distinct differences in guanine nucleotide binding and release characteristics, both between families and among members of the same family. The G_i's require added Mg^{2+} in the millimolar range to bind GTPγS and bind GTPγS much more slowly than the G_o's (Figure 1). G_{i2} required 20 min to achieve maximum binding at 2 mM Mg^{2+}, G_{i1}, 65 min, and G_{i3}, 90 min. Both G_{o1} and G_{o2} achieve maximum binding of GTPγS within 4 min in the absence of added Mg^{2+}, but binding to G_{o1} is approximately twice as fast as to G_{o2}.

G_o achieves maximum binding faster in the presence of micromolar free Ca^{2+} than in the presence of micromolar Mg^{2+} (Figure 2). Maximum binding was somewhat less with Ca^{2+} than with Mg^{2+}. The release of bound GDP from G_o's is also fast (<1 min). The release of GDP from G_{i2} is also fast. In comparison, release of GDP from G_{i1} and G_{i3} were slower, G_{i1} requiring 4 min and G_{i3} 2 min.

These results indicate that guanine nucleotide binding and release are separately regulated intrinsic propertics of the individual G-proteins and suggest that alterations in the ratios of these G-proteins or in intracellular ion concentrations may have a significant

effect on the kinetics and efficiency of the signal transduction process. This research was supported by NIDA Training Grant DA 07135.

Figure 1:
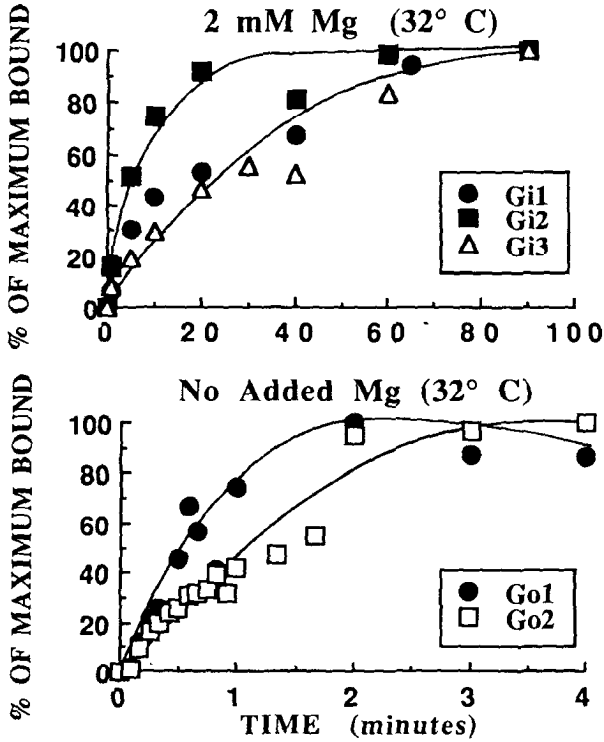
Figure 2:
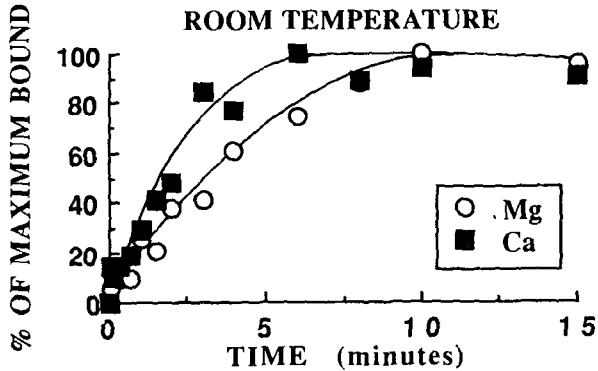
Department of Pharmacology, Mount Sinai School of Medicine, New York, NY.

Stereospecificity of Mixed-Action Opioid Agonist/Antagonists in Morphine-Tolerant Squirrel Monkeys

Linda A. Dykstra, Mitchell J. Picker, and Kelly Powell

Previous studies in squirrel monkeys responding under a schedule of food presentation have shown that monkeys develop tolerance to morphine as well as cross-tolerance to other morphine-like drugs. Moreover, morphine-tolerant monkeys become more sensitive to the effects of opioid antagonists such as naloxone. In the present study, the isomers of the mixed action opioids, (±) cyclazocine, (±) n-allylnormetazocine, and (±) pentazocine, were examined in squirrel monkeys responding under a fixed-ratio 30 schedule of food presentation. Dose-effect curves for all drugs were obtained prior to, during, and following a chronic regimen in which monkeys received 6 mg/kg/day of morphine. When compared to the dose-effect curves obtained prior to the chronic regimen, the morphine dose-effect curve obtained during the chronic regimen was shifted to the right 0.5 - 1.0 log unit whereas the naloxone dose-effect curve shifted over 3 log units to the left. No changes were observed between the prechronic and chronic dose-effect curves for (+) cyclazocine, (+) n-allylnormetazocine, and (+) or (-) pentazocine. The (-) isomers of n-allylnormetazocine and cyclazocine shifted 0.6 - 1.7 log units to the left. These results suggest that the stereoisomers of cyclazocine and n-allylnormetazocine can be differentiated in morphine-tolerant monkeys. Moreover, the leftward shifts in the dose effect curves for the (-) isomers of cyclazocine and n-allylnormetazocine suggest that these isomers have mu antagonist properties which are revealed during chronic morphine administration.

Authors

Linda A. Dykstra
Mitchell J. Picker
Kelly Powell

Departments of Psychology and Pharmacology
University of North Carolina
Chapel Hill, N.C.

The Effect of Prenatal Methadone Exposure of Development and Nociception During the Early Postnatal Period of the Rat

E. Karl Enters, Hongrhi Guo, Usha Pandey, Daijin Ko, and Susan E. Robinson

Methadone readily crosses the placenta and has been found to concentrate in the fetal brain, delaying postnatal brain growth in the rat. It is well-accepted that infants born to mothers maintained on methadone have low birth weights and undergo an abstinence syndrome. These same findings are duplicated in animal studies: prenatal exposure to methadone produces behavioral changes suggestive of neonatal abstinence followed by a more protracted disturbance in rest-activity cycles. Even more interesting and potentially of greater social impact, are reports that adult rats exposed prenatally to opioids, including methadone, demonstrate a difference in sensitivity to centrally-acting drugs with abuse potential and enhanced self-administration of morphine.

Nulliparous 100 day old female Sprague-Dawley rats (240-270g) were mated with male rats of the same strain. Food and water intake, as well as maternal weight, were measured daily. On day 8 of gestation, two groups of the female rats were briefly anesthetized with methoxyflurane and implanted subcutaneously with 14-day osmotic minipumps (Alza, Palo Alta, CA) filled with sterile water or methadone hydrochloride (initial dose rate, 9 mg/kg). An additional group of untreated control dams was also included. On either postnatal day 4 or 21, pup responses to opiate challenge (morphine: 0.4 mg/kg, s.c. on P4; 1.5 mg/kg, s.c. on P21). or blockade (naloxone, 1 mg/kg, s.c.) in the presence of a nociceptive stimulus were measured by use of the tail-flick test. An additional baseline group (saline, 20 µl, s.c.) was included. Methadone hydrochloride and morphine sulfate were obtained from the National Institute for Drug Abuse (NIDA) and naloxone hydrochloride was the kind gift of Endo Laboratories (Garden City, NY).

 Dams treated with water or methadone exhibited no significant differences in weight gain or food or water consumption from that of the untreated dams. Dams developed and retained dependence upon methadone through parturition. When challenged with naloxone (2 mg/kg, s.c), within 24 hours of delivery, dams and pups in the methadone group exhibited a withdrawal syndrome. There were no differences in litter size or sex distribution among the 3 treatment groups. Methadone exposure did not affect the number of implantation sites. Although there were no dead pups in the litters born to the untreated and water-treated dams, a small, but significant, number of dead pups were observed in the litters born to the methadone-treated darns. The total mortality rate was 16% in the methadone treated group, with all deaths occurring on or before day 1. Additionally, the weights of both female pups and male pups in the water and the

methadone treatment groups were slightly, but significantly, reduced on the first postnatal day, resolving by posmatal day 2 in males, but persisting until the third posmatal day in female pups.

As expected, morphine administration induced significant tail-flick analgesia in pups from all three treatment groups at both 4 and 21 days. However, methadone-exposed 4-day-old male pups exhibited a significantly enhanced analgesic response to the morphine challenge, with female pups exhibiting a similar, but statistically insignificant enhanced analgesic response to morphine. In contrast, methadone-exposed 21-day-old male pups exhibited a significantly reduced analgesic response to the morphine challenge, with female pups exhibiting a similar, although non-significant, trend towards a reduced analgesic response to morphine.

In summary, we have found that prenatal exposure to methadone administered to the dam via osmotic minipumps produces little effect on maternal health or litter size, but is sufficient to produce dependence in both the dam and pups. Furthermore, methadone-exposed pups exhibited a significantly enhanced analgesic response to morphine at P4, and a reduced analgesic response to morphine at 21 days postnatally.

AFFILIATION

Department of Pharmacology and Toxicology
Medical College of Virginia, Virginia Commonwealth University
Richmond, Virginia 23298-0613, U.S.A.

Design and Synthesis of Opioid Receptor Selective Enkephalin Analogues

Catherine A. Gehrig, Om Prakash, Geza Toth, and Victor J. Hruby

Using the method of conformational constraints we have designed and synthesized a series of highly delta-opioid receptor selective analogues of the enkephalin analogue DPDPE which has the sequence modified at the Phe4 position. DPDPE, which has the sequence H-Tyr-D-Pen-Gly-Phe-D-Pen-OH, contains a 14-membered ring via a disulfide bridge between the D-Pen[2] and D-Pen[5] side chain groups. This global conformational constraint restricts the conformational possibilities of the peptide, and permits us to use DPDPE as a template upon which to design the next generation of analogues with greater selectivity and potency at the delta-opioid receptor.

Preliminary modeling studies and the results of bioassays indicate the that the Phe4 residue is important for activity at the delta-opioid receptor (1,2). Local conformational constraints such as the addition of a methyl group at the beta carbon of the amino acid, dehydration of the $C\alpha$-$C\beta$ bond, or addition of a methyl group of the aromatic ring of phenylalanine, all further restrict the conformational space available for this side chain group. DPDPE analogues containing the unusual amino acid ß-Me-Phe (all four isomers), dehydro-Phe, and 2'-Me-Phe recently have been synthesized by the solid phase method.

In particular, bioassays (GPI and MVD) and binding data of the ß-methyl-Phe containing analogues demonstrate a wide range of potencies and selectivities for the delta vs. mu opioid receptors (See Table). Especially interesting is the extreme delta selectivity of [(2S,3R)-ß-Me-Phe[4]]-DPDPE. This analogue, while approximately 30-fold less potent at the δ receptor, is nearly completely inactive at the μ receptor. Its diastereoisomer, [(2S,3S)-ß-Me-Phe[4]]DPDPE, shows in vitro activity comparable to DPDPE itself at the delta receptor and a 5-fold drop in activity at the mu receptor.

Table: DPDPE Analogues Inhibitory Potency and Selectivity in MVD and GPI Bioassays

	PEPTIDE	IC50 (nM)		GPI/MVD
		MVD	GPI	Ratio
1	DPDPE	4.26	9110	2100
2	(2S,3S)-ß-Me-Phe^4-DPDPE	2.34	42,660	18,000
3	(2R,3R)-ß-Me-Phe^4-DPDPE	72.4	>10,000	>140
4	(2S,3R)-ß-Me-Phe^4-DPDPE	148	>1,500,000	>10,000
5	(2R,3S)-ß-Me-Phe^4-DPDPE	>10^6	32,500	<0.03

ß-Me-Phe was chosen as a residue for study because of the different topographical properties it will provide a conformational template structure (3). Hence the conformations of these analogues became of critical importance and have been examined in detail by NMR and other physical methods. The similarities between the NMR data for these compounds suggest that the conformation of the peptide backbone has not been greatly altered. However, the change in stereochemistry at the ß-carbon of ß-Me-phenylalanine has a profound effect on the topographical disposition of the phenyl group at position 4. Therefore, it can be concluded that the large changes in biological activity can be attributed to changes in the topography of the side chain groups but not to changes in the conformation of the peptide backbone. These results further elucidate the topographical features required for delta vs. mu receptor selectivity. Supported by USPHS Grant NS19972 and the National Science Foundation.

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AFFILIATION

Department of Chemistry, University of Arizona, Tucson, AZ 85721

Pharmacological Properties of (+)-Buprenorphine and (+)-Diprenorphine

N.A. Grayson, R.B. Rothman, H. Xu, and K.C. Rice

(-)-Buprenorphine and (-)-diprenorphine are semisynthetic opium derivatives with established pharmacological profiles produced by interaction with classical opioid μ, δ, and κ receptors. (-)-Buprenorphine is a potent analgesic which demonstrates narcotic agonist-antagonist properties and (-)-diprenorphine is a narcotic antagonist capable of reversing the agonist actions of pure agonists and some mixed agonist-antagonists. More recently, (-)-buprenorphine has been found to suppress cocaine self-administration in monkeys through mechanisms which are unclear at this time.

Although the unnatural (+)-opiate enantiomers traditionally have insignificant affinity for classical opioid receptors in comparison to their (-)-counterparts, some of these compounds have been found to bind to other CNS sites and could have potential clinical utility. For example, the (+)-opiate-related dextromethorphan displays neuroprotective properties in CNS ischemic injury models and is a nonprescription antitussive with low affinity for the classical opioid receptors. Examination of the pharmacological profile of the (+)-isomers of buprenorphine and diprenorphine is therefore important both for defining the role of the (-)-isomers at the opioid receptor and in the search for new therapeutic agents. In addition, (+)-buprenorphine is a potentially valuable research tool in mechanistic studies of suppression of cocaine self-administration by the (-)-isomer.

EXPERIMENTAL

Both (+)-buprenorphine and (+)-diprenorphine were synthesized from (+)-thebaine, an intermediate available from the NIH Opiate Total Synthesis. Mu binding sites were labeled using [³H]DAGO and rat lysed-P2 membranes. Delta binding sites were labeled using [³H][D-ala², D-leu⁵]enkephalin and rat lysed-P2 membranes. Nonspecific binding was determined using levallorphan. Kappa₁ binding sites were labeled using [³H]U69,593 and guinea pig brain membranes depleted

of mu and delta binding sites by pretreatment with BIT and FIT
Nonspecific Kappa$_1$ binding was determined using U69,593.

RESULTS AND DISCUSSION

Our results indicate that the μ, δ and κ binding sites exhibit affinity in the pM range for the (-)- isomers of buprenorphine and diprenorphine. Conversely, as expected, none of these receptors possesses high affinity for either of the (+)-isomers. The opioid receptor subtype binding enantioselectivity ratio $[K_i(+)/K_i(-)]$ exceeds 10^6 for these compounds. These results are important for evaluating the safety and specificity of these compounds as potential medicinal agents (e.g. antitussives).

Previously, the preference of the μ receptor for (-)- over (+)-etorphine (K_i ratio = 3.5 x 10^5) was thought to be one of the most extreme examples of receptor enantioselectivity. However, the δ receptor demonstrates an extremely high relative affinity for (-)-diprenorphine in comparison to (+)-diprenorphine (K_i ratio = 7 x 10^7) The enantioselectivity of the δ receptor for (-)- over (+)-diprenorphine exceeds that of the μ receptor for (-)- over (+)-etorphine and is the largest enantioselectivity ratio of which we are aware.
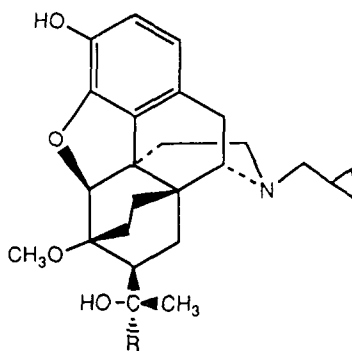
R = CH$_3$ = (+)-Diprenorphine
R = C(CH$_3$)$_3$ = (+)-Buprenorphine

AFFILIATIONS

Laboratory of Medicinal Chemistry, NIDDK (N.G., K.C.R.) and Laboratory of Cell Biology, NIMH (H.X., R.B.R.), National Institutes of Health, Bethesda, MD 20892

Factors Influencing Self-Administration of Aerosol Sufentanil in Rats

A.B. Jaffe, L.L. Weinhold and L.G. Sharpe

Previous investigators have reported that environmental and biological factors may influence the initiation and maintenance of drug-seeking behavior in animals. For example, it has been reported that individually-housed rats self-administer more i.v. heroin during the acquisition phase than do rats housed in groups (Bozarth et. al., 1989). Piazza et. al. (1989) observed a positive relationship between activity levels and drug self-administration rates. Panksepp et. al. (1984, 1985) reported that the administration of morphine to juvenile rats increased their frequency of pinning opponents upside-down.

We have recently reported (Jaffe et. al., 1989) that rats will reliably self-administer a mu selective opiate (sufentanil citrate) in aerosol form. In the present investigations, we examined the relationships between sufentanil aerosol self-administration and: housing conditions, activity levels and pinning opponents upside-down (pinning behavior).

The general procedure consisted of training adult male Sprague-Dawley rats (N=40) to perform a nose-poking response that resulted in a 2 sec delivery of sufentanil aerosol (SF) under an FR 5 schedule of reinforcement. Six rats in the pinning behavior experiment had access to water vapor (WV). Training consisted of 5 overnight sessions (14-16 hr) in which the reinforcement schedule was changed from FR 1 to FR 5 over the 5 days. After training, the animals were shifted to 2 hr daytime sessions (FR 5). At least 48 hr lapsed between the vapor self-administration sessions.

In the housing condition experiment weaning rats were either housed individually (n=6) or with a weight-matched cagemate (n = 3 pairs). Drug training began approximately 85 days later. The conditions were 5 alternative series of SF-DW exposures during the daytime sessions.

For the activity level experiment, rats were either housed individually (n=8) or with a weight-matched cagemate (n=4 pairs). The conditions during daytime sessions were either SF-DW or DW-SF. Activity levels were measured immediately after the vapor sessions.

During the experiment to monitor pinning behavior, rats were housed with a weight-matched cagemate (N=6 pairs). The conditions consisted of 6 rats on water vapor and 6 rats on sufentanil vapor during 6 daytime sessions. Videotapes of pinning behaviors between pairs of rats were recorded 10 min before and after the vapor sessions.

The results showed that: 1) rats housed individually self-administered more sufentanil vapor during the acquisition phase than did rats housed with a cagemate, 2) locomotor counts after the sessions were positively related to the amount of sufentanil and water vapor self-administered and 3) the duration of pinning behavior was positively related to the amount of sufentanil but not water vapor self-administered.

The relationships between the amount of sufentanil aerosol self-administered and the durations of pinning behaviors are as follows: (1) More sufentanil was self-administered by rats that had encountered greater differences in pinning durations before the vapor sessions (r=0.36). (2) Rats that self-administered more sufentanil also spent more time pinning their opponents after the vapor sessions (r=0.39). (3) Rats spent more time pinning their opponents after than before the sufentanil self-administration sessions. In contrast, rats spent less time pinning their opponents after than before the water vapor self-administration sessions.

In conclusion, housing conditions, locomotor activity and pinning behavior influence sufentanil vapor self-administration but it is not clear whether these are mutually exclusive determinants in drug-taking behavior. Sufentanil but not water vapor self-administration led to increases in pinning behaviors, indicating that some form of aggression is initiated by sufentanil in certain types of social situations. Our results agree with those using oral and intravenous opiate self-administration in rats, findings that may lead to understanding what biological and environmental factors are important to the vulnerability of drug-seeking behavior. Aggressive behavior in humans during childhood and adolescence is a predictor of adult drug abuse, but in our present animal model we do not know to what extent aggression contributed to the self-administration of sufentanil aerosol.

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AFFILIATION:

NIDA, Addiction Research Center P.O. Box 5180, Baltimore, MD

Effects of Oxymorphindole on Morphine Induced-Antinociception in Mice and Rats

Q. Jiang, Kenner C. Rice, Brain deCosta, and Frank Porreca

Recent investigations have demonstrated that the opioid δ receptor can directly mediate antinociception in the mouse. Additionally, however, data also have been accumulated which suggest that [Leu[5]]- and [Met[5]]Jenkephalin, the putative endogenous δ ligands, can indirectly modulate u-mediated effects such as antinociception through actions at the δ receptor. Evidence that these modulatory effects are mediated via a δ receptor includes recent observations that the δ selective antagonist ICI 174,864 prevents both the increase and the decrease of morphine antinociception produced by sub-effective doses of [D-Pen[2,5]]Jenkephalin (DPDPE) and [D-Ala[2],Met[5]]enkephalinamide (DAMA), respectively. Further, studies *in vitro* have demonstrated apparent noncompetitive interactions between μ and δ binding sites. Based on these types of observations, it was proposed that the modulatory effects of δ agonists on μ-mediated antinociception occur through a δ binding site of an opioid receptor complex involving some μ and some δ receptors. Thus, it is hypothesized that δ receptors can exist either separately or in a functionally- or physically-associated state with μ receptors, termed the δ non-complexed and δ complexed receptors, respectively. In the present study, the direct agonist and modulatory profile of the non-peptide δ opioid, oxymorphindole, was compared with that of the peptide δ agonist, DPDPE in the mouse and rat.

Acute antinociceptive effects of oxymorphindole, DPDPE and morphine were evaluated in the 55° C warm-water tail-flick test in both species; all compounds were given by the intracerebroventricular *(i.c.v.)* route and antinociception tested after 10 min (mice) or 20 min (rats). Morphine produced dose-related antinociception in both species which was not blocked by ICI 174,864 while DPDPE produced dose-related antinociception only in the mouse; this effect was blocked by co-administration of ICI 174,864 (4.4 nmol). In contrast to DPDPE, direct antinociceptive effects of oxymorphindole could not be demonstrated in either species under these conditions. In spite of a failure to

demonstrate direct antinociceptive actions, oxymorphindole was able to modulate morphine antinociception in both species. In the mouse, co-administration of oxymorphindole (6.38 nmol) or a threshold dose of DPDPE (1.55 nmol) significantly increased morphine antinociceptive potency, while in the rat, co-administration of oxymorphindole (21.25 nmol) or DPDPE (15.5 nmol) antagonized morphine antinociceptive potency; both the positive (mice) and negative (rats) modulation of morphine antinociception (but not the direct agonist actions of morphine) were blocked by ICI 174,864 (4.4 nmol).

These data are consistent with the possibility that oxymorphindole may act selectively at the putative $\delta_{complexed}$ receptor, while DPDPE, and the δ antagonist ICI 174,864 act at both $\delta_{complexed}$ and $\delta_{non-complexed}$ receptors. Further experimentation under differing conditions of nociceptive stimulus will be necessary before this hypothesis can be fully tested. Nevertheless, these observations provide further support for the concept of functionally complexed μ and δ opioid receptors and suggest the possibility of identification of selective compounds at these hypothesized sites. These types of compounds will be of critical importance in determining the validity of the hypothesis of coupled μ and δ receptors.

AFFILIATIONS

Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, and Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

Involvement of Spinal Adenosine in Descending Antinociception Produced by Supraspinal Receptor-Selective Opioid Agonists

Zhiwei Li, Gary E. DeLander, and Frank Porreca

The possible involvement of adenosine in opioid antinociception has been supported since Ho et al., (J. Pharmacol. Exp. Ther. **185:** 336-346, 1973) first reported that theophylline, a nonselective adenosine antagonist, reduced the antinociception produced by subcutaneous *(s.c.)* morphine in the rat. Subsequently, DeLander and Hopkins (J. Pharmacol. Exp. Ther. **239:** 88-93, 1986) showed that intrathecal *(i.th.)* theophylline produced a dose-dependent antagonism of the antinociception produced by *i.th.* or intracerebroventricularly-given *(i.c.v.)* morphine in the mouse tail-flick and hot-plate assays. Recent studies by Sweeney et al. (J. Pharmacol. Exp. Ther. **248:** 447-454, 1989). have directly demonstrated morphine-evoked release of adenosine from synaptosomes of rat spinal cord *in vitro* and following morphine administration *in vivo*.

While these studies are supportive of the involvement of adenosine as a secondary neurouansmitter in the antinociceptive effects of morphine at both spinal and supraspinal sites, it is unclear whether adenosine can be associated with the result of activation of other opioid receptor subtypes. Our previous studies have shown that *i.th.* theophylline antagonized the effects of *i.th.* morphine, [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO), [D-Pen^{2,5}]enkephalin (DPDPE) and ß-endorphin (ß-END) in the mouse tail-flick, but not hot-plate, test, implicating adenosine as a secondary neurotransmitter in local spinal antinociceptive processes. The present study now extends these observations by examining the possible involvement of spinal adenosine in the antinociception resulting from supraspinally-administered opioid receptor-selective agonists acting at opioid receptor subtypes. such as the μ and δ, as well as the putative ε receptor. Additionally, it was unclear whether adenosine involvement could be implicated in other (i.e., non-antinociceptive) actions of opioids. These questions were addressed by studying antinociceptive and gastrointestinal effects of receptor selective opioid agonists following *i.c.v.* administration to mice in the presence or absence of concurrently-given *i.th.* theophylline.

Male, ICR mice received direct intracerebroventricular *(i.c.v.)* injections of graded doses of morphine, DAMGO, DPDPE or ß-END concurrently with *i.th.*

theophylline (55 nmol), and antinociception was determined after 10 min using the 55°C warm water tail-flick test. In all cases, *i.th.* theophylline blocked the antinociceptive actions of the *i.c.v.* agonist. In the presence of *i.th.* theophylline, parallel rightward shifts were observed for *i.c.v.* morphine and DAMGO; *i.th.* theophylline produced an increase in the *i.c.v.* morphine and DAMGO A_{50} of 14 and 210-fold, respectively. In contrast, the dose-response lines for *i.c.v.* DPDPE and ß-END after *i.th.* theophylline were relatively flat and did not achieve a 100% effect; approximate estimates of the rightward displacement of *i.c.v.* DPDPE and ß-END by *i.th.* theophylline were 8- and 66-fold, respectively. The order of greatest rightward displacement of the *i.c.v.* antinociceptive dose-response lines by *i.th.* theophylline was DAMGO > ß-END > morphine > DPDPE. In contrast, *i.th.* adenosine did not antagonize the gastrointestinal actions of *i.c.v.* opioids, suggesting that spinal adenosine is not involved in the outflow pathways to the gut which are activated by opioids. These data support the local involvement of spinal adenosine in descending antinociceptive processes resulting from the activation of supraspinsl μ and δ, as well as the putative ε, opioid receptors.

AFFILIATIONS

Department of Pharmacology, University of Arizona, Tucson, AZ 85724 and *Department of Pharmacology, College of Pharmacy, Oregon State University, Corvallis, OR 97331

Oxide-Bridged 5-Phenylmorphans as Probes for Narcotic Receptor Mediated Phenomena

J.T.M. Linders, T.R. Burke, A.E. Jacobson, and K.C. Rice

5(*m*-Hydroxyphenyl)-2-methylmorphan (**1**) synthesized 35 years ago by May and Murphy (1955), has proven to be a compound with a remarkable pharmacological profile. In its racemic form the compound shows morphine-like activity, but although both the (+)- and (-)-enantiomers possess strong antinociceptive activity, the (-)-antipode is not morphine-like in a number of *in vivo* tests, suggesting its interaction with different (non-μ) opioid receptors.

1
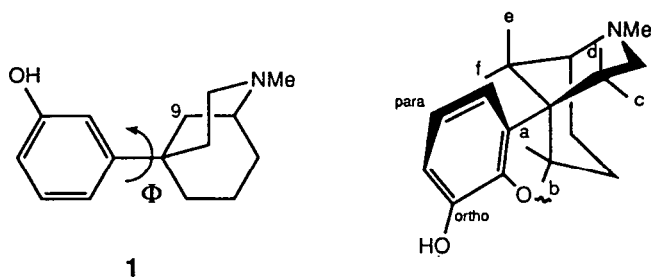
Compound **1** is conformationally heterogeneous. The three low-energy conformers, typified by the phenyl ring eclipsing each of the three C-C bonds, are not equally populated. Introduction of a methyl group in the 9α-position restricts the freedom of rotation and the number of accessible conformations even more, and leads to decreased agonist and increased antagonist activity (Awaya et al. 1984).

In order to correlate the pharmacological activities of 5phenylmorphans with their conformations (i.e., the orientation of the phenyl ring relative to the bicyclo[3.3.1]nonane skeleton) the torsion angle Φ was fixed by anchoring the aromatic moiety with an epoxy bridge. Two sets of six compounds can be envisaged, having the oxide bridge either *ortho* or *para* relative to the other oxygen function. Connection of the epoxy bridge to positions a, d, and f of the bicyclo[3.3.1]nonane part of the molecule afforded compounds which showed relatively low affinity for the opioid receptors (IC_{50} = 1766, 1000, and 96 nM, respectively, compared with the IC_{50} of **1** = 5.2) (Burke et al. 1986).

We have now begun a study directed towards the synthesis of the *ortho*- and *para*-substituted "e"-isomers **6** and **7**. Ketone **3** was synthesized from 2-(*m*-methoxyphenyl)cyclohexanone in four steps (May and Murphy 1955). Stereoselective reduction to the alcohol **4,** followed by partial reduction of the aromatic nucleus under Birch conditions gave cyclohexadiene **5.** Preliminary experiments suggest that hydrolysis to the conjugated ketone and bromination with *N*-bromoacetamide led to formation of the desired oxide bridge. Aromatization will give 5-phenylmorphan **6.** Depending on the conditions used for the hydrolysis, it may be possible to convert **5** into the non-conjugated ketone, which can serve as starting material for the *ortho*-substituted **7.**
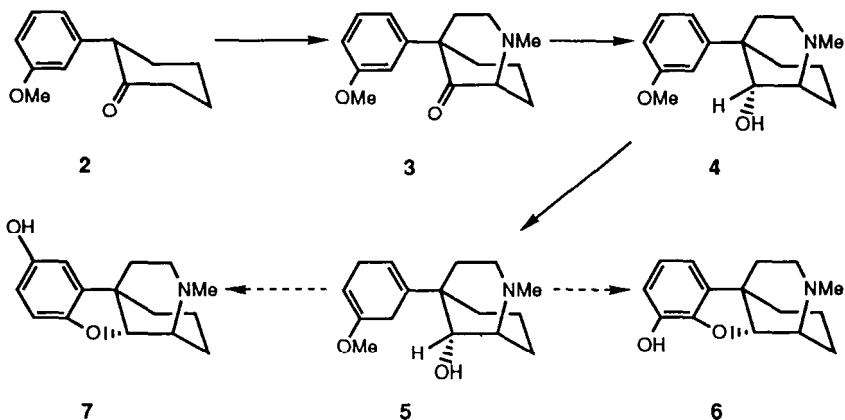
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AFFILIATIONS

Laboratories of Medicinal Chemistry, NIDDK (JTML, AEJ, and KCR), and DCT, NCI (TRB), National Institutes of Health, Bethesda, MD 20892.

Antagonism of Mu and Kappa Opioids: Effects on Schedule-Controlled Behavior in Pigeons

Sondra R. Mattox, Mitchell J. Picker and Linda A. Dykstra

Mu opioid agonists, such as morphine, and kappa opioid agonists, such as U50,488, decrease rates of food-maintained responding in pigeons. In the present investigation, naltrexone's potency as an antagonist of these effects was examined. Four pigeons were trained to key peck under a Fixed Ratio 10 schedule of food presentation. When a stable baseline of responding was established, a cumulative dosing procedure was used to obtain dose-effect curves for morphine (1.7-17.5 mg/kg), l-methadone (0.01-3.0 mg/kg), U50,488 (0.3-17.5 mg/kg) and bremazocine (0.001-0.3 mg/kg). In addition, dose-effect curves were obtained for each agonist in the presence of doses of naltrexone that did not decrease response rates (0.01-10 mg/kg). It was seen that the dose-effect curves for morphine, l-methadone and bremazocine were shifted in a dose-dependent manner by naltrexone. In contrast, no dose of naltrexone tested shifted the U50,488 dose-effect curve. These results demonstrate that: a) naltrexone is more potent in antagonizing the rate-decreasing effects of the mu opioid agonists than the kappa opioid agonists and b) that the rate-decreasing effects of bremazocine may be mediated via the mu receptor. (Supported by USPHS grant DA-02749).

AFFLIATION

Departments of Psychology and Pharmacology, University of North Carolina, Chapel Hill, N.C. 27599-3270.

[D-Met2]-FMRFamide (DMFa): Production of Naloxone-Sensitive Antinociception in Mouse Tail-Flick Test

Robert B. Raffa and Charlene D. Connelly

ABSTRACT

Considerable data support the hypothesis that mammalian FMRFamide (Phe-Met-Arg-Phe-NH$_2$) or mammalian FMRFamide-related peptides (FaRPs) function as endogenous antiopiates (for review see Raffa, 1988). We report here that central administration (i.c.v.) of a FaRP with D-amino acid substitution in the second position, i.e. [D-Met^2]-FMRFamide (DMFa), produces dose-related, naloxone-reversible antinociception in the mouse tail-flick test. Hence, this modification appears to confer agonist-like activity.

INTRODUCTION

In contrast to other endpoints in which they act as functional antagonists (e.g., Tang et al., 1984; Yang et al., 1985; Kavaliers and Hirst, 1985; Lake et al., 1989; Malin et al., 1989), despite low affinity for opioid receptors (Zhu and Raffa, 1986; Zadina and Kastin, 1986), we found that i.c.v. FMRFamide or FaRPs mimic, and do not block, morphine or enkephalin analogs on mouse colonic propulsive motility (Jacoby et al., 1987). This action is antagonized by naloxone (Raffa and Jacoby, 1989). DMFa was particularly active (Raffa and Jacoby, 1990). The purpose of the present study was to examine the action of DMFa on an antinociceptive endpoint in mice.

MATERIALS AND METHODS

Male, pathogen-free albino Swiss CD-1 mice, 18-24 g (Charles River Laboratories) were used. All testing was performed in accordance with the recommendations and policies of the IASP and NIH guidelines. DMFa (Bachem Inc.), naloxone hydrochloride and morphine were prepared fresh daily in HPLC pure water. I.c.v. injection was by freehand (5 µL) into the lateral ventricle of gently restrained mice based on the method of Haley and McCormick (1957). Antinociception was assessed with a modified version of the tail-flick test (D'Amour and Smith, 1941). Percent of maximum possible effect (%MPE) was determined using %MPE = 100x(test

latency-PL)/(20-PL), with the pretest latency (PL) of each animal
and a cutoff time (20 sec) established to prevent injury.

RESULTS

DMFa (i.c.v.) produced dose-related antinociception that was
antagonized by naloxone (FIG. 1). The morphine antinociception
dose-response curve was shifted to the right by DMFa (and
FMRFamide) (FIG. 2).

Fig. 1
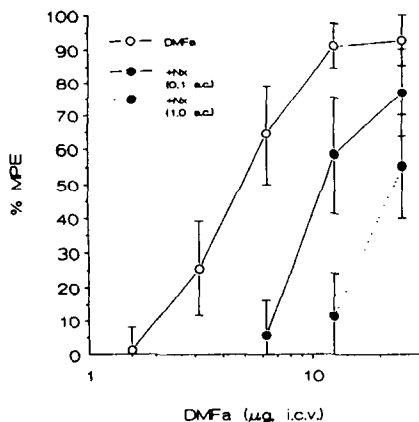
Fig. 2
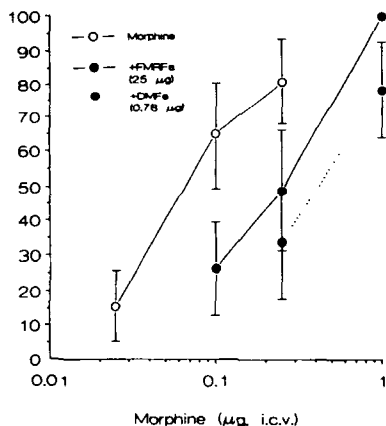
CONCLUSIONS

1. [D-Met^2]FMRFamide (DMFa) produced supraspinal (i.c.v.) opioid-
mediated (naloxone-sensitive) antinociception in the mouse tail-
flick test.
2. DMFa (and FMRFamide) may be partial agonists at opioid
receptors.

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AFFILIATION: CNS Research, R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776.

A Computer-Aided Investigation into the Role of Hydrogen Bonding in the Binding Conformation of the Endo-Ethenotetrahydrooripavine, PEO

Simon F. Semus

INTRODUCTION

A conformational study on a series or oripavine derivatives using the PCILO semi-empirical quantum mechanical method suggested an important interaction between the hydrophobic side-chain substituents at C19 and a possible lipophilic receptor site. Low energy conformers were found with intramolecular hydrogen bonding between the proton of the hydroxyl side-chain and the C6 oxygen. A molecular mechanics conformational search has been reported where intramolecular hydrogen bond constraints were employed without a parallel search omitting the constraints[2]. It was suggested that such intramolecular hydrogen bonding directed the hydrophobic side-chain to the lipophilic site. However, the high activity of 6-desmethoxy analogues appear to contradict this hypothesis[3]. In order to account for these observations, together with the stereoselectivity for this series of compounds, two binding sites have been proposed[4]. Together with the lipophilic site, a second hydrophilic site has been suggested to receive the C19 hydroxyl group above the c-ring.

RESULTS AND DISCUSSION

In this study a molecular mechanics conformational search has been undertaken on 7a-[1(R)-1-hYdroxY-1-methYL-3-phenylproyl]-6,14-endo-ethenotetrahydrooripavine (PEO) in order to determine the preferred lowest energy state. Molecular volume maps were then generated to investigate possible hydrophilic binding sites on the receptor.

A conformational search was performed using the SYBYL 5.3 suite of programs, employing the Tripos molecular mechanics force field. The search was initially performed on the rotatable bonds τ_1 through τ_6 (Figure 1) at 30° increments, in the range 0 to 3. This initial search generated an extended side-chain conformation with an energy of 37.755 kcal. A subsequent search was performed on this conformer at 5° increments, at ±30° range around rotatable bonds τ_1 through τ_6. The search generated a conformation with an energy of 35.156 kcal, which was optimised to 34.868 The lowest energy conformation (32.233 kcal) was obtained by placing distance constraints of 1.8 to 2.8Å between the proton of the hydroxyl side-chain and the C6 oxygen,
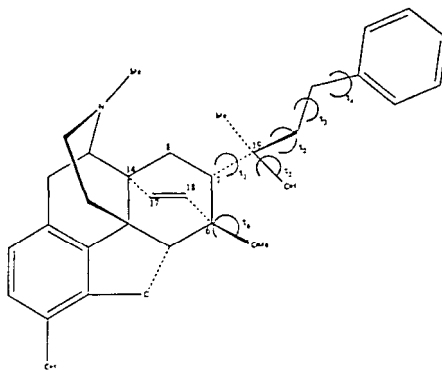
FIGURE 1. REPRESENTATION OF PEO, SHOWING TORSION
ANGLES τ_1 THROUGH τ_6.

thus forcing intramolecular hydrogen bonding between
the two moieties. The search was performed in two
stages as above.

In order to determine possible hydrophilic receptor
sites a dummy hydrogen bond donor was placed 2.8Å from
the PEO oxygen at C19, in the both the extended side-
chain and intramolecular binding conformation, and
volume maps corresponding to the Van der Waals surface
were calculated according to methods previously
described[5]. These maps showed no volume overlap between
PEO in the intramolecular hydrogen bonded conformation
and the dummy atom, indicating a possible hydrogen
bonding site on the opiate receptor. In contrast, the
extended side-chain conformation showed considerable
volume overlap with the dummy atom.

Thus, the lowest energy conformation of PEO is that
where hydrogen bonding occurs between the C19 hydroxyl
proton and C6 oxygen functions. Additionally, the C19
oxygen of PEO, when in this state, may be hydrogen
bonded to a donor atom in a hydrophilic site on the
opiate receptor.

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AFFILIATION

Division of Biomedical Engineering,
Medical College of Virginia,
Box 694, M.C.V. Station,
Richmond, Virginia 23298.

394

Effects of Opiate Agonists and Antagonists on Cerebral Metabolic Activity in the Conscious Rat

Elliot A. Stein and Scott A. Fuller

INTRODUCTION

Opiate agonist agents have multiple actions both centrally and peripherally. Among their most prominent central effects include potent analgesia, sedation, decreases in body temperature, increase in food and water consumption and their ability to influence or produce reinforcement mechanisms. It is likely that this multiplicity of actions require activation at multiple independent brain sites or circuits.

Many techniques have been employed to define central loci of opiate actions including electrophysiologic recording, microinjections of pharmacologic agents, and selective lesions or neurochemical measures. Autoradiographic techniques ate able to reflect activity in numerous and widespread regions simultaneously.

Local cerebral glucose utilization (lCGU) studies have consistently reported decreases in neuronal activity after opiate administration. However, electrophysiologic recordings as well as neurotransmitter turnover studies have routinely observed both regional increases and decreases in activity. We recently reported heterogenous increases in regional cerebral blood flow 60 sec after a single dose of heroin (1). Since opiate action is both dose and time dependent, time constraints of each method may help explain these disparities. As such, we have performed a complete dose-response study, in an attempt to reconcile these studies and as a first attempt to determine those specific opiate-induced effects.

METHODS

Thirty male, Sprague-Dawley derived rats had chronic catheters implanted into the right jugular vein and externalized dorsally. After recovery, rats were placed into operant chambers within sound attenuated rooms. Following a 5 min acclimation period, rats received an IV injection of either heroin HCl at 1) 0.0 mg/kg, 2) 0.1 mg/kg, 3) 0.3 mg/kg, 4) 1.0 mg/kg, 5) naloxone at 1.0 mg/kg or 6) a cocktail of 0.3 mg/kg heroin plus 1.0 mg/kg naloxone in 0.25 ml saline. One min later, 160 μCi/kg of [1-^{14}C] Octanoic acid was delivered IV in 0.25 ml saline. Two min later, rats were sacrificed, brains rapidly removed frozen in isopentane (-40°C), sectioned at 16 μm, thaw mounted onto glass slides, dried on a warming tray and apposed to X-tay film (MR-1; Kodak) for 8-10 weeks.

Autoradiograms were analyzed by computer densitometry using an MCID Image Analyzer (Imaging Research, St. Catherine, Ont.). Optical densities (OD) were converted to a relative optical density score (2). Data were subjected to analyses of

variance followed by Students Neuman Keuls tests. Significance was set at p<05.

RESULTS

Sixteen of 58 structures analyzed were significantly altered by either heroin or naloxone. Heroin-induced increases in blood flow were seen in the caudate nucleus at the low dose, claustrocorte at 0.3 mg/kg and laterodorsal nucleus of the thalamus and dentate gyrus only at the highest dose. Heroin-induced decreases in blood flow were seen at the lowest threshold in the bed nucleus, after 0.3 mg/kg in the dorsomedial hypothalamus, dorsal raphe and entorhinal cortex, and only after the highest heroin dose in the cingulate cortex, basolateral nucleus of the amygdala and the medial preoptic and paraventricular nuclei of the hypothalamus. Naloxone-induced increases in labeling were seen in the olfactory tubercle and paraventricular nucleus, while decreases were seen in the posterior cingulate cortex and basolateral nucleus of the amygdala

DISCUSSION

Results from this experiment bridge those obtained previously using the blood flow marker iodoantipyrene (IAP) and the metabolic marker 2-deoxyglucose (2-DG). All four regions activated by heroin in this experiment also demonstrated an increase in rCBF previously (1). In contrast, while the decreases in octanoate labeling seen in the present study does not generally reflect the IAP results, they do agree with both our previous octanoate study (3) and reports of decreases in lcgu (4,5). Only one previous report has found increases in lcgu (in SNr and mammillary body) after sq morphine (6). Similarly, while we found four regions altered by naloxone (two each decreased and increased), no alterations in lcgu have been reported for any forebrain structure examined, although naloxone-induced decreases in lcgu were seen in 18 brainstem nuclei (7) and rCBF decreases seen in entorhinal cortex (1).

Most of the structures inhibited by heroin are constituents of or receive projections from limbic structures. Many hypothalamic nuclei were inhibited including the PVn which, with its outputs to the median eminence and posterior pituitary, may reflect inhibitory influence of opiates on neuroendocrine functions. Four regions were activated by heroin. At least three ate strongly interconnected with the dentate and Id thalamus communicating with the hippocampus and entorhinal cortex. The significant of naloxone-induced alterations in blood flow is not clear. It would seem to imply a high endogenous opioid tone within neurons in these regions.

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AUTHORS

Elliot A. Stein, Ph.D., Scott A. Fuller, Departments of Psychiatry and Pharmacology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226

Opioid and Non-Opioid Behavioral Actions of Dynorphin A and the Dynorphin Analogue DAKLI

Keith A. Trujillo and Huda Akil

Of the three opioid peptide families, peptides from the prodynorphin family produce the most distinct and unusual behavioral effects. Proopiomelanocortin and proenkephalin peptides tend to produce actions typical of opiate drugs, such as positive reinforcement and analgesia. Prodynorphin peptides, in contrast, have been found to produce unusual effects, including dramatic postural changes and motor abnormalities. The actions of prodynorphin peptides on pain sensitivity are currently unclear; while some studies have shown these peptides to produce analgesia, others have shown them to have no effects, or to inhibit morphine analgesia. Several of the atypical actions of dynorphin peptides have been found to be due to non-opioid effects--these actions are not affected by opioid receptor antagonists, and are produced by fragments of dynorphin, such as dynorphin A 2-17 (Dyn A 2-17), which do not bind to opioid receptors. The present studies were initiated in order to further examine the opioid and non-opioid actions of dynorphin, particularly in regards to sensorimotor function and analgesia. In addition, we wished to to determine if the recently developed dynorphin analogue, DAKLI ([Arg[11,13]] dynorphin A-(1-13)-Gly-NH(CH$_2$)$_5$NH$_2$), might be a useful ligand for the study of behavioral actions of dynorphin peptides. This peptide, a C-terminally modified analogue of Dyn A was developed by Goldstein and coworkers (PNAS, 85:7375-7379, 1988) as a ligand for use in receptor binding assays. We also wished to examine a second form of this dynorphin analogue, boc-DAKLI, for its potential use as a probe for non-opioid behavioral actions of dynorphin. Boc-DAKLI has a t-butoxycarbonyl group attached to the N-terminal tyrosine. Since opioid peptides require a free amino-terminal tyrosine in order to bind to opioid receptors, boc-DAKLI should not produce opioid actions.

METHODS

Experimental subjects were adult, male Sprague-Dawley rats stereotaxically implanted with stainless steel cannulas aimed at the lateral ventricles. For sensorimotor evaluation, animals were injected i.c.v. with Dyn A 1-17, Dyn A 2-17, DAKLI or boc-DAKLI (5 or 25 nmoles i.c.v.), and a series of neurological tests performed at 10 minute intervals. For evaluation of analgesia the tail-flick response was used. After baseline tail-flick latency was determined, animals were injected i.c.v. with boc-DAKLI or saline. Tail-flick latency was determined immediately after injection, and at 20 minute intervals for 80 minutes. Injections of morphine sulfate (1.5 mg/kg s.c.) were administered immediately after the zero, 20 min and 40 min time points, in a cumulative dosing paradigm.

RESULTS AND DISCUSSION

Each of the four peptides produced dramatic, dose-dependent (5 or 25 nmoles i.c.v) impairment of sensorimotor function as determined by performance on the neurological tests, including impairment of righting reflex, corneal reflex, muscle tone, hindlimb function, visual-motor coordination and locomotion. Close examination of the behavioral effects of the peptides has led to the identification of two distinct syndromes. The first was produced primarily by Dyn A 1-17 and DAKLI, and thus appears to be either opioid-mediated, or dependent on an interaction between opioid and non-opioid effects. This syndrome began with the animal turning contralateral to the side of the injection; at moderate expression this was followed by rotational behavior, while at extreme expression bizarre postures and barrel rolling were seen. The second syndrome was observed following administration of each of the four peptides; since it was produced by the non-opioid peptides Dyn A 2-17 and boc-DAKLI, in addition to Dyn A 1-17 and DAKLI, this syndrome apparently reflects the non-opioid actions of dynorphin peptides. Soon after injection a decrease in activity was observed, developing until the animal became mostly inactive. The animal became extremely flaccid, layed flat on its belly with its hindlimbs splayed, and any further movement occurred primarily with the head and forelegs. Extreme salivation and ptosis were seen, and the animal appeared to be sedated. Despite the apparent sedation, however, the animal was hyper-reactive to sharp sounds or to touch, and became temporarily aroused upon handling. The latter, non-opioid syndrome, often followed the opioid syndrome when Dyn A 1-17 or DAKLI were administered. The results demonstrate that Dyn A peptides produce severe neurological impairment by a non-opioid-mediated action, and suggest that postural abnormalities and circling behavior may distinguish opioid from non-opioid effects of these peptides. The dramatic neurological impairment seen in the present studies suggests that caution should be used in interpreting behavioral studies on dynorphin peptides, since the animals may be non-specifically impaired. In addition, the present results suggest that DAKLI and boc-DAKLI may be useful tools for the examination of opioid and non-opioid behavioral actions of dynorphin.

Two studies were performed in order to more carefully examine potential interactions between opioid and non-opioid effects of dynorphin peptides. In the first, naltrexone (10 mg/kg s.c.) was found to increase the sensorimotor impairment produced by DAKLI (25 nmoles i.c.v.), but decrease postural abnormalities and circling behavior. The fact that the opioid receptor antagonist increased sensorimotor impairment suggests that opioid effects may interfere with the non-opioid-mediated debilitating actions of dynorphin peptides. In addition, as above, the results suggest that postural abnormalities and circling behavior may result from opioid actions of dynorphin peptides, or from interactions between opioid and non-opioid effects. The second study found that boc-DAKLI (25 nmoles i.c.v.) produced hyperalgesia, and suppressed the analgesic effects of morphine (1.5-4.5 mg/kg s.c). These results are very similar to those reported previously for Dyn A 2-17, and suggest that non-opioid actions of dynorphin may be antagonistic to opioid effects. Taken together with the above results, these data suggest that opioid and non-opioid actions of dynorphin peptides may be mutually antagonistic. (Supported by NIDA NRSA DA05336 to K.A.T., and NIDA Grant DA02265 and NIMH Grant MH422251 to H.A. DAKLI and boc-DAKLI were generously provided by Dr. J. Nestor).

AFFILIATION

Mental Health Research Institute, The University of Michigan
205 Washtenaw Place, Ann Arbor, Michigan 48109-0720

Involvement of a Peripheral Component in the Antinociceptive Effects of Opioids in a Model of Tonic Pain

Helen Wheeler-Aceto and Alan Cowan

INTRODUCTION

Opioid receptors have been found on peripheral neuroterminals of small diameter primary afferents. Moreover. opiates inhibit plasma extravasation and neuropeptide release following antidromic C-fiber stimulation. Recent evidence suggests that these peripheral sites may be involved in the modulation of inflammation-induced hyperalgesia by opioids. Mu and *kappa* receptors are thought to have a role in modifying responses to noxious heat in carrageenan inflamed tissue (Hargreaves *et al.,* 1988). In addition, mu, *kappa* and delta receptors can mediate the effect of exogenously administered opioids on the response to noxious pressure in adjuvant-induced inflammation (Stein *et al.* 1989). However, endogenous activation of mu and *delta* receptors by ß-endorphin may be most important (Stein *et al.,* 1990). We have previously found PD 117302. the selective karma agonist. to be as efficacious as morphine against tonic flinching after s.c. administration in the rat paw formalin test (Cowan *et al.,* 1989). Certain kappa agonists may, however, possess aversive properties in man (e.g. Pfeiffer *et al.,* 1986). The development of peripherally active kappa agonists might provide new generation analgesics without psychotomimetic side-effects. With this in mind, we investigated possible peripheral effects of PD 117302 and morphine, against formalin-induced pain. following administration directly into the paw. ICI 204448, a selective *kappa* agonist. with limited access to the CNS (Shaw *et al..* 1989). was also studied. Overall our work suggests that the antinociceptive actions of *mu* and *kappa* agonists are not mediated exclusively by the CNS when formalin is used as the noxious stimulus.

METHODS

Tests were performed in male Sprague Dawley albino rats (70-90 g) as previously described (Cowan et *al.,* 1989). Morphine (10-300 µg) or PD 117302 (3-300 µg) was administered into the dorsal surface of a hind paw, in a volume of 10 µl, either 10 min before (acute phase) or 10 min after (tonic phase) formalin (50 µl, 5% in isotonic saline). Behavior. recorded as the number of flinches/shakes of the injected paw, was assessed between 0-10 min or 20-35 min post-formalin for acute and tonic phases, respectively. The activity of ICI 204448, given s.c. 10 min before formalin, was determined in the tonic phase of the response. Results are expressed as mean percent antagonism of formalin ± SEM. In addition. the activity of morphine (10-300 µg) and PD 117302 (3, 30 and 300 µg) was assessed in the 50°C warm water tail-dip test at 10 and 20 min after administration into the paw. Percent maximum possible effect was calculated based on a 15 s cut-off time. All doses are expressed as

free base. A50s and 95% CLs were determined by linear regression analysis. Statistical analysis was by means of ANOVA followed by Dunnett's test.

RESULTS

Morphine and PD 117302 produced dose-related antinociception against both acute [morphine 41.9 (31.9-56.9) µg and PD 32.7 (17.1-62.7) µg] and tonic [morphine 44.7 (28.0-67.5) µg and PD 15.1 (7.2-33.5) µg] flinching responses. The (+)-enantiomer of morphine (300 µg) was ineffective. However. in the case of morphine, antinociceptive activity was recorded in the tall-dip test (% MPE at 20 min = 32.8±4.4: p<0.01) after 300 µg into the paw. While no antinociceptive activity was observed in the tail-dip test with PD 117302. there was evidence of behavioral depression (splayed posture, erect tall) which was most marked followlng 300 µg intra-paw. ICI 204448 also produced dose-related antinociception against late phase flinching [6.9 (3.9- 12.9) mg/kg, s.c.]. but was of slightly lower efficacy than morphine or PD 117302 given either *into* the paw or s.c. There were no marked behavioral effects at any of the doses tested. In contrast. when ICI 204448 (30 and 100 µg) was given by the icv route of administration it caused profound and long lasting (> 3 hr. compared to 20-30 min with 30 µg of PD 117302 icv) behavioral effects. These consisted. initially, of depression splayed posture, erect tail and vertical nystagmus similar to those seen with PD 117302. Later (between 2-3 hr). depression was replaced by "dopamine-like" behavioral activation very similar to that seen following oral administration of PD 117302 (unpublished observation). The occurrence of such profound behavioral effects after 30 µg of ICI 204448 icv. with minimal overt behavioral changes at doses up to 300 mg/kg, s.c. (equivalent to ~ 24.000 µg per rat), further indicates the low CNS penetration of this compound and strongly favors a peripheral site for the antinociceptive activity observed in this study.

CONCLUSIONS

Both morphine and the selective *kappa* agonist PD 117302 produced dose-related antinociception in the rat paw formalln test after s.c. administration into the paw in a volume of 10 µl. However, activity in the warm water tail-dip test and behavioral depression with morphine and PD 117302, respectively. at the highest doses tested indicate that these agents are not restricted to the periphery following localized injection. Nevertheless, the relative potency of morphine in the formalin and tall-dip tests after intra-paw administration and the antinociceptive activity of ICI 204448 against formalin [tonic phase) suggests a role for peripheral opioid receptors in this test. Furthermore, our observations support the use of ICI 204448 as a tool to study selective activation of peripheral or central *kappa* receptors.

A complete list of references may be obtained from the authors.

AFFILIATION

Alan Cowan, Ph.D. and Helen Wheeler-Aceto, B.Sc.
Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.
HWA is a NIDA pre-doctoral fellow.

Differential Effects of Calcitonin Gene-Related Peptide In Tests Utilizing Thermal Versus Nociceptive Stimuli

S.P. Welch, D.L. Stevens, and W.L. Dewey

CGRP intrathecally administered (i.t.) has been shown to attenuate the antinociceptive effects of i.t. morphine, DPDPE, and calcium in the tail-flick test. Lanthanum (La+++), which has previously been shown to produce opiate-like effects following intraventricular administration (icv.), produces opiate-like antinociception following i.t. administration (ED_{50}=30μg) in the tail-flick and hot-plate tests. These effects were blocked by both i.t. CGRP (2 μg) and naloxone (1 μg). These results are consistent with the recent reports that mu and delta opioids produce antinociception by decreasing the release of CGRP spinally. However, in the p-phenylquinone stretch test (PPQ test), a test using a chemical/visceral nociceptive stimulus, i.t. CGRP produces robust antinociceptive effects (ED_5=1 μg) which are not reversed by naloxone (10 μg i.t. or 1 mg/kg s.c.) the delta antagonist ICI-174864 (4 μg i.t.), or the kappa antagonist nor-BNl (10 μg/i.t.). In tests employing visceral nociceptive stimuli, morphine and Ca++-induced antinociceptive effects are additive with those of CGRP. These results indicate that i.t. morphine may decrease the release of CGRP spinally in those pain pathways involved in thermal nociception, this producing aminociception, but does not appear to produce similar decreases in those pathways mediating viscera/chemical stimuli. These data may indicate a difference in the neuromodulators involved in mu and delta versus kappa opiate receptors. (Supported by grants DA-01647 and DA-05340, and the Commonwealth of VA Center on Drug Abuse Research).

AFFILIATION:

DEPARTMENT OF PHARMACOLOGY
        AND TOXICOLOGY
VIRGINIA COMMONWEALTH UNIVERSITY
MEDICAL COLLEGE OF VIRGINIA
RICHMOND, VA 23298-0613

Lack of Irreversible Antagonism of Opioid & Receptors by [D-ala^2, Leu^5, Cys^6] Enkephalin in the Mouse Isolated vas Deferens Preparation

Kenneth D. Wild, Qi Jiang, Maria Iannone, Wayne Bowen, and Frank Porreca

Previous research has suggested the possibility of differences between opioid δ receptors in rat brain and in mouse vas deferens (MVD). Shimohigashi et al. (FEBS Lett. 222: 71-74, 1987) assessed the binding of [D-Ala^2, (2R,3S)-VEPhe^4,Leu^5]enkephalin and found that while it had high affinity for δ receptors in the rat brain, it was almost inactive as an agonist or an antagonist in both the MVD and guinea pig ileum bioassays. Vaughn et al. (Eur. J. Pharmacol. 177: 99-101, 1990) assessed binding of a similar enkephalin analogue, [D-Ala^2,(2R,3S)-VEPhe^4, Leuslenkeph-alin methyl ester (CP-OMe) and [D-Pen^2,pCl-Phe^4,D-Pen^5]enkephalin (pCl-DPDPE) in both rat brain and MVD. While pCl-DPDPE recognized δ sites in brain and MVD with equal affinity, CP-OMe showed a 30-fold lower affinity for the MVD δ site than for the brain δ site. Both of these studies are strongly suggestive of differences between opioid δ receptors in rat brain and in the MVD.

Radioligand binding studies have shown that [D-Ala^2, Leu^5, Cys^6]enkephalin (DALCE) can covalently bind to opioid δ receptors in rat brain preparations *in vitro* via thiol-disulfide exchange (Bowen et al., J. Biol. Chem. 262: 13434-13439, 1987). Subsequent work by Calcagnetti et al. (Peptides 10: 319-326, 1989) demonstrated that DALCE acted as a short-term agonist and a long-term antagonist at opioid δ receptors using behavioral tests *in vivo* in the rat. Work by Jiang et al. (in press) has examined the time course of DALCE's agonist antagonist actions in mice. DALCE, given *i.c.v.,* produces antinociception in mice using the 55°C hot water tail flick test for up to one hour. This antinociception was antagonized by *i.c.v.* ICI 174,864, a δ selective opioid antagonist. However, when DALCE was given as an *i.c.v.* pretreatment, antinociception produced by [D-Pen^{2,5}]enkephalin (DPDPE), a δ selective opioid agonist, was antagonized for 6 to 72 hours later (Jiang et al., in

press). These data are also suggestive of irreversible antagonism at the opioid δ receptor.

The present study extended these findings by determining whether DALCE could produce irreversible antagonism of δ receptors in the MVD. Following acute application to the MVD, DALCE acted as a reversible δ agonist (IC_{50} of 44.8±7 nM) which was selectively antagonized by the δ antagonist, ICI 174,864, at a dose which did not antagonize DAMGO (µ-selective opioid agonist). In order to assess the possibility of subsequent irreversible antagonist properties, the tissues were incubated with DALCE in oxygenated, magnesium-free Krebs (pH = 7.4) for 20, 30, 60 and 120 min at concentrations ranging from 1 - 30 µM. Tissues were also exposed to DALCE (30 µM) for 20 or 30 min without oxygenation during the incubation period in order to prevent possible DALCE oxidation. Additionally, mice received intraperitoneal DALCE (10 mg/kg), and the vas deferens tissues were removed and tested after 16 or 24 hr. In no case did DALCE produce significant antagonism of either the DPDPE or DALCE concentration-effect curves. These data suggest that, under the conditions of the present study *in vitro,* DALCE cannot covalently bind to the δ receptor in the MVD. The difference in antagonist profile of DALCE in antinociceptive tests *in vivo* and in the MVD *in vitro* are suggestive of differences in δ receptor characteristics in the brain and the MVD and is consistent with previous suggestions of subtypes of δ receptors.

AFFILIATIONS

Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724 *Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912.

Nonpeptide Opioids: In Vivo Effects on the Immune System

Wanda Williams, Kenner C. Rice, and Richard J. Weber

INTRODUCTION

Opioid agonists alter immune function both in vitro and *in vivo* (see Weber et al., this volume). In a study of the immunologic status of heroin addicts, Brown et al., (1974) reported that peripheral blood lymphocytes from these subjects exhibit reduced proliferative responsiveness to phytohemagglutinin, pokeweed and Concanavalin A, in addition to increases in IgM levels. Subsequent studies demonstrated that splenic lymphocytes from mice injected repeatedly with morphine displayed a reduced proliferative' response to Concanavalin A and that this suppression could be partially blocked by the opioid antagonist naloxone. More recent systematic *in vivo* investigations also have shown that certain opioid agonists suppress antibody production (Weber et al., 1987), alter leukocyte mitogenic responses (Bryant et al., 1987), and decrease NK cell activity (Shavit et al., 1986). Furthermore, opioids result in increased susceptibility to bacterial and fungal infections (Tubaro, et al., 1983), as well as murine retroviral infections (Watson et al., 1988), and decreased survival in tumor-bearing animals (Lewis et al., 1984), effects thought to be related to the immunosuppressive potential of opioids.

Based on these and other observations, we have extended our earlier findings to examine the structure/activity relationships of opioids and components of the immune system. We have determined the effects of a battery of opioid agonists on natural killer (NK) cell activity and report here our results using (-)-buprenorphine hydrochloride, a semi-synthetic opioid derived from natural thebaine.

METHODS

Animals. Female BALB/c mice were injected intraperitoneally with either pyrogen-free saline, (-)-morphine sulfate, or (-)-buprenorphine HCl. Animals were killed three hours post-injection for the study of the acute effects of drug administration, while for the effect of chronic effects, single injections were performed daily for three days and animals killed one hour post-injection on the third day. Spleens were removed and single cell suspensions prepared (see below). Male Fischer 344N rats were injected intraperitoneally with either saline, (-)-morphine sulfate, or (-)-buprenorphine HCl.

NK cell cytotoxicity assay. Spleens were surgically excised under sterile conditions, mechanically dissociated into a single cell suspension. Splenic lymphocytes were washed twice in RPM1 1640 and suspended to a concentration of 2 x 107 cells per ml in RPM1 1640 supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 50U/ml penicillin, and 50ug/ml streptomycin. NK cell activity was assessed in a standard 4 hour chromium release assay using YAC-1, a murine lymphoma cell line as targets. YAC-1 cells were harvested, washed, adjusted to 10^7 cells/ml and labeled at 370 for 2 hours with 400 uCi sodium-chromate (^{51} Cr). Cells were washed twice, counted, and suspended to 5 x 104 cells per ml and 100uL added to micro-titer plates containing 2-fold serial dilutions of 1OOuL spleen cells at various concentrations to give effector /target ratios ranging from 400:1 to 50:1. The plates were centrifuged at 50 x g for 5 minutes and incubated for 4 hours at 370 in 5% $CO2$. Following the incubation period, plates were centrifuged at 400 x g, and supernatant harvested using a Skatron Supernatant Collection System (Skatron, Sterling, VA), and counted in a gamma counter (LKB 1270). Maximal isotope release was determined by incubating targets with 0.1 N HCl. Spontaneous release was the amount released in the presence of media alone.

RESULTS

(-)-Buprenorphine HCl. Significant suppression of NK cell activity was shown in mice injected with acute doses of (-)-buprenorphine HCl equimolar to those of (-)-morphine sulphate which suppress NK cell activity *in vivo.* However, similar doses of (-)-buprenorphine HCl administered chronically showed no suppressive effects on murine splenic NK cell activity. Acute equianalgesic and 5X-analgesic doses of (-)-buprenorphine HCl (compared to (-)-morphine sulphate), demonstrated no suppressive effects on NK cell activity. Analgetic equivalents and 5X doses were calculated based on the determination of an ED_{50} comparing (-)-morphine to (-)-buprenorphine in the hot plate test (ED_{50} for buprenorphine = 0.35 or 34 times more potent than morphine).

Therefore, (-)-buprenorphine HCl, a potent opioid analgesic with mixed agonist-antagonist properties, does not suppress NK cell activity when given in equianalgesic or 5X higher dose compared to (-)-morphine sulphate. Acute equimolar, administration of (-)-buprenorphine-HCl, (compared to (-)-morphine sulphate), however, produces a dramatic suppression of NK cell activity, an effect which diminishes following repeated chronic injection.

Table 1
Comparison of the Effects of (-)-Morphine-SO$_4$ and (-)-Buprenorphine HCl on Mouse NK Cell Activity

Effect of (-)-Buprenorphine HCl on NK Cell Activity

Acute Injection			Chronic Injection
EA	5X	EM	EM
NE	NE	S	N E

Tabulated are the effects of various doses of (-)-buprenorphine-HCl in comparison to a dose of (-)-morphinesulphate which suppresses NK cell activity *in vivo.* "EA" means dose necessary to produce an analgetic equivalent, "5X" means five times the dose required to produce the analgetic equivalent, and "EM" means equimolar or approximately 34 times the analgetic equivalent, all in comparison to (-)-morphine sulphate.

Analgetic equivalents and 5X doses were calculated based on the determination of an ED_{50} comparing (-)-morphine to (-)-buprenorphine in the hot plate test (ED_{50} for buprenorphine = 0.35 or 34 times more potent than morphine). "Ne" means no effect and "S" means suppression of NK cell activity.

These preliminary studies in the murine system suggest that certain nonpeptide opioids can produce analgesia without immunosuppressive effects when given in equianalgesic or higher doses compared to (-)-morphine sulfate. Data also suggest that the participation of specific opioid receptor subtypes in opioid-induced suppression (see Weber, et al., this volume). The study of the structure/activity relationships of opioids and immune function should provide a better understanding of the neuroanatomical, pharmacological, and immunological parameters which are involved in opioid-induced suppression of immune function and to guide the synthesis of novel opioids which produce analgesia without immunosuppression.

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AFFILIATION

Neuroimmunology Unit, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Distribution of Opioid Peptides in Brain and GI Tract: Age- and Species-Specific Differences

E.M. Unterwald, F. Nyberg, L. Terenius, and M.J. Kreek

Recently the possible impact of advanced age on the endogenous opioid system has become a focus of attention in both basic and clinical research. Alterations in the endogenous opioid system have been implicated in the mediation of age-related changes in basal nociceptive threshold, temperature control, gastrointestinal motility, and response to morphine administration. These age-related changes may be due to age-induced alterations in the levels of the endogenous opioid peptides. The present study quantitates the levels of Met-enkephalin-Arg^6-Phe^7 (MEAP). the terminal heptapeptide of the preproenkephalin gene product, in the brain and gastrointestinal (GI) tract of young adult and naturally aged guinea pigs and rats. Species- specific differences were also investigated.

Male Hartley guinea pigs, three months or 33 to 36 months old, and male Fischer rats, two months old, have been used to date in this ongoing study. Peptides were extracted from tissues of interest by homogenization in acetic acid followed by elution from SepPak C18 columns. Peptide levels were determined by radioimmunoassay (RIA) using an antibody raised against the sulfoxide heptapeptide Met-O-enkephalin-Arg^6-Phe^7. Cross-reactivity with Met-enkephalin or other proenkephalin- or prodynorphin-derived peptides was less than 0.1%.

In young adult guinea pigs, the levels of MEAP were greatest in the hypothalamus, followed by the central grey and nucleus accumbens. Moderate levels were detected in the pituitary and frontal cortex. The hippocampus, striatum, and cerebellum contained low levels of this peptide. In the GI tract, the colon contained the highest levels of MEAP. The amount in colon was in the-moderate to high range when compared with the levels in the brain areas assayed. The antrum of the stomach, duodenum, jejunum, and ileum all contained low levels of this enkephalin peptide. In comparison with the young guinea pigs, the aged guinea

pigs had a somewhat higher content of MEAP in the
hypothalamus and pituitary. Lower levels were found in
the cerebellum. The GI tract of the old guinea pigs
contained similar amounts of MEAP except in the colon
where the levels were substantially lower.

In young adult rats, the levels of MEAP were high in the
hypothalamus, nucleus accumbens, central grey, and
striatum. Moderate levels were detected in the
hippocampus, frontal cortex, and pituitary. Moderate to
low amounts were measured in the cerebellum. The levels
in rat brain were similar to those in guinea pig except
the amount in rat striatum was four times higher than in
guinea pig striatum. The greatest levels of MEAP in the
GI tract of the young adult rats were found in the colon
and ileum. Low levels were detected in the antrum of
the stomach, duodenum, and jejunum. In comparison to
the GI tract of the young guinea pigs, the rat colon
contained three times less Met-enkephalin-Arg[6]-Phe[7].
The other regions of the GI tract contained similar
levels of this peptide in both species.

In conclusion, MEAP was greatest in the hypothalamus of
both species. Age-specific alterations in MEAP content
were detected in several brain and GI regions.
Potenially important species-specific differences in
MEAP levels were also detected in the brain and GI. In
particular, the content in rat striatum was four times
higher than in guinea pig striatum. Levels in rat colon
were three times lower than in guinea pig colon.

These results indicate that the expression of MEAP is
different in these two species. This finding has
implications for the selection of an animal model when
studying the physiology and function of the endogenous
opioid system. The alterations in peptide content seen
during senescence may contribute to the age-related
changes in pain thresholds and in the response to
morphine administration that is seen in elderly animals
and humans.

AFFILIATION

The Rockefeller University, New York, NY (E.M.U. and
M.J.K.); University of Uppsala, Uppsala, Sweden (F.N.);
The Karolinska Institute, Stockholm, Sweden (L.T.).

Pseudoirreversible Binding of Opiates to Opioid Receptor Subtypes in Rat Brain: A Quantitative Study

Heng Xu, Chong-Ho Kim, Y.C. Zhu, Richard J. Weber, Kenner C. Rice, and Richard B. Rothman

INTRODUCTION   Previous studies demonstrated that preincubation of membranes with (+)-cis-3-methylfentanyl ((+)-cis-MF) produced a wash resistant inhibition of mu receptor binding, a phenomenon we termed "pseudoirreversible" inhibition (1). The primary purpose of the present study was to define the mechanism(s) of pseudoirreversible inhibition and to begin a structure activity study of pseudoirreversible inhibition.

METHODS   Frozen rat brains were homogenized with a polytron in ice-cold 10 mM TRIS-HCl, pH7.4 and centrifuged at 40,000 x g for 15 min. The pellets were resuspended with an equal volume of buffer and recentrifuged. The membranes were resuspended with 50 mM TRIS-HCl, pH 7.4, containing 3 mM $MnCl_2$. Aliquots were distributed to test tubes prefilled with various drugs. After a 60 min incubation at 25° C, the membranes were washed once by centrifugation, and resuspended in 10 mM TRIS-HCl, pH 7.4, containing 200 mM NaCl and 50 μM GppNHp. After a 60 min incubation at 37° C, the incubation was terminated by centrifugation, and the membranes washed an additional 3 times by centrifugation. The final pellets were kept at -70° C. Mu binding sites were labeled with either [3H]FOXY (SA= 40.1 Ci/mmol) or [3H]ohmefentanyl ([3H]OHM, SA=76 Ci/mmol) using previously described methods (1). Higher affinity [3H][$Dala^2$,D-leu^5]enkephalin (SA=39.3 Ci/mmol) binding sites were also labeled as previously described (1). Experiments designed to determine the dissociation rate of [3H]OHM used a previously described centrifugation method (2). (+)-cis-MF and (-)-cis-MF were synthesized as described (3).

RESULTS AND DISCUSSION   Morphine, naloxone, fentanyl, (+)-cyclazocine, and (-)-cis-MF at 1 μM did not act as pseudoirreversible inhibitors of mu or delta binding sites. As reported in Table 1, lofentanil, (+)-cis-MF, ohmefentanyl and sufentanil all produced a dose-dependent pseudoirreversible inhibition of mu binding sites. They had no effect on delta binding sites.

TABLE 1
IC50 (±SEM) Values of Drugs as Pseudoirreversible Inhibitors of Mu Binding Sites

	[³H] FOXY	[³H]OHM
Lofentanil	1.38±0.26	1.34±0.52
(+)-Cis-MF	3.96±0.96	11.7±6.0
Ohmefentanyl	6.25±2.95	44.6±24
Sufentanil	22.2±8.1	92.3±46.2

Saturation binding studies using control and membranes pretreated with 500 nM OHM demonstrated that the pseudoirreversible inhibition of [³H]OHM binding by this agent was due to a 7-fold increase in the K_d, not a decrease in the B_{max}, a result compatible with the presence of residual drug. To directly address the issue of residual drug, membrane suspensions were centrifuged, rather than filtered, and the supernatants assayed for inhibitory activity in the binding assay. Membranes pretreated with 500 nM OHM had enough inhibitory activity to account for a 2-fold increase in the K_d, suggesting that residual drug plays a minor role in the pseudoirreversible increase of the K_d of mu binding sites. Dissociation experiments compared the dissociation of [³H]OHM from membranes pretreated with 0 nM (control membranes) or 500 nM ohmefentanyl (OHM-membranes). [³H]OHM dissociated so slowly from control membranes that a dissociation rate constant could not be calculated. However, [³H]OHM dissociated measurably faster from OMH-membranes.

Viewed collectively, these data suggest that the fentanyl analogs produce pseudoirreversible inhibition of mu receptor binding via an allosteric mechanism. During the preincubation phase of the protocol, the drugs bind tightly to an allosteric site, which might be, for example, a subdomain of the mu receptor. This produces a conformational change in the mu receptor, which is detected as an increase in the K_d and an increase in the dissociation rate. Since the fentanyl analogs which produce pseudoirreversible inhibition are also considerably more potent in tests of antinociception than their apparent affinity for the mu receptor predicts, these data suggest that their capacity to bind to this allosteric site might contribute to their extraordinary potency in vivo.

References are available upon request from the authors.

AFFILIATIONS

Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892;
Shanghai Institute of Materia Medica, Shanghai 200031 China
Unit on Receptor Studies, LCS, NIMH, Bethesda, MD 20892.

Modulation of NK Activity: Role of Neuroendocrine Status

G. Bodner, K.M. Soda, J. Kennedy, H. Albeck, and M.J. Kreek

We have observed a significant reduction in natural killer cell (NK) cytotoxicity activity as well as other abnormalities in immune function in heroin addicts. We have also observed multiple abnormalities of neuroendocrine function in heroin addicts. We have shown that these physiological abnormalities are reversible in former addicts stabilized on steady dose, long-term methadone treatment.

We hypothesize that alternations in NK activity may be modulated, in part, by changes in neuroendocrine status. In this study we have examined the possible link between normal circadian changes, or any abnormal changes, in plasma levels of cortisol (F), and the levels of NK activity. The effects of acute manipulation of neuroendocrine status on NK activity in normal non-drug abusing volunteers have been studied.

Eleven volunteers (8M, 3F, mean age 30±3y) were studied. Baseline (BL) neuroendocrine and immunological studies were performed, followed by a metyrapone (M) test (2.25g single dose po), and a dexamethasone (D) suppression test (1mg po) in each subject. Plasma concentrations of (F), total lymphocyte (L) counts, absolute numbers of L subsets, and NK activity, were determined on blood specimens obtained both at 9am and 10am on the BL study day, and again on each of the two provocative study days (time 0 for M test, or 10h after administration of D), and at 10am on the M study day (1h after administration of M). The 10am levels for each index were compared with the 9am values on the BL and M study days. The two 9am levels obtained on BL and M study days were averaged and compared with the 9am values on the D study day. NK activity was determined by measuring ^{51}Cr release by K562 target cells (1:100 E:T ratio). Plasma F levels were measured by a radioimmunoassay technique.

BL study results were within normal range. The absolute NK activity at 10am was significantly higher compared with the 9am results (P<0.05) on both BL and M test

days. The difference in NK activity 10h after D administration as compared with the average 9am results of BL and M test days was insignificant. Also, there was no significant difference in the 9am to 10am change in NK activity during the M test as compared with BL study. However, a significant difference in the change in NK activity (a smaller increase) was found 10h after administration of D (P=0.0003) as compared with the change from 9am to 10am in the BL study day. The change in NK activity was also higher between 9am and 10am following M administration as compared with the change before and 10h after D administration (P=0.001). Plasma F concentration was reduced by 90% by the D suppression test and by 69% 1h following M administration (P<0.05). No change in L counts or L subsets has been observed during either test.

Our previous observations have shown reductions in NK activity and impairments in hypothalamic-pituitary-adrenal function in heroin addicts. These functions are normalized in patients receiving long-term, steady dose methadone maintenance treatment. In this study, the following observations were made: 1) On BL study day, NK activity was increased between 9am-10am, an interval characterized by the normal circadian decline in F levels. 2) During M study day, NK was significantly increased 1h after M administration, a time when F levels were reduced to a greater extent than the reduction due to circadian rhythm alone. 3) In the D study day, an insignificant change in NK activity was observed 10h after D administration. In this test, F levels were suppressed even more than by M. However, since D is itself a potent glucocorticoid (G), total G activity was probably much greater at 10h after D administration than at any other time point studied.

These findings suggest that F (or total G activity) may naturally contribute to the modulation of NK activity and that in the setting of normal or provoked reduction in F levels, NK activity may increase. Some role in enhancement of NK activity may be played by the POMC related peptides, which become increased in the setting of M administration. This would account for the even greater increment in NK activity 1h after M administration. Others have reported that B-endorphin (BEP) enhances NK activity in vitro. Further studies are in progress in attempt G elucidate the effects of normal circadian rhythm on NK activity in healthy humans as well as in patients with addictive diseases, and to determine the possible role of both BEP and ACTH on NK activity.

AFFILIATIONS:
The Rockefeller University, New York, New York 10021

Dependence-Producing Drug Testing in Opiate Users in Warsaw, Poland

T.L. Chrusciel and H. Tobolska-Rydz

Diagnosis of drug dependence in Poland has been made on the basis of clinical data only, until 1988, when immunochemical reagents in health laboratory services have been introduced.

In our laboratory, testing for dependence-producing drugs in body fluids of drug users has been undertaken in 1988:

1.   To check the applicability of immunochemical reagents supplied by a foreign company in our specific and atypical drug scene (the Polish drug users administer illicit home produced extracts from opium poppy (termed "Compotte" and "Makiwara") for varying periods of time).;
2 .   To collect data on levels of drug other than opiates, concomitantly taken;
3 .   To establish mean levels of opiates following varying degrees and duration of use.

MATERIAL AND METHODS

Samples of blood and urine (supervised) have been collected and saved for not longer than 7 days, until assay in -20°C.

Testing was done with Abbott manufactured reagents for immunodetection of opiates, barbiturates, benzodiazepines and amphetamines, using a TDx detector (Abbott).

Patients were long-term users of dependence-producing drugs. Reporting for counseling to the out-patient unit or treated and rehabilitated in treatment and rehabilitation centers.

RESULTS

Group #1 consisted of 63 out-patients reporting for counseling to the out-patient station of municipal health services.

In 56 patients opiates were found in serum the level was 0.11 -3.14 ng/ml. In urine samples the content of opiates was less than 100 ng/ml (n=16); 100 - 1000 ng/ml (n=65); or more than 1 mug/ml (n=3). In 12 patients barbiturates

(0.15 - 15.4 mug/ml) and benzodiazepines (n=17, 0.18 - 3.85 ng/ml) were detected.

In group #2, consisting of 98 patients of a detoxification ward of <u>Nowowieski</u>-Hospital, opiates in urine were detected only at the admission examination (n=15, 16 - 312 ng/ml; n=16, traces of opiates only). During detoxification, lasting for up to 10 days, sedative drugs were occasionally administered: hence the barbiturates (n=60, 0.5 - 38 mug/ml and benzodiazepines (n=57, 120 - 300 ng/ml) have been detected.

Group #3 consisted of 128 patients of the hospital-based treatment and rehabilitation drug free unit in garwolin. Control examinations at admission disclosed in 4 patients 438 - 2450 ng/ml concentration of opiates in urine. Random urine checking failed to discover opiates in other than at admission tests. Since sedatives were occasionally administered. Barbiturates (n=44, 0.1 - 34 mug/ml; n=8, traces only) and benzodiazepines (n=17, 172 - 2600 ng/ml, N=10, traces only) have been found in urine.

Group #4 consisted of 108 patients of a treatment and rehabilitation unit in Grzmiaca. Random urine testing revealed 2 cases (n=2, traces only) of opiates use and some cases of barbiturates (n=16, 0.5 - 2.8 mug/ml, n=4, traces only) and benzodiazepines (n=23, 112 - 1750 ng.ml, n=8, traces only) use.

Group #5 consisted of 35 patients of the out-patients counselling unit, randomly checked for amphetamines on the basis of natural history of drug use data.
In 14 patients amphetamines were excreted in urine (n=14, 0.36 - 7.8 ng/ml).

DISCUSSION

We have found that in both "Compotte" and "Makiwara" users opiates can be detected by means of standard immunochemical techniques, applied in other countries, with the application of reagents destined for testing users administering different than used in Poland illicit opiate products.

The presence of numerous substances of herbal origin contained in illicit home-made opiate products did not interfere with opiates assay in biological fluids of addicts.

The level of opiates found in biological fluids indicates that the level of use varies considerably.

Most frequently the level of opiates found in serum was 20 - 3000 ng/ml.
The large distribution of data did not permit comparison among duration an/or degree of use versus level of drugs detected. Further studies are in progress.
It is noted that in drug-free communities the level of trespassing the rules is very low.

AFFILIATION:

Department of Clinical and Social Pharmacology, Postgraduate Medical Education Center, PL-00-0653, Warsaw, Poland

Metyrapone-Induced Withdrawal Symptoms: Symptoms in Methadone-Maintained Patients

J.A. Kennedy, N. Hartman, R. Sbriglio, E. Khuri, H. Albeck, and M.J. Kreek

The metyrapone test is widely used in endocrinological testing to test the integrity of hypothalamic-pituitary-adrenal axis function; we have used it to study the metabolic basis of addictive disease and opioid dependence. We have observed that metyrapone administration in long-term methadone-maintained patients and in patients undergoing slow dose reduction to drug-free status following chronic treatment induces a narcotic withdrawal-like syndrome. Although metyrapone is known to produce mild adverse reactions in non-opiate-dependent subjects, narcotic withdrawal-like symptoms have not been previously reported. The metyrapone test was administered to 15 former heroin addicts: 10 (8M±2F) in steady-state methadone maintenance therapy (30-90 mg/d) and 5 (3M±2F) in the final phase of a slow methadone dose reduction procedure (0-10 mg/d). Twelve out of 15 subjects exhibited a narcotic withdrawal-like syndrome ranging from "mild" to "severe," occurring within one hour after metyrapone administration, and resolving within two hours of onset. No significant symptoms were seen in 9 normal volunteers (7M±2F). The mechanism by which metyrapone induces symptoms resembling narcotic withdrawal in opiate-dependent individuals is unknown but physicians performing this test should be aware of the possible response.

[a]The Rockefeller University, New York, New York 10021 (USA) and [b]The New York Hospital-Cornell Medical Center, New York, New York 10021 (USA)

416

Cardiovascular Components of the Response to Morphine

Mary Beth Pretorius, Conrad J. Wong, and David B. Newlin

The purpose of the present study was to define the cardiovascular response to moderate doses of morphine in humans. We particularly wanted to determine the extent to which the HR response to morphine was mediated by the parasympathetic nervous system. The subjects were 12 male opiate abusers who were not currently dependent on opiates. After a first session in which there was no injection, they then received intramuscular injections of 20 mg morphine in the first 4 sessions and in the 6th session, and placebo injection in the 5th session.

We used time series analysis of the electrocardiogram (ECG) which quantifies HR variations in the respiratory sinus arrhythmia frequency band (centered at approximately 0.33 Hz) in order to provide a noninvasive measure of tonic vagal influences on the heart. We also quantified HR variations in the frequency band centered around 0.10 Hz, which is associated with blood pressure regulation and baroreceptor activity.

The HR response to morphine was biphasic. The drug initially increased HR, then decreased HR 90 - 100 min post-administration relative to the no-drug control condition. The HR increase was significant in the first 10 min ($F(1,527) = 9.2$, $p<.003$) and 20 min ($F(1,527) = 4.1$, $p<.05$) after the injection. However, HR was significantly ($F(1,527) = 4.5$, $p<.05$) decreased 90 to 100 min after the injection. The placebo nonsignificantly decreased HR, so that the final HR decrease was not significant when compared to the placebo control rather than the no-injection session. The initial HR increase was associated with significant decreases in vagal tone index (V). Compared to no-injection, morphine decreased vagal tone significantly in the first 10 min ($F(1,527) = 4.6$, $p<.05$) and second 10 min periods ($F(1,527) = 3.8$, $p<.05$). However, the final HR decrease was not associated with a significant vagal tone change ($F(1,527) = 0.6$, n.s.). Similar results were found in comparison with the placebo control. This indicated that the initial HR increase was vagally-mediated but that the subsequent HR decrease was not. Morphine consistently and strongly decreased THM throughout the recording period, whether compared to the no-injection or placebo controls; this indicated sustained effects of morphine on baroreceptor activity. The correlation of initial change from baseline in the ARCI MEG euphoria scale score with change in vagal tone index (V) was -0.43, indicating that morphine-induced euphoria was most closely associated with change in vagal tone.

We are currently investigating a "linkage hypothesis" concerning the relationship between drug-induced reward mechanisms and cardiovascular change. We hypothesize that there is central linkage between activation of the dopaminergic

reward system of the mesolimbic area, psychomotor stimulant activation (Wise and Bozarth, 1987) and drug-induced tachycardia. Obrist's (1979) "cardiac-somatic coupling" theory proposes that vagally-mediated changes in HR are coupled to motor activity as the brain changes HR to anticipate metabolic demand of the skeletal muscle system. We propose that drug-induced tachycardia is a correlate of the this mechanism in the sense that vagally-mediated HR changes to rewarding drugs are coupled to psychomotor stimulant activation. Our findings that the initial tachycardia to morphine was vagally-mediated (at least in part), and the best correlate of MBG euphoria was vagal tone change, tend to support this linkage hypothesis. We are currently investigating the limits of the linkage hypothesis with drugs such as cocaine, marijuana, amphetamine, alcohol, nicotine, and barbiturates.

NIDA Addiction Research Center, Box 5180, Baltimore, MD 21224

Factors Influencing Assessment of Opioid Miosis in Humans

L.L. Weinhold and G.E. Bigelow

Pupillary constriction in man is caused by morphine and most mu and kappa opioid agonists (Jaffe & Martin, 1985) and is a commonly-used index of opioid agonist activity. Pupillary diameter, however, is affected by numerous other stimuli (Tyron, 1975). This study examined the effect of lighting intensity on the detection of opioid-produced pupillary constriction. No prior studies have examined the effects of a broad range of visual field luminance levels on the detection of drug-induced alterations in pupil diameter.

This study also examined the differences in pupil diameters measured with one eye open as compared with both eyes open. The evidence is sparse but consistently reported that pupil diameters of adults are larger when only one eye is open as compared with both eyes open. No prior studies have examined the influence of measuring pupil diameters with one eye open vs. both eyes open on the detection of drug-induced alterations in pupil diameter.

Participants were seven methadone maintenance patients who received their usual daily dose of methadone (50-60 mg p.o.) in single 3.5 hr laboratory sessions. Pupillary diameter measures were obtained photographically pre- and 5, 30, 60, 90, 120,180 min post-methadone. At each time point, and after 5 min exposure to dim light, the pupil was photographed under ascending lighting intensities (visual field luminances of 4, 16, 40, 80, 160 and 240 foot-lamberts [fl]). At each fl pupil photographs were obtained with both eyes open and one eye closed in counterbalanced order.

Peak miosis occurred 90 min after methadone and was easily detected under dim (4, 16 fl) but not bright (160,240 fl) lighting conditions (see Figure 1).

On average, pupil diameters were 0.35 mm larger with one eye open than with both eyes open. The data further suggested an interaction in which methadone may reduce the differences in pupil diameters with one eye open vs. both eyes open.

We conclude that methadone miosis is best detected under dim lighting and that it is important to control whether pupil photographs are obtained with one eye open or both eyes open.

Acute Methadone Miosis
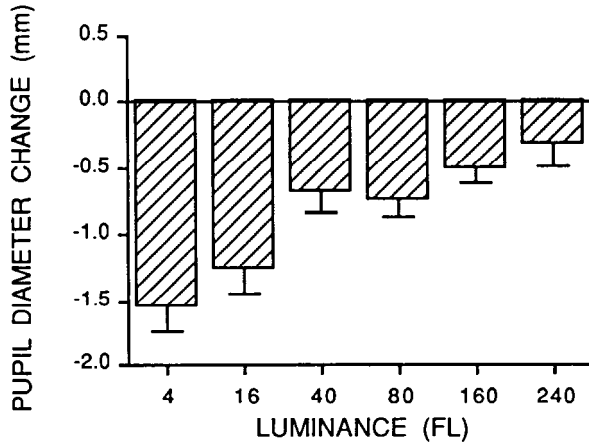
Figure 1. The magnitude of methadone-induced pupillary constriction is shown as a function of the luminance of the visual field. Values are mean changes from pre-methadone to 90 min post-methadone (time of peak effect) Brackets show SEM.

ACKNOWLEDGEMENTS

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AFFILIATION

The Johns Hopkins University School of Medicine,
Francis Scott Key Medical Center,
Baltimore, Maryland 21224

The Evaluation of Synthetic Cannabimimetic Congeners For Discriminative Stimulus and Cataleptogenic Effects in Rats

William R. Prescott and Billy R. Martin

Rats were trained to distinguish between i.p. injections of 3 mg/kg delta-9-THC and vehicle in a standard two lever, food reinforced drug discrimination paradigm using "repeat tests" 30 and 90 mins. after injection. The cataleptogenic effect of the tested compounds was quantified in the discriminators by measuring the immobility time of the animals when placed on elevated rings over a 5 minute period. A series of compounds were tested based on 3-phenylcyclohexanol (cis-3[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-cyclohexa-1-ol) with the gem-dimethyl side chain length varying from 2 - 11 carbons. Only the heptyl (CP-47,497) and hexyl (CP-49,752) compounds were shown to fully substitute for the delta-9-THC discriminative stimulus, the former being more potent.

Analogs of the nantradol nucleus with either a methyl-furan (CP-50,350) or a methyl-cyclopropyl (CP-50,414) substituent on the nitrogen also resulted in generalization. A 9-nor-9-hydroxy-hexahydrocannabinol with a 1-methyl,4-phenylbutyl-oxy side chain (CP-42,096) demonstrated the most potent and complete substitution with clear separation between doses allowing drug discrimination and those resulting in catalepsy or non-specific CNS depressive effects. The maximum dose of each drug tested was 10 mg/kg and the relative potency of the generalizing compounds was: $42,096 \rightarrow \rightarrow 47,497 \rightarrow 49,752 \approx 50,414 \approx 50,350$.

AFFILIATION:

Department of Pharmacology and Toxicology
Virginia Commonwealth University
Medical College of Virginia
Richmond, Virginia 23298-0613

Spinal Action of Cannabinoid-Induced Antinociception

Aron H. Lichtman and Billy R. Martin

Δ^9-THC, a naturally occurring psychoactive chemical present in marijuana, as well as other cannabinoid compounds, have been demonstrated to produce potent antinociception in laboratory rodents (Little et al., 1988). Although few studies have addressed the issue of site of action, there is some indication of a spinal mechanism of action (Yaksh (1981). The purpose of the present investigation was to examine the relative contributions of spinal and supraspinal mechanisms of cannabinoid-induced antinociception by assessing tail-flick latencies after either intravenous (i.v.) or intrathecal (i.t.) administration of cannabinoids in spinally transected and intact rats. The degree to which spinal ligation attenuated cannabinoid-induced antinociception was used to infer the relative contribution of supraspinal structures, since direct neural communication between the brain and spinal cord was completely severed. Conversely, any residual antinociception in the spinal animals was attributed to spinal action.

Methods

Sprague-Dawley male rats weighing approximately 350 g served as subjects. Animals designated for spinal transection were given a midthorasic laminectomy and a 0.5 cm section of the cord was aspirated. The potent cannabinoid analog CP-55,940 was administered via the tail vein in a 1: 1: 18 (ethanol-emulphor-saline) vehicle. For i.t. drug administration, subjects were surgically implanted with chronic spinal catheters that were 8.5 cm in length. The tips of the catheters were placed just rostral to the lumbar enlargement. CP-55,940, levonantradol, and Δ^9-THC were administered in a 10 µl DMSO vehicle followed by a 10 µl saline flush into the catheter. To examine whether the cannabinoid-induced antinociception was stereoselective, CP-56,667, the inactive (+)-enantiomer of CP-55,940, was also administered i.t.

Results

As indicated in Figure 1, i.v. administration of CP-55,940 significantly elevated tail-flick latencies. More importantly, spinal transection attenuated this antinociception.
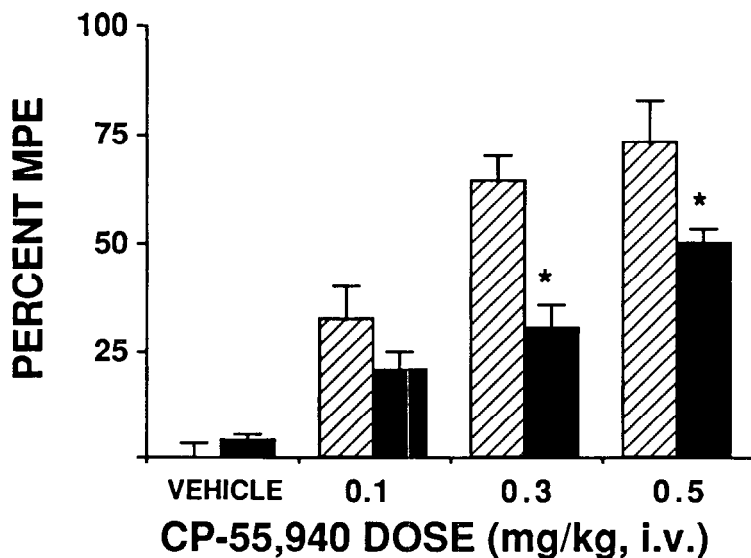
Figure 1. Antinociceptive activity of CP-55,940 in ▨ intact and ■ spinal rats. The tail-flick response was measured 15 min after an i.v. injection, and the results are presented as means ±SEM (n=6-8 per group) of %MPE. * Significantly different from the intact group.

All three psychoactive compounds administered i.t. produced antinociception (see Table 1). However, Δ^9-THC was considerably less potent than either levonantradol or CP-55,940. Spinal transection significantly reduced the antinociception produced by 100 μg of CP-55,940, suggesting that the drug may have diffused rostrally to supraspinal sites. On the other hand, spinal transection failed to reduce antinociception in animals treated with 40 μg of levonantradol.

TABLE 1. Antinociceptive activity of intrathecahy administered cannabiioids in intact and spinal rats 30 min after injection (n=68 per group).

DRUG	%MPE INTACT (± SEM)	% MPE SPINAL (± SEM)
CP-55,940		
DMSO	6 ± 2	1 ± 1
30 μg	34 ± 13	22 ± 11
100 μg	59 ± 12	42 ± 13
Levonantradol		
DMSO	0 ± 2	0 ± 2
40 μg	74 ± 13	78 ± 15
Δ^9-THC		ND
DMSO	0 ± 3	
100 μg	33 ± 14	
300 μg	58 ± 15	
1,000 μg	43 ± 20	

Finally, CP-56,667 failed to elevate latencies above baseline (data not shown), indicating that the effect is stereoselective. These results demonstrate that cannabinoids inhibit the tail-flick response to radiant heat by either i.v. or i.t. route of administration. More importantly, cannabinoids appear to produce antinociception through multiple mechanisms at the spinal and supraspinal levels of the CNS.

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AFFILIATION

Department of Pharmacology and Toxicology
Medical College Virginia
Virginia Commonwealth University
Richmond, VA 23298-0613

Aminoalkylindoles (AAIs): A New Route to the Cannabinoid Receptor?

Susan J. Ward, E. Baizman, M. Bell, S. Childers, T. D'Ambra, M. Eissenstat, K. Estep, D. Haycock, A. Howlett, D. Luttinger, M. Miller, and M. Pacheco

INTRODUCTION

The present study demonstrates that aminoalkylindoles (AAIs) are a new class of antinociceptive compounds that interact with a G-protein coupled receptor at which cannabinoid ligands also act. AAIs have been described previously as typified by the analgesic pravadoline (Haubrich et al., 1990). Pravadoline has a dual mechanism of action; inhibition of cyclooxygenase, and agonist activity at the AAI/cannabinoid G-protein coupled receptor. The molecules used in the present study were all devoid of cyclooxygenase inhibitory activity.

METHODS

AAIs were synthesized as described previously (Bell et. al,, 1990). $[^3H]$-Win 55212-2 and $[^3H]$-CP 55940 binding assays were performed as described by Haycock et al. (these proceedings) and (Devane et. al. 1988) respectively. The mouse vas deferens (MVD) preparation and antinociceptive and rotorod assays were performed as described by Haubrich et al. (1990). Adenylate cyclase activity was measured using the method of (Childers and LaRivier, 1984). Win 55212-2 is(+)-[2,3-Dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl] (1-naphthalenyl)methanone monomethanesulfonate.

RESULTS

The potency of Win 55212-2 and structurally related AAIs in $[^3H]$-Win 55212-2 (MI), $[^3H]$-CP 55940 (CP), MWD, adenylate cylclase (cA) antinociceptive and rotorod assays are shown in Table 1.

Table 1 illustrates that agonist potency in cA and MVD assays parallels binding affinity in both the AAI and cannabinoid binding assays. Similarly, agonist potency in vitro was predictive of antinociceptive potency in vivo. The activity of AAIs was stereospecific in each assay, with the (R) enantiomer of Win 55212 being active, and the (S) enantiomer inactive.

425

Table 1
Profile of Activity of AAI Agonists

	IC50 (nM)				ED50 (mg/kg)		
	AAI	cA	MVD	CP	ACh (iv)	ACh (po)	AA (po)
pravadoline	3155	3600	348	88%	2.8	41	15
analog 1	390	2300	83	353	1	39	15
analog 2	19	750	5	28	0.5	97	30
analog 3	7.0	460	4	48	0.15	16	7.4
analog 4	1.2	–	0.17	0.76	0.03	9.4	0.7
(R)Win55212-2	2.8	320	0.19	1.1	0.03	33	7.5
(S)Win55212-3	8000	>10,000	>3,000	90%	>5.5	>173	>100
THC	17.1	–	4	1.6	1.2	32	5
levonantradol	0.68	400	0.93	1.55	–	0.7	–

Assays: ACh (ACh-induced writhing), AA (acetic acid-induced writhing, rat).

Several cannabinoids, represented by delta-9-tetrahydrocannabinol (THC) or levonantradol in Table 1, were also inhibitory, not only in the [^{3}H]-CP 55940 binding assay, but also in the [^{3}H]-Win 55212-2 binding, adenylate cyclase and MVD assays in vitro, and in the ACh writhing assay in vivo. In each instance, the potency of cannabinoids fell on the line of the correlation of potency of AAIs in any two assays (data not shown). These data suggest that the AAI receptor may be a cannabinoid receptor. The ability of MI antagonists to attenuate both AAIs and THC in the MVD preparation (Casiano et al., these proceedings) supports this conclusion.

It should be noted that the inhibition of adenylate cyclase by AAIs is via a G-protein since the effect is GTP-dependent and pertussis toxin-sensitive (Pacheco et al., these proceedings). The antinociceptive activity of AAIs, like those of cannabinoids, is accompanied by "sedation/ataxia" (Luttinger et al., these proceedings).

In summary, it is concluded that AAIs interact with a G-protein coupled receptor, and that this receptor is probably a cannabinoid receptor.

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AFFILIATIONS

Departments of Neurosciences and Medicinal Chemistry, Sterling Research Group; Department of Physiology. Wake Forest University, Bowman Gray School of Medicine; and Department of Pharmacology, St. Louis University.

Aminoalkylindoles (AAIs): Structurally Novel Cannabinoid-Mimetics

M.A. Eissenstat, M.R. Bell, T.E. D'Ambra, K.G. Estep, D.A. Haycock, E.M. Olefirowicz, and S.J. Ward

Pravadoline (**1**) is an aminoalkylindole (AAI) which has demonstrated analgesic activity against post-operative pain in man. Other papers at this conference have described the data showing that pravadoline and other AAIs bind to a cannabinoid binding site. Structure-activity relationships for this activity have been generated.

On the 3-aroyl group there is no preference for para-substitution over ortho- or meta-. Increasing lipophilicity as in the para-ethyl analog (**2**) increased potency by an order of magnitude. Replacement of the monocyclic aromatic ring by a bicyclic aromatic ring as in compound (**3**) provided another order of magnitude increase in potency. At the 2-position on the indole replacement of methyl by H as in (**4**) gave a further 2-fold improvement in potency. An aminoethyl 1-substituent was optimum.
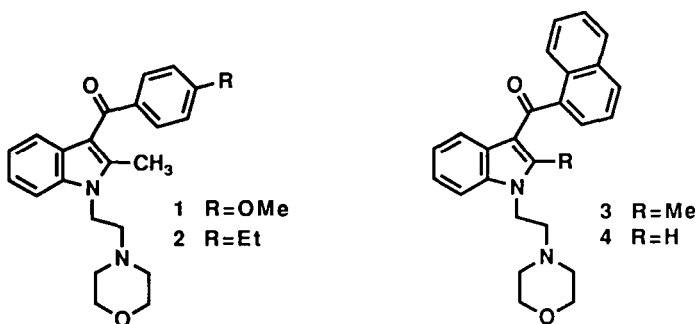
Compound (**4**) represents an AA1 with cannabinoid binding potency comparable to Δ^{9}-THC. Published cannabinoid SAR has

disclosed highly lipophilic structures based on a dibenzopyran nucleus to which is attached an aliphatic side-chain and one or more hydroxy groups. It is not obvious how these structural features fit with the SAR described above. Potential commonality of these apparently divergent structural classes is being explored. Physicochemically, AAIs are less lipophilic than cannabinoids and the presence of the basic amine functionality allows for enhanced aqueous solubility.

A cannabinoid antagonist has long been sought by workers in this field. During the course of exploring substitution at C_4-C_7 of pravadoline several compounds have been synthesized which bind to the cannabinoid binding site, but lack agonist activity as measured by inhibition of electrically stimulated contractions of the mouse vas deferens. Evaluation of such compounds as potential cannabinoid antagonists is ongoing.

AAIs thus represent a new class of compounds which will both enhance our understanding of what structural features are important for binding to a cannabinoid binding site and be a source of research tools which will help further characterize the binding site and its physiologic relevance.

AFFILIATION

Sterling Research Group, Rensselaer, N.Y. 12144

Importance of Rectiwe Metabolites to the <u>In Vivo</u> Hepatic Clearance of Phencyclidine (PCP)

M.A. Zorbas and S.M. Owens

INTRODUCTION

It has been suggested that PCP metabolite covalent binding could be a direct or indirect cause of the long-lasting schizophrenic-like effects attributed to PCP. Conceivably, these effects could be produced by a prolonged half-life of the drug in some individuals. This prolonged half-life could be caused by suicide inhibition of the enzymes which metabolically inactivate PCP. Alternatively, prolonged effects could be caused by PCP metabolite covalent binding to critical macromolecules, presumably in the central nervous system. The purpose of these studies was to determine if PCP covalent binding to liver microsomal en es could produce a reduced clearance of the drug, which could lead to long-lasting effects through a prolonged PCP half-life.

METHODS

For the <u>in vitro</u> studies Sprague-Dawley rats were dosed with phenobarbital for 4 days at 75 mg/day/kg to induce microsomal enzymes. The livers were then removed from phenobarbital-induced and control rats. Microsomal enzymes were isolated by ultracentrifugation The isolated microsomal enzymes were metabolically incubated with 0.1 mM of PCP and tracer doses of [^{3}H]PCP in the presence or absence of NADP$^+$. The addition of NADP$^+$ was used to determine the amount of metabolically-dependent covalent binding. [^{3}H]PCP covalent binding was determined by liquid scintillation spectrometry after TCA protein precipitation and multiple washes to remove unbound drug. The M_r of the [^{3}H]PCP-protein adducts was determined by SDS-PAGE and autoradiography.

For the <u>in vivo</u> pharmacokinetic studies four different groups of Sprague-Dawley rats were infused 9 days with PCP doses ranging from 2.5-21 mg/day/kg (n=4 for each dose group). At the end of dosing a blood sample was collected from each rat for determination of PCP concentration by radioimmunoassay. From the plasma concentrations and infusion rate or PCP, steady-state clearance values were calculated for each animal.

RESULTS

No covalent binding was found in the metabolic incubation mixtures without $NADP^+$. PCP covalent binding in the complete metabolic incubation mixtures was 1.09 and 1.7 PCP-equivalents/mg microsomal protein for the control and phenobarbital-induced microsomal enzyme preparations, respectively. Based on the SDS-PAGE results the predominate covalent binding was to a protein of approximately 50,000 KDa, although much lower amounts of covalent binding were found in two larger molecular weight proteins.

The averages PCP plasma clearance in the four dose groups were not statistically different. The grand average for all four dose groups was 114 ± 28.2 ml/min/kg (n = 16).

CONCLUSIONS

These studies showed that PCP reactive metabolites can bind to phenobarbital-induced and normal rats in vitro but accumulation of reactive metabolite(s) in non-induced rats was not sufficient to alter PCP systemic clearance. These data also showed PCP clearance was independent of dose in this animal model. Therefore, we would not expect the half-life to be prolonged in these animals.

Although PCP pharmacokinetics in rats were not affected by the high doses of PCP used in this study, it does not completely rule out the possibility of an extended biological half-life in some humans. It is possible that genetic variations in the metabolism of PCP (i.e., different isoenzymes) could cause an extended biological half-life and duration of action which would not be found in a genetically homogeneous population of rats.

AFFILIATIONS

Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205

Influence of Phencyclidine (PCP) Pharmacokinetics on PCP Depencence After i.v. and s.c. Routes of Administration

William D. Wessinger and S. Michael Owens

While animal studies clearly show that PCP administered at high doses can produce physical dependence, few reports describing abstinence syndromes in humans have appeared. Our studies of the dependence producing properties of PCP have focused on lower, more clinically relevant doses and utilized animal behavior as an index of dependence. Chronic dosing with i.v. PCP at doses as low as 5.6 mg PCP·HCl/day/kg for as few as 10 days reliably produces disruptions in rat operant behavior when the drug is withdrawn (Wessinger and Healey, 1989).

The purpose of the present studies was to investigate the pharmacokinetics of PCP under the same dosing regimen used to investigate the dependence producing properties of PCP and to determine how the behavioral effects of starting and stopping infusions correlate with the blood concentrations. Because of the inherent difficulties of implanting and maintaining i.v. catheters, relative to the ease of using osmotic minipumps for s.c. administration, it was of interest to compare the pharmacokinetics using these two routes to determine if other planned studies could utilize the s.c. route of administration.

At present, PCP pharmacokinetics have been studied in 6 rats. Under pentobarbital anesthesia they were implanted with i.v. catheters for blood sampling which entered the inferior vena cava via the femoral vein. Three of the rats were also implanted with catheters in the external jugular vein for i.v. drug administration. Following recovery from surgery, PCP infusions at the rate of 8.7 mg PCP (base)/day/kg for 10 days were administered via the jugular catheter for i.v. studies (3 rats) or via s.c. implanted osmotic minipumps for s.c. studies (3 rats). During the onset of infusions, 100 µl blood samples were taken at 0, 2, 4, 8 and 24 hr. Thereafter, samples were taken every 48 hr until the end of the infusion period at 240 hr. At this point PCP infusions were terminated by switching to saline in the i.v. rats or by removing the osmotic pumps from the s.c. rats. During the offset of PCP infusions, samples were taken at 2, 4, 8, 12 and 24 hr. PCP serum concentrations were determined using a PCP-specific RIA which employed a high affinity goat anti-PCP serum, a $[^3H]$-PCP radioligand, and a rabbit-goat solid phase second antibody separation method (Owens et al., 1982; 1987).

There was a very high correlation between the log of the PCP concentrations produced by i.v. administration determined in this study and behavioral effects (as measured by operant response rates) seen in rats chronically treated with i.v. PCP under the same treatment regimen. However, there were qualitative

differences in the relationship between PCP concentration and behavioral effects depending on the time-point the correlations were determined. During the onset of PCP infusion, PCP concentrations were inversely related to behavioral effects (i.e., as PCP concentration increased, operant response rate decreased). PCP concentrations plateau after about 24 hr of infusion and remained steady for the duration of the infusion period. During this time rats become tolerant to the behavioral suppressant effects of PCP with behavior recovering to baseline levels within 4 to 5 days. During the offset of PCP infusions, PCP concentrations were directly related to behavioral effects (i.e., as PCP concentration fell, response rate also decreased). This dramatic change in the relationship between PCP concentration and behavioral effects that occurs during chronic administration is interpreted as evidence that dependence on PCP had occurred. However, the physiological/biochemical nature of the change is unknown.

PCP pharmacokinetics determined in rats receiving i.v. PCP and in rats receiving s.c. PCP indicates that the pharmacokinetic parameters and steady state concentrations for these two routes of administration are virtually indistinguishable and very reproducible. The table below lists the steady state concentration (C_{ss}), volume of distribution (V_{ss}), systemic clearance (Cl_{ss}), and the terminal elimination half-life ($t_{1/2}$) for each route (averaged values ±S.D., except that $t_{1/2}$ is the harmonic mean):

Parameter	i.v. infusion		s.c. infusion	
C_{ss} (ng/ml)	90.6	(±17.4)	82.5	(±6.9)
$t_{1/2}$ (hr)	4.1		3.9	
V_{ss} (L/kg)	25.4	(±7.8)	24.9	(±2.7)
Cl_{ss} (ml/min/kg)	68.5	(±14.5)	73.6	(±6.4)

These data suggest that using the s.c. route of administration via osmotic minipumps should be an acceptable alternative for PCP dependence studies. Indeed, preliminary studies comparing the behavioral effects of PCP dependence using the i.v. and s.c. routes of administration produce remarkably similar behavioral profiles.

ACKNOWLEDGEMENTS

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AFFILIATION

Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Withdrawal from Nicotine Fails to Produce a Conditioned Taste Aversion to Saccharine in Rats and Mice

Heidi F. Villaneuva, John R. James, Shahwali Arezo, and John A. Rosecrans

There is substantial evidence from this laboratory and others that chronic administration of nicotine produces tolerance in rats. Nicotine abstinence in animals previously administered nicotine has been shown to produce increases in plasma corticosterone levels (1), moderate disruption of operant responding has been demonstrated during nicotine abstinence in mice (4) and rats (2), and (3) have shown that nicotine withdrawal generalizes to the discriminative cue produced by pentylenetetrazol in a two-lever drug-discrimination procedure. Nicotine abstinence, however, does not produce a powerful withdrawal syndrome in animals and a nicotine withdrawal syndrome has been difficult to demonstrate and to reproduce. The present studies attempted to measure any effects of nicotine withdrawal in rats and mice by pairing the cessation of daily drug administration with a novel taste. Previous studies have characterized this taste aversion paradigm by pairing the novel taste of saccharine with the cessation of chronic opiate administration in rats (5,6).

In experiment 1, male Sprague-Dawley rats were randomly assigned to one of three groups- morphine, nicotine or saline (N = 8). Using a stair-step technique, the morphine dose was increased from 20 to 160 mg/kg per day. Similarly, the nicotine group was increased from 0.3 to 2.4 mg/kg. 24 hours after the last injection on day 28, a post-drug saccharine preference test was carried out for 21 days. Forty-eight hours after the last injection, the morphine group demonstrated a conditioned aversion to saccharine. The nicotine group, however, was not different from the saline group, suggesting that nicotine does not produce physical dependence in the rat.

In experiment 2, male ICR mice were used in a manner identical to the rats in experiment 1, except the final dose of morphine was 240 mg/kg and the final dose of nicotine was 3.2 mg/kg. In this experiment, there was no conditioned aversion to the saccharine in either the nicotine or morphine groups.

In experiment 3 male ICR mice were implanted with 50 mg morphine pellets or placebo pellets. After 3 days, pellets were removed and animals were tested for conditioned aversion to saccharine. Again, no aversion to the novel taste of saccharine was demonstrated.

Previous studies have demonstrated that opiate abstinence produces a conditioned aversion to the novel taste of saccharine in rats (5,6), but abstinence from amphetamine does not produce a conditioned aversion (5). Experiment 1 confirmed that morphine abstinence produces a robust conditioned taste aversion, but abstinence from nicotine administration did not produce a conditioned aversion. These results suggest that compounds with stimulant properties do not produce a physiological withdrawal syndrome analogous to morphine withdrawal in this paradigm. Experiments 2 & 3 suggest that the mouse model of conditioned aversion is not analogous to the rats model, although further studies are needed to confirm this lack of conditioned aversion in the mouse.

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Affiliation: Virginia Commonwealth University- Medical College of Virginia, Department of Pharmacology & Toxicology, Richmond, Virginia 23298.

[3H]AHN 070: A High Affinity Irreversible Radioligand for Characterization of Peripheral Benzodiazepine Receptors

A.H. Newman, R.T. McCabe, J.A. Schoenheimer, and P. Skolnick

The peripheral-type benzodiazepine receptors (PBR) have been characterized with two selective ligands, Ro 5-4864 and PK 11195. Although both compounds display high affinity and selectivity for PBR, their chemical structures and pharmacological profiles differ. The benzodiazepine Ro 5-4864 exhibits high affinity (low nM) binding to PBR in rodent (e.g. rat, mouse, guinea pig) tissues but binds with low affinity to tissues from other species (e.g. cow, rabbit, human). The isoquinoline PK 11195 antagonizes the pharmacological actions of Ro 5-4864 in some but not all paradigms, and binds with high affinity to PBR in all mammalian tissues examined. The ethylisothiocyanato-derivatives of these ligands, AHN 086 and AHN 070 respectively, were synthesized and selectively acylate PBR. We previously demonstrated that [3H]AHN 086 selectively acylates a 30 kDa protein in rat pineal.

[3H]AHN 070

435
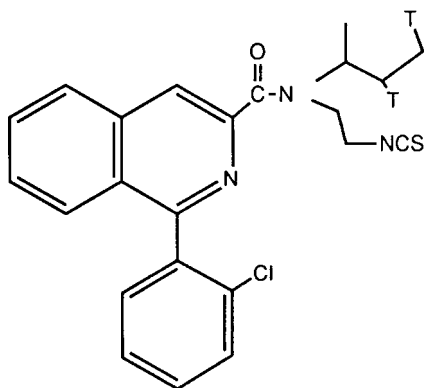
[^{3}H]AHN 070 was prepared in order to characterize its binding to PBR and compare the Mr of the protein(s) labeled in rat kidney and pineal with that observed using [^{3}H]AHN 086 and the PBR photoaffinity label [^{3}H]PK14105. [^{3}H]AHN 070 (S.A. 60 Ci/mmol) was synthesized in five steps via an N-methylallyl intermediate. This radioligand proved to bind irreversibly and selectively to PBR as demonstrated by: 1) The presence of specific binding after repeated washes, heat denaturation and treatment of the labeled tissue with trypsin and 2) the ability of a variety of PBR ligands and not centrally active benzodiazepine ligands to displace [^{3}H]AHN 070 binding. Although a true dissociation constant cannot be be calculated for an irreversible ligand like [^{3}H]AHN 070, under a standard set of conditions, the ligand appears to occupy 50% of the receptors at approximately 16 nM.

Preliminary experiments using SDS-polyacrylamide gel electrophoresis demonstrated that [^{3}H]AHN 070 primarily labeled both two proteins with approximate Mr of 18 and 30 kDa, in rat kidney. Electrophilic irreversible ligands notoriously acylate many proteins nonspecifically. Thus, we believe the specific binding will increase in a preparation containing primarily purified receptor from an affinity chromatography or ion exchange column.

Since [^{3}H]AHN 070 and [^{3}H]AHN 086 appear to label PBR to a different extent (18 and 30 kDa), studies using these two high affinity ligands may provide important information regarding the molecular architecture and physiology of the PBR. The ability to irreversibly label PBR provides an excellent technique to investigate these binding sites devoid of the limitations of reversible ligands.

AUTHORS

Amy Hauck Newman, Department of Applied Biochemistry, Walter Reed Army Institute of Research, Washington, D. C.

R. Tyler McCabe, Joyce A. Schoenheimer and Phil Skolnick, Laboratory of Neuroscience, NIDDK, National Institutes of Health, Bethesda, MD

Synthesis and Evaluation of Novel BTCP Based Affinity Ligands for the Dopamine Reuptake Complex

**Brian R. deCosta, Richard R. Rothman,
Hyacinth Akunne, Arthur E. Jacobson, and
Kenner C. Rice**

The dopamine (DA) transporter protein is the major site through which the behavioral effects of cocaine and related drugs are elicited. In order to further characterize this site, we wished to develop selective affinity ligands for the DA transporter with an isothiocyanate (NCS) moiety; the NCS moiety has been used to prepare affinity ligands for elucidation of the structure and function of a number of CNS receptors (Rice et al. 1983; Rafferty et al. 1985). Because N-[4-(2-benzo[b]thienyl)cyclohexyl] piperidine (BTCP, **1**) is a highly potent and selective ligand at the DA-transporter (Vignon et al, 1988), we decided to use it as a template for our putative affinity ligands **2** and **3** . Compound **2** was synthesized in 7-steps from cyclohexanone; **3** was synthesized in 8-steps from cyclohexanedione monoethylene ketal. A key step in the synthesis of **3** relied upon a stereospecific low temperature $LiAlH_4$ reduction of 4-azido-4-[2-(benzo[b]thienyl)]cyclohexanone to give 1-[2-(benzo[b]thienyl)]-4-hydroxy cyclohexylamine with, as yet, undefined stereochemistry.

The capacity of **2** and **3** to cause wash-resistant inhibition of [3H]BTCP (Vignon et al, 1988), and [3H]GBR12935 (Rothman et al. 1989a) (both label the DA-transporter), as well as [3H]TCP (Rothman et al. 1989b) and [3H]cocaine binding (Akunne et al.) in whole guinea pig brain was evaluated. The amine precursors to **2** and **3** , compounds **4** and **5** , were used as controls.
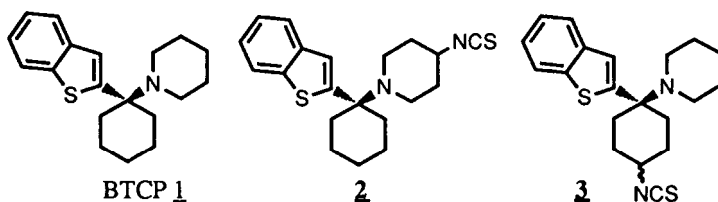
The results showed that **2** displaced [3H]BTCP with an IC_{50} of 350±21 nM and [3H]GBR12935 with an IC_{50} of 188±4 nM. Compound **2** displaced [3H]cocaine very weakly, and it failed to displace [3H]TCP. At a 10 µM concentration, **2** caused a 79% wash-resistant inhibition of [3H]GBR12935

binding, an 89% inhibition of [^{3}H]BTCP and a 23% inhibition of [^{3}H]cocaine binding, and failed to affect [^{3}H]TCP binding. Compound **3** displaced [^{3}H]BTCP with an IC$_{50}$ of 629±27nM, [^{3}H]GBR12935 with an IC$_{50}$ of 3694±335 nM and [^{3}H]cocaine very weakly. It failed to displace [^{3}H]TCP. A 10 μM concentration of **3** resulted in a 44% wash resistant inhibition of [^{3}H]GBR12935 binding, a 71% inhibition of [^{3}H]BTCP binding, an 85% reduction in [^{3}H]cocaine binding and showed little or no effect on [^{3}H]TCP binding. Amine precursor, **4** was tested in the same way as above and it produced apparent wash resistant inhibition of [^{3}H]BTCP (93%), [^{3}H]GBR12935 (89%) and [^{3}H]cocaine (73%) binding; this phemomenon will be the subject of a larger study (Akunne et al.). Amine **5** failed to affect [^{3}H]BTCP or [^{3}H]GBR12935 binding.

In conclusion, we have shown that the two NCS derivatives **2** and **3** are apparent affinity ligands at the DA-transporter protein. They should allow a better understanding of the functional role of this site, and may lead to its isolation and purification.

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AFFILIATIONS

Laboratory of Medicinal Chemistry (BRdeC, AEJ, and KCR), and Unit on Receptor Studies (RRR and HA), NIDDK, National Institutes of Health, Bethesda, MD 20892

Role of NMDA Receptors in Methamphetamine-Induced Changes in Extrapyramidal and Limbic Neuropeptide Systems

G.R. Hanson, N. Singh, L. Bush, and J.W. Gibb

Administration of methamphetamine (METH) causes substantial increases in the tissue content of neurotensin (NT) and dynorphin A (Dyn) in extrapyramidal and limbic structures. Thus, 18 hr after 5 doses of METH (10 mg/dose; 6 hr-intervals), levels of NT-like immunoreactivity increased to 254%, 173% and 300% of control in the striatum, nucleus accumbens and substantia nigra, respectively: in the same METH-treated animals, the corresponding levels of Dyn-like immunoreactivity increased to 560%, 197% and 408%, respectively. Recently, it has been reported that blockade of N-methyl-D-aspartate (NMDA)-type glutamate receptors attenuates or blocks METH-induced changes in monoaminergic neurochemical parameters; consequently, we conducted experiments to determine if NMDA receptors also participate in the response of NT and Dyn systems to this stimulant. Rats were treated with the noncompetitive antagonist, MK-801, prior to multiple s.c. injections of METH, as described above. METH-induced increases in levels of NT and Dyn in extrapyramidal and limbic structures were attentuated with MK-801 doses as low as 0.01 mg/kg/dose and, for the most part, totally blocked with MK-801 doses of 0.5 mg/kg/dose. As these METH-induced changes in neuropeptide levels are likely associated with the pharmacological actions of this stimulant, we propose that the NMDA receptor plays an important role in mediating some of the METH effects. (Supported by grants DA 00869 and DA 04222).

Affiliation: Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

The Relationship Between Cocaine Venous Blood Levels and the Cardiovascular and Subjective Effects of Smoked and Intravenous Cocaine

Richard W. Foltin and Marian W. Fischman

Although limited, data indicate that cocaine smoking produces rapid rises and peaks in cocaine blood level and subjective effect, similar to intravenous administration. In the present study two doses of cocaine were given during experimental sessions and heart rate, blood pressure, subjective effects, and cocaine venous blood levels were sampled repeatedly. The relationship between venous blood level, route of administration and the effects of cocaine on the various dependent measures was determined on the ascending and descending limb of the cocaine venous blood level concentration curve.

Ten adult male research volunteers, with histories of iv and smoked cocaine use participated in four sessions. Cocaine was smoked using modified corn-cob pipes. Subjects were instructed to inhale the cocaine in their usual manner, or intravenous cocaine in a volume of 1 ml was injected over 10 sec. All subjects were tested with two doses each of smoked [25, 50 mg (base)] and iv cocaine 116, 32 mg (hydrochloride)]. Each dose was given twice during a single session with the first dose administered at time zero and the second dose given 14 min later. Bloods were collected and a series of subjective effects questionnaires were completed prior to, and repeatedly after cocaine administration.

The high doses produced significantly greater cocaine blood levels than low doses, and there were no differences between smoked and iv cocaine. Blood levels 44 min after the first dose were similar to those observed 4 min after the first dose. High doses produced significantly greater elevations in heart rates than low doses, and there were no differences between smoked and iv cocaine. The significance of the linear regression between cocaine venous blood level and dependent measures at each time point that blood samples were collected was calculated. When analyzed 4 min after the first dose, cocaine blood level accounted for 85% of the variance in mean heart rate. The variance accounted for by cocaine blood level decreased with time during the session so that by 60 min after the first smoked cocaine dose there was no significant relationship between blood level and mean heart rate. The predicted mean change in heart rate on the descending limb (44 min) of the cocaine blood level

curve was less than on the ascending limb (4 min). Thus after the initial increase in cocaine venous blood level and heart rate, the same cocaine blood level at later times was associated with smaller increases in heart rate.

There were no significant main effects of route of administration on any subjective effects measure. Both low and high doses increased VAS ratings of "stimulated." High doses increased LSD scores of the ARCI while VAS ratings of "hungry" increased during the session. Cocaine venous blood level accounted for up to 71% of the variance in "stimulated" scores 4 min after the first dose, and up to 25% of the variance 30 min after the first dose. This amount of variance is similar to that observed for heart rate. Cocaine blood level accounted for 24-70% of the variance in LSD scores over the entire session. In contrast to the cardiovascular effects, variance in LSD scores accounted for by cocaine blood level remained stable throughout the session. There were significant relationships between cocaine venous blood level and changes in VAS ratings of "hungry" at the end of the session, not the beginning. The predicted mean change in "stimulated" score 4 min after the first dose was larger than the predicted mean change 44 min after the first dose when blood levels were comparable. A different pattern of results was obtained with the LSD and "hungry" scores. Predicted changes in LSD scores were similar 4 min and 44 min after the first dose when blood levels were similar. The predicted mean change in "hungry" score 4 min after the first dose was smaller than the predicted mean change 44 min after the first dose, when blood levels were comparable.

Similarities between smoked and intravenous cocaine were not always the rule, however, as significantly greater after-session ratings of drug "liking" were obtained following smoked cocaine compared to iv cocaine.

The results of the present study demonstrate that smoked or iv cocaine produced dose-dependent increases in heart rate, and subjective effects in human subjects. The potency of smoked cocaine was about 60% of that of iv cocaine in this regard, i.e., 50 mg smoked cocaine had effects similar to 32 mg iv cocaine. For both routes of administration the cardiovascular and some subjective effects were significantly smaller on the descending limb of the venous blood concentration curve compared to the ascending limb, demonstrating acute tolerance.

Acknowledgement

Authors

Division of Behavioral Biology, Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, 600 N. Wolfe St, Houck E-2, Baltimore, MD 21205, U.S.A.

Low Doses of Caffeine Can Serve as Reinforcers in Humans

A.H. Oliveto, J.R. Hughes, S.L. Pepper, W.K. Bickel, and S.T. Higgins

A recent investigation in our laboratory showed that among subjects with previous exposure to higher doses (100-200 mg) of caffeine, many self-administered 50 and/or 25 mg of caffeine in preference to placebo under double-blind conditions. The present experiment was conducted to replicate these findings in subjects without prior exposure to higher caffeine doses (i.e., $>$ 100 mg). Eleven moderate coffee drinkers (1.5-7.0 cups/day) were tested under 25- and 50-mg dose conditions, using a randomized cross-over design. Then subjects were tested under a 12.5-mg and/or 100-mg dose condition. Each dose was tested against placebo across six independent trials. Each trial consisted of a 2-day exposure period followed immediately by a 2-day, concurrent-access choice period. During the exposure period, subjects consumed their usual number of cups of one coffee (e.g., decaffeinated coffee) on Day 1 and of the other coffee (e.g., decaffeinated coffee plus caffeine anhydrous) on Day 2. During the choice period, subjects had concurrent access to the same two coffees and drank as many of either as they wished. Subjects also were not required to drink a minimum number of cups. Reinforcing effects were inferred from the relative rates at which subjects self-administered caffeinated coffee and decaffeinated coffee during this concurrent access. Over the 6-trial period, the 12.5-, 25-, 50-, and 100-mg dose of caffeine consistently served as a reinforcer in 0 of 2, 2 of 11, 5 of 11, and 5 of 10 subjects, respectively. These results replicate prior results and suggest that caffeine, at doses commonly found in teas and sodas, can function as a reinforcer.
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Human Behavioral Pharmacology Laboratory, University of Vermont, Department of Psychiatry, 38 Fletcher Place, Burlington, Vermont 05401

Assessment of Mazindol for Abuse Liability

W.B. Pickworth, S.A. Klein, E.B. Bunker, and J.E. Henningfield

Mazindol, a compound used for the treatment of obesity, is chemically dissimilar from amphetamine and cocaine but "bind to" cocaine-sensitive dopamine reuptake sites. Animals self administer mazindol, but a human study indicates no significant abuse potential at therapeutic doses. In a preliminary study, mazindol reduced cocaine craving in patients being treated with methadone for opiate dependence. Our purpose was to compare the abuse liability of mazindol (3 and 6 mg) with methylphenidate (30 mg) in volunteers with histories of stimulant use. Seven residential male subjects participated in the study. Mazindol and methylphenidate increased blood pressure and heart rate, however, their subjective effects differed. Mazindol caused decreases in dose-related measures of vigor, increased measures of fatigue and increased scores on the LSD and PCAG scales of the ARCI. (Those scales measure drug-induced hallucinations/dysphoria and apathetic sedation, respectively). Neither drug increased the MBG scale scores, a measure of drug-induced euphoria. Methylphenidate did not cause the sedative-like effects seen after mazindol. Both drugs reduced hunger and increased visual analog measures of drug liking and disliking. These data indicate that at three times the therapeutic dose mazindol poses little abuse potential; however, its dysphoric effects may limit the use of mazindol in the treatment of cocaine dependence. The lack of drug-induced euphoria after methylphenidate may be due to the relatively low dose or a protacted effect of chronic stimulant abuse.

Affiliation: NIDA Addiction Research Center

Low-Dose Caffeine Discrimination in Normal Humans

Kenneth Silverman and Roland R. Griffiths

Few studies have demonstrated behavioral effects of low dietary doses of caffeine. A recent study utilizing a drug discrimination procedure has provided the clearest demonstration to date of reliable behavioral activity of low dietary doses of caffeine in humans. Behavioral pharmacologists serving as both investigators and subjects in that study discriminated doses of caffeine from placebo that were lower than those previously thought to be behaviorally active. The present study was undertaken to determine if similar procedures could establish low-dose caffeine vs placebo discriminations in 15 minimally-instructed, normal volunteers with histories of daily caffeine consumption.

Subjects were told that they could receive any two of a variety of drugs found in coffee, tea, chocolate, and soft drinks or one of those drugs and an inactive placebo. They were not told specifically which two compounds they would receive. Instead, the two compounds, caffeine and placebo, were identified by letter codes (e.g., O and Cl) that were unique for each subject. Each day, under double blind conditions, each subject sequentially consumed two capsules 60 minutes apart; one contained 178 mg caffeine and the other placebo. The order of the capsules was randomized each day. Fifteen, 30 and 45 minutes after consuming the first capsule, the subjects guessed which of the two letter-coded compounds they had received. Correct guesses at the 45-minute time point earned $10. For each subject, discrimination accuracy was analyzed using the binomial probability distribution. Significant discrimination performance was defined as correctly identifying the capsule content on 15 or more of the last 20 sessions (i.e., $\geq$ 75%; P < .05).

Five subjects acquired the 178 mg caffeine vs placebo discrimination within the first 20 sessions. Eleven of the 15 subjects ultimately acquired the discrimination after continued training or dosing manipulations. Compared to placebo, 178 mg of caffeine decreased ratings of sleepy and increased ratings of alert, desire to talk, motivation to work, and energy/active. On the Addiction Research Center Inventory, caffeine increased scores on the MBG and LSD scales and decreased scores on the PCAG scale.

Nine of the 11 subjects who acquired the discrimination were exposed to progressively lower doses of caffeine until their discrimination accuracy fell below 75% correct for 20 sessions. Subjects varied considerably in the

lowest discriminable dose. The lowest discriminable doses were 178 mg for 1 subject, 100 mg for 4 subjects, 56 mg for 3 subjects, and 18 mg for 1 subject. Two of these subjects are still participating in the experiment. Onset of the discriminations varied from 15 to 45 minutes.

This study shows that low doses of caffeine can be established as discriminative stimuli in minimally-instructed, normal human subjects and further documents the behavioral activity of low doses of caffeine.

AFFILIATlON

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21224

Low-Dose Caffeine Physical Dependence in Normal Subjects: Dose-related Effects

Suzette M. Evans and Roland R. Griffiths

Although caffeine is the most widely used behaviorally active drug in the world, caffeine physical dependence has been only moderately well characterized in humans. In a recent study caffeine withdrawal from 100 mg/day caffeine was demonstrated in 7 subjects previously trained to discriminate low doses of caffeine.

The present experiments were designed to determine the generalizability of low dose caffeine physical dependence in normal subjects. Subjects were normal healthy volunteers who were daily, moderate consumers of caffeine-containing foods. During the experiments subjects were required to abstain totally from all sources of dietary caffeine. Experiment 1 investigated the role of dosing interval on caffeine physical dependence. Using a double-blind cross-over design, 15 subjects were maintained on 300 mg/day caffeine in capsules either as a single 300 mg dose in the morning or as 100 mg t.i.d. Placebo was intermittently substituted for periods of 2 consecutive days. Experiment 2 investigated the role of caffeine maintenance dose on the magnitude of withdrawal symptoms. Using a double-blind cross-over design, 17 subjects were maintained on either 100,300, or 600 mg/day caffeine in capsules given b.i.d. Placebo was intermittently substituted for periods of 2 consecutive days.

The results of both experiments demonstrated that caffeine physical dependence occurs in normal subjects; compared with periods on which subjects were maintained on caffeine, substitution of placebo was associated with increased ratings of headache and drowsy/sleepy, and decreased ratings of energy. In Experiment 1 there were no significant differences between the two dosing intervals with regards to caffeine withdrawal symptoms.

Experiment 2 demonstrated significant withdrawal symptoms after administration of the lowest dose of 100 mg/day. Furthermore, Experiment 2 showed a dose-related caffeine withdrawal syndrome both in terms of magnitude and range of symptoms. The magnitude of headache ratings on placebo days was related to maintenance dose, i.e., compared to the lowest maintenance dose (100 mg/day), the magnitude of headache produced by placebo substitution at the two higher maintenance doses was significantly increased. Also, compared to caffeine days, placebo was associated with a dose-related increase in the number of significant withdrawal symptoms.

This is the first prospective human study to demonstrate the dose-dependent nature of caffeine withdrawal.

AFFILIATION

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

The Effects of Deprivation on the Reinforcing Efficacy of Smoking

L.H. Epstein, C.M. Bulik, K.A. Perkins, and A.R Caggiula

This study assessed whether the reinforcing value of smoking increased under conditions of smoke deprivation. Six women (ages 18-19) who smoked >15 cigarettes/day for at least the past two years were provided access on concurrent schedules to smoking or money during two-hour sessions conducted over a period of four days. On half of the days subjects were smoke deprived (no smoking from 11 pm prior; $COa \leq$ 8ppm), while on the other days subjects smoked at their usual rate before coming to the lab. Order of deprivation/non-deprivation conditions was randomized. Smoking was presented on a concurrent schedule with another reinforcer (money) available. Response rate and time allocation served as measures of the relative reinforcing value of smoking. The computer generated task presented trials in which smoking or money was available on progressive concurrent variable ratio schedules (VR4, 8, 12, 16, 20, 25, 33, 50). Completion of schedule requirements earned one puff or $0.10. Trials involved earning two reinforcers. Results showed that subjects spent a significantly greater percentage of their time working for cigarettes under deprived (Mean = 57.5) than non-deprived (mean = 38.0) conditions (F=24.59, p=.00) across all schedule comparisons. There were no significant differences in either the order of presentation or study day. In addition, a significant interaction between conditions by schedules was observed, with time significantly increasing over schedules when subjects were deprived, and time significantly decreasing over schedules when subjects were not deprived. These results indicate that the concurrent schedule paradigm used showed smoking's reinforcing value increased under deprivation and this paradigm can be used to examine other factors that regulate the reinforcing value of smoking.

University of Pittsburgh School of Medicine

Acute Opioid Physical Dependence in Humans: Effects of Varying the Morphine-Morphine Interval

Kimberly C. Kirby and Maxine L. Stitzer

Recently we determined that morphine (18mg/70kg) and naloxone (10mg/kg) i.m administrations must be separated by a minimum interval of 45 minutes and a maximum interval of 24 hours in order to observe precipitated withdrawal in humans (Heishman et al., 1989; Kirby et al., 1990). Absence of acute withdrawal at 36-hour and longer intervals suggests that the temporal spacing of multiple agonist exposures may be important in the development of physical dependence. In the present study, we delivered multiple morphine injections prior to the naloxone injection to examine the effect of the interval between agonist injections on intensity of the precipitated withdrawal response.

METHOD

Participants were 10 nondependent males with a history of regular opiate use. They were currently using 3-4 times a week. Participants lived on an 8-bed inpatient research unit during the study and 1-2 volunteered at a time.

The study involved four experimental conditions. In each condition the subject received a total morphine pretreatment of 54mg/70kg administered i.m. in three doses of 18mg/70kg each. Conditions differed only in the interval between morphine injections (12, 24, 48, or 72 hours). In every condition, a naloxone challenge (10mg/kg) was administered i.m. 24 hours following the last of the three morphine doses. The interval between the last morphine injection and the naloxone challenge was kept constant at 24 hours. In addition to the four experimental conditions, a control condition was employed in which a single 18mg/70kg morphine pretreatment was followed 24 hours later by a naloxone challenge. This allowed a within subject comparison of the intensity of precipitated withdrawal after a single injection and that precipitated after multiple injections. Conditions were counterbalanced across subjects. A 2-3 day washout period occurred between conditions.

Fifteen minutes prior to the naloxone injection, and at 5, 15, 30,45 and 60 minutes post naloxone, subjects were observed and asked to report signs and symptoms of the withdrawal syndrome. Observer measures consisted of six traditional Himmelsbach signs, such as yawning, perspiration, and restlessness. Severity of each sign was rated from 0 (not present) to 9 (most severe). The withdrawal symptom questionnaire was completed by the subject and employed the same 0-to-9 scale. It contained 15 adjectives describing typical withdrawal symptoms such as chills, irritability, and backache.

RESULTS

The composite observer rating, which was derived by summing the ratings of the Himmelsbach signs, showed an orderly increase in the severity of post naloxone withdrawal as the interval between morphine injections was shortened. When each sign was analyzed separately, only one of them, runny nose, showed significant injection spacing effects. Post hoc comparisons indicated that only the pretreatments spaced at 12 and 24 hours resulted in more intense withdrawal than that precipitated after a single pretreatment.

Subject ratings supported the observer rated results. The composite subject rating, which was derived by adding the ratings of 15 adjectives on the withdrawal questionnaire, showed an orderly increase in the severity of the withdrawal as the interval between morphine pretreatments was shortened. The 12 and 24 hour ratings were both significantly elevated in post hoc comparisons with the rating from the single injection condition. Seven of the adjectives showed statistically significant injection spacing effects. These items included irritability, rhinorhea, restlessness, lacrimation, chills, backache, and bothered by noises. Six items showed significant elevations over the single pretreatment condition when injections were spaced at 12 hours. Only 2 items were significant at the 24 hour condition (irritable, bothered by noises).

CONCLUSION

To summarize, withdrawal precipitated after three injections spaced at 12 or 24 hour intervals is reliably greater than that precipitated after a single morphine pretreatment, but when multiple injections are spaced at intervals longer than 24 hours, the effects are not reliably greater than those occurring from a single pretreatment. Thus, only closely spaced pretreatments, appear to produce incremental advances in development of physical dependence. This does not imply that widely spaced exposures are devoid of physical dependence potential, since even a single isolated exposure produces acute dependence, as seen with the antagonist challenge procedure. Rather the difference between 12-24 hour and longer exposure intervals is in intensity of withdrawal, not occurrence of withdrawal.

Linear effects were observed in withdrawal intensity, suggesting that examining both shorter and longer pretreatment intervals would be necessary for determining the full range of the exposure interval effect. In addition, it would be interesting to manipulate the number of morphine pretreatments. While spacing 3 injections by 72 hours does not appear to lead to increased dependence, the effect of a larger number of pretreatments spaced at 72 hours might be quite different. In general, however, these findings support clinical observations that human drug abusers can use opiate drugs intermittently without developing significant overt signs and symptoms of physical dependence.

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Dept. of Psychia. & Beh. Sci., Johns Hopkins University School of Medicine

Acute Physical Dependence in Opiate-Naive Humans

Julian L. Azorlosa and Maxine L. Stitzer

The purpose of this study was to determine whether acute physical dependence to an opiate agonist could be demonstrated in subjects lacking opioid dependence histories and whether the intensity of antagonist-precipitated withdrawal effects would be influenced by the number of prior opioid agonist exposures (one versus two exposures). Morphine sulphate (15mg/kg, I.M.) was administered in a double-blind procedure to 20 male volunteers with little or no history of opiate exposure. Subjects were excluded if they had taken a prescribed opiate for more than 2 weeks or if they had taken any opiate less than 3 months before participating in the study.

Subjects were randomly assigned to one of two experimental conditions. On Day 1, all subjects received a naloxone challenge (30mg/kg). On the morning of Day 2, subjects in Group 1 (n=10) received saline and the subjects in Group 2 (n=10) received morphine. In the afternoon of Day 2, both groups received saline. The following morning, (Day 3) both groups received morphine. That afternoon, (4.3 hours later) both groups received naloxone. Respiration, skin temperature, pupil diameter, subjective effects and observer rated withdrawal were recorded.

There was no reaction in either group to the first naloxone challenge. Morphine produced pupillary constriction, respiratory depression and an increase in skin temperature. Subjects in both groups reported a significant drug effect when morphine was given. There were no differences between the groups on Day 3 when Group 1 received the first morphine injection and Group 2 received its second morphine injection.. Following the naloxone challenge on Day 3, both groups had a significant increase in pupil size. compared to the pre-injection baseline but only Group 2 had significant elevations on subjective ratings of bad drug effect. Both groups had significant elevations on a withdrawal symptom adjective checklist but scores were markedly higher in Group 2. Both groups exhibited withdrawal signs, as rated by a blind observer, with perspiration prominent in Group 1 and perspiration, runny nose and restlessness prominent in Group 2 which, overall, had much more intense withdrawal symptoms. These results show that naloxone-precipitated withdrawal after a single acute morphine treatment can be elicited in subjects lacking an opioid abuse or dependence history and that the intensity of naloxone precipitated withdrawal effects is greatly enhanced by two as compared with one prior exposure to morphine.

AFFILIATION

The Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine

Relative Abuse Liability and Pharmacodynamics of Transnasal and Intramuscular Butorphanol

John T. Sullivan, Kenzie L. Preston, Margaret P. Testa, and Donald R. Jasinski

Butorphanol is a mixed agonist/antagonist opioid with kappa agonist activity. It is currently marketed as an analgesic and is available in an intramuscular preparation only. In order to compare the pharmacodynamics of intramuscular to transnasal butorphanol, a double-blind, double-dummy latin square study was conducted. The subjects were 7 male opioid abusers and they received placebo, transnasal and intramuscular butorphanol doses of 1, 2, and 4 mg on consecutive days. Pharmacodynamic measures included subjective, behavioral and physiologic responses including Addiction Research Center Inventory scales, symptoms, signs and miosis. The profile of effects for the 1 and 2 mg doses did not differ across the two routes The onset of the 4 mg transnasal butorphanol was similar to the 1 and 2 mg doses but was both slower and of a lesser magnitude on most measures compared to the 4 mg intramuscular butorphanol. qualitative measures were similar across all active drug doses and included miosis, identifications as opioids and ratings of disliking. Dysphoria was most prominent with the 4 mg intramuscular dose. The results demonstrate a flattened dose effect relationship of transnasal (compared to intramuscular) butorphanol possibly due to limited absorption. The abuse potential of transnasal butorphanol is low and is not different from intramuscular butorphanol. Abuse of multiple transnasal doses may be limited by a ceiling effect.

AFFILIATION

Department of Medicine (JTS, MPT, DRJ), Department of Psychiatry and Behavioral Sciences (KLP) and Johns Hopkins University School of Medicine, Baltimore, MD 2 1224

Changes in Mood, Craving and Sleep During Acute Abstinence Reported by Male Cocaine Addicts

**William W. Weddington, Barry S. Brown, Edward J. Cone,
Charles A. Haertzen, Elizabeth M. Dax, Ronald I. Herning,
and Barry S. Michaelson**

We examined changes over 28 days in mood states, craving for cocaine, and sleep during short-term abstinence reported by 12 male, predominately intravenous-using cocaine addicts in a research facility. For comparison, we examined 10 non-addict controls.

There were no significant differences between cocaine addicts and controls regarding demographics and selected DBM-III-R psychiatric diagnoses other than psychoactive substance use disorder and antisocial personality disorder. There were significantly higher scores of psychiatric symptoms reported by cocaine addicts one week prior to admission. Mood-distress and depression scores recorded at admission and during acute abstinence were significantly greater than those reported by controls. Addicts' mood-distress scores and craving for cocaine were greatest at admission and decreased gradually and steadily during the 28-day study. There were no significant differences regarding sleep other than addicts' reporting more "difficulty falling asleep" and less "clear-headedness on arising." Although there were significant differences in resting heart rate at admission and over time, there *were* no significant differences in weight gain or blood pressure.

We conclude that symptoms of cocaine addicts were most severe on Day 1 on the residential unit. There was a gradual, persistent improvement in craving, mood states, and sleep. No discreet phases or cycles of changes in psychological or physical reports were observed. Thus, short-term abstinence from cocaine in cocaine addicts is distinct from "withdrawal" of alcohol, opiates, benzodiazepines, and nicotine. Also, abstinence from cocaine in controlled, residential settings is distinct from outpatient cessation, perhaps because of the availability of conditioned cues and cocaine to outpatients. our findings are limited by small sample

size; reliance on self-reports to assess mood, craving for cocaine, and sleep factors; the experimental environment in which the study was conducted: and the length of the study. Our findings are thus preliminary as well as limited insofar as their generalizability to other cocaine addicts who initiate abstinence in controlled environments.

Further research is needed to better delineate acute abstinence in cocaine addicts. In particular, studies to examine daily diurnal measures of craving, mood, physical symptoms, and sleep are need to better clarify the first ten days of abstinence. By administering a fixed dose of cocaine intravenously at the onset of a study of cocaine abstinence, one may achieve a uniform abstinence-initiation point. Findings from such research will help to better delineate the clinical course of cocaine addiction, acute abstinence, and relapse in order to diagnose cocaine addicts more specifically and to develop focused interventions of demonstrated efficacy.

Given the absence of a classical "withdrawal" pattern, "short-term abstinence syndrome" may be a more appropriate classification of psychological and physical phenomena experienced by cocaine addicts who initiate abstinence in a controlled environment.

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Addiction Research Center/National Institute on Drug Abuse, Baltimore, Maryland

Double Blind Assessment of Buprenorphine Withdrawal in Opiate-Addicts

L. Sam, J. Camí, T. Fernandez, J.M. Ollé J.M. Peri, and M. Torrens

The dependence potential of buprenorphine was assessed in opiate addicts who switched from heroin to oral or intravenous buprenorphine. Twenty-nine buprenorphine consumers who fulfilled DSM-III criteria for opiate addiction were eligible for inpatient detoxification. Five patients were excluded because of simultaneous consumption of heroin and buprenorphine, and two refused hospitalization. Twenty-two patients were randomly assigned to double-blind administration of placebo (n = 11) or methadone (n = 11) for 13 days after abrupt withdrawal of the drug. Methadone was administered according to four pre-established dosing schedules depending on the previous amount of daily consumed buprenorphine (7.5 mg of methadone for 1 mg of buprenorphine). The Opiate Withdrawal Checklist was used to assess the severity and course of withdrawal symptoms. Changes in pharmacological treatment-as well as completion of detoxification treatment were also evaluated. None of the methadone-treated patients required modification of the therapeutic regimen, whereas 8 of 11 placebo-treated patients needed treatment with methadone. Buprenorphine withdrawal syndrome was of opiate type, began somewhat more slowly, showed a peak between days 3 and 5 after withdrawal, and was showed prolonged but less intense than heroin withdrawal syndrome. We conclude that methadone but not placebo was an appropriate agent in suppressing buprenorphine withdrawal symptoms. The occurrence, time-course, and characteristics of buprenorphine withdrawal syndrome obliges to reconsider the abuse potential of this analgesic and to control its prescription in drug- addicts.

AFFILIATION:

Section of Toxicomanias, Hospital del Mar and Institut Municipal d'Investigació Médica. 08003 Barcelona, Spain

Use and Abuse of Prescription and Recreational Drugs by Chronic Pain Patients

Deborah L. Haller and Stephen Butler

Certain of the Axis I psychiatric disorders have known high prevalence among chronic pain patients. While incidence varies from study to study, the rates for narcotic and sedative-hypnotic abuse, alcohol abuse, depression, somatiform disorders, and anxiety disorders are generally elevated. Axis II personality disorders are being studied as well and several types have been found to occur in 10% or more of the study samples (namely dependent, passive aggressive, and histrionic). However, there is nothing in the literature which pertains specifically to use of recreational drugs. It was therefore our intent to study the use of cocaine and marijuana by pain patients and to relate their recreational drug use to prescriptive drug use, psychological and treatment variables.

A total of 184 chronic pain outpatients were classified into one or more of the following groups based on self-report, urine toxicology, and substance abuse consultation findings: Rx abusers (N=40), recreational (marijuana and/or cocaine) users (N=54), ETOH abusers (N=35), Rx non-abusers (N=55), and no drug use controls (N=56). The overall sample statistics were as follows: age=42.8 years, 74.2% white and 25.3% black, 48.4% male and 51.6% female, 11.8 years education, IQ=87, and Visual Analogue Scale Score (VAS) = 6.11.

Findings from Chi-Square and T-tests revealed a 45.9% overlap in group membership for recreational users and ETOH abusers and a 54.1% overlap for Rx abusers and ETOH abusers. More men comprised the recreational (70.4%) and ETOH (75.7%) groups. Mood disorder was associated with sedative-hypnotic use (63.2%) and personality disorder was associated with Rx abuse (29.5%). Compared to the controls, the Rx abusers evidenced the greatest psychopathology (as measured on the MMPI), most pain, and showed the least improvement. Furthermore, they tended to drop out of treatment prematurely. The recreational users and ETOH abusers presented similar MMPI and pain profiles and fell in the middle in terms of overall severity of pain problem and response to

treatment. The Rx non-abusers were more like the controls who
were lowest on all dimensions. Interestingly, it was Scale 8 on
the MMPI (schizophrenia) which differentiated all of the drug use
groups from the control group despite the fact that all five groups
generated a 1-3-2 profile type. When all the drug use groups were
combined and compared to their non-drug using counterpart, several
interesting findings emerged. First, the drug users were found
to be significantly younger (38.7 as compared to 44.3 years). They
had higher initial pain levels and evidenced more psychopathology,
specifically, secondary elevations on Scales 8 and 9 of the MMPI.

In sum, all chronic pain patients are not alike in terms of their
drug use and their patterns of use are related to other variables.
both medical and psychological. Of particular interest is the
finding that Rx abusers are the most psychologically disturbed and
most pained sub-group and are least responsive to help. Conversely,
the non-drug using controls have fewer problems to begin with and
have a better outcome. The ETOH abusers and Rx users fall somewhere
in the middle. This suggests that relative success can be predicted,
at least in part, by drug use status at time of admission to a
pain clinic. It also highlights the efficacy of using toxicology
screening as a diagnostic test.

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Available upon request from the authors

Affiliation

Medical College of Virginia
Division of Substance Abuse Medicine
Box 109. MCV Station
Richmond, Virginia 23298-0109

Nicotine Dependence in an Urban Population of Young Adults: Prevalence and Co-morbidity with Depression, Anxiety and Other Substance Dependencies

Naomi Breslau, Patricia Andreski, and M. Marlyne Kilbey

The Detroit Epidemiologic Study is a survey of 1007 randomly selected 21 to 30 year old members of a large HMO. Median age was 26; 61.7% were female and 80% white. The NIMH-DIS, revised to cover DSM-III-R disorders, was used in face-to-face interviews. Data were analyzed to determine the prevalence of nicotine dependence, its co-dependencies with alcohol, cannabis and cocaine and its co-morbidity with major depression and anxiety disorders.

PREVALENCE OF SUBSTANCE DEPENDENCIES BY SEX, RACE AND EDUCATION:

Sex: Lifetime prevalence of nicotine dependence was unrelated to sex (20.0% male, 20.5% female). Alcohol dependence was more than twice as prevalent in males (28.2%) as females (11.9%), and cannabis dependence was more than three times as prevalent in males (13.2%) as females (4.2%). Lifetime prevalence for cocaine dependence was 4.7% for males and 2.4% for females.

Race: Lifetime prevalence of Nicotine Dependence was over twice as prevalent in whites (22.9%) as blacks (9.3%), as was alcohol dependence (20.3% vs 9.3%). Rate differences in lifetime prevalence were smaller for cannabis (8.1% white vs 5.7% black) and cocaine dependencies (3.7% white vs 1.6% black).

Education: Persons with less than high school education had the highest rates of all substance dependencies. The relative risk for nicotine and cocaine dependence for persons with no college vs college education was significant (p<.05).

NICOTINE CO-MORBIDITY WITH SUBSTANCE DEPENDENCIES, DEPRESSION AND ANXIETY:

The co-occurrence of depression, anxiety disorders (inc. panic, OCD, PTSD, Phobia, GAD), and substance dependencies (inc. alcohol, cannabis and cocaine) with nicotine dependence are presented by level of dependence, i.e. mild or moderate. On the average, persons with moderate nicotine dependence began smoking one year earlier and smoked 10 more cigarettes daily than persons with mild nicotine dependence.

The associations of moderate nicotine dependence with depression and anxiety disorders are significantly (p<.05) greater than with mild. The sex-adjusted odds ratio for depression is 2.2 for mild vs 6.8 for moderate nicotine

dependence. The sex-adjusted odds ratio for anxiety disorders is 1.6 for mild vs 4.9 for moderate nicotine dependence.

TABLE ONE

Rates (in Percent) of Substance Dependencies, Depression, and Anxiety by Level of Nicotine Dependence

	Level of Nicotine Dependence		
	None (n=803)	(n=127)	Moderate (n=77)
Alcohol	15.8	21.3	37.7
Cannabis	5.1	18.1	16.9
Cocaine	1.9	4.7	15.6
Depression	10.8	21.3	44.2
Anxiety	26.4	36.2	62.3

A significantly ($p<.05$) increased risk for depression was found also, in association with other substance dependencies. The sex-adjusted odds ratio for depression was 2.5 in moderate alcohol dependence, 3.6 in cannabis dependency, and 4.6 in cocaine dependence. The association of moderate nicotine dependence with depression is not accounted for by other dependencies.

TABLE TWO
LIFETIME PREVALENCE OF DEPRESSION IN PERSONS WITH/WITHOUT NICOTINE OR NICOTINE PLUS OTHER SUBSTANCE DEPENDENCIES

	Depression	
	Number	Percent
No Nicotine Dependence (n=803)	87	10.8
Mild Nicotine Dependence (n=85)	15	17.6
Moderate Nicotine Dependence (n =38)	16	42.1
Nicotine Dependence plus Other Substance Dependencies (Alcohol, Cannabis, Cocaine, Other Drugs) (n = 81)	30	37.0

CONCLUSION:

In the population of young adults, nicotine dependence is more frequent in whites than in blacks and in persons with less than college education. The findings support the validity of the distinction between mild and moderate levels of nicotine dependence as persons with moderate nicotine dependence have increased rats of alcohol and cocaine dependency as well as depression and anxiety disorders.

AFFILIATION: Henry Ford Hospital, Department of Psychiatry and University of Michigan School of Medicine, Detroit, MI, Department of Psychology, Wayne State University, Detroit, MI; Henry Ford Hospital, Department of Psychiatry and Wayne State University, Department of Sociology, Detroit, MI

Drug Use and Abuse at Historical Black Colleges

Julius Debro

This is a pilot study in which we will compare drug use and abuse at historical black colleges among black students with drug use of black students at historically white colleges and universities. We will also examine rates of use among white students at historically white colleges and universities. The proposed study is guided by two hypothesis:

1.   Drug and alcohol use is significantly lower among black students at HBCU's than among black students attending predominantly major white colleges and universities.

2.   Black students at HBCU's use drugs and alcohol at a lesser rate than white students at white colleges and universities.

There are no reliable cross time data about drug use patterns of black students in colleges and universities. The National Household Survey has a section on trends in annual prevalence of drug use among college students 1-4 years beyond high school but the sample of blacks is so small that it is impossible to assess the general use in this population.

The only known study that had a substantial amount of black students was completed some ten years ago by the New York State Division of Substance Abuse Services. In that study of college students in New York, they found white students used drugs at a higher rate than any other group, followed by hispanics and then by blacks.

This pilot study will provide the foundation for a larger study looking at drug use among the black middle class. The impetus for this research came from a contract funded by NIDA to look at drug research at historical black colleges.

Drug use and drug violence is portrayed in the media as a major black issue. Most television shows portray blacks as the offenders and victims. The preliminary research reviewed for this study challenges the stereotypical image of blacks and drug abuse and evidences that blacks who attend colleges are <u>less</u> likely to abuse drugs/alcohol than their white counterparts.

This ongoing pilot study is based on a sample of four HBCU's and four major white colleges in the south. The larger study will include colleges and universities throughout the country. A major research problem will be the comparability of our sample colleges and universities. Most of the historical

black colleges and universities are located in the south. Most of the 4 year white colleges and universities are located in the north, east and west. We will attempt to compare public and private, religious and non-denominational as well as selective vs. non-selective colleges.

Preliminary findings from samples of HBCU's indicate that overall black undergraduate students use less alcohol and drugs on black campuses. None of the students have admitted to using heroin. In a sample of 240 at one black college, half of the students did not use alcohol or drugs. Most came from poor families in cities and most were from two-parent homes.

Black colleges seem to instill in students a degree of confidence which increases their self esteem and improves their feelings of self-worth. Drugs and alcohol are seen as an impediment which will restrict their growth as competitive citizens in a hostile world. Peer pressure seem to be strongly against the use of drugs.

We anticipate that our findings will support our hypothesis, i.e., that black students drug and alcohol use is significantly less at HBCU's than it is at historical white colleges and universities and that black college students as a group, regardless of affiliation, use less drugs and alcohol than their white counterparts.

AFFILIATION:

Narcotic and Drug Research, Inc.
11 Beach Street
New York, NY 10013

Drug Use in Women With Bulimia and Anorexia Nervosa

Cynthia M. Bulik, Leonard H. Epstein, Monica McKee, and Walter Kaye

Over the past several years both clinicians and researchers have noted high rates of substance use and abuse in patients with eating disorders and their family members. This study was designed to: 1) examine the hypothesis that the high rates of drug use in the eating disorders population were primarily due to the contribution of women with bulimia nervosa; and 2) to generate hypotheses to be tested in the laboratory regarding the relation between eating and substance use in this population.

We administered the Habits Questionnaire (unpublished instrument) to sixty-nine consecutively admitted female patients to the eating disorders unit at Western Psychiatric Institute and Clinic between 1988-1989. The sample consisted of 42 patients with bulimia nervosa (with or without current or past anorexia nervosa) (BN) and 27 patients with restrictor anorexia only (AN). All subjects were studied within two weeks of admission to the hospital as part of a standard intake assessment battery. The questionnaire asses drugs commonly associated with the eating disorder (e.g. laxatives, diuretics, emetics), licit drugs (e.g. alcohol, cigarettes and caffeine), and illicit drugs (marijuana, cocaine, etc.).

There was no significant difference in age between the AN (20.3 ± 10.5) and BN (22.2 ± 5.8) groups (t= 0.8, ns). The AN group presented at a significantly lower percentage of ideal body weight than the bulimics (72.8% ± 10.2 vs. 101.4% ± 19.0). Significantly more women with bulimia than anorexia regularly used cigarettes (52% vs. 14.8%), alcohol (45% vs. 11%), laxatives (62% vs. 19%), emetics (26% vs. 4%) and diuretics (33% vs 7%) using Chi square analyses. There was no difference in the percentage of bulimic and anorectic women who used caffeine. Bulimic women who smoked were significantly more likely to use alcohol and marijuana than their non-smoking bulimic counterparts.

The mean age of the four anorectic women who smoked was 45.6. All of these women developed anorexia later in life and may differ in other ways from the overall anorexia group. Bulimic women who used alcohol reported drinking significantly more drinks per sitting and experiencing

significantly more blackouts than anorectic women who drank. The average number of laxatives per week consumed by bulimic women (47.7 ± 58.3) was significantly higher than that for anorectic women (10.8 ± 17.5).

The majority of bulimic women (64%) who smoked reported that smoking decreased their appetite. Forty-seven percent of bulimic women claimed that alcohol acted to increase appetite (32% claimed alcohol decreased appetite and 21% no effect). Forty-six percent of bulimic women felt caffeine decreased appetite (43% no effect and 11% increase). Sixty-one percent of bulimic women claimed laxatives had no effect on appetite (31% decrease and 7% no effect. This finding could be due to the fact that the majority of women took laxatives prior to bedtime or that the amount of time between drug ingestion and drug effect is markedly longer with laxatives than the other drugs and a direct effect on appetite would not be expected. In general, women with anorexia nervosa tended to report that drugs had no effect on appetite which could be an artifact of the fact that at later stages of the illness these individuals have difficulty recognizing internal sensations of hunger.

The bulimic women who used laxatives reported the following side-effects: diarrhea (92%); cramping (85%); nausea (73%); dehydration (62%); bloating (85%). The most frequently listed withdrawal symptoms were constipation and rebound edema. Sixty-two percent of bulimic women reported an increase in the number of laxatives needed to achieve the desired effects.

In summary, women with eating disorders report high rates of drug use including drugs specifically related to the eating disorder as well as alcohol, cigarettes and caffeine. The high rate of use in this population appears to be primarily due to the contribution of women with bulimia nervosa. In fact, women with restrictor anorexia nervosa display strikingly low rates of use of all drugs except caffeine. We have hypothesized that the repeated fasting and dieting associated with bulimia increases the reinforcing efficacy of other reinforcers leading to the high rates of drug use in the bulimic women. The same logic may apply to the high rates of exercise seen in women with anorexia nervosa. In the absence of direct empirical evidence, the self-reports of women with bulimia nervosa suggest that tolerance and withdrawal to laxatives occur and that a large number of women continue to abuse these potentially harmful drugs even when experiencing uncomfortable and serious side-effects. We would like to suggest that laxative use may begin as part of the eating disorder but may develop into true laxative dependence which may require additional and specific treatment beyond the general interventions for the eating disorder. Finally, our self-report data suggest that women with bulimia tend to report that cigarettes and caffeine decrease appetite, that alcohol increases appetite and laxatives have no effect. The effects of these drugs on appetite and subsequent food consumption are rich areas for further laboratory study in this population.

University of Pittsburgh School of Medicine, Department of Psychiatry

Qat Use in New York City

Dallas L. Browne

Qat is an amphetamine-like central nervous system stimulant (Kalix 1981). It has been used in Africa since 2,000 B.C. (Rodinson 1977). Its use in New York City is limited to two immigrant communities, primarily Somali and Ethiopian Africans, and Yemeni immigrants from the Middle East. Unconfirmed reports date the use of qat in New York City to as early as 1904. With the exception of Kennedy (1987) and Varisco (1986). few American researchers know anything about this drug and none are writing about its use on American soil.

Using participant observation and open ended interviews, the habits, lifestyles, values and attitudes of 50 Somali immigrants who use qat at least twice weekly were studied for one year.

Users chew the young tender shoots of the qat tree which are flown into New York almost daily and distributed through a gypsy cab network to selling points citywide. The bitter juice of the qat plant contains the stimulant cathinone as well as norpseudoephedrine. These create a state of mild euphoria after chewing for one half hour. With continued chewing this high often lasts from four to six hours. During the first two hours sexual interest is aroused if the opposite sex is present, and the drug allegedly improves the sexual satisfaction and performance of new users. Prolonged use conversely can lead to sexual impotence. Throughout this first phase users are highly suggestible and business persons exploit this by taking clients to qat sessions when they want to encourage a difficult client to sign a contract or close a deal. After a few hours in a qat establishment they can successfully conclude their deal. These qat establishments in New York also double as social centers. Men arrange marriages during qat sessions and exchange information about political and social developments back in Somalia. Here, too, people learn of job, business, educational opportunities, and planned social gatherings. A few of the regulars are students at nearby City College and they use qat to help make them stay awake while studying for engineering exams. A majority of users in New York work as night security guards and claim that qat use on the job helps them remain vigilant and alert. Needless to say, they prize the drug. Since qat is an appetite suppressant, a ritual meal is recommended before each chewing session. Due to the dryness of the plant, users drink lots of very sweet tea or coffee while chewing, along with lots of water.

While qat is well established within Somali immigrant culture in New York City, there is little evidence that its use will spread to other ethnic groups. The fact that it is bitter and a taste for it must be acquired, the fact that it is used secretively, and the fact that few efforts are being made to convert Americans to its use, all suggest that it is unlikely to spread beyond current user groups in the near future.

AFFILIATION:

Social Science Division
YORK College-CUNY
94-20 Guy Brewer Boulevard
Jamaica, New York 11451

Patterns of Drug Abuse Treatment Utilization in a General Population

Rumi K. Price, Linda B. Cottler, and Lee N. Robins

The need to expand drug abuse treatment is well-recognized. However, strategic plans are lacking with respect to quantity of expansion as well as the improvement of quality and regimen of treatment. One reason for such a lack may be scarcity of unbiased epidemiological data on treatment utilization. The Epidemiologic Catchment Area Program (ECA), a recent multi-site psychiatric epidemiology survey, provides an opportunity to study the epidemiology of drug treatment utilization in a general population, because factors commonly thought to lead to treatment can be examined in relation to drug abuse treatment and other health care utilization.

METHODS AND RESULTS

In this paper, we analyzed a subsample of the ECA respondents drawn from 4 sites who reported to have used any illicit drug 6 times or more in lifetime. The sample size is 3,160 (28.2% of males and 17.6% of females). The information about symptoms, duration of drug problems, types of drugs, adult and juvenile criminal history were available from the questions contained in the NIMH Diagnostic Interview Schedule. The questions in the Health Services Questionnaire were used to generate detailed information on the lifetime utilization of inpatient and outpatient mental health care services including drug abuse treatment, alcohol abuse treatment, psychiatric specialist services and psychiatric services in general medical settings.

In this sample, only 4% of males and 2.9% of females ever used drug treatment; 4.3% of males and 1.9% of females ever used alcohol treatment. The low prevalence of substance abuse treatment is not surprising given that our sample includes a large proportion of non-problematic users. However, even among those who met DSM-III diagnosis of drug dependence or abuse, only 26% of males and 17.5% of females have ever gone to drug treatment, whether inpatient or outpatient. Over 30% of them have never used any kind of mental health services at all (Figure 1).

MALES (N=671)   FEMALES (N=366)

The result of logistic regression analysis shows that the severity of drug problems is only one of many factors leading to drug abuse treatment (Table 2). The drug-related variables (severity, type of drugs, duration of abuse) are strong predictors. Nevertheless, others have equal or even stronger effects on drug abuse treatment use. The strongest is whether or not the respondents talked to M.D. about their drug problems. Criminal history, both juvenile and adult, also increases the likelihood of ending up in drug abuse treatment significantly. The number of different types of mental health services use also increases the likelihood of entering into treatment, suggesting that drug treatment use is in part a function of heavy use of mental health care system. Interestingly, having comorbid psychiatric disorders other than substance abuse is a protective factor of drug treatment use. Race alone was not found to be a significant predictor, however, the combination of being a black and an opiate user was found to increase the likelihood of the entry into drug treatment.

Table 2: Predictors of Lifetime Inpatient/Outpatient
Drug Abuse Treatment Use:
6+ Times Drug Users from ECA 4 Sites

Variable	Beta	Prob.	95% C.I.
Age (per year)	-.06	.0001	.91-.98
Los Angeles	.62	.005	1.20-2.87
Severity of drug problems			
(per prob.)	.30	<.0001	1.18-1.55
Opiates	1.23	<.0001	1.91-619
Black *opiates	1.08	.004	1.42-6.08
Duration of abuse/dep. (5+ yrs)	.14	.001	1.33-3.28
Other psych. dxs (per dx.)	-.58	<.0001	.47-.68
Juvenile arrest	.57	.009	1.15-1.53
Felony conviction	1.18	<.0001	2.07-5.07
Talked to M.D.	2.01	<.0001	4.79-11.68
Other MH use (per type)	.45	<.0001	1.38-1.78

Goodness of Fit: .959

AUTHORS
Rumi K Price, Ph.D., M.P.E.; Linda B. Cottler, Ph.D., M.P.H.;
Lee N. Robins, Ph.D. Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Opening the "Black Box" of Drug Abuse Treatment-Measurement and Evaluation of the Treatment Domain

John C. Ball

It has been recognized for some time[1] that drug abuse treatment programs are a "black box" in that we commonly lack information about what occurs in the process of treatment. Recently, however, Moos and Finney[2] have developed a new conceptualization for opening the black box. How drug abuse treatment can be evaluated by this paradigm will be reviewed and discussed.

The objective of this session will be to present current evaluation methodology and research findings pertaining to the effectiveness of drug abuse treatment. The session will include a review and current status of evaluation with respect to methadone maintenance treatment, therapeutic communities and other modes of treatment for drug abuse. The focus will be upon presenting recently devised procedures for evaluating the effectiveness of specific drug abuse programs.

[1]NIDA Research Monograph 51: Drug Abuse Treatment Evaluation: Strategies. Progress and Prospects. 1984: Research on the Treatment of Narcotic Addiction: State of the Art (1983). Rockville: National Institute on Drug Abuse.

[2]Moos, R.H. and Finney, J.W. (1987-1988). "Alcoholism Program Evaluation: The Treatment Domain," Drugs & Society, 2(2): 31-51.

AFFILIATIONS

Addiction Research Center, National Institute on Drug Abuse
Baltimore, Maryland

NDRI
New York New York

Alerting Drug Abusers to Overdose Threats: The Media's Role

**James L. Sorensen, Julie London, Rachel Wolfe,
Donald Tusel, Allyson Washburn, Kyung-Hee Choi,
and Denyse Lee**

In Fall 1989 a potent supply of heroin triggered 50
hospitalizations and 3 overdose deaths during one weekend in San
Francisco. We interviewed 115 heroin abusers in three
outpatient drug treatment program during the next two weeks, to
understand how patients learned of the danger, and to inform
those who had not heard. Subjects were in an outpatient heroin
detoxication clinic (N=55) and two methadone maintenance
clinics (N=60), approached consecutively as they came to the
clinics for treatment. Mean age was 41 years, 78% were men, 43%
were ethnic minorities, and 92% were unemployed. Only 4% had
not yet heard of the overdoses at the time of the interview.

PATIENTS SOURCES OF OVERDOSE INFORMATION
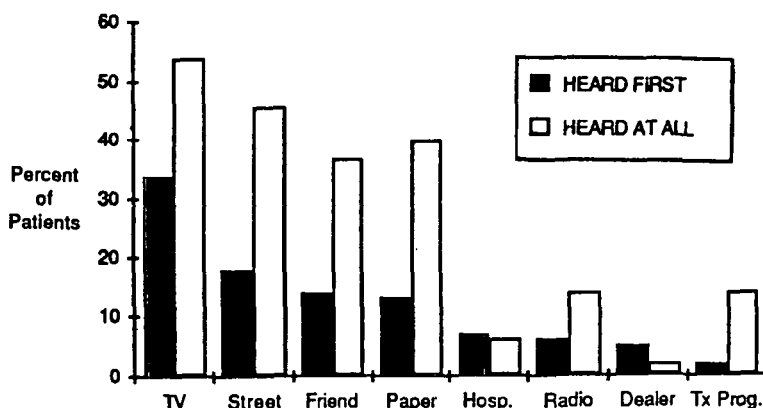
As the table illustrates, of the those had heard of the problem, 34% learned of it first from television coverage, 32% from friends of "on the street", and 13% from newspapers. When asked to indicate whether they had heard the overdoses at all from various sources (regardless of which was first), 54% indicated that they had heard from television, 46% "on the street", 40% from newspapers, and 27% from friends. Over three-fourths of the subject reported hearing about the overdoses from multiple sources, e.g. both television and on the street. This report revealed similar patterns across drug treatment programs in patterns of learning about the overdoses

The study is limited by the retrospective self-report methodology. In addition, attempts to inform drug users of such public health dangers should recognize that knowledge does not necessarily lead to risk reduction: There was also a wide variety in subjects' responses to learning about the overdoses, ranging from seeking to purchase the potent heroin to seeking drug treatment and recovery.

The mass media is an important source of information in an overdose epidemic. Altogether 53% of heroin abusers heard first of the overdoses from the mass media. We recommend that public health planners design systems that will collaborate with the media when such problems begin to appear. In addition, increasing the linkages between emergency rooms and drug treatment programs could increase the likelihood that drug users learn early about such potential threats.

AFFILIATIONS: University of California, San Francisco (Sorensen, London, Wolfe, & Choi); San Francisco VA Medical Center (Tusel); City & County of San Francisco (Lee)

A Historical Perspective on British and American Drug Policies

John J. Rouse and Bruce D. Johnson

The current understanding of British and American drug policies raises the issue of historical perspective on the evolution of these respective policies. It is important to understand that the status quo does not represent the epitome of a static continuum but merely one phase of continuously shifting moral paradigms which underlie policy.

Both Britain and the U.S. have had constantly shifting and adaptive attitudes towards the drugs which are currently illicit. It is also true for both countries that at times more than one moral paradigm influenced policy at the same time. In order to understand how these two countries have arrived at their respective drug policies today it is necessary to see how they have changed and evolved over the years.

The nineteenth century witnessed a convergence of British and American drug policies predicated on a commercial morality. The British fought two Opium Wars with China, in 1840-42 and 1956, largely over British and American desire to maintain open trade in China for their merchants, including legalizing the opium trade. The primary consideration was the economic importance of this trade. Concurrently, in both Britain and America in the nineteenth century patent medicines and painkillers containing opium were widespread and protected by commercial interests. (Berridge and Edwards, 1981; Courtwright, 1981; King, 1982).

In the early part of the twentieth century consensus grew against commercial opium and its widespread availability. A public health morality started to predominate with the convening of international conferences, first in Shanghai in 1909 and then in The Hague in 1912 and 1913. These conferences were convened in order to reach international agreements regulating the manufacturing, distribution and sale of opium products.

While these agreements at the international conferences did not have the force of law, they required legislation in the U.S. and Britain for regulating the manufacturing, sale and distribution of opium, including licensing all involved in the trade.

British and American policy began to diverge with the passage of the Harrison Narcotic Ace in 1914 in the U.S. This act was a public health and revenue law but it became a law enforcement measure by the Narcotic Division of the Prohibition Unit within the Treasury Department. Physicians who dispensed opiates to their addicted patients began to be harassed and intimidated by law enforcement authorities. Successful morphine maintenance clinics were closed. Several lawsuits made it all the way to the U.S. Supreme Court, which ultimately decided in favor of the physicians' right to prescribe opiates to addicts (Musto,

1973). The earlier pattern of harassment and intimidation had taken its toll; many physicians were frightened from dispensing these drugs. The US. had adopted a prohibition/criminalization morality regarding opiates, particularly heroin. This approach continues to this day. The black market for heroin and other drugs grew, particularly in the 1950's and 1960's. (Courtwright, Joseph, DesJarlais, 1989; Brecher, 1972).

The British experience followed a different pattern in the 1920's. The Rolleston Committee in 1926 recommended that the decision to prescribe opiates should be left to physicians, thus the British institutionalized a public health morality concerning opiates, but this is shifting in the 1980's. This public health morality exists along with a vice regulation morality which maintains that although a certain behavior is considered to be immoral it will be permitted in order to avoid other adverse social consequences which would occur if prohibition were enforced.

Especially in the U.S., a rehabilitation morality emerged toward addicts. Therapeutic communities started in Britain in the 1940's, but have been more widespread in the U.S. These residential communities promote abstinence from illicit drugs and attack the defenses and justifications of the clients. Starting in the 1960's, methadone maintenance also became widespread in the U.S.

In the 1960's Britain started government clinics for the dispensing of heroin and injectable and oral methadone for registered addicts. Although still legal for private physicians to prescribe opiates, most physicians send new addicts to the government clinics (Johnson, 1976; Pearson, 1987). These clinics were planned as a way of containing the British addict population within the context of a public health perspective. But in 1980-2, large supplies of illegal heroin entered and widespread illegal heroin sales occurred. Despite the British medical model and the American criminalization model the two countries' policies have converged more than at any time since the nineteenth century. Both countries have laws against black market sales of illicit drugs and both countries attempt to treat and limit their addict populations through government-sponsored treatment and maintenance centers.

Both the U.S. and Britain face new challenges in the future. In the U.S. the National Drug Policy will attempt to expand the national War on Drugs through increased federal spending, largely in the area of law enforcement. Britain is faced with the prospect that, in 1992 when the member nations of the European Community eradicate tariffs and trade barriers, there will also be some movement toward uniformity of drug laws. Whether this will follow the example of liberalization and the harm reduction philosophy followed in the Netherlands, or the strict law enforcement approaches of Germany and Sweden is unknown at this time.

Both the U.S. and Britain have been influenced by different moral paradigms at different times in their respective histories. In the future both countries will have to rethink the moral bases and underlying promises of their drug policies as circumstances change. Perhaps a reappraisal of historical trends and perspectives can lend some wisdom to future policy makers.

AFFILIATION: New York City Department of Probation and Narcotic and Drug Research, Inc., New York

Predictors of HIV Seropositivity in Newark and Jersey City i.v. Drug Users Not Currently Enrolled in Treatment

**Martin Y. Iguchi, Mitchell Rosen, Harvey Musikoff,
Harvey Kushner, John French, Robert Baxter, Victor Lidz,
Jerome J. Platt, and Christine Grant**

The purpose of this study was to examine high-risk drug-related and sexual behaviors in IVDUs and to determine the relationship of these variables to HIV serostatus. The focus of this study was on IVDUs residing in Newark and Jersey City who were not currently enrolled in a drug treatment program. This study describes 2 of 63 community-based outreach projects which have been established in 50 cities throughout the U.S. by the National Institute on Drug Abuse (NIDA), as part of its National AIDS Demonstration Research Program (NADR).

METHODS

From May to December 1989, 1397 IVDUs in Newark and Jersey City, were recruited to storefront locations by indigenous outreach workers and by word of mouth. Subjects were asked to provide a blood sample for HIV testing (drawn by venipuncture or finger stick, tested by ELISA, and confirmed by Western Blot) and to answer questions from a structured questionnaire (NIDA AIDS Initial Assessment v. 8.0). In Newark, 812 IVDUs (76% male) were interviewed, with a mean age of 36.5 years. Blacks constituted 83.2% of the sample; 14.6% were hispanic; 2.2% were white or other. In Jersey City, 585 IVDUs (76.6% male) were interviewed, with a mean age of 33.8. Blacks constituted 68.3% of the sample; 17.4% were hispanic; and 14.3% were white or other. In Newark, 432 of 765 subjects (55.8%) tested positive for HIV. In Jersey City, 260 of 554 subjects (46.9%) tested positive for HIV. Subjects received $15 for their participation and for providing a blood sample. Subjects also received a coupon redeemable for either 21- or 90- days (randomly determined) of free methadone treatment.

SELECTED RESULTS

Over 500 variables related to risk behavior from the AIA were examined with over 200 found to be significantly associated with HIV serostatus. Using both logistic regression and discriminant function analyses with a Bonferroni adjustment, 13 variables were identified as significantly and independently related to HIV seropositivity. The significant discriminators, with the standardized discriminant-function coefficients included in parentheses, are provided below. Significant IV drug use variables included: years of IV drug use (.30); frequency of injecting heroin and cocaine together (speedball) in the past 6 months (.24); and frequency of injecting cocaine in the past 6 months (.20). Significant non-IV

473

drug use variables which were inversely related to HIV seropositivity included: use of crack cocaine in the past 6 months (-.27) and non-injection heroin use in the past 6 months (-.21). Significant health-related variables included: the individual's own assessment of their likelihood of developing AIDS (.37) and reporting more than one of eight HIV-risk associated health problems in their lifetime, including: endocarditis; syphilis; tuberculosis; pneumonia; hepatitis; genital herpes; gonorrhea; and chlamydia (.14). Three other significant variables included: the number of times in jail in the past 5 years (.29); a history of abusing glue/paint (.23); and the presence versus absence of sexual partners in the past 6 months (-.22). Demographic variables significantly associated with HIV seropositivity included: completion of high school (-.18); being non-black versus black (-.15); and female (.12).

Employing a discriminant classification analysis with the thirteen variables, 416 of 611 cases (68.1%) were correctly classified as HIV seronegative, while 486 of 667 cases (72.9%) were correctly classified as HIV seropositive. Overall, 902 of the 1278 cases (70.6%), were correctly classified with respect to HIV serostatus. Using the discriminant function coefficients, a clinical risk index was calculated for each case, ranging from -9.27 to 28.98. The relative odds ratio for testing HIV positive between the 94 cases assigned a risk score 220 (80 HIV+, 14 HIV-) and the 100 cases assigned a risk score of <1.0 (15 HIV+, 85 HIV-) was 40.82.

DISCUSSION

With the exception of the variable associated with the self-perception of risk, the key estimators were those directly associated with the use of IV drugs, including, years of IV drug use and injection frequencies of speedball and/or cocaine alone. This might not be the case in areas of lower HIV seroprevalence in that individuals may frequently engage in such high-risk needle use behaviors without exposure to the virus in their given social/drug using network. Interestingly, a high frequency of non-injection drug use decreased the relative odds of testing HIV+. This was found both for crack use and for the use of non-injection heroin (primarily snorting), and is probably directly related to a decreased frequency of IV use. The role of variables such as times in jail, a history of abusing glue/paint, and the reported absence of sexual partners in the past 6 months, is unclear at this time. We have speculated that jail and a history of abusing glue/paint might be related to some aspect of social pathology, while the absence of sexual partners may be related to heavy drug use and/or perhaps psychiatric co-morbidity. The demographic variables indicated a generally higher risk for blacks, females, and those who have not completed high school. Finally, the risk index derived above may be clinically useful for identifying IVDUs at highest risk for developing HIV, possibly allowing for effective treatment allocation in the form of client to treatment matching. We are currently estimating the effectiveness of this index in a prospective study.

AFFILIATIONS:

University of Medicine & Dentistry of New Jersey, School of Osteopathic Medicine, Camden, NJ, 08103 (MYI, VL, JJP); Hahnemann University, Philadelphia, PA (HK); and the New Jersey Dept. of Health, Trenton, NJ (MR, HM, BB, JF, CG).

Needle Sharing Behaviors Associated With HIV Among Intravenous Drug Users (IVDUs): A Two-year Study

Kenneth Foster, Tooru Nemoto, Lawrence S. Brown, Jr., and Robert J. Battjes

INTRODUCTION

Despite the life-threatening spectre of AIDS, the sharing of drug injection equipment continues among intravenous drug users (IVDUs) and is an increasing threat for the transmission and spread of the human immunodeficiency virus (HIV) among heterosexuals, women, children and ethnic minorities. To date, conventional interventions have not sufficiently curbed these unsafe practices. Shooting galleries persist as major vectors of transmission of HIV. As of April, 1990, over 20 per cent of the AIDS cases in the U. S. were attributed to IV drug use among females and heterosexual males. In New York City, however, IVDUs are the largest group at risk for infection, accounting for approximately 37 percent of the city's cases. The purpose of this study is to better understand the relationship of needle-sharing behavior and HIV transmission in order to design more effective strategies to help prevent the transmission of HIV.

METHODS

During the years 1987 and 1988, 218 and 223 subjects, respectively, participated in the study. The subjects were patients in methadone maintenance clinics in Brooklyn and Manhattan in New York City. New admissions for 1987 and 1988 represented 87 per cent and 77 percent, of the total sample, respectively. Participants did not differ from the patient populations at these sites, with regard to age, race or sex. Trained interviewers administered a standardized questionnaire. Data collected included demographics, drug treatment history, drug and sexual behavior. Medical personnel obtained blood samples and sera were screened and confirmed for presence of HIV antibodies.

RESULTS

In the 1987 cohort, 60 per cent tested positive for HIV, while the infection rate for 1988 was 51 percent. HIV serostatus had no significant associations with ethnicity, gender, or whether the subject was a new admission. Age group did have a significant

association with serostatus in both years, where the older groups were more likely to be seropositive. In 1987; needle sharing at shooting galleries was strongly associated with HIV status. The HIV positive subjects reported significantly higher frequencies of using shooting galleries, in the last 12 months and the last five years, than those who were HIV negative. Subjects reported a significant decrease in needle sharing behavior, including shooting galleries, during the last 12 months, compared with the last five years. There was also a significant increase in cleaning needles. Females who shared needles with different groups were significantly more likely to be HIV positive than those who shared with the same person. In 1988, during the last 12 months, shooting galleries were again significantly associated with serostatus. However, all needle sharing behavior decreased significantly and needle cleaning showed a significant increase.

DISCUSSION

Needle sharing at shooting galleries was a significant and persistent factor in predicting HIV status among IVDUs who were in the methadone treatment programs. Needle sharing behaviors during the last five years were significantly more likely to be associated with HIV status than behavior during the last 12 months. These IVDUs seemed to decrease their needle sharing behavior and increase the frequency with which they clean their needles. Female IVDUs who shared needles with different persons were more likely to be infected with HIV. Studies and reports indicate that IVDUs continue to be vulnerable to peer behavior, self-delusion and misinformation. While needle cleaning seems to be on the rise, it is neither the rule nor the exception. Data show that these factors have an overwhelming significance for-minorities, women and children. Study results amplify the need for future investigations to further explore these and other issues.

AFFILIATION:

Addiction Research and Treatment Corporation
Brooklyn, New York
National Institute on Drug Abuse
Rockville, Maryland

Ibogaine Fails to Reduce Naloxone-Precipitated Withdrawal in the Morphine Dependent Rat

L. Sharpe and J. Jaffe

In several anecdotal reports, ibogaine seems to alleviate opiate withdrawal in some humans. The apparent benefits from its use have fostered several ritualized underground treatment collectives in Europe. Only a few studies show that ibogaine partially reduces withdrawal from morphine in animals. The main pharmacologic effect of ibogaine (20-40 mg/kg, i.p.) in rats is the induction of fine head and body tremors. We investigated the effects of nontremorigenic and tremorigenic doses of ibogaine on several withdrawal signs precipitated by naloxone in the morphine-dependent rat.

Ibogaine (5, 10, 20 and 40 mg/kg, s.c.) was administered 15 min before naloxone (0.5 mg/kg, s.c.) in morphine-dependent rats (3 days after the s.c. implantation of a 75 mg morphine pellet). Of the 12 withdrawal signs scored,, teeth chattering was the only sign that changed (increased) after ibogaine (5 mg/kg, compared with saline controls).

In spite of ibogaine's apparent interaction with several neurotransmitter receptor systems, it does not alleviate opiate withdrawal in this animal model at nontremorigenic (5 and 10 mg/kg) or tremorigenic (20 and 40 mg/kg) doses. However,! the benefits from ibogaine may stem not from the suppression of withdrawal symptoms but from its psychedelic (hallucinogenic) action to alter the addiction process. Such claims were made for LSD in the 1960's for treating alcoholism but such assertions could not be substantiated in controlled clinical trails.

AFFILIATION

NIDA, Addiction Research Center, P. O. Box 5180, Baltimore, MD 21224

HIV Infection in Intravenous Drug Abusers (IVDAs) in NYC Drug Treatment Clinics: 1985-1989

L.S. Brown, Jr., R.Y. Phillips, D. Nurco, B. Primm, R. Battjes, and T. Nemoto

INTRODUCTION:

Studying the changing patterns of the acquired immunodeficiency syn-
drame (AIDS) has brought increasing attention to intravenous (IV) drug
use and its role in the spread of the AIDS virus (HIV: human immuno-
deficiency virus). Based on information from the Centers for Disease
Control (CDC), IV drug use represents the second most frequent behav-
ior associated with AIDS. As part of a continuing clinical, epidemi-
ological and behavioral project, vie have bean monitoring trends in HIV
seroprevalenoe in IV drug users (IVDUs) in selected drug treatment
clinics in New York City (NYC).

METHODS:

Subjects for the study were recruited from patients enrolled in metha-
done maintenance clinics located in the boroughs of Brooklyn and Man-
hattan in New York City. following informed consent, subjects were ad-
ministered a questionnaire about patterns of drug use (type of drugs,
routes of administration, duration/frequency of use, etc.) and sexual
behaviors. Serum was collected for HIV serology by members of the
clinic medical staff. HIV-1 antibody was assessed by enzyme linked
immunosorbent assay (ELISA) (Genetic Systems). Repeatedly positive
ELISA. specimens ware confirmed by Western Blot (Dupont). Sera with
antibodies to p24 and/or gp41 plus gp120 or gp160 bands ware consid-
ered positive. Statistical associations were determined using uni
variate and multivariate methods of analysis.

RESULTS:

There ware no significant differences observed among the five cohorts
in study population characteristics (see table below). Over the five
years, the HIV seroprevalence was 55%. An increase in seroprevalence
was observed between the '85 and '86 charts, with a steady decline
over the 3 subsequent years; The changes, however, did not prove to be
significant. Age was significantly associated with HIV seropositivity
in '85 (p=.038), '87 (p= .021) and '89 (p=.001). In '85 and '86, fe-
male subjects had a higher HIV infection rate than their mate counter-
parts; however, the difference in the infection rate between males and
females only reached a statistical difference in 1389 (p=0.027).

DEMOGRAPHICS AND HIV SEROPREVALENCE BY COHORT YEAR

COHORT YEARS

	1985	1986	1987	1988	1989
N	469	262	218	223	548
MEAN AGE (yrs)	34.6	33.9	33.4	33	34.9
RACE/ETHNICITY					
blacks (%)	48	41	44	56	50
Hispanics (%)	43	45	47	37	45
whites /others (%)	9	14	9	7	5
GENDER					
Males (%)	70	62	65	59	59
Females (%)	30	38	35	41	41
HIV SEROPOSITIVE					
Number	255	159	131	115	254
Percent	54	61	60	52	46

Between '85-'87, blacks and whites showed an increase in rate.
Hispanic subjects oily showed an increase in 1986. Each race/ethnic
group showed a decline in seroprevalence between '87-'89. The dif-
ferences in seroprevalence rates over the five years, however, were
not significant. In '85, subjects who used heroin and cocaine in
combination, were more likely to be HIV infected than subjects who
used any other combination of illicit drugs (p=.048). Far 1986,
intravenous use of both heroin (p=.043) and cocaine (p=.001), were sig-
nificantly associated with HIV infection. In '87, the frequency of
heroin use (p= .026), and the frequency of visits to shooting gal-
laries (p=.002), were associated with HIV infection. The frequent use
of cocaine (p=.011), speadballing (p=.003), sharing of needles (p=
.021) and visits to shooting gallaries (p=.001), were all shown to be
associated with HIV infection in '88. The data did not show a statis-
tically significant correlation between sexual behaviors and HIV in-
fection over the five years.

DISCUSSION:

The lack of significant changes in the overall HIV infection rates
between the five years studied, may suggest that HIV infection in this
population of IVDUs may be reaching a saturation point. Monitoring
HIV infection, provides greater opportunities to examine associated
risk behaviors and assist in the development of rational plans for
health and social services.

AFFILIATIONS:

Division of Evaluation and Medical Affairs, Addiction Research and
Treatment Corporation, Brooklyn, New York; Department of Medicine,
Harlem Hospital and College of Physicians and Surgeons, Columbia
University, New York, N.Y.; Friends Medical Science Research Center,
Baltimore, Ml.; National Institute on Drug Abuse, Rockville, MD.

The Role of Cocaine Use in the HIV Transmission Among IVDUs: 1987 and 1988 Cohort Study

Tooru Nemoto, Lawrence S. Brown, Jr., Robert J. Battjes, Alvin Chu, and Kenneth Foster

Sharing needles and other paraphernalia, particularly at shooting collieries has been documented as a strong predictor of HIV seropositive status among intravenous drug users (IVDUs). Furthermore, intravenous cocaine use has teen strongly associated with HIV infection. However these epidemiological studies did not investigate the association between HIV infection rates and intranasal cocaine or crack use compared with intravenous cocaine use. The present study focused particularly on the role of cocaine use for HIV infection among cohorts of IVDUs in 1987 and 1988.

METHOD

A total 440 participants (218 in 1987 and 222 in 1988) were recruited from the methadone maintenance program which maintains about 2,100 patients a year and runs six clinics in minority concentrated areas in Manhattan and Brooklyn, New York City. All participants who were admitted to the selected clinic sites were contacted by trained interviewers, and asked to participate voluntarily in the study, in which participants could obtain HIV results and counseling, if they so desired. The characteristics of participants were: 62% males and 38% females; 51% blacks, 42% Hispanics, and 7% whites and others; mean age = 33 years. Eighty-seven percent and 77% of the participants were recruited from the newly admitted patients in 1987 and 1988, respectively. After obtaining informed consent, a structured questionnaire was administered by interviewers. Also, blood was collected and tested for HIV anti-body by ELISA and confirmed by Western Blot techniques.

RESULTS

The overall HIV infection rate was 60% and 51% in 1987 and 1988, respectively. No significant association was found between HIV serostatus and ethnicity, sex, type of admission (new and re-admission), and levels of education. However, there was a significant associations between HIV serostatus and age groups in both cohorts,

that is, older groups were more likely to be seropositive. The IVDUs who used cocaine intravenously or used speedball during the last five years were significantly more likely to be HIV positive than those who did not vise cocaine intravenously. Whereas, those who had taken cocaine by snorting or striking were significantly less likely to be HIV positive than those who had not. A significantly larger number of older IVDUs reported intravenous cocaine use during the last five years compared with younger IVDUs. Instead, a significantly larger proportion of young IVDUs used cocaine by snorting or smoking. These consistent findings in both 1987 and 1988 seemed to explain the significantly higher HIV infection rates among older IVDUs. Analysis of covariance with age as a covariate revealed that Hispanics and blacks used cocaine by snorting substantially more often than other ethnic groups. One IVDUs who frequently to use cocaine by snorting were more likely to use crack with similar frequencies. These results further indicated the separation between IV drug and non-IV drug users. Discriminant analysis revealed that the frequencies of IV* cocaine use has the strongest power to differentiate the HIV positive IVDUs from the negatives.

DISCUSSION

The IV cocaine users were characterized by older age. Young blacks and Hispanics, especially females, were more frequently engaged in using ermine by snorting or crack. It is unknown whether these young crack or intranasal cocaine users had changed their drug taking behaviors because of an awareness of the high risk of IV drug use for HIV infection. However, this study indicated that future intervention programs for preventing HIV infection among IVDUs should focus particularly en older IVDUs who continue to use cocaine and heroin intravenously. Crack or intranasal cocaine users were significantly less likely to be HIV positive, based en univariate analysis. When considering demographic variables and the other drug taking variables in the discriminant analysis, these variables did not have significant magnitude to enter the discriminant function. This study showed that ethnicity was significantly associated with using cocaine by snorting or smoking. And, female IVDUs were more likely to be engaged in snorting or smoking cocaine. It is very interesting that particular ethnic, sex, and age groups have been using cocaine with certain modes of behavior. It is unknown whether these modes of behavior are influenced by cultures of urban ethnic groups or by distinctive reactions toward the AIDS epidemic. Further research should investigate in-depth the type of cocaine use in relation with other drug behaviors, perception of risks for HIV infection, sexual behaviors and knowledge and attitudes toward protective behaviors for HIV infection.

AFFILIATIONS
Addiction Research and Treatment Corporation, Brooklyn, New York
National Institute on Drug Abuse, Rockville, MD

Effects of Education on High Risk HIV Transmission Behaviors

Donald A. Calsyn, Andrew J. Saxon, George Freeman, Jr., and Stephen Whittaker

The impact of a 90 minute AIDS prevention education presentation on reducing high risk HIV transmission behaviors was assessed in 218 iv drug users in treatment, Sample demographics were age $\bar{x} = 39.1, \sigma = 7.8$; sex, male=67.9%, female=32.1%; race, white=71.1%, black=22.9%; primary drug abused opiates = 80.6%, cocaine = 7.4%. Subjects were randomly assigned to education, education plus optional HIV antibody testing, and 4 month wait list conditions. The educational package covered the description of the virus, routes of transmission, disease progression, pros and cons of antibody testing, a three tiered risk reduction strategy, needle cleaning and condom demonstrations, and provision of bleach and condom starter packs. Assessments via structured interview were conducted prior to group assignment and 4 months later.

During the year prior to the intervention 69% of the sample reported involvement in high risk behavior (sharing needles without cleaning with bleach or multiple sexual partners without using condoms). During the 4 month follow up 23% reported involvement in high risk behavior. Of those involved in high risk behaviors at initial assessment 71% reported changing to low risk behaviors during the follow up (McNemar p < .001). Of those reporting sharing needles during both assessment periods more cleaned their needles with bleach "always" or "usually" at the follow up (47%) than initially (17%, McNemar p < .001). More males with multiple sexual partners reported condom use greater than 50% of the time for vaginal intercourse at follow up (38%) than initially (9%, McNemar p < .001). There were no significant differences in the percent changing to low risk behaviors, increasing bleach use or increasing condom use based on educational condition.

Subjects continuing to be involved in high risk behaviors were younger ($\bar{x} = 36.1, \sigma = 6.7$) and more likely to have a sexual partner who uses iv drugs (43%) than those changing from high to low risk ($\bar{x} = 39.4, \sigma = 7.9$; 27%) and those at low risk at both assessments ($\bar{x} = 40.8, \sigma = 8.1$, F=5.4, p < .01; 15%, $\chi^2 = 10.8$, p < .01). Those at low risk at both assessments had been in treatment more months ($\bar{x} = 38.3, \sigma = 35.8$) and were less likely to have been incarcerated in the prior 5 years (34%) than those continuing high risk behaviors ($\bar{x} = 20.9, \sigma = 31.4$; 84%) and those changing from high to low risk $\bar{x} = 16.7, \sigma = 31.5$, Kruskal-Wallis $\chi^2 = 15.4$, p<.001; 69%, $\chi^2 = 24.1$, p<.001).

Subjects continuing at high risk and those changing from high to low risk
used alcohol, heroin and cocaine iv more days during the 30 days prior to
initial assessment than subjects continuing at low risk. Subjects continuing
at high risk used heroin and cocaine more days during the 4 month follow up
period than those changing from high to low risk and those continuing at low
risk (see table 1).

Table 1

Substance use and change in risk behavior

Initial Assessment **Groups**

Days using prior 30	Continued high risk		High to low risk		Continued low risk	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
Alcohol	2.7	6.8	2.2	5.6	0.6	2.9***
Heroin	4.8	9.1	3.2	7.0	1.6	5.5***
Cocaine iv	2.7	6.8	2.2	5.6	0.6	2.9**
Cocaine smoke	0.8	2.7	0.4	2.7	0.1	0.9**
Follow up Days using prior 120						
Alcohol	12.6	24.7	14.0	29.6	8.9	27.1**
Heroin	21.3	35.0	10.3	25.9	6.8	22.2***
Cocaine	11.0	21.8	6.1	15.9	4.9	19.9***
Cocaine smoke	3.5	15.0	2.3	13.8	1.1	7.8

** $p<.01$, *** $p < .001$ using Kruskal-Wallis 1-way ANOVA

Results suggest many iv drug users are changing their behaviors, but the
efficacy of a one time education presentation could not be demonstrated.
Possible explanations for the negative findings include contamination of the
wait list group by other educational efforts, the subjects were already
knowledgeable, those for whom knowledge was sufficient to initiate change
had already done so, and more than information giving is needed to help the
highest risk individual change their behaviors. The results do support drug
abuse treatment as an effective tool in combatting involvement in high risk
HIV transmission behaviors.

Affiliation: [1]Veterans Affairs Medical Center and University of
Washington School of Medicine. [2]People of Color Against AIDS Network,
Seattle, Washington.

New York City's Needle Exchange Experiment: Policy Implications From Its First Year

Cherni L. Gillman

The outcome of New York City's needle exchange experiment exposes the fallacy of presuming that science will inform public policy.

The Centers for Disease Control estimates that at least half of New York City's injection drug users (IDUs) are HIV antibody positive. In response to their plight, the City Department of Health (DOH) established the experimental needle exchange program. Begun on Election Day 1988 (to minimize publicity, according to sources at DOH), and closed by Mayor Dinkins in February 1990, it operated within stultifying restrictions. Due to political concessions, clients could be given only ONE sterile needle per visit. The office was a lead-lined former x-ray room that was later converted to a closet. Its location was adjacent to the "heart" of the War on Drugs --- next to Criminal Court; Family Court; Civil Court; the Supreme Court; the "Tombs" prison; and what has been characterized as the largest concentration of narcotics detectives in the western world.

Yet contrary to the public perception that the experiment failed, the data reveal a credible performance. Surpassing its quota of 200 participants, 318 clients enrolled. Out of a sample of 250 clients, 78% accepted referral to a drug treatment program. Two-thirds were male; one-third were homeless; the mean age was 33. After initial enrollment, 44% returned, while an additional 14% did not return because they entered drug treatment on the same day as their initial visit. At intake, 11% had infectious syphilis. Half (51%) tested HIV antibody positive.

However, the data from this scientific experiment had no impact on the decision to terminate it. Why? Because it was never specified by those who determined its fate what the experiment had to accomplish to prove its value. Since it lacked this agreement before undertaking the test project, the findings were ignored. No criteria existed on which to debate its merit. Nowhere in DOH's official report does it state what the objectives of the needle exchange pilot program were.

Although the rhetoric was given that we needed scientific data to demonstrate the effectiveness of needle exchange to inform public policy, there is every evidence that public policy does not respond to scientific data. Instead, another perspective dominates that, ideologically, suppresses all debate. According to the United States Secretary of Health, "there is a danger that the controversy surrounding needle exchange will overshadow the greater issue of drug abuse in society. We cannot have a solution become part of the problem". Health Secretary Sullivan's

position contrasts sharply with the Chief of Police in San Francisco. He says, "I don't arrest people who give out needles. I've got real crime to worry about. These people aren't part of the problem. They're part of the solution."

Thus, politics has shifted the issue into an ideological choice between combatting AIDS or addiction. Mayor Dinkins said he didn't want to "give people the paraphernalia to continue using drugs", so he closed the test project. Given the ideological opposition to the concept of needle exchange, there are no criteria, no data, no findings that could influence policymakers to retain the pilot program.

Where does science fit in this? Sterile needles are the most expedient agent that can stop the AIDS virus from being transmitted by injection. Yet our government prohibits people from implementing the best information we have for slowing the AIDS epidemic.

The response to AIDS among IDUs has not been based on effective epidemiological strategies. Instead, it is based on politics. And we cannot presume that politicians will seek rational, data-based policies, or that logical consistency and rules of inference will be principles that guide how they formulate policy. There are rigid ideological stances that refuse to brook new evidence or information. The lesson from the needle exchange is that unless political agreement is reached that specifies acceptable criteria to conduct an objective evaluation, no policy -- whether it is based on science or not --- will be accepted or approved.

AFFILIATION:

Narcotic and Drug Research, Inc.
New York, NY

Diseases Commonly Found in the Drug Abuser: Is There More Than HIV?

M.J. Hickson, L.S. Brown, R.Y. Phillips, D.A. Ajuluchukwu, and T. Nemoto

INTRODUCTION

AIDS has become the most challenging medical phenomena at the close of the 20th century. As a result, there has been a wave of concern over consequences of drug dependence, recognizing that certain drug behaviors places one at risk for HIV disease. Drug abuse however, is not synonymous with HIV/AIDS. There are other diseases commonly found in the drug user. Information on the medical disorders presented by drug abusers in the ambulatory setting is not readily available. While there have been several reviews and reports of medical disorders in drug dependent persons, none have provided a systematic analysis of the scope of medical disorders experienced by drug abusers. This report provides a cross-sectional examination of medical diseases among drug abusers enrolled in New York City drug abuse treatment clinics.

PATIENT AND METHODS

Physical examinations and medical histories were per nomad in 2102 patients enrolled in methadone maintenance clinics in the boroughs of Brooklyn and Manhattan during the calender year 1987. Of the 2102 patients, 1780 had the required laboratory data base for analysis. In accordance with an institutional (human subject) review board approved protocol, vie obtained and matched HIV results with medical history and other laboratory studies in 167 patients. Eligibility requirements were 18 years or older, with at least a 1 year history of opiate dependence. Laboratory evaluation included a complete blood count, serum electrolytes, liver function tests, hepatitis B sexology (Abbott) and syphilis by rapid plasma reagin. HIV-1 antibody was assessed by enzyme linked immunoasorbent assay (ELISA) (Genetic Systems). Repeatedly positive specimen by ELISA were confirmed using Western blot technique (Dupont). Sera with p24 and gp41 bands on Western blot were considered positive. All medical diseases were grouped into three categories: those associated with intravenous drug use, those associated with HIV infection and those Hi mama having no known association with IV drug use or HIV infection, but coo Tiring concurrently. Using univariate methods of analysis and analysis of variance (SPSS/PC+ statistical package), we evaluated the relationships between the indepen-

dent variable of drug abuse and the dependent variables of medical history, laboratory parameters and HIV serostatus.

RESULTS

Nearly 75% of the patients reported at least one medical condition; 49.7% reported at least two of the medical disorders. Patients with at least one abnormal laboratory value were more likely to be older than 34 years of age (p=.001), female (p=.053), African-American (p<.001), and users of IV heroin (p=.0003), IV cocaine (p=.0002) and alcohol (p=.042). Compared with patients with at least one medical condition, patients who denied any medical abnormality ware significantly younger (p=.001), more likely to be Hispanic (p=.001), less likely to report a history of alcoholism (p=.003), IV heroin use (p=.041), or IV cocaine use (p=.045). Three hundred and nine patients reported at least one disorder not associated with drug abuse or HIV infection. four hundred and eighty patients disclosed a history of at least one HIV-related medical disorder. These patients tended to be older (p= .001), more likely to be males (p=.001) and African- American, and more likely to be IV cocaine users (p=.043), as compared to persons without a Half-reported medical disorder. Two hundred and twenty two patients disclosed at least one drug abuse associated medical condition. As compared with patients who denied any medical disease, they were younger, female, and of African-American ethnicity. Among this population there were a number of disorders strongly associated with demographic information. African-American drug abusers tended to be at clear risk for pneumonia, tuberculin infection, hypertension, anemia, leukemia, diabetes, and skin ulcers/abusers. Write drug abusers tended to be at greater risk for seizures and decrease libido. Income was not significantly related to the prevalences of any reported medical disorders, but was clearly associated to hepatitis B antibody status. The three most commonly reported medical disorders ware pneumonia, hepatitis and anemia.

CONCLUSION:

This study provides information concerning the array of medical diagnoses seen in the drug abuser. It would appear that the provision of primary medical care in drug abuse treatment clinics, would have an important beneficial impact on these disease entities. The services should be tailored to the demography of the population served.

Department of Medicine, Harlem Hospital (falter, College of Physicians and Surgeons, Columbia University, New York, N.Y., and Division of Evaluation and Medical Affairs, Addiction Research and Treatment Corporation, Brooklyn, New York.

Intravenous Drug Abusers With Antisocial Personality Disorder: High Rate of HIV-1 Infection

Robert K. Brooner, George E. Bigelow, Lawrence Greenfleld, Eric C. Strain, and Chester W. Schmidt

Intravenous drug abuse is associated with high rates of psychopathology and with the Human Immunodeficiency Virus (HIV-1) infection. One of the more common psychiatric diagnoses made among intravenous drug abusers is Antisocial Personality Disorder (ASP). The co-occurrence of intravenous drug abuse and ASP is clearly related to poor drug abuse treatment outcome. More recently, Brooner et al. (in press) reported significantly more needle sharing and more needle share partners among IVDAs with versus without a diagnosis of ASP. These self-report data suggest that a diagnosis of ASP among IVDAs might be related to a potentially higher risk of HIV-1 infection. The present study compared the rates of HIV-1 infection among IVDAs with versus without a diagnosis of ASP.

Subjects (N=178) were IVDAs consecutively enrolled in a longitudinal study of drug use behavior and results of HIV-l blood testing. Fifty-five percent (N=98) were in methadone treatment at the time of study enrollment, the remaining 45% of the sample were not in drug abuse treatment at the time of study enrollment. Sixty-nine percent of the sample were male and the mean age was 35.7 years. Forty-two percent were white and 58% were black or hispanic. Quantitative drug use information was obtained with a detailed questionnaire covering the number of drug injections, number of needle shares and number of different needle share partners for each of the prior five years. Blood samples were analyzed by the ELISA test and HIV-1 positives were confirmed by the Western Blot. Diagnosis of ASP were made according to DSM-IIIR by use of a structured psychiatric interview. In all cases, the assessment of HIV-1 risk behaviors preceded the diagnostic interview.

Overall, 40% of the sample were classified as having a diagnosis of ASP. Ten percent of the sample were HIV-1 positive. HIV-1 infection was associated with having a diagnosis of ASP (18% versus 5%, p=0.03) and with being a being a member of an ethnic minority (17% versus 0%, p=0.01). Importantly, the infection rate among minority subjects without a diagnosis of ASP was 9% compared to 28% among minority subjects satisfying criteria for ASP (p=0.014).

The mechanism of increased HIV-l transmission among the ASP subjects was unclear. The higher rate of infection may have been related to the fact that ASP subjects reported more needle sharing and more needle share

partners than the non-ASP subjects across each of the five years prior to study enrollment, and more of the ASP subjects were positive for a current cocaine use disorder.

A diagnosis of ASP is a well known predictor of poor drug abuse- treatment performance. These data add a new dimension to the importance of this diagnostic subset of IVDAs. Replicating these findings in a larger sample of IVDAs and learning more about the mechanism(s) of HIV-1 transmission among IVDAs with ASP will be important to developing more specific and effective risk-reduction interventions.

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AFFILIATION

The Johns Hopkins University School of Medicine,
Francis Scott Key Medical Center,
Baltimore, Maryland 21224

Psychiatric Symptoms, High Risk Behaviors and HIV Positivity Among Methadone Patients

David S. Metzger, George E. Woody, Patrick Druley, Dominick DePhillipis, Helen Navaline, A. Thomas McLellan, and Charles P. O'Brien

INTRODUCTION

Despite widespread knowledge of the basic mechanisms of HIV transmission, a substantial number of drug users continue to share needles and engage in unprotected sex. Efforts designed to slow the spread of HIV must begin to target this high risk subgroup who represent a leading edge of the AIDS epidemic. Specifically, efforts must be made to identify the factors associated with high risk behaviors.

Our past work in assessing high risk behaviors among intravenous drug users in methadone treatment has generated a number of hypotheses about the characteristics which mediate the drug user's ability to reduce their risk of becoming infected with HIV. Specifically, we have noted a relationship between continued needle sharing and psychological distress. The study presented here was designed to test the hypothesis that high risk behaviors among methadone patients are associated with elevated symptoms of psychological distress.

METHODS

Three hundred seventy nine methadone patients were were surveyed as part of a longitudinal study of high risk behaviors and HIV infection. One hundred fifty two subjects were randomly selected to participate in ongoing serological studies. Nine percent were found to be positive for HIV and 22% tested positive for HTLV I-II.

These patients had an average age of 39. Fifty nine percent were black, 31% were white, and 8% were Hispanic. Most were male (72.5%) and only 23% were married and currently living with their spouse.

Subjects were first classified into risk groups based upon their involvement in needle use and unprotected sexual activity. Subjects in the resulting groups were then compared on a number of sociodemographic, behavioral and psychological characteristics

Analyses were conducted comparing the psychological symptomatology of the five risk categories. The Symptom Checklist - 90 (SCL-90) and the Beck Depression Inventory (BDI) were used to assess psychiatric symptom levels.

Patients who indicated that they were seropositive were excluded from these analyses given the likelihood that their serostatus would impact upon the psychological measures. Thus, the final study group totaled 355.

With regard to the behaviors associated with HIV infection, 32% indicated that they had shared a needle at least once during the preceding six months. Only 11% of the sexually active patients reported using condoms all of the time. These risk behaviors were so extensive that all but 18 subjects were able to be classified into one of the risk groups.

RESULTS

Subjects were first classified into the following five groups based upon the frequency of their needle sharing during the preceding six months: A=no injections [N=83 (23%)]; B= injected but no needle sharing [N=159 (45%)]; C= shared 1 - 2 times [N=34 (10%)]; D=shared 3 -6 times [N=42 (12%)]; E= shared more than 6 times [N=37 (10%)].

Data from both the SCL-90 and the BDI confirm our hypothesis. The needle sharing groups scored significantly higher on each of the nine subscales of the SCL-90. Depression as measured by the BDI was also significantly related to frequency of needle sharing. High risk sexual behavior was also significantly related to measures of psychological distress.

Seven other variables were found to be significantly associated with needle sharing: length of time in treatment; arrests during the past five years; past alcohol problem; gender: and, age of first marijuana use. When the relative strength of these relationships were tested using a stepwise discriminant function analysis, the psychological symptomatology was found to account for the greatest amount of variance. This variable remained significant even were all other variables were entered first. Alcohol use was the best predictor of high risk sexual behavior.

CONCLUSIONS

Psychological distress is strongly associated with high risk needle sharing behaviors among IVDUs in Methadone Treatment. In fact these symptoms of distress are the best predictors of needle sharing. Consequently, regardless of the causal relationships which underly this association, risk reduction programs targeted at IVDUs need to be designed with an awareness of the potential role of psychiatric factors. Reduction of symptoms may also reduce high risk behaviors. These data also suggest that the treatment of HIV infected IVDUs may require greater psychiatric input than the treatment of other infected groups.

University of Pennsylvania/Philadelphia/Veterans Administration Medical Center
Center for Studies on Addiction,
Philadelphia, Pennsylvania.

Preliminary Results of Polymerase Chain Reaction (PCR) Testing of Elisa Negative IVDUs in Methadone Treatment

George Woody, David Metzger, Brian Wu, Patrick Druley, A. Thomas McLellan, and Charles P. O'Brien

INTRODUCTION

The polymerase chain reaction (PCR) test provides a new potential for the early detection of exposure to HIV and other retroviruses. There are currently many questions about the sensitivity of this test and the clinical significance of PCR results. In addition to concern with HIV, there has been growing interest in HTLV-I/II infection rates. This interest stems from the observation that these retroviruses are also transmitted by sharing contaminated needles and through unprotected sexual contact. Further, there is particular concern that these viruses may serve as a cofactors in triggering disease activity among HIV infected individuals.

Thus far, there have been no systematic studies of HIV infection among IVDUs in Philadelphia. Nor is there available data on the HTLV-I/II infection rates. The purpose of this paper is to present findings of the first attempt to characterize the infection rate in this population using both ELISA/Western Bot and PCR testing.

METHODS

As part of a longitudinal study of seroprevalence among IVDUs in and out of methadone treatment in Philadelphia, 152 randomly selected patients and 103 opiate abusers not in treatment were tested for Human lmmunodeficiency Virus (HIV). These subjects have an average age of 40 yrs. Thirty percent were white; 60% black: and 7% latino. Seventy percent were male and 30% female.

All subjects completed high risk behavioral assessments and had their blood tested for HIV and HTLV-I/II via ELISA and Western Blot. PCR testing was also completed on 54 subjects with negative ELISA results for HIV and 21 subjects testing negative for HTLV I/II.

RESULTS

Overall, the HIV infection rate for this group was 12% (N=30). Those subjects selected from the methadone program had a 9% HIV infection rate while those subjects who had not been in treatment for the past year had an infection rate of 16%. The infection rate for HTLV-I/II was significantly higher at 22% (N=57). Seven subjects were positive for both viruses.

26% of the PCR tests were positive for HIV and 24% were positive for HTLV I/II. Since only those who were negative by negative by ELISA were tested by PCR, we can project that overall 35% of the study group had been exposed to HIV and 40% to

HTLV I/II. These projections are based upon an assumption of accuracy and must be cautiously   interpreted.

Analysis of the ELISA and Western BLOT results identified few sociodemographic characteristics associated with HIV infection. There was a strong linear relationship between age and HTLV-I/II infection (X2=34; df=12; p<.001). The infection rate for those under 30 years of age was found lo be 4% while for those 50 and older, the rate of HTLV-I/II infection was 52%.

We also examined the relationship between infection rates (as determined by ELISA and Western Blot) and recent high risk drug use and sexual activity. Overall, fifty percent of those infected with HIV reported sharing needles in the preceding six months. This did not differ significantly from the 41% rate of needle sharing among those testing negative. Significantly more of the HIV positive subjects reported unprotected sexual activity with multiple partners than those who tested negative (48% vs 32%; X2=13; df=6; p<.05).

There was no significant difference found between the needle sharing patterns of the HTLV-I/II infected subjects when compared lo those testing negative for this virus. Nor did these groups differ with regard to high risk sexual activity.

DISCUSSION

The PCR is currently being evaluated as a more sensitive test for the presence of HIV. These preliminary results suggest that there is a significant rate of HIV exposure among those who test negative by ELISA. The higher rate of positivity found by PCR raises important questions about the accuracy of this test and its role in the identification of HIV infection rates. Through prospective studies, the clinical significance of these test results will be determined.

A very high rate of HTLV-I/II was found among this sample. Overall 23% tested positive for antibodies to this virus. Given the nonspecific nature of the ELISA and Western Blot, we cannot precisely distinguish between the HTLV-I and the HTLV-II. Early results of PCR testing suggests that the majority of these infections are for HTLV II.

Perhaps the most important data reported here is the level of high risk behavior which continues despite widespread knowledge of the risks. Both needle sharing and unprotected sexual activity look place during the six months prior to testing among a majority of those who were subsequently found to test positive for HIV. When this finding is considered along with our preliminary PCR results and the current rates of infections, it suggests that Philadelphia is in the early stage of the AIDS epidemic.

Center for Studies on Addiction University of Pennsylvania/Philadelphia Veterans Administration Medical Center Medical College of Pennsylvania and Philadelphia VA Medical Center.

Pre-treatment Natural History of Cocaine Addiction

Hari K. Khalsa, M. Douglas Anglin, and Alphonso Paredes

The U.C.L.A. Drug Abuse Research Group's "Cocaine Treatment Evaluation" study was designed to evaluate the effectiveness of treatment for cocaine addiction and to study the natural history of cocaine dependence: the characteristics of users and their patterns and progression of use. Natural History results are reported here for the period between first cocaine use (FCU) and treatment entry (TE) for 285 cocaine dependent male veterans admitted for treatment to the VA Medical Center-West Los Angeles between April 1988 and September 1989.

Demographic Characteristics of the Sample
The mean age for these male veterans at the time of interview was 36.7 years; 32% were 40 or older and 11% were younger than 30. Two-thirds of the subjects were black, 26% white, and 7% Hispanic. Level of education was high: 44% had post-secondary schooling, 42% completed high school, and only 14% reported less than a 12th grade education. Level of education contrasts with the high proportion of unemployed subjects at the time of the interview (43%). Sixty-seven percent had been married at least once (67%) and 65% had fathered children. Eighty-six percent reported being arrested at least once in their lives, most of them (41%) before age 18 for the first time. The mean age first arrested was 20 years. At admission, 13% reported being homeless.

Drugs use history was extensive for this group: 98% had used marijuana or hashish (82% regularly), 69% amphetamines (32% regularly), 67% hallucinogenics (21% regularly), 65% PCP (17% regularly), 58% downers (21% regularly), 53% crystal methamphetamine (18% regularly), 40% heroin (16% regularly), 36% tranquilizers (8% regularly), and 26% glue (7% regularly). Mean age of first drug use was under 18 for marijuana and glue, between 18 and 20 for hallucinogenics, amphetamines, and downers, between 21 and 25 for crystal methamphetamine, heroin, tranquilizers and PCP.

Criminal History
The results showed a high rate of early deviant behaviors: 33% had run away from home before age 18; 58% were expelled from school; 50% had been drunk and/or high on drugs while in school; 13% stole from school; 11% had damaged school property; 25% had threatened an adult; and 17% had hit an adult. Those activities were most often committed for the first time between ages 13 and 15.

The proportion of subjects reporting stealing and/or shoplifting was high: 66% reported stealing from stores, 41% from wallets and purses, and 38% from their families. More than half of those who reported stealing and shoplifting did so before

using cocaine, approximately a fourth of them both before and after using cocaine and the other fourth only after. Involvement with the illegal economy was high a well: 46% of the subjects reported buying stolen goods and 24% selling and fencing them. There was a small increase in the proportion of subjects involved with stolen goods after initiating cocaine use.

Involvement with force and weapons was common: 49% reported carrying weapon: (increasing after cocaine use), 27% reported threatening with a weapon, 12% had used force for profit, and 9% reported having threatened for profit. Involvement with serious violence was less frequent: beating someone severely (29%) shooting someone (11%), and committing forcible rape (2%). Most of those activities occurred before the individuals had used cocaine.

Results showed a high degree of involvement in the drug subculture: 65% of the sample reported selling drugs and 47% carrying drugs for others. Although these activities commonly occurred before the use of cocaine there was an increase in these activities after the use of cocaine (of those who sold or carried drugs, 48% and 41% respectively did so only after using cocaine).

History of Cocaine Use

The mean age of first cocaine use was 24. For 76% of the sample, first cocaine use was influenced by a friend or acquaintance, for 8% by a parent or other relatives (excluding siblings), for 4% by a girlfriend or wife for 4% by a sibling and for 8% by either a dealer or prostitute or other. In 40% of the cases, the person intluencing the subjects' first cocaine use was a cocaine addict. The reasons reported for first cocaine use were: curiosity 36%, social reasons (at parties, for fun and celebration) 34%, psychological reasons (depression, loneliness, anxiety) 15%, wanting to get high 7%, reasons related to sex (sexual partner using, to have better sex and to get sexual partners) 3%, and easy availability of street cocaine 6%. The route of first administration utilized was intranasal for 77%, smoking crack for 11%, intravenous for 4%, freebasing for 3%, and other for 5% (primo[1], rails[2] or oral). For the first cocaine use, in 84% of the cases cocaine was given to the subjects, and 15% of the sample bought it themselves.

Pre-treatment Natural History

The average total time from FCU to TE (which was the first cocaine treatment for 95%) was 11% years (SD=5.9 years). At some point during their pre-treatment career 278 (98%) used cocaine at a severe level and the mean percent non-incarcerated time[3] using at this level was 40%. A high proportion of the subjects (86%) were abstinent at some point in this period and the mean % time abstinent was 37%, while 14% were never abstinent from FCU to TE. In other words, on the average, in the 11% years of the cocaine addiction career, subjects used cocaine at a severe level approximately 4% years, at a mild level for a year and 8 months, at a moderate level for over seven months, and were abstinent for almost 5 years. The average length of the period from First Severe Use (FSU) to TE was 83 months and the average longest (continuous) severe period was 37 months. During the period from FSU to TE, the mean % time using at **a** severe level increased to 70% while the mean % time of abstinence decreased to 18%.

For the overall pre-treatment career (FCU-TE), most of the subjects used more than one route of administration, the most popular being intranasal (IN) (74%) and crack (72%). Freebasing was less prevalent (38%) and intravenously (IV) (16%) **was** not common.

During the FSU to TE period, there was a shift in mode of administration from IN to crack. While 42% used crack in the 3% years after FSU, 70% did so during the 3% (non-overlapping) years before TE. The mean % time using crack increased as well, from 42% to 70% respectively. Other routes of administration remained stable.

From FCU to TX, alcohol was heavily used: 76% of the sample drank alcohol excessively (at least 4 ounces per day) for 50% time during their pre-treatment career. Marijuana was used for a mean % time of 25% at a daily level and 28% at a less than daily level. Narcotics and amphetamines were used by about a fourth of the sample, but at a lower mean % time than marijuana or alcohol (4.5 for narcotics and 4.8 for amphetamines). The mean % time using marijuana both daily and other than daily, drinking alcohol excessively, and using narcotics and amphetamines progressively decreased during the period from FSU to TE, particularly during the 3% years before TE.

While 61% reported dealing drugs at some point during FCU to TE, the mean % time doing so was low (22%). The proportion reporting dealing decreased to 44% during the 3% years before TE, but the mean % time dealing remained stable at 21%, suggesting that those dealing were doing so for longer periods. Prevalence of income generating illegal activities (property crimes) were reported by 35% in this period, but at a relatively low mean % time of involvement (under 10%). Overall, twice as many subjects reported dealing than committing property criminal activities.

The proportion of subjects employed was high (99%) during the entire pre-treatment career and decreased only slightly from 86% after FSU to 82% as subjects engaged in more severe cocaine use in the 3% years before TE. Eighty-two percent had been involved in marriage and/or common law relationships during FCU to TE with a mean % time of 50%. As subjects approached TE, the proportion of those so involved decreased to 64%, but the mean % time involved did not change.

Overall this sample represented a highly functional population, able to maintain employment and relationships during most of their cocaine-using career.

To explore different patterns of use, we divided the sample according to the sequence of the initial pattern of cocaine use. Table 1 shows four patterns of progression found for the initial cocaine career: mild-moderate-severe (group 1), mild-severe (group 2), moderate-severe (group 3), and instantly severe (group 4). The definitions used for mild, moderate and severe levels of cocaine use were based on the frequency and amount of cocaine used, and on the length of the abstinence between cocaine use episodes. The most prevalent pattern (44% of the sample) consisted of starting cocaine use at a mild level and subsequently jumping directly to a severe use level.

Overall the mean % time from FCU to TE was 141 months for groups 1 (mild-moderate-severe) and 2 (mild-severe), and 109 and 111 for groups 3 (moderate-severe) and 4 (instantly severe). For subjects who started using cocaine at a mild level, the average overall time to treatment admission was 2 years longer than for those subjects who started at a higher (moderate or severe) level. As table 1 shows, the shortest average length of time at any one stage corresponded to the group 1 progression from a moderate to severe level while the longest was the group 4 progression from FSU to TE. The results indicate that the majority of subjects were generally able to maintain mild use for a considerable length of time but once they reached a moderate level of use, escalation to severe use occurred comparatively rapidly.

TABLE 1 - INITIAL SEQUENCING PATTERNS

		N	%
(FCU)MILD ———— MODERATE ———— SEVERE ———— TE (GROUP 1) m.a.=24 m.a.=28 m.a.=30 m.a.=35 <52 months> <36 months> <54 months> SD=50 SD=29 SD=50		47	17
(FCU)MILD ————————————— SEVERE ———— TE (GROUP 2) m.a.=24 m.a=29 m.a.=35 <64 months> <78 months> SD=53 SD=59		125	44

——— (FCU)MODERATE ———— SEVERE ———— TE (GROUP 3)
     m.a.=26   m.a=29   m.a.=35
     <39 months>   <70 months>
     SD=45   SD=58
		28	10

———————————— (FCU)SEVERE ———— TE (GROUP 4)
       m.a.=27   m.a.=36
     <111 months>
     SD=81
		84	30

* m.a = mean age
*SD = standard deviation

Tables 2 and 3 show levels of cocaine use, routes of administration, other drug use, and different behaviors for the progressive stages represented by the four different groups. While the initial sequencing patterns of cocaine use was markedly different for the four groups, the progression related strifts in cocaine use, other drug use, and in the behavioral domains did not differ significantly. All groups were similar in terms of the types of shifts and distribution of levels among the cocaine routes of administration. That is, all showed increasing cocaine involvement over time in smoking routes, and less involvement in other drug use. The differences among groups typically occurred in the baseline levels of drug use and behavior.

Subjects in group 1 used less narcotics and amphetamines than subjects in other groups; they also used slightly less alcohol and marijuana. Furthermore, they were less involved in antisocial behaviors such as criminal activities and dealing, and were more involved in pro-social activities such as working. Although fewer group 1 subjects had a marriage/common law relationship, those who did were involved for longer periods of time.

Group 2 subjects were characterized by a slightly higher level of freebasing, by a low level of narcotic use, and a slightly lower level of excessive alcohol drinking. Group 3 subjects used cocaine severely and for longer periods, while fewer used mildly or were ever abstinent. For this group, the preferred cocaine form was crack and fewer used marijuana and alcohol.

Subjects in group 4 were characterized by a higher level of abstinence and lower levels of moderate use. This group was distinctive in that the IN and IV routes of administration were more prevalent than in any other group, while the proportions of crack and freebase users were lower. More subjects in this group used narcotics and amphetamines than in the other groups.

Overall, results show that the pre-treatment cocaine career lasted 11% years and subjects were able to maintain a severe level of cocaine use for 6½ years. Four different sequences of escalation to severe cocaine use were described. As subjects approached TE, the mean % time of severe use increased and a shift occurred to crack use from other routes of administration. Despite this progression, antisocial

TABLE 2

	GROUP 1: MILD - MODERATE - SEVERE (N=47)								GROUP 2: MILD - SEVERE (N=125)					
	Mild to Moderate 52 months		Moderate to Severe 36 months		Sev. to Midpoint 27 months		Midpoint to TE 27 months		Mild to Severe 64 months		Sev. to Midpoint 39 months		Midpoint to TE 39 months	
	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time
Cocaine Use														
None	79	54	57	46	22	7	49	15	72	53	45	18	61	21
Mild	100	46	36	32	17	11	27	9	100	48	18	4	31	8
Modera	---	---	100	23	12	4	24	8	---	---	15	4	22	5
Severe	---	---	---	---	100	78	100	69	---	---	100	74	98	55
Cocaine: route of administration														
IN	79	40	57	31	39	23	24	9	76	33	46	25	29	12
CRACK	11	3	36	10	46	38	73	51	76	33	44	25	69	41
FB	6	0	24	6	27	16	27	17	17	2	29	18	35	17
PRIMO	9	1	5	3	5	1	0	0	6	3	11	6	7	3
IV	2	0	7	2	12	8	15	4	9	2	7	3	8	3
OTHER	4	1	2	1	15	7	7	4	9	0	7	2	10	3
Marijuana Use														
Daily	53	32	52	36	42	31	27	13	42	42	46	29	36	18
Less than daily	55	38	43	33	37	25	46	33	50	32	40	24	45	19
Excessive Alcohol Drinking	67	56	64	58	59	52	59	40	68	55	63	49	58	39
Any Narcotic Use	9	1	5	2	5	2	2	1	12	3	7	3	7	3
Any Amphetamine Use	13	4	10	5	7	3	10	4	17	6	12	5	6	3
No Drug Use (excluding cocaine)	38	26	38	25	49	39	85	62	34	22	55	40	82	56
Dealing	32	20	19	13	22	11	37	15	40	25	47	31	45	22
Criminal Activites	11	5	12	6	15	10	20	8	14	6	16	9	23	11
Working	94	90	95	92	98	94	93	83	98	93	94	84	93	80
Relationships	55	41	48	44	46	46	56	44	65	53	88	49	66	48

Note: % people will add to > 100% since may have periods primarily involving several different routes.

TABLE 3

	GROUP 3: MODERATE-SEVERE (N=28)						GROUP 4: INSTANTLY SEVERE (N=84)			
	Mod to Severe 39 months		Sev. to Midpoint 35 months		Midpoint to TE 35 months		Sev. to Midpoint 56 months		Midpoint to TE 56 months	
	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time	% people	mean % time
Cocaine Use										
None	50	33	34	10	57	14	58	24	64	34
Mild	18	5	11	2	11	3	19	6	29	7
Moderate	100	63	18	7	29	8	10	3	21	6
Severe	0	0	100	81	96	75	100	67	94	64
Cocaine: route of administration										
IN	66	39	39	24	21	9	57	29	31	16
CRACK	16	16	54	48	62	62	34	25	66	39
FB	7	5	18	13	18	9	19	8	25	11
PRIMO	4	4	4	0	4	1	6	3	4	2
IV	0	0	4	2	4	4	12	7	14	27
OTHER	7	4	4	4	7	1	8	3	6	3
Marijuana use										
Daily	50	35	29	15	21	18	43	22	26	13
Other than daily	46	35	36	27	25	16	49	28	46	20
Excessive Alcohol Drinking	71	69	71	58	64	47	68	54	66	48
Any Narcotic Use	11	7	14	6	11	6	21	9	17	6
Any Amphetamine Use	14	8	7	6	7	6	17	7	13	3
No Drug Use (excluding cocaine)	36	24	68	49	68	56	55	35	77	55
Dealing	39	28	39	27	46	24	51	31	44	21
Criminal Activities	14	10	11	8	25	10	17	7	24	12
Working	100	93	93	83	93	80	98	87	95	65
Relationships	54	50	64	47	61	44	69	50	68	46

behaviors (dealing, and property crime) did not increase, pro-social activities (working and relationships) did not decrease significantly, and excessive alcohol drinking and other drug use decreased. Generally, subjects' pre-treatment functional levels were high.

One Year Follow-up: Preliminary Results
One hundred fifty-two subjects have been followed for one year after TE. Relapse to daily use occurred for 23%, 52% reported relapsing to other than daily use, and 25% did not report any cocaine use. If the three month pre-interview period is considered, 16% used cocaine daily, 24% used other than daily, and 61% did not use. Most relapses (51%) occurred within the first month after treatment discharge.

FOOTNOTES

[1] Primo is smoking cocaine combined with marijuana.

[2] Rails is smoking cocaine with tobacco.

[3] Mean % time is the number of months involved in a behavior or in a specified status divided by the number of months between the critical dates of interest.

Authors:
Hari K. Khalsa, M.D.[1], M. Douglas Anglin, Ph.D.[1], Alphonso Paredes, M.D.[2]

[1] UCLA Drug Abuse Research Group
Neuropsychiatric Institute
1100 Glendon Ave., Suite 763
Los Angeles, CA 90024-3511
(213) 825-9057
(213) 825-4779 (FAX)
(213) 825-3736 (Bulletin Board)

[2] Brentwood VA Medical Center
Alcohol and Drug Treatment Program
(213) 824-3164

Behavioral and Cardiovascular Effects of Cocaine and Alcohol Combinations in Humans

S.T. Higgins, W.K. Bickel, J.R. Hughes, M. Lynn, and M.A. Capeless

Polydrug use is quite prevalent, yet much remains to be learned concerning the behavioral and cardiovoscular effects of drug combinations. This study examined the behavioral and cardiovascular effects of intranasally administered cocaine (4, 48, 96 mg/70 kg) and orally administered alcohol (0, 0.5, 1.0 g/kg) in 6 recreational cocaine users. Alcohol was administered in five drinks across a 30 minute period. Cocaine was inhaled 45 minutes after subjects completed drinking. Cocaine and alcohol alone produced orderly, dose-related behavioral and cardiac effects. For example, cocaine enhanced performance on the Digit Symbol Substitution Test (DSST), while alcohol impaired DSST performance. Both compounds increased heart rate. The effects of combining these compounds differed across measures, and cannot be characterized as either "antagonistic" or "additive" without reference to particular dependent measures. For example, cocaine attenuated the rate-suppressing effects of alcohol on DSST performance, exacerbated alcohol's effects on heart rate, and left subject ratings of drug-produced "high" unchanged. Such differential effects are consistent with findings from a prior study conducted in our laboratory examining d-amphetamine-alcohol combinations, thereby extending the range of conditions under which cocaine and &amphetamine produce comparable effects. Additionally, results from both studies illustrate the complex nature of drug interactions, and underscore the need for careful investigation to understand the behavioral and cardiovascular consequences of polydrug use.

Human Behavioral Pharmacology Laboratory, Department of Psychiatry, University of Vermont, 38 Fletcher Place, Burlington, VT 05401

Relapse to Cocaine Use Following Treatment: Preliminary Findings on the Role of Social Support

Barbara E. Havassy, Sharon M. Hall, and David M. Wasserman

In this prospective study, we examined links between social support and abstinence from cocaine in a sample of 104 persons completing treatment for cocaine dependence. We studied both structural support (the existence of supportive others) and functional support (the quality of support). Higher levels of structural and functional support were hypothesized to predict abstinence during posttreatment follow-up.

Subjects were recruited into the study during their last week of treatment at one outpatient and four inpatient programs. Ss were assessed at study intake and followed weekly for 12 weeks, starting the week after treatment completion. Ss were assessed again 6 months after treatment completion regardless of their cocaine use during the 12-week follow-up period. Ss were mostly male (73.1%) and white (51.9%). The mean age was 31.7 years (SD=6.3). Most Ss (78.8%) reported employment during the past 6 months.

Posttreatment structural support, assessed at the first follow-up, was operationalized as a 7-point index of social integration reflecting the existence of a spouse or partner, a best friend, and other friends and relatives; the frequency with which friends and relatives were seen; and membership in groups. Functional support was measured at all assessments with three self-report scales tapping perceived emotional, instrumental, and "negative" support. Abstinence was assessed by self-report and confirmed by urine assays.

One-third of the sample (33.7%) used cocaine primarily intranasally, 48.1% primarily smoked crack or freebase, and 5.8% used intravenously. The remaining 12.4% of Ss both snorted and smoked cocaine. Males were more likely than females to use crack/freebase or to administer cocaine intravenously; females were more likely to use intranasally. White Ss, compared to black Ss, were more likely to be intranasal or intravenous users, whereas black Ss were more likely to use crack/freebase.

In the 12 weeks following discharge, 42 Ss (40.4%) were continuously abstinent from cocaine. Fifty Ss (48.1%) reported cocaine use or submitted

a positive urine specimen at one or more follow-up assessments. The median number of weeks elapsing before the initial instance of use was 3 weeks. Twelve Ss (11.5%) were lost to follow-up before reporting any cocaine use. At the 6-month assessment, 33 Ss (31.7%) who had been abstinent during the first 12 weeks still were abstinent by self-report and urine assays. Fifty-six Ss (53.8%) had used cocaine since treatment completion and 15 Ss (14.4%) were lost to follow-up.

In the first week following treatment, 30.8% of Ss reported a current cocaine user in their "immediate" social network, that is, a spouse or lover, other household member, or best friend. Another 35.6% had no current users but at least one former user in their immediate network. Cocaine use was most common among best friends: 37.7% of best friends currently used cocaine, and 36.2% were former users.

Twelve-week results are reported here. The analytic model for predicting the first instance of cocaine use during the 12-week posttreatment period from social-support variables was Cox's proportional hazards regression. Ss lost to follow-up were coded as returning to use the week following the last assessment completed. Covariates were chosen using stepwise variable selection. With gender and race forced into the model, route of administration was the only other significant covariate. Models were hierarchical, with covariates entered first, then the social-support variable, and finally the covariate X social support interaction set.

Social integration did not have a main effect on abstinence during the 12 weeks. There was, however, an effect for the interaction set, $p=.0535$. This was due to an interaction of race with social integration, χ^2 (1, $N = 104$) = 4.94, $p = .0263$: greater support predicted a decreased risk of cocaine use in white but not black Ss.

Greater functional support predicted a decreased risk of relapse. Higher weekly levels of perceived emotional support predicted a decreased risk of cocaine use, χ^2 (1, $N = 102$) = 7.01, $p = .0081$. Instrumental and negative support were unrelated to cocaine use (ps > .30). No interactions were present between the covariates and functional support.

These findings indicate that social integration and emotional support may facilitate abstinence in cocaine users who complete treatment. The interaction of social integration with race suggests that the protective elements of this type of support may be different for white and black cocaine users. Elements of social integration for black users need further exploration. Perceived emotional support, however, appears to protect against early lapses to cocaine use across race and gender groups.

AFFILIATIONS

Barbara E. Havassy, Ph.D.
University of California, San Francisco
School of Medicine, Department of Psychiatry
San Francisco General Hospital (7M)
1001 Potrero Avenue
San Francisco, CA 94110

Sharon M. Hall, Ph.D.
University of California, San Francisco
San Francisco Veterans Administration Medical Center
Psychiatry Service (116A)
4160 Clement Street
San Francisco, CA 94121

David A. Wasserman, Ph.D.
University of California, San Francisco
San Francisco Veterans Administration Medical Center (116W)
4160 Clement Street
San Francisco, CA 94121

Effects of Bromocriptine on Subjective and Autonomic Responses to Cocaine-Associated Stimuli

Henry R. Kranzler and Lance O. Bauer

<u>INTRODUCTION</u>

After detoxification and standard treatment, cocaine-dependent patients have been found to be highly reactive (using subjective and physiologic measures) to both videotaped and audiotaped sequences of cocaine use and to the handling of cocaine paraphernalia. Preliminary results suggest that the most common response to these stimuli is an increased desire for the drug, which occurs in 60-65% of those tested. Decreases in skin temperature and skin resistance occur less frequently (Childress <u>et al.</u> 1988).

Medications used to treat cocaine dependence include the dopamimetic, bromocriptine, which has been shown to be superior to placebo in both the experimental reduction of craving and the treatment of cocaine dependence. Unfortunately, these trials were limited by an overreliance on self-report to assess outcome and by the absence of a successfully blinded methodology.

<u>METHODS</u>

In order to replicate and extend previous work, we examined the autonomic reactions and self-reported symptoms of 20 abstinent, cocaine-dependent males exposed to videotaped sequences of cocaine-associated stimuli and neutral stimuli in a controlled laboratory setting. Sessions were conducted during the first week of inpatient rehabilitation and on a second occasion one week later. During the one-week interval between sessions, patients received either bromocriptine (1.25 mg b.i.d) or placebo in double-blind fashion. Before and during the presentation of both films during both sessions, measures of ANS activity [heart rate, skin temperature, skin conductance, pulse transit time, respiratory sinus arrhythmia (RSA)] and subjective ratings (desire for cocaine, intensity of cocaine intoxication and withdrawal symptoms, and the POMS) were obtained.

The two patient groups (i.e. bromocriptine, placebo) did not differ in age, cocaine use in the preceding month, severity of

cocaine dependence, or anxiety, depression or sociopathy. The bromocriptine group had been abstinent from cocaine for a significantly (p < .05) longer period of time prior to the study. However, the duration of abstinence did not significantly influence results of the analyses.

RESULTS

During the first laboratory session, the cocaine film evoked greater increases in self-reported desire for cocaine (p < .0001), depression (p < .03) and hostility (p < .03) and significantly greater decreases in RSA (p < .05), than did the neutral film. The cocaine film also evoked increases in the intensity of several symptoms of cocaine intoxication (p < .01), and of cocaine withdrawal (p < .06). During the second laboratory session, many of these effects either diminished or disappeared (Session X Film: Desire, p < .01; RSA, P < .03; intoxication symptoms, p < .03; depression, p < .03; hostility, p < .02). This decrease in reactivity, however, was not attributable to bromocriptine: the drug had no significant effect on desire, RSA, or symptom severity, other than to increase self-rated hostility (p < .06). It also produced an increase in heart rate (p < .06) in response to the cocaine film, which was not detectable in patients who received placebo. Subjects in both treatment groups were unable to identify correctly the agent they received.

CONCLUSIONS

Insofar as we observed increased desire and autonomic changes in response to cocaine-associated, but not neutral stimuli, these results provide partial replication of the work of Childress and colleagues (1988). Bromocriptine did not, however, decrease the desire for cocaine, as was reported by Dackis and colleagues (1987). Because the blind was maintained throughout our study, we are confident that the expected benefits of treatment with an active medication did not influence subjects' self-reported symptoms. Based on these findings, we conclude that the capacity of bromocriptine to reduce cocaine craving is due, in large part, to positive expectations, rather than pharmacologic effects.

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Department of Psychiatry, University of Conn. School of Medicine

Effects of Cocaine Alone and in Combination With Bromocriptine in Human Cocaine Abusers

Kenzie L. Preston, John T. Sullivan, Eric C. Strain, and G.E. Bigelow

The present study was conducted to determine whether bromocriptine modulates the pharmacologic effects of cocaine in humans. Bromocriptine is a dopamine agonist suggested in some clinical trials to be useful in the treatment of cocaine abusers. Eight current users of IV cocaine who were not seeking treatment for their cocaine abuse participated while inpatients on a research unit. Twelve drug conditions were given in randomized order under double-blind, double-dummy conditions and included cocaine 0, 12.5, 25, and 50 mg (IV) alone and in combination with bromocriptine 1.2 and 2.5 mg (given orally 2 hr before cocaine injection). Physiological and subject- and observer-rated responses were measured. Cocaine alone significantly increased pupil diameter, heart rate and blood pressure and ratings of drug effect, good effects, liking and rush. The effects of cocaine when combined with bromocripine were not significantly different on any measure from cocaine alone. Clinically significant adverse effects, fainting, occurred in two subjects after bromocriptine alone. The effects of cocaine were not modified by pretreatment with bromocriptine in any way that might indicate either a therapeutic benefit or a safety concern. However, bromocriptine itself produced undersirable effects that should be considered before administration to outpatient cocaine abusers. Any possible therapeutic benefits of acute administration of bromocriptine in cocaine abuse are not likely to be due directly to modulation of the acute effects of cocaine. (Supported by USPHS grant DA 05196.)

AFFILIATION

Department of Psychiatry and Behavioral Sciences (KLP, ECS, GEB) and Department of Medicine (JTS), The Johns Hopkins University School of Medicine, Baltimore, MD 21224

Concurrent Effects of Acute Intravenous Cocaine in Context of Chronic Desipramine in Humans

Glenn Pickett, Thomas R. Kosten, Frank H. Gawin, R. Byck, J. Fleming, D. Silverman, T.A. Kosten, and P. Jatlow

Desipramine (DMI) reduces cocaine use and craving in double blind outpatient studies. Three questions have arisen about the use of DMI for cocaine treatment: 1. does it block cocaine euporhoria? 2. does it reduce cocaine craving? 3. what is the medical safety of using cocaine while treated with DMI chronically? This study addresses these questions by using intravenous cocaine in weekly challenge sessions: four sessions on placebo DMI and four on active DMI. At each of these four sessions patients received intravenous cocaine in a randomized order: 0 mg, 0.125 mg/kg, 0.25 mg/kg, or 0.5 mg/kg. Desipramine was given at a fixed dosage of 150 mg/day for ten days before the first cocaine challenge to allow time for DMI induced receptor changes. In the cocaine challenge laboratory, blood pressure and heart rate as well as subjective report scales were monitored for at least a one hour at baseline and then for four hours following cocaine challenge.

Five subjects completed the challenge series, 80% were male, all were white with a mean age of 30 +/- 1.8 years. 60% were freebase users and 40% were i.v. users of cocaine. The average grams per week of cocaine used was 3.1 (range = 0.2 - 5.5) with the average days per month of use being 7.5 (range = 3 - 20). Alcohol was used an average of 9 days per month (range = 2 - 18).

At all doses of cocaine there were no significant differences between placebo and DMI treated patients in their systolic or diastolic drug pressures, but heart rate response was different, due to higher baseline heart rate in the desipramine treated subjects (10-15 bpm). The peak heart rate induced by cocaine was equivalent between the two groups, but the percentage change from baseline was substantially attentuated in the DMI treated condition. The slope of heart rate change versus cocaine dose was tenfold higher in the placebo condition.

The subjective responses to cocaine were altered by
desipramine. Although the "high" was not attenuated during the
DMI condition, the desire for cocaine appeared to be attenuated
during the DMI condition at the highest cocaine dosage (0.5
mg/kg). "Desire" for cocaine dropped below baseline within
sixty minutes on active desipramine and an equivalent drop took
240 minutes on the placebo DMI.

In conclusion, although textbooks describe a potentially
adverse cardiovascular interaction between DMI and stimulants,
we did not observe this with cocaine abusers treated
chronically with DMI. Although baseline heart rate is
increased, heart rate response to cocaine is blunted. DMI
appears to reduce cocaine abuse without blocking cocaine
euphoria, instead it may reduce the duration of "desire" for
cocaine, thereby reducing the "priming" effect whereby a "slip"
with a single use of cocaine leads to relapse.

Glenn Pickett, M.D.
Thomas R. Kosten, M.D.
Frank Gawin, M.D.
Robert Byck, M.D.
Julia Fleming, M.D.
David Silverman, M.D.
Therese A. Kosten, Ph.D.
Peter Jatlow, M.D.

Substance Abuse Treatment Unit
Department of Psychiatry
27 Sylvan Avenue
New Haven, CT 06519

Amantadine and Desipramine in the Treatment of Cocaine Abusing Methadone Maintained Patients

T.R. Kosten, C.H. Morgan, and R.S. Schottenfeld

Several pharmacological treatments for cocaine abusing methadone maintained patients have been proposed including desipramine and amantadine (1). Both amantadine and desipramine have been found to reduce cocaine craving and use in methadone maintained patients, although the one controlled study of amantadine found it no better than placebo (2). In the current double blind, randomized clinical trial, once daily dosing with amantadine at 300 mg and desipramine at 150 mg were each compared to placebo. Medication levels were monitored and compliance assured by daily observed medication ingestion at the methadone program. Methadone dosage was stable with an average of 45 mg daily. Outcomes included twice weekly urine for benzoylecgonine and self reported cocaine use and craving. Sixty-one patients were treated, including twenty-four on amantadine, twenty-four on desipramine, and twenty-seven on placebo. The three groups were comparable in race (70% white), sex (50% male), age (32 years), percent anti-social personality (22-35%), and baseline usage of cocaine ($735 to $944) with eight years of cocaine use. Treatment retention was excellent, with 100% retention at six weeks, 70% retention at the end of the trial, and no differential dropout across the three groups. Cocaine craving substantially decreased for the amantadine group to 70% of baseline, but not for the desipramine or placebo groups. Dollars spent on cocaine also substantially decreased for the desipramine and amantadine groups, down to 40% of baseline use, while the placebo group initially increased their cocaine use to 140% of baseline up to week five and then finally declined to 75% of baseline. Sustained cocaine abstinence, based on urine toxicologies was attained by 45% of the amantadine group and 60% of amantadine patients who completed all twelve weeks of the study. Abstinence rates were only 20-25% in the desipramine and placebo groups. In examining the fifty-six methadone patients who did not have anti-social personality disorder, cocaine craving showed a more substantial decline among the amantadine patients to 50% of baseline, and cocaine use dropped to 20% of baseline. The non-ASP patients treated with desipramine showed a similar marked

reduction in cocaine use. In examining blood levels of
desipramine we found no association between desipramine blood
levels and treatment response, but an inverse relationship
between amantadine blood levels and treatment response. There
was a greater decrease in cocaine use among patients with lower
amantadine levels (range 120-1200 ng/ml). In summary, non-ASP
patients showed a good treatment response to either amantadine
or desipramine, while patients on amantadine showed a good
treatment response regardless of ASP diagnosis.

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Kosten, T.R.
Morgan, C.H.
Schottenfeld, R.S

Department of Psychiatry
Substance Abuse Treatment Unit
Yale University School of Medicine
27 Sylvan Avenue
New Haven, Connecticut 06519

Clinical Trail of Multiple Treatment Agents for Cocaine Dependence: A Placebo-Control; Elimination Study

Forest Tennant

Beginning in the early 1980's, attempts were initiated to identify medical treatment agents for cocaine dependence. Since that time, several agents, including amantadine, bromocriptine, carbamazepine, desipramine, levodopa. tryptophan and tyrosine, have been reported to be clinically effective for treatment of cocaine dependence. In 1988, our group reported that the combined, step-wise use of these agents was successful in detoxifying approximately 65% of cocaine addicts. The criteria for success was based on the achievement of a urine test during treatment that did not contain cocaine metabolite when tested by enzyme immunoassay (EMIT) at a threshold of 300 ng/ml. A repeat study of 457 cocaine addicts in 1989 showed, that only 22.5% were able to detoxify when the criteria for success was the achievement of a cocaine metabolite urine concentration of less than 30 ng/ml when determined by Polarized Fluorescent Immunoassay (PFI, Abbott TDX). A review of our previous studies as well as others reported in literature revealed that the criteria for detoxification success has been primarily based on insensitive urine tests with a minimal testing threshold of 300 to 500 ng/ml and/or the use of reduction of subjective craving and withdrawal symptoms. Consequently, a search for new treatment agents and a reevaluation of others by a placebo-controlled study, was launched in February, 1990.

The following 11 agents were selected for evaluation and comparison with placebo: amantadine, bromocriptrine, buspirone, carbamazepine, desipramine, levodopa, L-deprenyl, omega fatty acids, pergolide, phenmetrazine, and prazosin.

Subjects are self-reported cocaine addicts who voluntarily seek medical treatment and demonstrate cocaine metabolites in urine by PFI. They are sequentially assigned to receive one of the treatments agents or a placebo capsule which is identical in appearance to omega fatty acids. All subjects receive a standard set of nutritional supplements consisting of amino acids and vitamins; attend an outpatient clinic 3 to 5 times a week, and submit an observed urine specimen on each visit. One dose of study medication is given in the clinic and the remainder is taken outside the clinic. Between February and April, 1990, 125 subjects were admitted (10 or 11 in a group, except pergolide) and the results compared to placebo (n = 11). An additional 24 subjects applied to the study, and even though they claimed cocaine addiction, did not demonstrate cocaine metabolite in urine.

The 11 placebo subjects have showed the following: 4 (36.4%) converted their urine to zero (< 30 ng/ml of cocaine metabolite); remained a mean of 15.9 days in treatment, and 5 (45.5%) remained in treatment over 30 days. Fouragents, carbamazepine, desipramine, pergolide, and phenmetrazine have been eliminated from further study because no subject converted their urine to cocaine metabolite negative and/or remained in treatment over 30 days. In addition, pergolide provided such severe side-effects of nausea and dizziness that only four subjects were studied. Re-evaluation by urine conversion and treatment retention will be done after 5 additional subjects are treated with each agent. Continuation in the study will require that an agent perform equal or better than placebo. Treatment agents that remain in the study have various and, in some cases, unique biochemical properties. For example L-deprynl or selegeline is a MAO-B inhibitor that makes dopamine and norepinephrine more available to receptor sites, and it is partially metabolized to amphetamine and methamphetamine. Buspirone is a serotonin receptor agonist, and omega fatty acid are prostaglandin precursors. The target date for completion is calendar year 1990.

Affliation:

Community Health Projects, Inc.
Research and Education Division
336½ S. Glendora Ave.
West Covina, CA 91790

Mazindol Treatment of Cocaine Abuse. A Double-Blind Investigation

I.A. Diakogiannis, M. Steinberg, and T.R. Kosten

The efficacy of mazindol, a dopamine reuptake blocker, for 19 cocaine abusers on methadone maintenance was investigated in a crossover study. They had a mean age of 32.4, 10 were white and 9 were males. They were given Mazindol 2 mg daily for 1 week followed or proceded by 1 week of placebo. The weekly quantity of cocaine used, the quality of euphoria, the craving for cocaine, Beck and Hamilton inventories and SCL were reported and urine toxicology for cocaine was tested 3 times a week. One patient discontinued Mazindol on the 3rd day while most described feeling jittery or agitated. No significant change in Beck, Hamilton or SCL scores was found. Seven patients bought less cocaine while on Mazindol compared to placebo. Craving appeared to increase in Mazindol versus placebo treatment periods (7.2 vs. 5.7). No significant difference in the quality of euphoria was reported. Two patients gave clean urines on Mazindol and two on placebo. These results suggest that Mazindol is not an effective rapid treatment for cocaine, and efficacy may need to be investigated for longer than a week period. Supported by NIDA Training Grant 5T32-DA07238.

I.A. Diakogiannis, M.D.
Marlene Steinberg, M.D.
Thomas R. Kosten, M.D.

Substance Abuse Treatment Unit
Yale University
27 Sylvan Avenue
New Haven, Connecticut 06519

Evaluation of the 5HT3 Antagonist Ondansetron (O) for Cocaine (C) -Like Activity and Abuse Potential

Donald R. Jasinski, Kenzie L. Preston, Margaret Testa, and John T. Sullivan

Ondansetron is proposed for use as an IV antiemetic. Evaluation of ondansetron for cocaine-like abuse potential was done since both ondansetron and cocaine are competitive antagonists at 5HT3 sites. In addition, ondansetron is being investigated as a treatment drug for various forms of drug abuse since ondansetron reduces central dopaminergic activity and also modifies withdrawal in animals. Our initial study involved 8 post addicts who were first given cocaine 20mg and placebo IV over 2 minutes on 2 consecutive days. On the next 2 days, they were given ondansetron 40mg and placebo IV over 15 minutes. Subjects clearly discriminated cocaine 20mg from placebo, reported euphoria and showed a pressor response and tachycardia. Under these same conditions ondansetron 40mg could not be distinguished from cocaine by any subjective, behavioral or physiologic measure. These observations are consistent with other studies in animals and humans that indicate no abuse potential for cocaine. There are no contraindications to the further study of ondansetron as a treatment for substance abuse.

AFFILIATION

Department of Medicine (DRJ, MT, JTS) and Department of Psychiatry and Behavioral Sciences (KLP), The Johns Hopkins University School of Medicine, Baltimore, MD 21224

Fluoxetine for Cocaine Abuse in Methadone Patients: Preliminary Findings

Steven L. Batki, Luisa B. Manfredi, James L. Sorensen, Peyton Jacob, Roland Dumontet, Reese T. Jones

Cocaine use is a serious problem among patients in methadone maintenance treatment (MMT) (1) and may be an added risk factor in HIV infection (2). There are no clearly effective treatment strategies for this problem. Group therapy alone may not be effective (3), and current pharmacotherapies, such as desipramine (4), may also have only limited utility. The present study examined the utility of an open trial of fluoxetine added to group therapy.

METHOD: Sixteen cocaine-dependent methadone maintenance patients were treated with a combination of fluoxetine and weekly group therapy. All subjects met DSM-III-R criteria for both opioid and cocaine dependence. Most subjects had serious medical problems, and eleven (69%) were HIV-infected. Nine subjects (56%) were male and 13 (81%) were black Average age was 41 years, average length of heroin dependence and cocaine dependence were 20 years and 14 years, respectively. Ten subjects (63%) had a lifetime history of Major Depressive Disorder. Fluoxetine was administered each morning in an open outpatient nine week trial, with doses ranging from 20 to 60 mg. per day. Average final fluoxetine dose was 45 mg. per day. Average methadone dose was **52** mg. per day.

RESULTS: Analysis of outcome measures showed reduced cocaine use at the end of treatment. Comparison of intake to Week 9 demonstrated a major decrease in cocaine use by self-report, from an average of 14.4 times per week to 1.2 times/wk (p<0.01) (Fig. 1).

Figure 1

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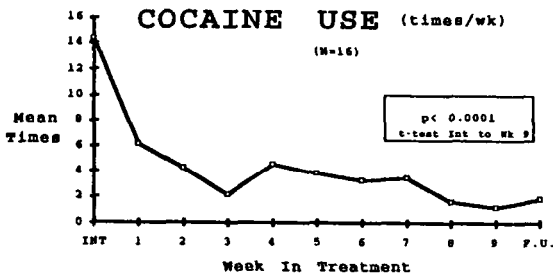
Qualitative analysis of weekly urines showed insignificant decreases in positives for all drugs (from 66% at intake to 53% at Week 9) and in positives for cocaine (from 53% at intake to 40% at Week 9). However, quantitative analysis of urine cocaine and benzoylecgonine (BE) levels revealed significant decreases ($p < 0.01$ and $p < 0.05$, respectively) from intake to Week 9. Plasma cocaine levels also declined significantly ($p < 0.01$).

Figure 2
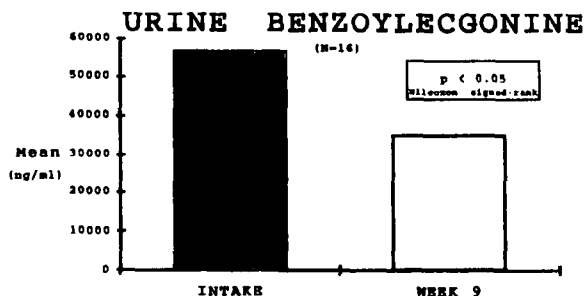
Cocaine craving decreased from 14.2 to 4.1 (possible range = 0 - 24,) a highly significant change ($p<0.0005$). Money reported spent on cocaine decreased from $228 per week at intake to $15 at Week 9. Subjects who still used cocaine at Week 9 reported a decrease in their "high", from 4.6 to 3.1 (range = 0-8) ($p < 0.05$).

Fluoxetine was well-tolerated in combination with methadone. Few adverse effects were noted, and no subjects had to discontinue fluoxetine.

DISCUSSION: In summary, fluoxetine appears to be a promising treatment approach for cocaine-using methadone patients when combined with group therapy, even among a severely ill patient cohort.

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AFFILIATIONS: UCSF Dept. of Psychiatry, San Francisco General Hospital, Substance Abuse Services and Drug Dependence Research Center, Langley Porter Institute.

Identification of a Novel Class of Highly Potent Sigma Ligands Related to the Kappa-Selective Agonist U50,488

Lilian A. Radesca, Brian R. deCosta, Wayne D. Bowen, Lisa J. DiPaolo, and Kenner C. Rice

Sigma receptors are non-opioid, non-phencyclidine (PCP) and non-dopaminergic binding sites for some opiate-, PCP- and D_2-dopamine antagonist-related compounds. Although their functional role is not fully understood, they have been implicated in the mediation of physiological effects which include the regulation of motor behavior, the dystonic side effects of antipsychotic drugs as well as in neuroprotection in ischemia models.

We have recently shown that the *cis* diastereomers (-)-1 and (+)-2 of the kappa-selective agonist U50,488 3, are potent ligands for sigma receptors with K_i values of 81 and 221 nM respectively, in competition with [^{3}H]-(+)-PPP.[1] However, 1 and 2 exhibited weak affinity for kappa receptors. This observation led us to further investigate the structure-activity relationships of 1 and 2.

(-)-1   (+)-2   (±)-3
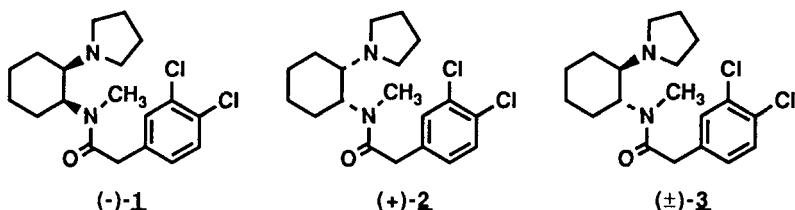
A systematic structure-activity study was performed involving the synthesis of more than 40 compounds.[2] This was done by varying the arylacetyl moiety and leaving the rest of the molecule unchanged. The strategy followed was first to test the racemic analogs for their affinity at sigma and kappa receptors. The enantiomers of the most potent and selective ligands of the series were then examined for their binding at sigma, kappa, PCP and D_2-dopamine receptors. Of the analogs examined, the compound with the highest affinity for sigma receptors was diamine 4 (R=CH$_3$). The 1R,2S-(-)-4 (R=CH$_3$) enantiomer, BD737, showed a K_i value of 1.3 nM and the 1S, 2R-(+)-4 (R=CH$_3$), BD738, one

of 6 nM. Both of them had very little or no affinity for kappa, PCP and D_2-dopamine receptors.

When tested in rats, no behavioral effects were observed after intracerebroventricular (ICV) injection of BD737 and BD738 at concentrations up to 100 nM/rat.[2] However, both BD737 and BD738 showed neuroprotective effects[3] in a spinal cord ischemic injury model.[4]
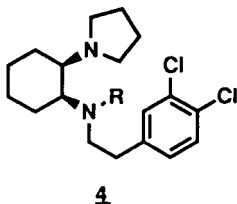
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With these encouraging results, the present study was initiated to determine the influence of the size of the side chain R in (-)- and (+)-4 on this affinity for sigma receptors. Both enantiomers of the R = H, ethyl, propyl and cyclopropylmethyl analogs were prepared and tested for affinity at guinea pig brain sigma receptors using 3 nM [^{3}H]-(+)-PPP as the radioligand. All compounds showed very high sigma receptor affinity with most of the K_i values being lower than 100 nM. The most active analog was found to be the least substituted diamine **4** (R=H) of the 1 R,2S enantiomer with a K_i of 0.51 nM. This compares with values of 3.8, 20.5 and 27.4 nM for the prototypic sigma ligands haloperidol, DTG and (+)-3-PPP, respectively. Affinities of the members of this series for kappa, PCP and D_2-dopamine receptors are currently being determined, and studies in various biochemical, behavioral and neuroprotective assays are in progress.

In conclusion, we have shown that the *cis*-N-[2-(1-pyrrolidinyl)cyclo hexyl]arylethylamines exhibit high affinity for sigma receptors. Furthermore, the highest affinity sigma ligand is to our knowledge the arylethylamine 1R,1S-*cis*-N-[2-(3,4-dichlorophenyl) ethyl]-2-(1-pyrrolidinyl)cyclohexylamine. We hope that this new class of sigma ligands will allow a better understanding of the functional role of these receptors.

Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892 and Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912

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Comparison of Binding Affinities and Adenylate Cyclase Inhibition for the Determination of Efficacy at μ-Opioid Receptors in a Neuroblastoma Cell Line

Lawrence Toll

The potency of an opioid ligand is influenced by several factors. These include lipophilicity, pharmacokinetics, receptor affinity and selectivity, and intrinsic activity, or efficacy. Recent studies have shown that μ agonists with low efficacy may prove promising as analgesics with low abuse liability, or even as candidate drugs to treat drug abuse. Relative efficacy can be determined by measuring some biochemical or physiological response at a known receptor occupancy. It has been difficult to accurately determine efficacies, particularly at the μ receptor for lack of a system in which activity at a single site and occupancy can both be measured under similar conditions. One system in which both parameters can be studied is the μ-containing neuroblastoma cell line SH-SY5Y. In this cell line, both binding affinity and a measure of activity (inhibition of cAMP accumulation) can be measured in intact cells under identical conditions. Furthermore, studies- in intact cells will give information about the affinity of opioid agonists at the receptor under *in vivo* binding conditions.

Affinities of opioid ligands were determined using the μ-selective peptide antagonist [3H]CTOP. Binding was conducted on intact cells attached to 35 mm culture dishes. Incubations were for 1 h at room temperature, at which time the plates were washed with buffer prior to harvesting of the cells for scintillation counting. Naloxone was used to determine nonspecific binding. As a measure of activity, IC_{50} values were determined for opioid inhibition of forskolin stimulated cAMP accumulation in intact cells. These experiments were also conducted at room temperature with cells attached to 35 mm culture dishes.

Binding is rapid, reaching equilibrium within 30 min. and dissociation is rapid, with a $t_{1/2}$ of 5 min. At low [3H]CTOP concentration specific binding is 80-90% of total binding. From saturation analysis it was determined that [3H]CTOP has a Kd of 6.5 nM, and a Bmax of approximately 140 fmol per mg protein. Analysis with the curve-fitting program LIGAND indicated a single binding site, suggesting that binding is to a single low agonist affinity

conformation of the μ receptor. As seen in table 1, the affinity is low for standard μ agonists morphine and DAGO, but high for the potent analgesic etorphine. as well as the partial agonist buprenorphine. the agonist/antagonist nalorphine and the antagonists naloxone and CTOP. Binding does not appear to be to δ or κ,, as the affinity of DPDPE and U50,488 are both quite low.

Forskolin at 10 μM was found to stimulate the cAMP accumulation at least 100 fold in 10 min, and this could be inhibited a maximum of 50-70% by opioid agonists. cAMP accumulation was inhibited a maximum of 25% by buprenorphine. and not inhibited by naloxone. IC_{50} values were determined for the μ agonists morphine, DAGO and etorphine and the nonselective agonist EKC (table 1). In each case IC_{50} values for activity were 6-8 fold lower than IC_{50} values for inhibition of [^{3}H]CTOP binding. This indicates that binding to approximately 15% of the μ receptors will produce a maximum response. As seen in table 1 activation of δ receptors by DPDPE can also inhibit cAMP accumulation.

This study has produced several surprising results. In contrast to findings in other systems, in neuroblastoma cells each of the full agonists have approximately equal efficacy at the μ receptor, as determined by the ratio of binding IC_{50} to activity IC_{50}. This includes the "κ -agonist" EKC, which apparently is a potent agonist as well. These studies also indicate that the high potency of etorphine. and presumably other potent analgesics, is to a large extent due to very high binding affinity even under low agonist affinity conditions.

Table 1. AFFINITIES AND ACTIVITIES OF OPIATES
IN INTACT SY5Y CELLS

| Compound | IC50 (nM) | |
	Binding	Inhibition of cAMP Accumulation
Morphine	300	50
Etorphine	1.3	0.3
DAGO	130	30
EKC	16	2.0
DPDPE	> 100,000	1.5
U50,488	7,500	-
Buprenorphine	0.4	* *
Nalorphine	7.5	* *
CTOP	10.0	* *
Naloxone	1.3	# #

*Inhibited less than 50%.
#No agonist activity.

AFFILIATION

SRI International
Menlo Park, California 94025

In Vitro d-Amphetamine and Cocaine Increase Opioid Binding in Mouse Brain Homogenate

B.C. Yoburn, K. Lutfy, and V. Sierra

Interactions between CNS stimulants and opioids have been reported previously. For example, cocaine and d-amphetamine potentiate the effects of morphine (Nott, 1968; Sasson et al., 1986; Blumberg and Ikeda, 1978) and buprenorphine has been shown to attenuate cocaine self-administration in Rhesus monkeys (Mello et al., 1989). Recently, Ishizuka et al. (1988) and Hammer (1989) have reported that in vivo treatment with cocaine significantly increases binding of ^{3}H-naloxone in certain areas of the brain. In the present study, we report an in vitro method for assessing drug effects on opioid binding.

MATERIALS and METHODS

Yale Swiss-Webster mice were used throughout. In a dose-response study, mice were injected s.c. with saline or 50mg/kg cocaine and 15min later received morphine s.c. (0.25-3.0mg/kg). Animals were tested after 30min for analgesia (tailflick). In binding studies, mice were decapitated, brains removed and homogenized in 10 volumes of 50mM ice-cold potassium phosphate buffer (pH 7.2). Homogenate was brought to 20 volumes with buffer, or 0.1-1mM cocaine or d-amphetamine. Homogenate was incubated (0-90min, 37°C), centrifuged (15,000rpm, 15min) and resuspended twice, incubated (30min, 25°C), centrifuged and resuspended, and then assayed in triplicate for ^{3}H-opioid binding.

RESULTS

Cocaine produced a 3.6-fold increase in morphine's analgesic potency (ED_{50} 2.71, 0.74mg/kg, control & cocaine-treated). In binding studies, cocaine (Fig 1) and d-amphetamine produced a significant (*p<.05) dose-dependent increase compared to control in 1nM ^{3}H-naloxone and ^{3}H-DADLE binding. Desipramine and amitryptiline produced no increase in binding. Displacement studies showed that cocaine displaced 1nM ^{3}H-naloxone and ^{3}H-DADLE at high concentrations (IC50's ≥'7001tk4). Saturation studies using ^{3}H-DAGO after pretreatment of the homogenate with 1mM cocaine or d-amphetamine indicated a significant increase in µ opioid receptor density (25% and 30%, respectively) with no

change in K_d. Time course studies indicated that both cocaine and d-amphetamine prevent a decline in binding during preincubation as compared to control.

DISCUSSION

Cocaine enhanced morphine's analgesic effects and produced an increase in opioid binding in mouse whole brain homogenate. The method described here for preincubation of homogenate with cocaine or d-amphetamine is an approach that allows <u>in vitro</u> experimentation for the interactions between cocaine, d-amphetamine and opioid binding sites. These results suggest the possibility that the potentiation of morphine analgesia by cocaine and d-amphetamine may be mediated, in part, through modulation of opioid receptor density. Furthermore, it is likely that the apparent increase in receptor number after <u>in vitro</u> exposure to cocaine or d-amphetamine is due to a protective effect of these agents; although the mechanism of this action is not known. Cocaine and d-amphetamine may prevent degradation of receptors during incubation.

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AFFILIATION
College of Pharmacy, St. John's University, Queens, NY 11439.

Figure 1
Effect of Cocaine on Brain Opioid Binding
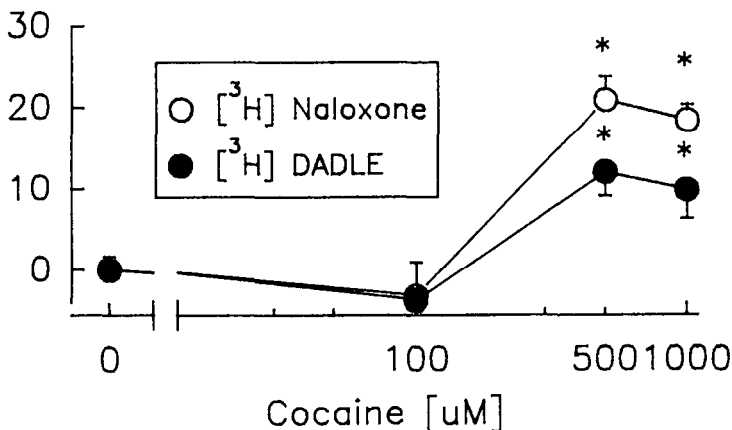
Opiate-Induced Inhibition of Calcium Flux in Immune Cells

Yoshitatsu Sei, Todd D. McIntyre, Ester Fride, Kohji Yoshimoto, Phil Skolnick, and Prince K. Arora

An increased risk of infection has been reported in intravenous substance abusers (1-4). While the sharing of injection paraphernalia and other substandard hygienic practices undoubtedly contribute to this phenomenon, the immunosuppressive effects of the abused substance may also increase susceptibility to infection. In animal models, both acute and chronic administration of morphine has been reported to effect a wide range of immunological changes including a decreased lymphocyte content in spleen and thymus, inhibition of mitogen-stimulated T and B cell responses, altered antigen-specific antibody production, and atrophy of the spleen and thymus (5-8). These findings are consistent with reports that opiate addicts have a depressed number of total T cells and a depressed lymphocyte proliferative responses (9-10). The sequence of cellular events mediating this opiate-induced immunosuppression has not been deduced. In the present report, we demonstrate a robust suppression of Ca^{2+} influx in CD4+ T cells and B cells, mediated by glucocorticoid-dependent and -independent mechanisms, respectively.

MATERIALS AND METHODS

Animals. C57BL/6J mice (6-8 weeks old) were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in 32 x 25 x 15 cm transparent plastic cages with woodchip bedding material and maintained in a temperature and humidity controlled facility on a 0600 - 1800 lights-on schedule. Rat chow and water were freely available. All animals were treated according to NIH-AALAC guidelines.

Dexamethasone (DEX) treatment. Animals were injected i.p. with DEX (10mg/kg) (Sigma, St. Louis, MO) in 60 % ethanol/saline or 60% ethanol/saline as vehicle control.

Morphine pellet implantation and adrenalectomy. Animals were given subcutaneous implants of either a single 75-mg morphine pellet (National Institute on Drug Abuse, [NIDA], Rockville, MD) or a single

microcrystalline cellulose placebo pellet (NIDA) (11). In some experiments, the subcutaneous implantation of either a single 10-mg naltrexone pellet (NIDA) or a single cholesterol-tristearin placebo pellet (NIDA) was additionaly employed. At 24, 48, 72, or 96 hr following implantation of either the placebo or morphine pellet, spleen cells were prepared for mitogen-stimulated Ca^{2+} influx assay. In some experiments, animals were subjected to either adrenalectomy (Adx) or sham-operation (adrenal glands were touched following laparotomy) and immediately implanted with morphine or placebo pellet implantation under pentobarbital anesthesia.

Ca^{2+} influx assay. Ca^{2+} influx was directly measured by changes in the fluorescence intensity of fluo-3 loaded cells (12-14). Cells (2 x 10^6/ml) were loaded with 1 µM fluo-3 as the acetoxy methyl ester (Molecular Probes, Eugene, OR) by incubation in subdued light (60 min; 25° C). The cells were subsequently washed twice with Hanks' balanced salt solution (HBSS), and stained with either Phycoerythrin (PE)-conjugated L3T4 (CD4) mAb (Becton-Dickinson, Mountain View, CA), or Lyt2 (CD8) Biotin/Avidin PE mAb (Becton-Dickinson), or PE-conjugated Thy 1.2 (Caltag, San Francisco, CA). The cells were washed an additional two times with HBSS and analyzed by flow cytometry using FACSCAN (Becton-Dickinson). In each experiment, the fluo-3 loaded cells were analyzed to obtain an unstimulated baseline. Cells (10^4) were analyzed every 60 sec after the addition of Con A (2.0µg/ml) + $CaCl_2$ (1 mM) at rates of 400 - 1000/sec. fluo-3 histograms of PE_+ cells were obtained by gating PE positive clusters in a flue-3 vs PE dot-plot display for analysis of fluo-3 shift in CD4+ or CD8+ cells. The percentage of responding fluo-3+ cells was then calculated and analyzed.

RESULTS

Morphine-induced depression of mitogen-stimulated Ca^{2+} influx: time course. At 24, 48, 72, or 96 hr following implantation, spleen cells were analyzed for mitogen-stimulated Ca^{2+} influx assay. There were significant reductions in Ca^{2+} influx in B cells at both 24 hr [52 % reduction relative to placebo, $p< 0.005$] and 48 hr [53% reduction. $p<0.0005$] following implantation of a morphine pellet. No significant suppression of Con A-induced Ca^{2+} flux in B cells was found at 72 and 96 hr post-implantation. Ca^{2+} influx in the CD4+ T cell population was also significantly inhibited at 24 [42% reduction, $p< 0.03$] and 48 hr [73% reduction, $p< 0.004$]. However, there was no significant change in Ca^{2+} influx in the CD8+ T cells during the observation period.

Serum corticosterone level following implantation of morphine pellet. Serum corticosterone levels increased significantly 24 and 48 hr following morphine pellet implantation. At 48 hr post implantation, the

levels were 58.1±13.1 and 3.5 ± 0.9 µg/dl in the morphine and placebo groups, respectively (p< 0.004, Students' t-test).

Effects of dexamethasone treatment on Ca^{2+} influx. A significant inhibition of Ca^{2+} influx in CD4+ T cells was seen 48 hr following injection of dexamethasone (10 mg/kg) compared to a placebo group (% responding cells: 8.7±2.5% versus 17.3±1.5%, p< 0.04). Administration of dexamethasone, however, did not affect mitogen-stimulated Ca^{2+} influx in either CD8+ or B cells.

Effects of adrenalectomy on morphine-induced inhibition of Con A-stimulated Ca^{2+} influx in B and CD4+ T cells: Mice were either bilaterally adrenalectomized or sham operated prior to implantation of either a morphine or placebo pellet. Two-way analysis of variance (ANOVA) revealed significant effects of morphine on Ca^{2+} influx in B cells (F=31.8; df=1, 25; p<0.0005), but no significant operation (adrenalectomy) effects (F=0.4; df=1, 25; NS), and no significant morphine by operation interaction (F=0.01; df=1, 25; NS). In contrast, adrenalectomy abolished morphine-induced inhibition of Ca^{2+} influx in CD4+ cells. ANOVA revealed a significant effect of morphine (F=24.3; df=1, 25; p<0.0005), a significant operation effect (F=21.3; df=1, 25; p<0.0005), and a significant morphine by operation interaction (F=5.1; df=1, 25; p< 0.04). Single degree of freedom comparisons showed that morphine suppressed Ca^{2+} influx in CD4+ T cells (morphine: 6.8±3.4 vs 16.0±3.4 in the placebo group, p<0.05, Fisher's LSD) but had no effect in adrenalectomized mice (15.6±2.0 vs placebo; NS).

Effects of naltrexone administration on Ca^{2+} influx and serum corticosterone level. Naltrexone blocked morphine-induced inhibition of Ca^{2+} influx in B cells (F=7.2, df=1, 13; p< 0.02, ANOVA). Naltrexone itself had no effect on Ca^{2+} influx in B cells. Individual comparisons indicated that morphine suppressed Ca^{2+} influx in B cells (% responding cells: 13.6±1.0 vs 30.1±1.4 in the placebo group, p< 0.05) but morphine had no effect in mice treated with naltrexone. In contrast, naltrexone had little or no effect on morphine-induced inhibition of Ca^{2+} influx in CD4+ T cells (F=0.6; df=1, 13; NS, ANOVA). Naltrexone itself had no effect on Ca^{2+} influx in CD4+ cells. Naltrexone blunted but did not abolish the morphine-induced increase in serum corticosterone levels (naltrexone+morphine=17.4±7.4. versus placebo 3.5±0.9µg/dl).

DISCUSSION

Male C57BL/6J mice were subcutaneously implanted with a single morphine pellet. This procedure has been extensively employed as a model to study the effects of morphine in vivo, since it rapidly induces tolerance and physical dependence (7, 11). Morphine produced a robust inhibition of

Concanavalin A (Con A) stimulated-Ca^{2+} influx into B (Thy1.2-) and CD4+ (L3T4+) cells within 24 hr after implantation. This inhibition was fully manifest in both B and CD4+ T cells by 48 hr, and started to return to control values by 72 hr after implantation. In contrast, Con A-stimulated Ca^{2+} influx in CD8+ cells was unaffected by morphine, indicating a differential sensitivity of CD4+ and CD8+ cells, which may lead to a functional reversal of the CD4+/CD8+ ratio in vivo. Unresponsiveness of CD4+ cells could explain the reduction in mitogen-stimulated lymphocyte proliferation in morphine-treated mice if cellular events subsequent to protein kinase-C activation are not differentially affected.

Since high affinity, stereospecific opiate binding sites are present on immune cells (15, 16), experiments were performed to determine whether similar effects could be obtained by incubating lymphocytes with morphine in vitro. Exposure of lymphocytes in culture to morphine (10^{-8} - 10^{-4} M) for various periods up to 24 hr did not affect mitogen-stimulated Ca^{2+} influx in either CD4+ or B cells. The results suggest that the effects of morphine on Ca^{2+} influx in lymphocytes is not mediated through a direct action on opiate receptors in these cells. Morphine administration has been reported to produce an activation of the hypothalamic-pituitary-adrenal (HPA) axis (17), resulting in a profound increase in serum corticosterone levels.   We have also seen that serum corticosterone levels increased significantly 24 and 48 hr following morphine pellet implantation.

To determine whether this increase in circulating corticosterone was responsible for the morphine-induced inhibition of Ca^{2+} influx, mice were either adrenalectomized or sham operated prior to morphine or placebo pellet implantation. Adrenalectomy abolished the morphine-induced inhibition of Ca^{2+} influx in CD4+ T cells, but did not diminish this effect in B cells. Moreover, a significant inhibition of Ca^{2+} influx in CD4+ T cells was also seen 48 hr following injection of dexamethasone (10 mg/kg) compared to a placebo group. Administration of dexamethasone, however, did not affect mitogen-stimulated Ca^{2+} influx in either CD8+ or B cells. These results strongly support the hypothesis that morphine-induced inhibition of Ca^{2+} influx in CD4+ T cells may be mediated by an increase in the circulating levels of corticosterone. Since glucocorticoid-inducible protein, lipomodulin, has been shown capable of inhibiting phospholipase C activity (18), the inhibition of phosphatidylinositol (PI) hydrolysis may account for glucocorticoid-mediated inhibition of Ca^{2+} influx in CD4+ cells. Our results suggest that the effects of morphine on inhibition of Ca^{2+} influx are mediated through glucocorticoid - dependent and - independent mechanisms in CD4+ and B cells, respectively.

While in vitro incubation of lymphocytes with morphine did not affect Ca^{2+} influx in either B or CD4+ cells, administration of the opiate antagonist, naltrexone blocked morphine-induced inhibition of Ca^{2+} influx in B cells, suggesting that this action may be opiate receptor mediated, possibly at sites in the central nervous system (CNS). In contrast, while the morphine-induced activation of the HPA axis may be mediated via opiate

receptors in the CNS, naltrexone had little or no effect on morphine-induced inhibition of Ca^{2+} influx in CD4+ T cells. Naltrexone blunted but did not abolish the morphine-induced increase in serum corticosterone levels. Thus, naltrexone used in these studies was not sufficient to completely abolish the elevated serum corticosterone levels produced by morphine, while it was sufficient to minimize neurological signs (hyperactivity, jumping, etc) of morphine-dependence evaluated by naloxone challenge.

Ca^{2+} influx has been proposed to be one of the early events in a series of intracellular processes which culminate in lymphocyte activation and proliferation(19). Our findings suggest that the morphine-induced inhibition of Ca^{2+} influx in immune cells may be one of the early events mediating opiate-induced immune suppression.

SUMMARY

Administration of morphine as a subcutaneous implant inhibits the initial influx of calcium (Ca^{2+}) induced by mitogens in mouse splenocytes. This effect was not reproduced by incubation of splenocytes with morphine (10^{-8}-10^{-4}M). Within T cell subpopulations, CD4+, but not CD8+ cells were affected. Adrenalectomy abolished this effect of morphine in CD4+ but not B cells. Moreover, simultaneous administration of the opiate antagonist naltrexone blocked the effect of morphine in B cells, but not in CD4+ cells. These data indicate that inhibition of Ca^{2+} influx by morphine may be mediated through distinct glucocorticoid-dependent and independent mechanisms. The morphine-induced inhibition of Ca^{2+} influx in immune cells reported here may be an early event mediating opiate-induced immunosuppression.

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AFFILIATON

Laboratory of Neuroscience, NIDDK
Building 8, Room 111
National Institutes of Health
Bethesda, Maryland 20892

The Effect of Morphine Tolerance-Dependence and Abstinence on mu, delta, and kappa Opiate Receptors of Discrete Brain Regions ans Spinal Cord of the Rat

Hemendra N. Bhargava and Anil Gulati

INTRODUCTION

Since the discovery and characterization of opiate receptors in mammalian systems, attempts have been made to implicate them in processes leading to the development of tolerance and physical dependence on morphine. The results, however, have been inconsistent. Some of the reasons which may account for the divergent results are: (a) the animal species used, (b) the method of delivering morphine and thus the degree of addiction, (c) whether or not the animals were undergoing withdrawal, (d) the use of tissue homogenate or the slices, and (e) the specificity of the opiate agonist or antagonist ligand used to characterize the specific receptor systems (please see Bhargava and Gulati 1990b). Majority of the studies have been done on whole brain of nonabstinent rats or mice. The binding of opiate ligands was found to be increased (Pert and Snyder, 1976; Rothman et al. 1987) or decreased (Davis et al. 1979) in the rat. More recently, in the cortex of morphine tolerant guinea pig, the binding of ^{3}H-DAMGO was found to be decreased (Werling et al 1989). In this report, the effect of morphine tolerance-dependence and abstinence on the binding of ^{3}H-DAMGO, ^{3}H-DSTLE and ^{3}H-EKC (following suppression of μ and δ sites) to seven brain regions and spinal cord of the rat has been described.

METHODS

Male Sprague-Dawley rats were rendered tolerant to and physically dependent on morphine by implanting 6 morphine pellets (75 mg in each pellet) for 7 days. Rats serving as controls were implanted with 6 placebo pellets (Bhargava and Gulati 1990a). Opiate receptors were characterized in tolerant-dependent (pellets left intact) and abstinent (pellets removed) rats. The binding of ^{3}H-DAMGO, ^{3}H-DSTLE and ^{3}H-EKC to membranes prepared from discrete brain regions (hypothalamus, hippocampus, amygdala, cortex, pons and medulla, midbrain and striatum) and spinal

cord was carried out as described earlier (Bhargava et al. 1989).

RESULTS

The binding of ^{3}H-DAMG0 to μ opiate receptors on cortex, pons and medulla and spinal cord membranes of morphine tolerant rats was decreased. This change was due to reduction in B_{max} values rather than the K_d value. The binding of ^{3}H-DAMGO to spinal cord and brain regions of morphine-abstinent rats did not differ from placebo abstinent rats. The binding of ^{3}H-DSTLE to δ opiate receptors on cortex of morphine tolerant rats was lower than placebo tolerant rats. The decrease was due to changes in the B_{max} value; the K_d value remained unchanged. δ-Opiate receptors of spinal cord or brain regions of morphine abstinent rats did not differ from placebo controls. The binding of ^{3}H-EKC to k-opiate receptors in brain regions or spinal cord of morphine tolerant or abstinent rats did not differ from their respective placebo controls.

DISCUSSION

The studies presented here clearly indicate that the development of tolerance to morphine in the rat is associated with down-regulation of μ-opiate receptors in discrete brain regions (cortex and pons and medulla) and spinal cord, and of δ opiate receptors of the cortex. In both cases the number of binding sites were decreased but the affinity or the apparent dissociation constant of the ligand to bind to the receptors did not change. k-Opiate receptors of brain regions and spinal cord were unaffected in morphine tolerant rats. The down-regulation of μ-receptors in cortex in the present study is consistent with the study of Werling et al. (1989) in guinea pigs and of Davis et al. (1979) and Rogers and El-Fakahany (1986) in the rat. The reduction in μ receptors appears to be due to their uncoupling from associated G proteins. These results are at variance with those of Pert and Snyder (1976) and Rothman et al. (1987) who found an upregulation of μ-receptors in the whole brain of morphine tolerant rats. In the present studies s-opiate receptors labeled by ^{3}H-DSTLE of cerebral cortex of morphine tolerant rats were down-regulated. This observation is consistent with results of Law et al. (1983) who demonstrated that chronic exposure of NG 108-15 neuroblastoma-glioma hybrid cells to etorphine down-regulates δ opiate receptors. In conclusion, morphine tolerance appears to be due mainly to down-regulation of μ-opiate receptors of spinal and supraspinal structures and of a δ-opiate receptors of cerebral cortex, but the abstinence from morphine does not affect μ, δ and k-opiate receptors in the rat (supported by DA-02598).

References will be furnished on request.

AFFLIIATION

Department of Pharmacodynamics
University of Illinois
Chicago, IL

Sustained Release Injectable Naltrexone Microcapsules

E.S. Nuwayser, D.J. DeRoo, P.D. Balskovich, and A. G. Tsuk

Chronic administration of opioid antagonists is one of two major pharmaco-therapeutic approaches used in treatment regimens for post addicts. Naltrexone, the only opioid antagonist currently approved by the FDA for treatment of post addicts, is taken orally 3 times per week. A high drop out rate, skipping doses to permit a "high", and the inconvenience of a rigid oral dosing schedule are major problems.

BIOTEK is developing a sustained action formulation of naltrexone based on microcapsules which release the drug over a period of 30 days. Naltrexone free base was encapsulated with poly-L-(-)lactide-co-glycolide (PLA-PGA, 65/35 mole ratio) biodegradable polymer using BIOTEK's microfluidized bed coating process. In vitro release studies of the naltrexone free base microcapsules in pH 7.4 buffered saline showed the microcapsules released naltrexone base for 55 days by zero order kinetics.

Groups of five mice treated with naltrexone microcapsules were challenged with 5 mg/kg morphine sulfate by the s.c. route. Tail flick latencies indicated complete antagonism to the opioid for a period of 24 days. Opioid antagonism then declined rapidly and was not observable on day 25. As expected, tolerance developed when mice were treated with morphine alone in parallel with naltrexone microcapsule treated mice (morphine controls). Agonist scores fell to 20 by day 18. A group of 5 naltrexone control mice. was pretreated with naltrexone hydrochloride, 1 mg/kg, s.c. 10 minutes before each morphine challenge. This treatment fully antagonized any morphine effect on tail flick latency. To test for tolerance development, the naltrexone control mice received naltrexone hydrochloride immediately after morphine treatment on days 4, 14, and 21. These unprotected challenges gave agonist scores of 100 compared to 20 in the morphine control mice. These data suggest that when naltrexone is present to antagonize the effects of a 5 mg/kg, s.c. morphine dose, tolerance development does not confound the opioid antagonism assay.

A batch of naltrexone free base microcapsules was administered to 3 New Zealand white rabbits by the subcutaneous route at a dose of 400 mg/kg of naltrexone free base. Plasma naltrexone levels reached a peak within a few hours after injection but just as rapidly fell to a steady state level of about 100 ng/ml after 24 hours and remained at this level for a period of 37 days. The dose requirement to achieve 100 ng/ml naltrexone plasma level in rabbits would

suggest that the dose of naltrexone microcapsules required to maintain 2 ng/ml therapeutic plasma levels in humans for one month may be less than 8 mg/kg of naltrexone free base.

In vivo release of naltrexone was followed after intramuscular administration of 27.65 mg/kg of naltrexone from a microcapsule suspension to 3 monkeys. Plasma naltrexone hydrochloride reached a mean peak level of 24 ng/ml after 4 days. In all three animals the concentration of naltrexone hydrochloride reached a steady state level between 8-16 ng/ml for a period of 32 days. The level slowly fell from day 32 to day 55 reaching the therapeutic level of 2 ng/ml between day 42 and 45 (6 weeks after naltrexone administration). The monkey studies suggest a dose of 4.6 mg naltrexone free base would be required to maintain 2 ng/ml plasma concentration for one month. The treated monkeys were also challenged with morphine sulfate at two week intervals starting two weeks before naltrexone microcapsule administration and for 10 weeks thereafter. The monkeys showed the usual behavioral syndrome (ataxia, body sag, jaw sag, drowsiness, slowing, and scratching) when challenged with morphine 2 weeks before naltrexone. Morphine challenges 2 and 4 weeks after naltrexone elicited very few signs suggesting complete block. Six weeks after naltrexone, the monkeys began showing some signs especially scratching. Then, the 8 and 10 week morphine challenge elicited the full syndrome. It would seem that the naltrexone preparation was effective for approximately 6 weeks and perhaps longer.

Tissue irritation was reported to be a significant problem in a recent clinical study of naltrexone sustained release beads. Therefore mice and rabbits treated with PLA-PGA microcapsules were sacrificed at the conclusion of the in vivo duration study. Injection sites were dissected and examined. There were no gross signs of tissue irritation such as edema or erythema. During the study mice showed no clinical signs of irritation or sensitivity at the injection site.

CONCLUSIONS - Naltrexone free base microcapsules were prepared from poly-(L-)lactide-co-glycolide (PLA-PGA, 65/35 mole ratio) polymer. A zero order release profile for the drug was obtained from the microcapsules in vitro in pH 7.4 buffered saline at 37°C for a period of 55 days. In vivo evaluation of the microcapsules was performed in mice, rabbits, and rhesus monkeys. Tail flick latency studies in mice indicated complete antagonism to morphine sulfate challenge for 24 days. Plasma naltrexone hydrochloride concentration in rabbits reached steady state level 24 hours after s.c. injection of the microcapsules and remained steady for a period of 37 days. There was no evidence of tissue irritation in mice or rabbits. Similar pharmacokinetic studies in 3 rhesus monkeys gave steady state level from day 4 through day 32 after injection. The level slowly fell thereafter reaching a therapeutic level of 2 ng/ml after 42 days. Complete block of morphine sulfate challenges at a dose of 3 mg/kg was observed for a period of 6 weeks after naltrexone microcapsule administration. The above results suggest that the BIOTEK microcapsule formulation will block opioid effect for a minimum period of 30 days.

Acute Effects of Secobarbital and Ethanol as a Function of Family Alcoholism History

Mary E. McCaul, Jaylan S. Turkkan, Date S. Svikis, and George E. Bigelow

Response differences following administration of ethanol have been demonstrated between males with a positive (FHP) versus negative (FHN) family alcoholism history, possibly reflecting differences in vulnerability to alcohol dependence. It is of interest to determine whether FHP subjects also show increased risk for other drug dependencies. Specifically, the present study examined responses to secobarbital, a drug cross-tolerant with ethanol. Dose-effect functions were determined for a variety of physiological (heart rate, skin conductance), subjective (analog scales, Subjective High Assessment Scale), and psychomotor measures (tremor, body sway, hand-eye coordination, DSST) in FHP and matched FHN males following administration of secobarbital (0, 100 and 200 mg p.o.) and ethanol (1 g/kg p.o.). At equivalent blood alcohol levels FHP subjects reported greater effects of ethanol than did FHN subjects on almost all subjective measures. Following the high dose of secobarbital, FHP but not FHN subjects showed elevated subjective responses; these effects were substantially less and were evident in fewer measures than following ethanol. In contrast to effects on the subjective measures, ethanol and secobarbital produced comparable impairment in both groups of subjects for most psychomotor responses. Group differences were not obtained on any physiological measures. These data suggest that subjects with a family alcoholism history demonstrate a unique subjective response to alcohol that may partially generalize to other drug classes.

AFFILIATION

Department of Psychiatry and Behavioral Sciences
The Johns Hopkins University School of Medicine,
and The Department of Psychiatry
The Francis Scott Key Medical Center
Baltimore, Maryland

Neurofunctional Consequences of in Utero Cocaine Exposure

**Bernard Z. Karmel, Judith M. Gardner, and
Catherine L. Magnano**

As part of an ongoing project to study infants at risk for CNS in-
sults, we have been studying infants assigned to the Neonatal In-
tensive Care Unit (NICU) on a variety of clinical and experimental
neurofunctional procedures. Each infant is evaluated after 30 wks
gestational age (GA) with brainstem auditory evoked responses
(BAERs) that are repeated serially (if necessary). Just prior to
discharge, we evaluate infants with a neurobehavioral (NB) exam
and with an experimental visual preference (Vpref) task as a func-
tion of different levels of arousal. Stimuli for Vpref are un-
patterned lights presented at 2, 4, and 8 Hz. Infants view all
possible pairs of stimuli for 20 sec each in 3 arousal conditions:
(1) less aroused - swaddled after feeding; (2) more aroused:
endogenous - unswaddled before feeding; and (3) more aroused:
exogenous - swaddled after feeding but receiving additional visual
stimulation at 8 Hz prior to the onset of each trial. We also
measure infants' salivary cortisol responses under three specific
conditions reflecting different degrees of stress (basal, 30 min
after NB exam, and 30 min after heel prick for blood sample). We
review infants' EEGs (if performed) and brain imaging films (typi-
cally cranial ultrasounds (US) but also CT scans and MRIs), and
relate diagnoses to our BAER, NB, Vpref and cortisol findings
(Gardner et al, 1990: Devel Psych, 26; Karmel et al., 1988: EEG &
clin Neurophysiol, 71; Magnano et al., 1990: Child Devel, 60).

This report describes contrasts between a group of infants as-
signed to the NICU (n=21) known to have been cocaine-exposed (CE)
in-utero who were assessed in a manner similar to other NICU in-
fants (non-CE; n=99). Analysis of demographic data indicated that
birthweight (BW), GA, head circumference (HC), and body length
(BL) were lower in CE than in non-CE groups with approximately 90%
of CE vs 70% of non-CE being below the 50th percentile. Incidence
and severity of CNS pathology were comparable between the CE and
non-CE populations. BARR components after Wave I when evaluated
in the sub-population of infants without US-detected abnormalities
showed tendencies toward neuronal transmission speeds that were
markedly faster than normal. This finding is similar to effects
seen in experimentally-induced IUGR in fetal sheep (Gluckman et
al., 1988, In C.T. Jones (Ed.): Perinatology Press, 220-228).

NB evaluation indicated a higher proportion of motor problems than found in normal infants, but the distribution of abnormalities was comparable to that found in non-CE NICU infants as a whole with the exception that CE infants tended to demonstrate less head lag and hypotonicity in the upper extremities and more hypertonicity (and tremors) in both upper and lower extremities. We observed little or no incidence of the state control or feeding problems that others have reported.

On the other hand, CE infants showed diminished arousal and stress responses compared to non-CE infants. Manipulation of arousal level caused differential Vprefs to occur in a markedly atypical manner, indicating differences both from normal infants and from infants who suffered CNS structural damage. Since the Vpref data for the 2 more aroused conditions were the same, they are combined in the figure below. Normal infants show a marked interaction in their Vpref curves as a function of arousal level. When less aroused, they prefer more stimulation (the fastest frequency). When more aroused, they prefer less stimulation (the slowest frequency). Neither severely insulted non-CE infants nor CE infants showed this degree of modulation of attention with arousal. Infants with severe CNS involvement tended to prefer slower frequencies even when less aroused. In contrast, CE infants showed the opposite behavior, tending to prefer faster frequencies even when more aroused.

Salivary cortisol findings were consistent with arousal effects on attention. Although CE infants showed similar basal cortisol levels as non-CE infants, they showed suppressed responses (diminished increases) to both stressors (see figure below). No sex differences or interactions with sex were found.

We conclude that one primary effect of CE during pregnancy, documented in NICU infants, is on arousal and attention mechanisms as indicated by Vpref and cortisol responses to stimulation or stress. However, it is uncertain whether our demographic, BAER, US, and NB findings are due to direct or indirect effects of CE.
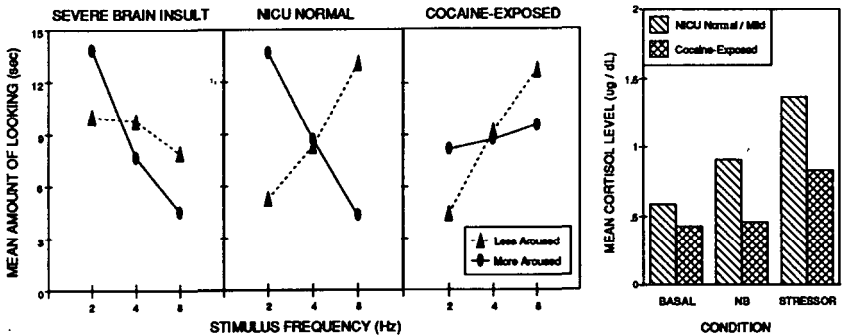
AFFILIATION: New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, 10314, USA; and St. Vincent's Medical Center of Richmond County, Staten Isl., NY.

New Trends in Infant Pain Management: Potential for Opiate Dependence

C.R McLaughlin, J.G. Hull, and C.P. Cramer

The area of neonatal pain and pain management has recently become an important and controversial topic. A review of the literature suggests a limited use of analgesics in the management of pain in neonates. This controversy caused us to survey neonatologists in the United States on their attitudes and practices in this area.

Our sample was selected from the Compendium of Medical Specialties (2nd Ed., 1988/1989). We sent surveys to those physicians who were board-certified in neonatal-perinatal medicine and who had completed a fellowship in neonatology (n = 976). A total of 362 surveys were completed and returned. Taking into account the 100 returned as undeliverable, this resulted in a 41% response rate.

Eighty-seven percent of the respondents indicated that attitudes about pain and pain management in neonates and infants have changed recently. When asked specifically whether very premature infants (gestational age < 28 weeks) feel pain, 22 responded "no" or were not sure while, 328 indicated "yes." When asked about neonates greater than one week of age, all respondents indicated that these infants were capable of feeling pain. These responses are in marked contrast to earlier surveys of this type (Purcell-Jones et al., 1988). Several questions in our survey addressed the assessment of pain in neonates. Our respondents indicated that it was difficult to assess pain in very premature infants (gestational age < 28 weeks). They cited increases in heart rate and blood pressure as being the best indices. The respondents indicated that pain assessment was somewhat easier in older infants (1-12 months postnatal age), however, and suggested that behavioral factors, such as crying and facial expression seem to gain in importance for this age group.

We next inquired about medicating practices during the intraoperative and post-operative period. These categories were further subdivided into major (intracavity, abdominal or thoracic procedures) and minor (hernia repair, orchidopexys and other short procedures) surgeries. Almost all of the respondents reported using anesthesia during the intraoperative period regardless of surgery type, major or minor. Again, this is in contrast to earlier reports. There were slight increases in reported anesthesia and analgesia use in older infants. Most of the variability in reported analgesia use, however, was in the post-operative period and 23 respondents, 8% of those adding written comments to their surveys, specifically cited the use of opiates in their units.

The more respondents agreed that physiological stress associated with pain can be more dangerous than analgesia, the more likely they were to strongly endorse using analgesia in the post-operative period. The small minority of respondents who indicated that analgesia is too dangerous to use in neonates, on the other hand, report using less medication in the post-operative period. Finally, it is interesting to note, that there were no gender effects in any of the survey questions.

Five survey questions were combined to form a composite variable "less pain." As the name implies, all of these questions assessed whether the respondents believed that neonates experienced pain to a lesser degree, less intensely or for a shorter duration than adults. Respondents who indicated that neonates feel less pain tend to see fewer signs of pain and report using less medication in the post-operative period. Conversely, respondents who indicated that neonates do not feel less pain (the majority of respondents) tended to see more signs of pain and reported using more medication in the post-operative period.

In conclusion, our results suggest that both attitudes and practices have changed in the area of neonatal pain and pain management. Almost all respondents indicated that even the youngest infants feel pain and most employed anesthesia during the intraoperative period. The variability in attitudes toward pain experiences in neonates is associated with variability in reported post-operative medicating practices and a review of the survey comments and the current literature suggests that opiates are the drugs most commonly employed in the post-operative period. Finally, the implications of these findings merit further investigation into the development of opiate dependence in infants.

Virginia Commonwealth University, Richmond, VA and Dartmouth College, Hanover, NH.

Cocaine, Catecholamines and Cardiac Toxicity

**Gabriel G. Nahas, Renaud Trouvé, and
William M. Manger**

In the awake Sprague Dawley rat, fitted with an intra-arterial caudal catheter, selected calcium antagonists (nitrendipine, nicardipine, diltiazem, flunarizine) will act as antidotes to a lethal dose of cocaine (60 mg/kg I.P.). This dose will produce in control animals behavioral and cardiovascular anomalies as well as convulsions and death within an average time of 10 min. Antidotes are administered 5 minutes after the lethal dose of cocaine. Besides selected calcium antagonists, a converting enzyme inhibitor enalaprilat, associated with diazepam is also an antidote to lethal cocaine intoxication. In the present study, the effects of a lethal dose of cocaine on plasma catecholamine concentration was investigated in the rats untreated or treated with either nitrendipine or enalaprilat associated with diazepam. Twenty-four fasting rats (295±28g) are fitted under pentobarbital anesthesis with a catheter in the caudal and carotid arteries. The caudal catheter is connected to a constant microinfusion pump and to a recorder for on line recording of blood pressure which is processed by microcomputer for on line display of heart rate and pulse pressure. Four groups of 6 animals are studied as follows:

	↑		↑		↑		↑
TIME	0		4'		5'		10'
TREATMENT							
	I = SALINE I.P.			I,II = SALINE I.A.			
	II,III,IV = COCAINE I.P.			III = Nitrendipine I.A.			
	(60 mg/kg)			IV = Enalaprilat +			
				Diazepam I.A.			
			↑				↑
BLOOD SAMPLING			A				B

Group I (control) is administered saline I.P. and 5' later saline intra-arterially (I.A.). Groups II. III and IV are administered 60 mg/kg cocaine I.P. Five minutes after, group II is administered I.A. saline; group III nitrendipine I.A. (7.2μg loading dose and 1.2μg/kg/min); group IV diazepam (0.7 mg/kg) and enalaprilat I.A. (0.3mg/kg). Two 0.8ml samples of blood are withdrawn from

the carotid artery in all groups: one (A), 4' after saline or cocaine administration and the second (B) 5' after treatment with saline or antidotes. Volume of blood removed is replaced with dextran. Measurements of catechols are performed by the radio-enzymatic method of Peuler and Johnson. Results are summarized in the following table (pmoles/ml):

Catechol	Group I	Group II	Group III	Group IV
(A)Dopa	91±30	95±22	185±129	226±22
(B)Dopa)	100±42	223±82	188±58	239±114
(A)Epi	364±83	579±255	775±778	572±419
(B)Epi	1368±753*	2430±649**	1318±36	1264±883
(A)Nore	323±195	603±121	673±380	776±508
(B)Nore	704±201**	1538±702***	1223 ±551	1475±700
Signif. of	*p<0.015	*p<0.006		
paired	**p<0.002	**p<0.002		
t-test		***p<0.04		

Catecholamine concentrations were compared between groups (analysis of variance). These concentrations, especially of epinephrine, were significantly higher in group I as compared to group II ($p<0.003$), indicating that cocaine releases catechols from the adrenal gland. Four minutes after cocaine interaction, epinephrine concentration (A) was not significantly different in group II, III and IV. Following treatment (B) either with nitrendipine (III) or with enalaprilat and diazepam (IV) epinephrine was significantly lower ($p<.02$) than in the untreated rat (II) and similar to control (I).

As previously reported, rats administered cocaine had increases in blood pressure, arrhythmias and at autopsy myocardial lesions. Administration of the previously defined antidotes to cocaine restored blood pressure and cardiac rhythm to normal, and prevented the occurrence of morphologic changes in the heart.

Central and peripheral stimulation by cocaine of the sympathetic and renin-angiotensin systems appears to be a major component of the acute toxicity of this alkaloid, which produces the uncontrolled escape of physiological mechanisms useful in normal regulation. Antidotes to cocaine will correct this drug induced deregulation.

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AFFILIATION

Department of Anesthesiology
Columbia University College of Physicians and Surgeons
New York, NY 10032

Chronic Cocaine Administration and Withdrawal from Cocaine Modify Central Neurotensin Receptors in Rats

N.S. Pilotte, W.M. Mitchell, L.G. Sharpe, E. De Souza, and E.M. Dax

Neurotensin (NT) is a peptide in part colocalized with dopamine (DA) within the midbrain and the direct application of NT there activates these mesocorticolimbic DA neurons. The mesocorticolimbic DA neurons have been implicated as essential in maintaining substance dependence. We assessed whether chronic treatment with cocaine would alter the number of NT binding sites in the mid- and forebrain and whether these effects persisted following withdrawal.

Rats were infused (i.v.) with isotonic saline (1 ml/kg) or 1 mg/kg cocaine in saline every 12 min for 2 hr over 10 days (passive administration) and then killed within 15 min of or 10 days after the last infusion (withdrawal). Brains were removed, immediately frozen, and sectioned at 10 μm at levels containing DA and/or NT perikarya or terminals and processed for NT receptor autoradiography.

In general, cocaine reduced NT binding. Binding was restored to initial levels during withdrawal in regions where NT and DA were not colocalized. Furthermore, cocaine affected the NT binding differently in regions in which DA cell bodies or terminals predominated. NT binding was decreased by two-thirds in the parabrachial pigmentosal area of the ventral tegmental area (VTA) of rats killed immediately after or 10 days after their final infusion session relative to that observed in saline-treated animals. In contrast, NT binding in the paranigral nucleus of the VTA was unaffected by cocaine or its withdrawal. Dopaminergic neurons originating in the VTA project to the prefrontal cortex (PFC) and to the nucleus accumbens (NAcc). There were more NT binding sites in the PFC when cocaine was present than when it was not. Binding of NT in the NAcc was unaffected by cocaine or its withdrawal. Although a modest reduction in NT binding occurred in the substantia nigra, pars compacta (SN Co) after cocaine, NT binding was enhanced significantly in rats abstinent from cocaine for 10 days. In addition, NT binding was modestly enhanced in the caudate-putamen (CP), the projection area of the SN Co. Cocaine also decreased NT binding in the dorsal hypothalamic area, a region without DA innervation, but this reduction was reversed by withdrawal. Increases in NT binding occurred in cortical areas

after chronic cocaine and tended to increase further during withdrawal.

Of the regions where NT and DA are colocalized, cocaine modified NT binding specifically in the VTA and SN Co and in their projection areas (PFC and CP, respectively). The alterations persisted for at least 10 days after cessation of cocaine in these areas previously proposed as critical to the development of sensitization. In other regions where DA and NT are not found together, cocaine-induced changes were transient.

Affiliation:

Addiction Research Center, NIDA, Baltimore, MD 21224

Effects of Chronic Cocaine on the Behavioral and Immunological Effects of Chronic Stress in Mice

David J. Mokler, Kathleen M. Correia, and James F. Novotny

The effects of drugs of abuse on the immune system has been extensively studied due to the increased risk of HIV infection as a consequence of i.v. drug abuse. The interactions of stress and the immune system also has implications as a health issue. Given the occurrence of these factors in many individuals there is justification in asking what the interactions of chronic stress and chronic administration of drugs of abuse on behavior and the immune system might be. Previous investigations have shown that there are interactions between stress and cocaine in motor activity; specifically, exposure to stress sensitizes animals to the stimulant effects of cocaine. Immunologically, both stress and cocaine may alter immune function in a number of ways. Thus, some treatments may increase immune function while others decrease parameters. The purpose of the present experiment was to examine the effects of chronic cocaine administration on the behavioral and immunological effects of chronic stress.

Male C57/B16J mice were prebled by orbital sinus puncture 2 weeks prior to the beginning of the stress paradigm. One week prior to stress animals were injected with 1% bovine serum albumin as antigen. Animals were exposed on a daily basis for 14 days for 1 hr/day to a 105 dB, 8 kHz sound for 1 sec on a VI 60 sec schedule. Locomotor activity was measured in 5 min intervals throughout the session. Control animals were placed in activity cages for one hour and activity measured without sound. Animals in cocaine groups received 5 mg/kg cocaine i.p. in home cages after the session for 14 days. Thus, there were four experimental groups; no stress - vehicle (NS), chronic stress - vehicle (ChS), no stress - chronic cocaine (NSCo), and chronic stress - chronic cocaine (ChSCo). One day following the last session animals were again bled. Blood was centrifuged and plasma frozen at -70° until analyzed. Concentrations of immunoglobulins IgG_1, IgG_2a, IgG_2b, IgM and IgA were determined qualitatively using an immunodiffusion assay. Additional determinations of immunoglobulins were done using SDS-polyacrylamide gel electrophoresis to separate serum proteins.

Locomotor activity was altered by cocaine and chronic stress. In control animals, activity decreased at a steady rate throughout the session (accommodation). Animals exposed to chronic stress or cocaine or chronic stress/chronic cocaine showed a similar pattern of accommodation during the initial days of the experiment (Figure). By Day 5 the ChS groups showed a decrease in activity compared to NS, whereas Co groups (receiving cocaine after the session) showed increased activity. During the session, animals started at
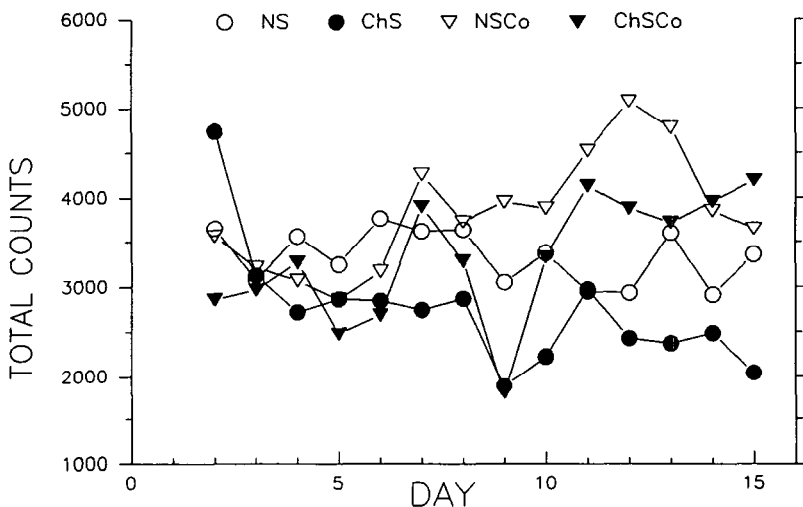
approximately the same level of activity. Animals in the ChS groups accommodated more quickly than NS animals. NSCo and ChSCo animals did not accomodate across the session. These differences continued throughout the experiment. Thus, during chronic stress, animals show a hypoactivity compared to unstressed animals. This has been shown following other stressors and in animals conditioned to show a hypoactivity compared to unstressed animals. This has been shown following other stressors and in animals conditioned to stress (conditioned emotional response). Animals given chronic cocaine show increased levels of activity. Animals in the ChSCo group show increased activity. Animals in the ChSCo group show increased activity compared to controls although these levels are slightly less animals exposed to cocaine alone. The increased activity in animals exposed to cocaine and stress suggest that the sensitivity of animals to stress is not increased by exposure to cocaine: this is in contrast with the increased sensitivity to the motor stimulant properties of cocaine following previous exposure to stress. The increased activity in animal given chronic cocaine may also be due to a conditioned stimulation since animals were given cocaine in their home cages following the stress session.

Qualitative changes in levels of immunoglobulins were seen in animals exposed to stress or cocaine. Using a diffusion assay serum levels of the immunoglobulins were decreased in animals exposed to ChS or ChSCo. Further work has been done to quantitate the levels of Igs using SDS-PAGE. Preliminary results confirm our initial observations on the direction of changes in Igs. These preliminary results suggest that chronic stress and/or chronic cocaine may compromise immune function as determined by Ig levels in mice.

AFFILIATION:

Departments of Pharmacology[1] and Microbiology[2], University of New England, College of Osteopathic Medicine, Biddeford, ME 04005

Sensitization to Cocaine Produced by Injection of Pertussis Toxin into the A10 Dopamine Region

J.D. Steketee and P.W. Kalivas

The progressive augmentation of the motor stimulant response to cocaine, termed sensitization, occurs with daily treatment and can last for months or years in rodents and humans. Sensitization has been suggested to result from a long-term change within the A10 dopamine region, in particular, via alterations in the tonic inhibition of dopamine neurons. Tonic inhibition involves both D_2 autoreceptors and $GABA_B$ receptors and is expressed via a G protein-mediated increase in K^+ efflux. The G protein which couples the D_2, and possibly the $GABA_B$, receptors to K^+ channels is pertussis toxin (PTX) sensitive. In this study we examined the possible role of PTX-sensitive G proteins within the A10 region in the development of sensitization.

Rats received simultaneous bilateral injections of PTX (1.0 µg/µl saline, 0.5 µl/min, 0.5 µl/side) or saline into the A10 region (A/P 2.6 mm, M/L 0.6 mm and D/V -2.4 mm). The dose of PTX used led to a ribosylation of G proteins in the A10 region which lasted for at least 2 weeks and an increase in dopamine synthesis and metabolism within the A10 region and nucleus accumbens which lasted for up to 4 days after intra-A10 injection. Immediately after PTX injections, some animals had 14 mm cannulae (26 gauge) bilaterally implanted 1 mm above the A10 region for microinjections or a 12 mm cannula (20 gauge) implanted 3 mm above the nucleus accumbens (A/P 9.1 mm, M/L 1.7 mm and D/V 0.0 mm) for *in vivo* microdialysis.

The motor-stimulant response to cocaine (15 mg/kg i.p.) was significantly augmented in animals pretreated with PTX two weeks earlier. The time course of horizontal activity revealed that cocaine-induced motor activity was augmented in the first hour after injection, similar to the augmentation following daily cocaine treatment. Also, *in vivo* microdialysis revealed that the cocaine-induced increase in extracellular dopamine within the nucleus accumbens was augmented in PTX-pretreated animals. This result is also similar to the augmented increase in dopamine seen when rats are challenged with cocaine after daily cocaine treatment. Intra-A10 injection of the $GABA_B$ agonist baclofen (0.3 nrnol/µl, 0.5 µl/side) 5 min before

cocaine injection blocked the acute motor stimulant response to cocaine in saline-pretreated rats. However, baclofen pretreatment did not block cocaine-induced motor activity in PTX-pretreated animals. This result suggests that PTX pretreatment led to an uncoupling of the GABA, receptor from its G protein.

As a further test for sensitization, some of the animals used in the cocaine behavioral studies were tested for their response to stress. After 20 min of either sham or foot shock stress (0.35 mA, 200 msec/sec) rats were decapitated and the concentrations of dopamine, DOPAC and HVA in the prefrontal cortex were determined via HPLC. The increase in DOPAC and HVA concentrations in saline-preretreated animals after foot shock, was significantly augmented in PTX-pretreated rats. Thus, PTX pretreatment led to a sensitized response to stress.

In conclusion, the results of this study suggest that PTX-sensitive G proteins may play an important role in the development of behavioral sensitization. Thus, rats exhibited an augmented behavioral response to cocaine which was associated with an augmented neurochermcal response following PTX-pretreatment. The inability of baclofen to block the cocaine response in PTX-pretreated animals suggests that the tonic inhibition of dopamine neurons provided by $GABA_B$. receptors is lost, thus allowing the neurons to be more responsive to cocaine treatment.

AFFILIATION

Department of Veterinary and Comparative Anatomy, Pharmacology an Physiology, Washington State University, Pullman, WA 99164-6520.

Evidence for High and Low Affinity [3H]Cocaine Binding Sites Associated with the Serotonin Reuptake Complex in Guinea Pig Brain: Allosteric Modulation by Paroxetine

**Hyacinth Akunne, Brian R. deCosta, Kenner C. Rice,
Arthur E. Jacobson, and Richard B. Rothman**

INTRODUCTION. Cocaine is a psychomotor stimulant and a drug of abuse whose primary mechanism of action is the inhibition of the biogenic amine reuptake system. Membrane associated [3H]cocaine binding sites have been repotted in the central nervous system of various species, and, depending on the brain region and assay condition, [3H]cocaine has been shown to label both the dopamine (DA) and serotonin transporters (1-4). Although the interaction of cocaine with the DA transporter is of key importance to the reinforcing effects of cocaine in animals (5) the interaction of cocaine with the serotonin transporter undoubtedly contributes to the complex behavioral actions of cocaine observed in animals and humans. The present study was designed to determine the characteristics of [3H]cocaine binding to membranes prepared from whole guinea pig brain, which initial experiments indicated might permit selective labeling of the serotonin transporter.

METHODS. Membranes were prepared from frozen guinea pig brains with minor modifications of previously described methods (6). [3H]Cocaine binding proceeded for 20 to 60 min at 0° C (equilibrium) in 25 mM sodium phosphate buffer, pH 7.4 (buffer). One ml samples were filtered with a single manifold over Whatman GF/B filters presoaked in 1 % polyethylenimine, and were washed with two 5 ml aliquots of ice cold buffer containing 400 mM NaCl. Nonspecific binding was determined with 10 μM cocaine.

RESULTS AND DISCUSSION. [3H]Cocaine binding was specific and saturable. Binding surface analysis (7) resolved two binding sites, site 1 (Kd=15.2 nM, Bmax=183 fmol/mg protein) and site 2 (Kd=650 nM, Bmax=1880 fmol/mg protein). The Ki values of various drugs for the two sites are reported in Table 1. Cocaine had highest affinity for site 1, and paroxetine had the highest affinity for site 2. The potent DA reuptake inhibitors mazindol, benztropine and GBR12909 had low affinity for both site 1 and site 2. GBR12935 had unexpectedly high affinity for site 2.

TABLE 1

Ki Values (nM) of Drugs for Site 1 and Site 2

(-)-Cocaine	15.2	650
Paroxetine	18.9	1.8
Mazindol	154	2537
GBR12909	686	1062
GBR12935	2608	8.5
Benztropine	1564	564
(+)-Cocaine	5088	87549

Preincubation of membranes with 5 nM paroxetine followed by extensive washing, produced a wash-resistant ("pseudoirreversible") inhibition of [3H]cocaine binding. In parallel experiments, membrane suspensions were centrifuged, rather than filtered, and the supernatants assayed for inhibitory activity. The lack of inhibitory activity suggested that the wash-resistant inhibition could not be due to residual paroxetine. Saturation binding studies demonstrated that pretreatment of membranes with paroxetine produced an increase in the Kd, and no change in the Bmax. Dissociation experiments showed that pretreatment of membranes with paroxetine increased the dissociation rate of [3H]cocaine, consistent with an allosteric mechanism.

Viewed collectively, these data suggest that [3H]cocaine labels two binding sites associated with the serotonin transporter in membranes of whole guinea pig brain. The two sites could represent two states of a single receptor, two distinct binding sites associated with a single class of transporters, or perhaps two sites, each associated with a different class of transporters. The observation that paroxetine pseudoirreversibly inhibits [3H]cocaine binding via an allosteric mechanism suggests that paroxetine binds tightly to a site which allosterically controls the conformation of the [3H]cocaine binding site.

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Furnished upon request

AFFILIATION.

[1]Unit on Receptor Studies, LCS, NIMH, Bethesda, MD 20892
[2]Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892

Intrathecal Morphine and Insulin Cause Hypoglycemia in Mice by Different Mechanisms

D.A. Brase, H.L. Tripathi, and W.L. Dewey

Previous work in our laboratory indicated antagonism of intrathecal (i.t.) morphine-induced antinociception by hyperglycemia (F. Lux et al., Eur J Pharmacol 146: 337, 1988), whereas i.t. morphine caused a dose-related hypoglycemia at supra-antinociceptive doses in both normal and diabetic mice (F. Lux et al., J Pharmacol Exp Ther 245: 187, 1988) and a depletion of liver glycogen (F. Lux et al., J Pharmacol Exp Ther 249: 688, 1989). Insulin (0.02-0.08 U, i.t.) also produced a dose-related hypoglycemia (D.A. Brase et al., Pharmacologist 2: 126, 1989). In the present study, the hypoglycemic effects of morphine (40 μg, i.t.) were compared with insulin (0.08 U, i.t.) in non-fasted, unanesthetized male ICR mice. Serial (basal, 15, 30, 60, 120 and 180 min) blood samples were obtained from the retro-orbital sinus. Both agents caused a 70% decrease in blood glucose in 1 hr; i.t. insulin caused a faster decline. Both agents were antagonized by the cellular glucose transporter inhibitor, forskolin (10 mg/kg. i.p.), which itself caused a significant hyperglycemia. Insulin caused more than a 10-fold increase in serum levels of insulin immunoreactivity (IR) at 30 min, which was not due to its systemic absorption, because a comparison of i.t. with s.c. [125I]-insulin administration indicated that <0.01% of i.t.-injected radioactivity, compared with 1.76-2.64% of s.c.-injected radioactivity, appeared per ml of blood over a 3-hr period. In contrast to i.t. insulin, i.t. morphine tended to decrease serum insulin IR (-20 and -30 %, P>0.05). and significantly increased plasma glucagon IR (40 and 131 %) at 30 and 60 min after i.t. administration, respectively. Several receptor antagonists were screened in an attempt to identify possible neurogenic components of insulin- and morphine-induced hypoglycemia, including 15-min i.p. pretreatments with 2.0 mg/kg doses of atropine, mecamylamine, methysergide, prazosin and yohimbine, 10 mg/kg propranolol and 20 mg/kg naloxone. Atropine and methysergide had no significant effects. In mice given i.t. saline, mecamylamine and yohimbine caused moderate hypoglycemia, whereas prazosin produced hyperglycemia. Propranolol blocked the increase in blood glucose observed in mice given i.t. saline and appeared to delay recovery from insulin-induced hypoglycemia. Propranolol did not affect morphine-induced hypoglycemia. The effect of i.p. mecamylamine tended to be additive to that of both i.t. insulin

and morphine. Naloxone antagonized morphine only. and prazosin partially antagonized insulin only. Yohimbine potentiated insulin. but antagonized morphine. Antagonism of i.t. morphine by i.p. yohimbine occurred in a dose-dependent manner with an ED-50 of 2.0 mg/kg at 1 hr after i.t. morphine. The centrally and peripherally active alpha-2-adrenoceptor antagonist, L-657.743 (M.E. Goldman et al., <u>Drug</u> <u>Dev.</u> <u>Res.</u> <u>17:</u> 141, 1989). also antagonized i.t. morphine with an i.p. ED-50 of 8.9 mg/kg at 1 hr after morphine, but the peripherally selective alpha-2-antagonist L-659.066 caused little antagonism (<28%) at doses up to 20 mg/kg, i.p. It is concluded that i.t. morphine-induced hypoglycemia is mediated by an insulin-independent mechanism which involves the stimulation of a central pathway containing alpha-2-adrenoceptors and subsequent activation of glucose uptake into tissues, whereas i.t. insulin-induced hypoglycemia may involve a pathway containing alpha-1-adrenoceptors that appears to mediate the release of pancreatic insulin which, in turn, activates cellular glucose uptake. (Supported in part by a Commonwealth of Virginia Center Grant for Drug Abuse Research, and by USPHS grants DA-00490 and DA-01647.)

AFFILIATION

Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0613

Potentiation of Reward by Food Deprivation is Blocked by Antibodies to Dynorphin A

Kenneth D. Carr

An hypothesis that has withstood the test of more than 30 years of research is that lateral hypothalamic self-stimulation (LH ICSS) results from direct activation of a neuronal system that mediates the positive reinforcement of natural motivated behavior. There is evidence that drugs of abuse produce their rewarding effects by exciting elements within this system as well. One of the behavioral findings that links natural consummatory reward, drug reward, and LH ICSS reward is that all three are enhanced by food deprivation. This phenomenon may reflect the operation of a homeostatic mechanism that sensitizes the reward system in order to increase the range of goal stimuli that will elicit approach and reinforce ingestion, thereby leading to drive reduction. Using LH ICSS in rats as a model, we have previously obtained evidence that the potentiation of reward by food deprivation is opioid mediated (Carr & Simon, Brain Res. 297:369, 1984). To summarize, a dose of systemic naloxone that had no effect on ICSS threshold under baseline conditions reversed the threshold-lowering effect of 24 hr food deprivation.

When LH stimulation is delivered at frequencies below those that sustain ICSS, animals often ingest food during the stimulation. We have found that LH stimulation threshold for eliciting feeding is elevated by low doses of systemic naloxone and by lateral ventricular infusion of antibodies to dynorphin A (Carr et al., Brain Res. 422:384, 1987). In order to further compare the opioid mechanism that potentiates ICSS in food deprived rats with the opioid mechanism that mediates feeding, the present experiment tested whether the ICSS threshold of food deprived rats is elevated by the same dynorphin A antiserum that elevated the threshold for stimulation-induced feeding.

Rats were trained to press a lever to receive 0.5 sec trains of square wave cathodal pulses in their permanently implanted LH electrodes. The method of limits was used to determine frequency threshold for a criterion rate of 40 ICSS responses per one min. trial. Thresholds were determined twice per day; immediately prior to and then 2 hrs following lateral ventricular infusion of antibodies to dynorphin A or ß-endorphin. Rats were tested under baseline conditions, where they had been feeding and drinking on

551

an ad libitum basis in the home cage, and on another occasion following 24 hr food deprivation. Both lyophilized antisera were reconstituted and diluted 1:10 in sterile distilled water.

Under baseline conditions dynorphin A antiserum had no effect on ICSS threshold while ß-endorphin antiserum produced a 16.1% elevation which was significantly greater than the effect of vehicle (p<.01). When animals were deprived of food for 24 hrs, ß-endorphin antiserum produced a 10.8% elevation which did not differ significantly from the effect under baseline conditions. Thus, antibodies to ß-endorphin produce a small but significant elevation of ICSS threshold regardless of feeding condition. The present test cannot distinguish whether this reflects a nonspecific performance deficit or an actual reward decrement. In contrast to ß-endorphin antiserum, dynorphin A antiserum selectively elevated the thresholds of food deprived rats, typically reversing the threshold-lowering effect of food deprivation. The 14.7% elevation in ICSS threshold produced by dynorphin A antiserum in food deprived rats was significantly greater than the 0.4% reduction produced by the same antiserum under baseline conditions (p<.01). This effect matches the effect obtained previously with systemically administered naloxone and suggests that the potentiation of reward by hunger is mediated by dynorphin A.

The close functional relationship between LH ICSS and the feeding system suggests that the dynorphinergic mechanism that potentiates ICSS reward in the service of hunger may normally regulate the hedonic response to alimentary stimuli. Whether this mechanism has any bearing on the increased self-administration of drugs by food deprived animals (Carroll & Meisch, Adv. Behav. Pharmacol. 4:47, 1984) is a question that may be worthwhile to pursue.

Department of Psychiatry, NYU Medical Center, N.Y., N.Y., 10016

Immunosuppressive Agent Modulates the Severity of Opiate Withdrawal

N. Dafny, P.M. Dougherty, and D. Drath

Cyclosporine-A (CsA) induces a profound state of immune suppression by direct alteration of T-lymphocyte function and by modification of neuro-endocrine parameters. In previous studies (1,2) CsA was shown to induce a marked reduction in naloxone precipitated morphine withdrawal severity following i.p. injection. Since opiate withdrawal and antinociception are both closely associated with CNS activity (5,7,9) and opiate receptors are found in both the CNS and the immune system (4,8), it may be that opiate withdrawal is a syndrome which involves participation of both organ systems. The goal of this study is to determine the target of CsA in reducing withdrawal severity through two experimental approaches. The first experiment determined the effect of CsA upon opiate withdrawal following direct administration within the brain ventricles. The second experiment deteremined the effect of treateing immune cells alone upon opiate withdrawal severity using an adoptive transfer technique.

METHODS

Experiment A. 45 male Sprague-Dawley rats weighing 180-220 g were used. The animals were anesthetized by pentobarbital (50.0 mg/kg) and implanted with a permanent i.c.v. canula (3). After a recovery period of 3 to 5 days, narcotic dependence was elicited using a subcutaneous implant of 75 mg morphine base. Dependence was assessed 72 h later by i.p. injection of 1.0 mg/kg naloxone and scoring of eight signs of precipitated withdrawal behavior. A total of six groups were used, two which received either i.c.v. saline or CsA carrier for controls, and the remaining groups which received one of several doses of CsA. All injections were in 10 µl administered over 2-3 min per rat. Canula placement was verified at the conclusion of each experiment by visualization of dye injected within the ventricles.

Experiment B. 150 male Fischer 344 rats (160-180 g) were used. 68 of these animals were used as donors of immune cells, 36 of which were treated with CsA (15.0 mg/kg, i.p.), 16 of which were morphine pellet treated (as above), and 16 which were placebo (saline) treated. Drug treatments for each of the donor animals was 72 hours before harvest. The methods for spleen harvest were described previously (2).

The recipient animals were divided into four groups, two of which received CsA-treated immune cells, one which received the morphine-treated splenocytes, and the final group which received the placebo-treated immune

cells. All donors except those receiving the cells of morphine-treated donors were mad dependent upon morphine as described above. Each of the animals in the recipient groups were injected with the cells of two donors. Finally, there were four groups of control animals which recieved drug treatments only including a placebo treated group, a CsA-treated group, a morphine treated group, and a morphine plus CsA-treated group. Each of the recipient and drug-treated animals was injected with naloxone and withdrawal was scored as above.

RESULTS AND DISCUSSION

Experiment A. It was observed that injection of either saline or the CsA carrier did not result in any significant reduction in morphine withdrawal severity. In contrast i.c.v. injection of CsA resulted in attenuation of withdrawal severity, with the most effective dosage at the middle (1.0 µg) of the concentration range showing a greater effect than either the higher (100 µg) or lower dosages (0.1 µg) used, suggesting a U-shaped dose response relationship may exist. The middle dosage reduced six of the eight signs observed.

Experiment B. Transfer of CsA-treated splenocytes 24 h before injection of naloxone resulted in an attenuation of withdrawal that was nearly identical to that produced by a direct injections of CsA. This effect was not observed following transfer of placebo-treated cells to morphine dependent recipients and no withdrawal was observed following the transfer of morphine-treated cells to naive recipients.

The results obtained in experiment A demonstrate that CsA attenuates morphine withdrawal severity upon direct administration within the CNS. Since opiate withdrawal is closely associated with the activity of several brain structures which border the brain ventricular system, it appears that CsA alters the physiologic sequelae of opioids at these sites. However, our results also show that an attenuation of withdrawal may be obtained by transfer of CsA-treated immune cells alone underscoring the role of peripheral targets of action of CsA. So overall our results indicate that the brain and peripheral targets are important sites of action recruited by CsA to exert its optimal effect upon opiate withdrawal severity. It was observed (6) that CsA acts upon cultured lymphoid tissues to reverse the effects of chronic morphine treatment which may explain the effects of the transferred cells. The basis for the CsA effect upon administration to the CNS will require further investigation.

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AFFILIATION

University of Texas Medical School at Houston
Houston, Texas 77225

Opioid Self-Administration Contingent on Physiologic Parameters

Kenneth Grasing and Hazel Szeto

There is evidence that endogenous opioid activity is related to arousal, which is best reflected by visceral or autonomic parameters. Models of opioid self•administration are traditionally based on drug infusions administered contingent on lever-pressing, a skeletal response. Instrumental responding for opioic reinforcement may be qualitatively different if visceral rather than skeletal responses are reinforced. Morphine self-administration contingent on increaser in heart rate has been demonstrated (Life Sciences, 45, p. 1967). Because the EEG is a more consistent indicator of level of arousal, we sought to develop a system for self-administration based on changes in the total EEG power.

The object was to operantly condition changes in the EEG through reinforcement with morphine infusions. Three adult, male Sprague Dawley rats, with surgically implanted jugular catheters and cortical electrodes were studied. One week following surgery animals were placed in special chambers designed for chronic venous access and EEG recording, and were allowed at least five days to habituate prior to the start of experiments. EEG was continuously digitized at 256 Hz and analyzed on line by fast fourier transform of 1024 data points every four seconds. Desynchronized epochs were identified by total power in the lower 3.0 percent of the frequency histogram for that individual animal, while epochs in the upper 3.0 percent were designated as synchronized. Morphine, at doses of 3, 10, or 30 ug/kg-inf, was injected by a computer controlled pneumatic syringe.

Conditioning took place daily over a six hour period, during which epochs that met criterion for reinforcement were followed by a bolus injection of morphine. Animals were exposed to morphine administered contingent on desynchronization for several days followed by a period in which the same dose was administered contingent on a synchronized EEG for an equal number of days. Days in which drug was administered contingent on desynchronization were identified by an audible tone, while synchronization contingent administration was signalled by white noise. Two cycles of desynchronization and synchronization contingent morphine at a dose of 30 µg/kg-inf, were followed by two additional cycles in which doses of 3 and 10 µg/kg-inf were administered in a random order. Outcomes from the last two days of each contingency, at three doses were compared by ANOVA.

Both synchronization and desynchronization contingent morphine administration cause a dose-dependent decrease in total EEG power. However, animals

receiving synchronization contingent morphine have a more variable EEG pattern. With synchronization contingent administration, total power is elevated for short periods that produce from 5 to 20 infusions. These relatively brief periods of elevated total power occur every one to two hours, at irregular intervals, and are separated by periods of diminished total power. After a reduction in morphine dose, an increased number of infusions is obtained under either contingency. These findings are consistent with operantly conditioned changes in the EEG.
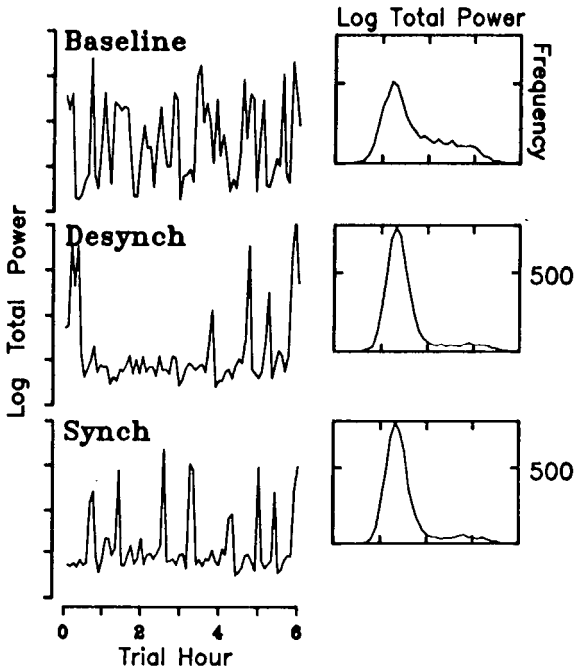
AFFILIATION:

Kenneth Grasing and Hazel Szeto, Department of Pharmacology, Cornell University Medical College, New York, NY

In Vivo Concentration-Response Relationships For Fentanyl: Absence of Acute Tolerance in the Rat Tail-Flick Test

Thomas H. Kramer, Elizabeth A. Ayres, and Thomas F. Burks

INTRODUCTION

Potency estimation and other in vivo pharmacologic endpoints involving opioid analgesics have traditionally not taken fully into account the pharmacokinetic behavior of the drug in the test animals or subjects. An alternative approach that is gaining increasing favor in clinical pharmacology is to simultaneously obtain pharmacokinetic and pharmacologic data from the same subject, and describe the relationship between drug concentration, usually in blood, and the effect of interest in the individual. This results in the elimination of interindividual variation in pharmacokinetics as a confounding factor, and in increased precision in the estimation of in vivo drug potency. We determined plasma fentanyl concentration-effect (CE) relationships for antinociception in the rat tail-flick test, and assessed the development of acute tolerance to fentanyl.

METHODS

Ten male Sprague-Dawley rats, 275-375 g, were implanted with jugular venous and femoral arterial cannulae under general anesthesia. After 3 days recovery fentanyl citrate, 10.35 µg/dose, was administered via the jugular cannula in two sequential constant-rate infusions of 30 min duration, spaced 60 min apart. During and after the infusions, multiple blood samples were obtained from the arterial cannula, and latencies to tail-flick (in sec) after tail immersion in warm (50° C) water were determined repeatedly over 6 hr (cutoff latency 30 sec). Plasma fentanyl concentrations were measured by RIA (FEN-RIA 200, Janssen Life Sciences). Plasma fentanyl concentration versus time data were analyzed by both compartmental (using generalized least squares nonlinear regression) and noncompartmental (integration by the trapezoidal rule) techniques to determine volume of distribution (V_β) and clearance (Cl). Concentration-effect (CE) relationships were assessed using traditional compartmental pharmacokinetic models and by nonparametric pharmacokinetic modeling (unconstrained cubic spline

interpolation) to estimate arterial fentanyl at the time of each effect measurement. Tailflick latency was plotted as a function of fentanyl concentration to produce the raw CE curves. After examination of the CE curves for evidence of hysteresis, 2nd order polynomials were fit to the CE data by unweighted nonlinear regression. The coefficients of the polynomials were constrained to be nonegative to ensure a monotonically increasing CE relationship. Fentanyl's potency was calculated from the fitted equation and was expressed as the concentration required to produce a 30 second latency.

RESULTS

For the parametric pharmacokinetic analysis, a two compartment pharmacokinetic model provided the best fit of the concentration data; use of a three compartment model did not result in statistically significant improvement in fit. However, both models demonstrated a significant bias relative to noncompartmental analysis, such that V_β and Cl were overestimated. The mean (± S.D.) values for V_β and Cl by noncompartmental analysis) were 1.75 ± 1.85 liters and 1.24 ± 1.12 ml/min, respectively. Because of the bias contained in the parametric models, cubic spline interpolation was used to estimate arterial fentanyl at the time of each tail-flick determination. When tail-flick latency was plotted as a function of fentanyl concentration, no clockwise or counterclockwise progression of the data with time (hysteresis or proteresis) was observed. A threshold phenomenon was observed, with minimal antinociception occurring below approximately 5 ng/ml. Above 5 ng/ml, the CE curves rose extremely steeply. 2nd order polynomials described the CE data well. The mean (± S.D.) concentration of fentanyl in arterial blood producing a 30 sec tail-flick latency was 15.3 ± 1.4 ng/ml.

DISCUSSION

The absence of consistent clockwise or counterclockwise progression of the CE data with time permits 2 general conclusions. First, the arterial blood appears to be in approximate equilibrium with the site of antinociceptive action of fentanyl, presumably the brain and spinal cord. This condition must be viewed in the context of the model used; larger animal species, different opioid drugs, and different effect endpoints might not display similar behavior. Secondly, the fentanyl CE relationship appears stationary, i.e., induction of acute tolerance does not occur within the time period of the experiment, with the fentanyl doses used here. The inter-animal variation in fentanyl potency was extremely low. Simultaneous pharmacokinetic and pharmacodynamic modeling yields highly precise estimates of fentanyl potency using small numbers of animal subjects, and is a powerful tool for future studies of opioid actions in vivo.

Epidural and Intrathecal Administration of Sufentanil, Alfentanil and Morphine in the Dog: A Comparison of Analgesic Effects and the Development of Tolerance

Paul J. Tiseo, Marc B. Sabbe, and Tony L. Yaksh

Sufentanil (Suf). alfentanil (Alf) and morphine (Mor) are *mu* receptor agonists with different receptor affinities, intrinsic efficacies, and lipid solubilities. There is clinical interest in the anilinopiperidines because of their higher liposolubility, the possibility of a more rapid onset of action than Mor, and a decreased risk of delayed respiratory depression secondary to bulk redistribution in the CSF. The purpose of this study was to evaluate the analgesic effects and the development of tolerance to these drugs following 14 day epidural (EP) or 28 day intrathecal (IT) administration. Beagles (-12 kg) were implanted with EP and IT catheters (PE-50) under halothane anesthesia. The nociceptive response was quantified by measuring the response latency of a thermally-evoked skin twitch produced by applying a thermal probe (62.5° C) to thoracic and lumbar areas of the hack.

 EP Suf (50,100 µg) and Alf (80,800 µg) as well as IT Suf (25,50 µg) and Alf (40,400 µg) produced a spinally induced analgesic response which was rapid in onset (< 5min) but reliably shorter in duration when compared to EP Mor (1, 10 mg) and IT Mor (0.5, 5 mg). In all cases the analgesic response was dose-related and produced 100% MPE at the higher doses. The development of tolerance was quantified by comparing the extent and the duration of the analgesic effect produced on Day 1 with that observed on the final da (Day-F) of the study. Results are expressed as a ratio of the AUC on Day-1/AUC on Day-F, where AUC = %MPE vs Time. The magnitude of tolerance observed for a given agent was similar regardless of the route of administration. Mor produced the greatest degree of tolerance over time with a ratio of 25. Alf and Suf demonstrated significantly less tolerance over time with ratios of 2 and 3 respectively. Such differences in the magnitude of tolerance may result from the fact that Suf and Alf are significantly more efficious than Mor and may require a lower receptor occupancy to produce their analgesic effects. As such, these agents would be less affected by the receptor downregulation that is suggested to take place during chronic drug administration. It is also possible that different drug kinetics or possible differences in drug pharmacodynamics may play a role in the different magnitudes of tolerance development. These results suggest that although the duration of action of Alf and Suf is significantly shorter than that of Mor, he more liposoluble and receptor specific anilinopipetidines produce a slower tolerance development which is of significance in the treatment of opioid-sensitive chronic pain.

University of California, San Diego Medical Center
Dept. of Anesthesiology, La Jolla, CA 92093

Intraspinal Opioids in Frogs: A New Behavioral Model For the Assessment of Opioid Action

Craig W. Stevens

Frogs have been a favorite subject of experimentalists since the beginning of biological studies. Within the field of neuroscience, it was an accidental stimulation of the frog crural nerve that sparked Luigi Galvani's landmark studies on "animal electricity" (in 1786). the frog spinal cord was the model that prompted Bell and Magendie to conclude that dorsal roots were sensory and ventral roots motor (1833), and it was on frog sciatic nerve that von Helmholtz first measured the conduction velocity of a nerve impulse (1852). These earlier studies were confirmed and extended in the present century on mammalian models as more advanced techniques for experimentation were developed (e.g. anesthetics, ventilators, microelectrodes, etc.). In the field of pain or nociceptive research in behaving animals, however, there has been an overwhelming focus on mammalian models which has only recently been extended downward in phylogeny to nonmammalian vertebrates. This brief paper describes some recent studies of opioid action in the common grass frog, *Rana pipiens,* after spinal administration and assessed with a simple algesiometric method, the acetic acid test.

All of the major opioid peptides isolated and characterized from mammalian CNS have been similarly found in amphibian CNS when examined (refs. in Stevens, 1988). This led Pezalla (1983) to begin a series of studies to develop a method to assess the nociceptive threshold in intact, behaving frogs. While early attempts to apply well-established algesiometric methods used in rodents (e.g. hot plate and tail flick) were unsuccessful, a method of applying dilute acetic acid solutions to the hindlimb of *Rana pipiens* proved to be a robust and reliable indicator of a nociceptive threshold. Termed the acetic acid test, 10 log-spaced concentrations of glacial acetic acid are made, numbered from lowest to highest concentration, and applied drop-wise from a Pasteur pipette to the dorsum of the frog's thigh starting from the lowest concentration. The nociceptive threshold (NT) is defined as the code number of the lowest concentration of acetic acid that causes a quick, ballistic wiping response of the ipsilateml or contralateral hindlimb to brush away the dilute acid solution. If no response is elicited within 5 seconds, the solution is thoroughly rinsed with distilled H_2O and a drop of the next higher solution is placed on the thigh of the other hindlimb.

The first studies of antinociceptive opioid action in frogs were assessed following systemic injections of morphine, however high doses were needed (100 mg/kg s.c.) to get a potent elevation of NT (Pezalla, 1983). These changes were most likely due to a tighter blood-brain barrier in frogs compared to rats, as shown for

other opioids by later studies (Carr <u>et al.,</u> 1984). The use of direct intraspinal (i.s.) injection of opioids at the lever the lumbar enlargement circumvented this problem and it was found that very low doses of morphine delivered in a 1 µl volume produced a potent, does-dependent, and naloxone-reversible elevation of the NT as measured by the acetic acid test (Stevens and Pezalla, 1983). This effect was stereospecific, as shown by administration of the enantiomers levorphanol and dextrorphan (Stevens and Pezalla, 1984). A potent dose-dependent effect was also seen after i.s. administration of beta-endorphin, met-enkephalin, and dynorphin (Stevens et al., 1987). The observation that i.s. dynorphin in frogs did not produce motor dysfunction as observed after i.t. administration in rats (Stevens and Yaksh, 1986) suggests that there may be a fundamental difference in the spinal dynorphinergic and/or kappa opioid systems in these two vertebrates. More recent studies have also shown that dynorphin and morphine and surprisingly ineffective after i.s. administration in fall frogs entering hibernation (Stevens and Pezalla, 1989).

In summary, in view of the frog's decreased neural complexity, the importance of a comparative approach to basic neurotransmitter systems in the spinal cord, and the contemporary concerns against employing mammalian models in pain research, future investigations of the spinal action of opioids and other antinociceptive agents may prove invaluable.

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AFFILIATION:

Craig W. Stevens, Ph.D.
Assistant Professor of Pharmacology
Oklahoma State University
College of Osteopathic Medicine
Tulsa, OK 74107-1898

Chronic Tolerance to Nicotine's Effects on Suppressing Hunger and Caloric Intake

Kenneth A. Perkins, Leonard H. Epstein, Richard I. Stiller, J.E. Sexton, and Rolf G. Jacob

INTRODUCTION

Body weight is inversely associated with smoking, as smokers weigh less than nonsmokers and gain weight after stopping smoking. Weight gain after cessation may be a cause of smoking relapse. Smoking may decrease weight in part because of nicotine's acute effects on reducing hunger and caloric intake. The present study was designed to: 1) determine effects of nicotine on hunger and satiety following consumption of a standard caloric load compared with no load; 2) examine nicotine's influence on ad lib caloric intake during a meal (particularly sweet, high-fat foods), and 3) determine possible differences in these nicotine effects between smokers and nonsmokers, which could reflect chronic tolerance.

METHODS

Male smokers and nonsmokers (n=10 each), abstinent overnight from smoking and food, participated in 4 sessions on 4 separate mornings. Sessions involved consumption of a caloric load (4.77 kcal/kg) or a water load, followed by administration of nicotine (15 ug/kg) or placebo via measured-dose nasal spray every 20 mins for 2 hrs (2 x 2 design). Hunger and satiety ("fullness") ratings were obtained prior to each dose presentation. At the end of the 2 sessions involving the caloric load (simulating breakfast), subjects were subsequently presented with a large array of typical lunch/snack food items varying in sweet taste and fat content and allowed to eat as much as they wished.

RESULTS

Nicotine enhanced the hunger-reducing effects of the caloric load for both smokers and nonsmokers. Hunger ratings were lower following nicotine compared with placebo during the caloric load session (p<.01), while there was no effect of nicotine during the water session. Furthermore, magnitude of hunger reduction

was greater in nonsmokers vs. smokers (p<.05). Smokers unexpectedly reported greater satiation than nonsmokers following the caloric load regardless of nicotine or placebo condition (p<01). There was no overall difference between smokers and nonsmokers in caloric intake. !As shown in Table 1, intake during the meal was significantly reduced following nicotine compared with placebo (p<.05), but this effect was significant only for nonsmokers (p<.02). The decrease in intake occurred in all food taste categories and was not specific to consumption of sweet, high-fat foods.

	SMOKERS	NONSMOKERS
NICOTINE	713±84	679±173
PLACEBO	781±68	913±106

Table 1. Mean±SEM caloric intake during ad lib meal following nicotine and placebo in smokers and nonsmokers.

DISCUSSION

These results indicate that nicotine reduces appetite under certain conditions, possibly helping to explain the influence of smoking on body weight. Nicotine decreased hunger in smokers, as well as nonsmokers, following consumption of a caloric load but not in the absence of such caloric consumption. Thus, nicotine does not appear to uniformly suppress hunger under all conditions but may do so only in conjunction with meal consumption. On the other hand, the present study also revealed that the suppression of hunger due to nicotine was greater in nonsmokers compared with smokers, and that nicotine significantly reduced caloric intake in nonsmokers but not in smokers, suggesting chronic tolerance. Chronic tolerance would suggest that nicotine exerts greater influence in suppressing hunger and caloric intake when smokers first adopt the smoking habit or have relapsed following some period of cessation, possibly serving as a particularly strong reinforcing effect of smoking. Further study of these effects may increase our knowledge of nicotine's role in alterations of energy balance and body weight due to smoking and cessation.

Acute Marijuana Smoking Reduces Vagal Tone

David B. Newlin, Mary Beth Pretorius, Conrad J. Wong, and Elizabeth M. Dax

The pronounced tachycardia from smoking marijuana has been assumed to be sympathetic in origin (Goodman and Gilman, 1965). We sought to determine whether there was a significant parasympathetic component (i.e., withdrawal of vagal inhibition) to this tachycardia. Use of vagal blockade for such an investigation is problematic because dramatic baseline cardiovascular changes may complicate interpretation of marijuana-induced heart rate changes. Therefore, we used vagal tone index (V), a noninvasive measure of tonic vagal inhibition of the heart using ECG monitoring. V quantifies rhythmicity in heart rate in the respiratory frequency band centered at approx. 0.33 Hz (i.e., respiratory sinus arrhythmia). Considerable research with animal models has validated vagal tone index (V) as a measure of tonic vagal inhibition of the heart. The THM wave quantifies heart rate variability at a lower frequency band (centered at approx. 0.10 Hz) and is associated with blood pressure homeostasis and baroreceptor activity.

24 male volunteers with a history of marijuana smoking were housed on the NIDA, ARC inpatient research unit. They were given either oral placebo, 10 mg THC orally, or smoked a 2.7% marijuana cigarette. Marijuana smoking increased heart rate from 79.9 to 94.3 bpm, and decreased V from 5.6 (SEM=0.14) to 3.6 (SEM=0.17) log units +30 min after smoking. Therefore, heart rate variability in the respiratory frequency band decreased markedly and respiratory sinus arrhythmia was attenuated after smoking. The THM wave decreased from 4.6 (SEM=0.16) to 2.9 (SEM=0.17) after smoking. Oral THC at this dosage increased static ataxia, but had no clear effect on the cardiovascular measures. We further studied 5 of these subjects before smoking, and +5 and +30 min after smoking a 2.7% marijuana cigarette. The +5 min recording yielded even greater decreases in vagal tone (-3.5 log units decrease) and THM (-2.4 log units decrease) than the +30 min recording. These were highly significant differences. In some subjects, the decrease in vagal tone after smoking marijuana was similar to that seen with atropine.

Therefore, we concluded that marijuana produces significant withdrawal of vagal inhibition, indicating that the cardiovascular effect of the drug may not be purely sympathetic. Markedly reduced vagal tone is a risk factor for sudden death after myocardial infarction (Reddy et al., 1990).

These results conform with the hypothesis that there is central linkage between dopaminergic reward mechanisms of the mesolimbic area and vagally-mediated tachycardia from abused drugs. We propose that this tachycardia may be a

concomitant of reward mechanisms that is related in part to psychomotor stimulant activation. However, this tachycardia can be measured noninvasively in humans who are restrained from motor activity. We are currently testing the limits of this hypothesis with other drugs that produce either euphoria or dysphoria.

NIDA Addiction Research Center, Box 5180, Baltimore MD 21224.

Short-Term D^9-tetrahydrocannabinol (THC) Does Not Affect Neuroendocrine or Immune Parameters

E.M. Dax, N.S. Pilotte, W.H. Adler, J.E. Nagel, and W.R. Lange

Extensive literature describes both endocrinological and immunological effects produced by marijuana smoking and/or THC (the most active cannabinoid). A new discipline has developed in the study of endocrine and immune system inter-relationships. The possible THC-induced interactions between neuroendocrine secretion and lymphocyte function were investigated concommitantly after short-term administration of low, but psychoactive doses of THC.

Male volunteers who smoked marijuana chronically were housed in the NIDA/ARC closed research ward for the duration of the study. After a washout period, subjects either ingested 10 mg THC tid, for three days or smoked THC cigarettes (approximately 2.7% or 18 mg THC inhaled tid for 3 days). One additional dose of the appropriate preparation was given to each subject on the 4th day. Plasma prolactin, ACTH, cortisol, luteinizing hormone and testosterone were assessed in blood samples drawn at 2 and 9 AM and at 12, 4 and 8 PM through two days before, during and up to seven days after the THC administration procedures. Immune parameters were assessed before, immediately after (4th Day), and up to seven days after the THC administration. Determinations were made of the total lymphocyte, T lymphocyte and B cell sub-populations, mitogen-stimulated cell proliferation, natural killer cell activity, and antibody-dependent cellular cytotoxicity.

In subjects who used THC heavily prior to admission, the mean plasma prolactin concentrations over 24 hr were lower than those found in light users (9.36 + .39 ng/ml and 12.16 + .62 ng/ml, respectively, $p < .0001$, N = 8, 8). This difference persisted through the study. Mean hormone concentrations were compared with pre-drug concentrations and were not significantly different in subjects either being administered or recovering from THC (ANOVA with repeated measures). No differences in immune parameters were seen between either heavy or light users or throughout the study in subjects administered THC.

Thus, we could not demonstrate alterations in immunological or neuroendocrine parameters following conservative but psychoactive doses of THC.

The mean 24 hr mean prolactin concentrations were lower in heavy users than in light users indicating that there may be chronic endocrinological effects of THC use. If THC use affects the immune system, more discriminating tests may be required to demonstrate these subtle differences at lower THC doses.

Affiliation:

Addiction Research Center, NIDA, Baltimore, MD 21224.
Gerontology Research Center, NIA, Baltimore, MD 21224.

Elevated Drug Saliva Levels Suggest A "Depot-Like" Effect in Subjects Treated With Sublingual Buprenorphine

E.J. Cone, S.L. Dickerson, W.D. Darwin, P. Fudala, and R.E. Johnson

The relative ineffectiveness of orally administered buprenorphine led to investigation of the sublingual route as an alternate means of drug administration in the treatment of opioid dependence. Sublingual buprenorphine has been used successfully for induction, short-term maintenance and detoxification of opioid-dependent subjects. Preliminary studies in our laboratory with human subjects following acute and chronic dosing suggest that sublingual administration of buprenorphine produces a depot of drug in the oral mucosa which is depleted over several days. Saliva and plasma levels were tested for buprenorphine following acute intramuscular and sublingual administration. After acute intramuscular dose administration, saliva levels were substantially less than plasma levels, whereas they were substantially elevated during the first twelve hours after sublingual doses. During chronic sublingual dosing, saliva levels were in the range of 20-200 ng/mL four hours after administration and were generally equivalent to plasma levels by 24-48 hours post drug administration and following the abrupt withdrawal of buprenorphine. The importance of this shallow compartment depot of buprenorphine should be considered in the future development of this drug for treatment purposes.

Affiliation: National Institute on Drug Abuse
                Addiction Research Center
                Baltimore, MD 21224

Interaction of Cocaine With Ethanol

M. Farre, M. Llorente, B. Ugena, X. Lamas, and J. Cami

Alcohol and cocaine are drugs frequently used in combination under social conditions. It is a common belief that cocaine antagonizes the intoxicating effects of alcoholic beverages.

Previous research in humans has shown that the combination of alcohol and cocaine produces greater increases in heart rate than that observed following cocaine alone. The effect of cocaine on the performance impairment induced by ethanol has not been well established. The present study investigated the effect of cocaine on the acute ethanol intoxication.

Nine healthy male volunteers ranging in age from 22 to 32 years participated. All had histories of recreational use of cocaine and ethanol without history of drug dependence.

Subjects were individually tested in seven experimental sessions. The first three sessions were pre-study sessions during which the subject could receive placebo, oral ethanol (lg/kg) or intranasal cocaine hydrochloride (100 mg). Subjects who discriminated correctly the effects of both drugs run into the study sessions.

In the four study sessions the volunteers received oral ethanol (1 g/kg), intranasal cocaine hydrochloride (100 mg) or placebo for both drugs in a double-blind, double-dummy, randomized, crossover design. There was a 72 hours washout period between sessions. After baseline measures, a drink containing ethanol or placebo was consumed over a 30 minutes period, followed by inhalation of cocaine or placebo.

Physiological parameters, subjective effects and psychomotor performance were measured at baseline and at different times during the experimental sessions. Visual analog scales were used to assess subjective effects. Performance tasks included Critical Flicker Fusion, an arithmetical task (Pauli test) and a Simple Reaction Time test. A Maddox wing device was used as a measure of exophoria.

Cocaine alone and in combination with ethanol significantly increased systolic and diastolic blood pressure. In addition concurrent administration of both drugs produced an increase in heart rate greater than that observed after cocaine or ethanol alone.

Subjects responded appropriately to the specific drug items, "high" which was significantly elevated only under cocaine conditions and "drunken" which was elevated only under ethanol

conditions. Ethanol and cocaine combination produced significant greater increases on subjective effects measures of "good effects" and "feeling good", than that produced by ethanol or cocaine alone. There was a significant increase of the subjective measure of "worse performance" following ethanol alone in comparison with placebo but no differences were found between the combination of both drugs and placebo.

Ethanol alone significantly decreased the total number of responses on the arithmetical task and increased the reaction time (motor and decision time) at the two evaluations done (45 and 90 minutes after the beginning of the session). Ethanol alone also increased the degree of exophoria (45 and 90 minutes test) and decreased the critical flicker frequency at 90 minutes. Cocaine alone decreased motor time and decision time at 90 minutes.

Cocaine significantly antagonized the ethanol-induced impairment on the arithmetical task at 45 and 90 minutes, reaction time at 45 minutes and critical flicker fusion at 90 minutes. The combination of both drugs did not modify the changes in exophoria produced by ethanol.

The results of the present study suggest that cocaine reverses some of the effects of ethanol on psychomotor performance and increases some of the subjective effects reported as pleasurable. It seems that ethanol and cocaine in combination produce greater cardiovascular effects than that observed following the administration of ethanol or cocaine alone.

AFFILIATION: Department of Pharmacology and Toxicology. Institut Municipal d'Investigació Medica. Barcelona, Spain.

Cocaethylene Inhibits Uptake of Dopamine and Can Reach High Plasma Concentrations Following Combined Cocaine and Ethanol Use

P. Jatlow, W.L. Hearn, J.D. Elsworth, R.H. Roth, C.W. Bradberry, and J.R. Taylor

Concurrent use of cocaine and ethanol is common. Anecdotal reports from users indicate prolongation of euphoria and moderation of acute withdrawal symptoms with the combination. Two reports in the literature have each described traces of cocaethylene (the ethyl ester of benzoylecgonine) and/or its metabolites in the urine of individuals who had used both cocaine and ethanol[1,2]. This metabolite presumably arises either through transesterification of cocaine, or ethylation of benzoylecgonine.

We have measured high concentrations of cocaethylene in blood from seven fatalities associated with cocaine use and consumption of ethanol. Cocaethylene concentrations, which were determined by HPLC ranged from 73 to over 1000 ng/ml, in association with ethanol concentrations of 20 to 240 mg/dl. In some instances the concentrations of cocaethylene exceeded that of the parent compound. Concentrations of cocaine in the same range as we found for cocaethylene are associated with significant pharmacological activity. We confirmed the identity of the compound measured in blood by gas chromatography/mass spectrometry. Cocaethylene, which we used as an analytical standard, and in subsequent neurochemical experiments was synthesized by esterification of benzoylecgonine in ethanolic HCl.

We also evaluated the neurochemical and behavorial activity of cocaethylene. Cocaethylene was equipotent to cocaine at inhibiting dopamine uptake into rat synaptosomes prepared from either nucleus accumbens or striatum (IC_{50} 250nM). Cocaethylene and cocaine were also equipotent in inhibiting the binding of [^{3}H]GBR, a dopamine uptake blocker, to striatal membranes (IC_{50} 300nM). Intravenous administration of 1 mg/kg of cocaethylene to rats caused a three fold increase in concentration of extracellular dopamine in the nucleus accumbens as determined by microdialysis. Rats treated with cocaethylene also showed increased locomotor activity.

Formation of this active metabolite may contribute to the toxicity and psychotropic effects which follow the combined use of cocaine and ethanol. Supported by NIDA grants P50 DA04050 and DA05119.

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AFFILIATION

Depts. of Laboratory Medicine, Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT and Dade County Medical Examiners Dept., Miami, FL.

Acute Tolerance to Cocaine Pressor Effects in Humans

John J. Ambre

Acute tolerance develops to the cardiac chronotropic and subjective effects of cocaine in humans. We have shown previously that tolerance to the chronotropic effect is incomplete, such that the heart rate decline in the presence of stable plasma cocaine concentrations approaches a plateau that exceeds the baseline heart rate (Clin Pharmacol Ther 44:1,1988). One possible mechanism for heart rate decline could be pressor-induced reflex slowing. We have investigated this phenomenon in intravenous (IV) cocaine users given prolonged steady state IV cocaine infusions, as described previously. We have found that the contour of the pressor response, under conditions of the study, is identical to that of the chronotropic response. Application of our kinetic-dynamic model gave a tolerance factor of 19 minutes, suggesting that approach to the plateau far exceeds the expected time course of cardiovascular reflexes or baroreceptor resetting. We also analyzed data presented in a report at variance with our conclusion (Drug and Alcohol Dep 22:169,1988). In that study heart rate, pressor and subjective effect data were collected after repeated intranasal doses of cocaine. We found that the data are describable by our model and, in fact, provide further evidence to support our view. We conclude that tolerance does indeed develop to the pressor effects of cocaine, that the response is similar to the tolerance to heart rate effect, and that our mathematical model of tolerance can also be used to describe the effects of cocaine taken in the more common manner, intranasally.

AFFILIATION

Northwestern University Medical School
Chicago, IL 60611

Pharmacologic Effects of Methamphetamine Vapor Inhalation (Smoking) in Man

Mario Perez-Reyes, Reid White, Susan McDonald, Judy Hill, Robert Jeffcoat, and C. Edgar Cook

The popularity of methamphetamine (ice) smoking is rapidly increasing. To investigate the clinical pharmacology of methamphetamine smoking a study was conducted in which normal volunteers were tested in a randomly-assigned, crossover design with the intravenous injection and the vapor inhalation (smoking) of methamphetamine HCl. Comparison between these two routes of administration was considered to be essential to discern possible differences inherent to the smoking process.

METHODS

Subjects: Six healthy, male, paid volunteers familiar with the use of amphetamines participated in the study. All were fully informed about the procedures, significance, and risks of the study. All signed a consent form approved by the Committee on the Protection of the Rights of Human Subjects of the University of North Carolina at Chapel Hill. Their age was 26.7 ± 1.7 years, their weight was 84.1 ± 5.2 kg, and their height was 176.4 ± 1.5 cm.

Vapor Inhalation Dosage: Thirty mg of methamphetamine HCl were placed in the bowl of a glass pipe heated to 300°C. The subjects were instructed to inhale the vapors produced completely and to hold them in their lungs for 15 secs. This process was repeated at one minute intervals until no drug or vapors were visibly present in the pipe (ca. 4 mins). This dose and method of inhalation were found in pilot experiments to produce distinct subjective and cardiovascular effects without untoward reactions.

Intravenous Injection: Fifteen mg of methamphetamine HCl were intravenously injected into the same subjects. This dose was dissolved in 3 ml of normal saline and injected over a 1 min interval.

Subjective Rating of Amphetamine-Like "High:" For this rating, the subjects were asked to estimate their level of "high" on a

scale of 0 to 100. Zero represented no drug effects, and 100 represented the "highest" they had ever been after taking amphetamines. Every time a rating was to be made, the subjects were given their previous ratings for comparison. This technique allowed the subjects to rate themselves as experiencing relatively more, less, or the same effects as those rated in the previous interval.

Cardiovascular Effects: Because methamphetamine is a sympathomimetic drug, it produces distinct changes in cardio-vascular function. The heart rate and the blood pressure were measured by an Accutracker Instrument (Suntech Medical Instruments Inc.). This instrument is portable and measures the heart rate and the blood pressure at any desired time interval, e.g., every 2 min or longer. The stroke volume, cardiac output, systolic time intervals, and measures of myocardial contractility were measured by a computer averaged impedance cardiogram. The total peripheral resistance was calculated from simultaneous deter-minations of the cardiac output and the blood pressure. All of these parameters were recorded at frequent intervals before and after drug administration, and always when the subjects were completely at rest. The data obtained are reported as the percent changes over baseline values.

RESULTS

Subjective Effects: The subjective ratings of "high" produced by the intravenous injection or the vapor inhalation of methamphetamine were more prominent during the first 30 minutes after drug administration. Peak effects were of similar magnitude between the routes and were considerably below the maximum that the subjects had previously experienced (41.5 ± 10.1 and 38.0 ± 9.8 percent for the intravenous and inhalation routes, respectively). The subjective effects of methampheta-mine completely subsided six hours after drug administration.

Cardiovascular Effects: Paralleling the subjective effects, cardiovascular changes were more prominent during the first 30 min after methamine administration (range 8.3 - 25.8 and 8.3 - 19.2 mins for the intravenous and inhalation routes, respectively). The magnitude and pattern of the cardiovascular effects produced by the intravenous injection or the vapor inhalation of methamphetamine were similar: heart rate was accelerated, systolic and diastolic blood pressures were elevated, stroke volume and cardiac output were increased, pre-ejection period, left ventricular ejection time, and total peripheral resistance were decreased. These effects are typical of those produced by the intravenous injection of epinephrine and not of those produced by the intravenous injection of norepinephrine.

DISCUSSION

The results indicate that the subjective and cardiovascular

effects produced by the intravenous injection of 15 mg of methamphetamine HCl were of similar magnitude to those produced by the vapor inhalation of 30 mg of the drug. This finding suggests that, under the experimental conditions used, the bioavailability of vaporized methamphetamine is approximately 50%. Theoretically, the following factors can account for this finding: pyrolytic degradation, deposition of the drug in the smoking apparatus, and trapping in the mucosas of the respiratory tract. Pyrolytic degradation does not appear to be a factor because the results of simulated smoking experiments indicate that no pyrolysis of the drug occurred. However, it was found that a constant amount of methamphetamine HCl (ca. 10 mg) was trapped in the cooler portions of the pipe and, hence, reduced bioavailability by approximately 30%. Moreover, a portion of the drug inhaled may be trapped in the mucosas of the respiratory tract further reducing its bioavailability.

The dosage of methamphetamine used in this study was selected to produce moderate subjective and cardiovascular effects to protect the well-being and safety of the volunteers. Therefore, the subjective effects produced by the vapor inhalation of 30 mg of methamphetamine were of modest magnitude and of relative short duration (ca. 6 hours). However, in preliminary experiments one of the subjects inhaled the vapors produced by the heating of 40 mg of methamphetamine. This subject experienced a pronounced hypomanic reaction that lasted approximately 2 hours, intense craving for further dosing, decreased appetite, difficulty in concentration, memory lapses, and insomnia. This reaction to a moderate increase in dosage (i.e., from 30 to 40 mg of methamphetamine) highlights the powerful effects that can be expected to occur from larger doses of smoked methamphetamine (i.e., 100 mg which is the usual amount of a "hit").

The cardiovascular effects of methamphetamine have been reported to be mediated by the release of norepinephrine at sympathetic nerve terminals. However, the pattern of the cardiovascular effects observed in this study, resembles those produced by epinephrine rather than norepinephrine release. This observation needs verification in placebo-controlled, dose-response experiments in which the levels of circulating catecholamines are measured before and after methamphetamine administration.

AFFILIATIONS

University of North Carolina at Chapel Hill, School of Medicine and the Research Triangle Institute.

Plasma Levels of Methamphetamine After Smoking of Methamphetamine Hydrochloride

**C. Egar Cook, A. Robert Jeffcoat, Mario Perez-Reyes,
Brain M. Sadler, Judith M. Hill, W. Reid White, and
Susan McDonald**

Unlike cocaine hydrochloride, which decomposes extensively upon heating and which must therefore be smoked in the form of the free base, the hydrochloride salt of methamphetamine volatilizes rather readily upon heating and the drug is smoked in this form. Experiments in our laboratory have shown that at temperatures of 300° over 90% of the methamphetamine hydrochloride placed in a pyrolysis furnace can be recovered as methamphetamine by trapping the volatiles from the pyrolysis apparatus (K. Davis, personal communication).

A glass pipe was designed for use in pharmacokinetic/pharmacodynamic studies of the smoking of methamphetamine hydrochloride. In preliminary experiments, a large volume syringe was used to simulate the smoker's lungs and to pull the vapors through a series of acid traps. When the amount of methamphetamine hydrochloride was 16 mg and the pipe was heated to 268°, 9 mg of the methamphetamine was recovered from the pipe, with the balance being drawn into the traps. When the amount of salt placed in the pipe was increased by 50%, the average amount remaining in the pipe was increased by only about 20%. The residue remaining in the pipe appears to be more a function of the area of cooler surface on which it can condense than of the absolute amount placed in the pipe.

Clinical studies were then carried out with methamphetamine hydrochloride (30 mg/dose, with the pipe heated in an aluminum block kept at 302-308°C). Plasma samples were taken at frequent intervals and were analyzed for methamphetamine and amphetamine. An internal standard, N-methylphenethylamine, was added to the plasma, which was then made basic and extracted with pentane. The pentane was back extracted with dilute sulfuric acid solution which was again made basic and reextracted, this time with methylene chloride. The methylene chloride solution was concentrated and then the amphetamine derivatized by addition of trichloroacetic anhydride followed

by heating at 60° for 10 min. Overall recoveries through the extraction process were monitored by use of radiolabeled compounds and were found average 69 -73%. The trifluoroacetamide derivatives were then chromatographed on a DB-5 gas chromatography column. The standard curve for methamphetamine was linear over a range of 1-225 ng. A standard curve was obtained from 1-15 ng for amphetamine.

Although the initial plasma concentrations of methamphetamine rose rapidly after the start of smoking, they essentially plateau over the first 3-4 h of the experiment, after which they begin to decline. It was not possible to fit a standard 1 or 2 compartment pharmacokinetic model with an absorption phase to these data. However, by use of a model-independent method, it was possible to determine an elimination half-life with an average value of 11-12 h and a range of 8-17 h. These values compare quite well with half-lives of 4-15 h which we have observed on oral administration of methamphetamine hydrochloride. Amphetamine is both a metabolite and pyrolysis product of methamphetamine and was analyzed in the same chromatogram as methamphetamine. However, levels of this compound were quite low.

There is a strong contrast between cocaine and methamphetamine plasma levels after the two drugs are smoked. Cocaine levels after smoking of cocaine free base rapidly peak and with some minor deviations also rapidly decline with a terminal half-life of ca. 56 min (Jeffcoat et al., 1989). Methamphetamine levels, although they rapidly approach peak concentrations, remain high for a considerable period of time before declining with a half-life of about 12 h. Regardless of the explanation for this phenomenon, this long plateau effect and the half-life of methamphetamine suggest considerable dangers in repeated smoking of methamphetamine since markedly higher plasma concentrations could be expected to occur if the dose is repeated, even at fairly long intervals.

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AFFILIATIONS

Research Triangle Institute and University of North Carolina School of Medicine.

Alcohol Effects on HCG Stimulated Gonadal Hormones in Women

Siew Kooh Teoh, Jack H. Mendelson, Nancy K. Mello,
Alicja Skupny, and J. Ellingboe

Chronic alcohol abuse is associated with derangements of reproductive function in women. Amenorrhea, anovulation, luteal phase dysfunction and early menopause appear to be associated with chronic alcohol use (Mello et al. 1989, Mello, 1988). The mechanism of increased risk for alcohol-related abortions and fetal alcohol syndrome is unknown. However, there has been very little research on alcohols effects on maternal hormones during early pregnancy. This omission is significant since the developing fetus is especially prone to drug induced malformations during the first trimester of pregnancy. The goal of this study was to determine if acute alcohol administration affected gonadal steroid hormone levels following administration of human chorionic gonadotropin (hCG). hCG was used to simulate the endocrine milieu of early pregnancy.

Ten Caucasian women between the ages of 21 and 33 provided informed consent for participation in the study. All women were studied during the mid-luteal phase of the menstrual cycle (between day 17 to 23). Plasma estradiol (E2), progesterone and prolactin levels were measured before and after simultaneous administration 5000 IU hCG(Profasi) and alcohol (0.7 g of alcohol/kg body weight) or placebo solution under double blind conditions.

There was a significant increase in plasma E2 ($P<0.001$) and prolactin levels ($P<0.01$) after hCG and alcohol administration but not after hCG and placebo administration. Plasma progesterone increased significantly ($P<0.01$) above baseline after hCG and placebo administration but this was not observed after hCG and alcohol administration.

It is possible that alcohols disruptive effects on maternal endocrine balance may contribute to fetal dysmorphologies. Our finding of increased estradiol levels suggests a possible mechanism of alcohol-related congenital anomalies in infants born to women who consume alcohol during early pregnancy when chorionic gonadotropin levels are high. Ovarian progesterone is essential for the maintenance of early pregnancy until placental progesterone production is established at the eighth gestational week. Alcohol's attenuation of the expected progesterone response to hCG stimulation could increase the risk for spontaneous abortion. The biological significance of alcohol-related increases in prolactin levels during gonadotropin stimulation has yet to be determined. Increased prolactin levels have been observed in alcoholic women during the 16-24th gestational weeks compared to abstinent control women at the same stage of

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AFFILIATION

Alcohol and Drug Abuse Research Center, Harvard Medical School-McLean Hospital, 115 Mill Street, Belmont, MA 02178

Medical Methadone/Interim Report

Edward Senay, Andrea G. Barthwell, Robert Marks, and Richard White

Common clinical experience indicates that there are many methadone maintenance patients who do well in treatment and who do not appear to need intensive care to maintain stability. Novick et al. found that such patients could be maintained on low levels of care and termed the regimen "medical maintenance." We studied "medical maintenance" in 135 methadone maintenance patients who were (a) between 21 and 60, (b) had no chronic relapsing illnesses, (c) on methadone for at least one year with the most recent six month period free of drug use or arrests and not on parole or probation, (d) good compliance with treatment, (e) employed or fulfilling homemaker or student roles, (f) no desire to detoxify for the coming year, and (g) signed an informed consent meeting all HHS criteria. Subjects were randomly assigned to (a) a one year period in the experimental clinic, or (b) to a one year period in which the first six months required them to continue in their clinic of origin under standard regulations for pick ups etc., followed by a six month period in the experimental clinic.

In the experimental clinic, methadone pick up was twice a month with one counseling session and one urine drop per month at the clinic of origin. In addition, true random urine screens were obtained on an infrequent but unpredictable schedule. The Addiction Severity Index (ASI) was administered at entrance and at six month intervals. Results indicate no significant differences between control and experimental conditions on the six criterion scales of the ASI at six months and at one year. No difference was found in the numbers of positive urines between control and experimental conditions. These results indicate that "medical maintenance," at least for a one year period, is a viable option for at least some subset of successful methadone maintenance patients. There were significant differences between units of service delivered in the control and experimental conditions indicating that the cost of treatment is reduced in the "medical maintenance" model.

A questionnaire was administered to all subjects inquiring about the subjects response to the experimental clinic. Without exception all subjects expressed satisfaction, citing most frequently the time freed and the absence of contact with "negative" people as the most salient factors in their satisfaction. Additional studies need to be carried out to examine replicability and to examine periods of time beyond one year. Most subjects indicated that they would rather continue in the experimental condition than to try to detoxify. The study will be

completed on October 1st of this year. The analyses reported above were carried out with an N smaller than we will have when all subjects complete the study.

Addictions Research Institute - DASA
100 West Randolph Street - 5th Floor
Chicago, IL 60601

The Effects of Buprenorphine in Methadone-Dependent Volunteers

E. C. Strain, G. E. Bigelow, K. L. Preston, and I. Liebson

The purpose of this study was to characterize the effects of buprenorphine in comparison to naloxone, hydromorphone, and placebo, in methadone-dependent volunteers. Participants were six inpatient male opiate abusers maintained on 30 mg of methadone daily, who underwent testing 2-3 times per week. Each test session consisted of baseline data collection, followed by a double-blind IM injection of either: buprenorphine (dose range 0.5-8.0 mg), hydromorphone (5-10 mg), naloxone (0.1-0.2 mg) or saline, followed by 2 hours of data collection. Injections were administered 20 hours after the last dose of methadone. Measures included physiologic monitoring, pupillary photos, and subject and observer ratings of drug effects.

Naloxone and hydromorphone produced characteristic antagonist-like and agonist-like effects, respectively, on both subjective and objective indices. In contrast, buprenorphine produced neither antagonist-like nor agonist-like effects. No dose of buprenorphine produced significantly increased subject ratings of drug effect, high, good effects, bad effects, liking, or sick. Likewise, no dose of buprenorphine produced significant changes in physiologic measures, pupillary diameter, or observer ratings of drug effects. Subjects did not identify buprenorphine as belonging to any particular drug class on a drug class identification questionnaire. Furthermore, subject and observer ratings using adjective checklists did not significantly distinguish any of the doses of buprenorphine as either agonist-like or antagonist-like. Observer ratings for signs of opiate withdrawal were not significant for buprenorphine. These results suggest buprenorphine produces minimal effects in methadone-dependent patients. This has implications for the use of buprenorphine in drug abuse treatment, and also suggests buprenorphine in this dose range has a low abuse potential in methadone-dependent patients. Relative to other opioid mixed agonist-antagonists buprenotphine appears to have a low antagonist potency relative to its analgesic potency.

AFFILIATION

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Francis Scott Key Medical Center, Baltimore, MD 21224

Outpatient Comparison of Buprenorphine and Methadone Maintenance. I. Effects on Opiate Use and Self-Reported Adverse Effects and Withdrawal Symptomatology

Rolley E. Johnson, Paul J. Fudala, and Jerome H. Jaffe

INTRODUCTION. One-hundred sixty-two opiate-dependent volunteers participated in an outpatient study conducted at the NIDA Addiction Research Center (Baltimore, MD) to assess the efficacy and safety of buprenorphine compared to methadone for the maintenance and detoxification of opiate addicts. Based on previous data obtained by the authors (1-3) and others (4-6) the working hypothesis of the study was that buprenorphine, at a daily dosage of 8 mg administered sublingually, would be more effective than methadone 20 mg given orally and not different in efficacy from methadone 60 mg. Preliminary results of that study are presented here.

METHODS AND RESULTS. Participants were stratified into treatment groups by age (21 to 35 and 36 to 50) gender, and Clinical Institute Narcotic Assessment (CINA; 7) scores obtained from a naloxone challenge test. The naloxone challenge test was given to all participants immediately prior to entering the study to ensure that one treatment group was not overly represented by individuals having average levels of opiate dependence greater or lesser than another. All participants met DSM-III-R criteria for opiate dependence. Participants were offered, but not required to attend 30 to 60 minutes of individual counseling weekly. For the purpose of evaluating the medical safety of buprenorphine, hematology and blood chemistry panels, urinalysis, and vital signs were monitored periodically. Patient report forms and medical records were maintained **for** each participant. Certified urine samples were collected three times weekly on Monday, Wednesday, and Friday. Study participants were required to come to the clinic daily to receive their medication. Since this was was a pharmacologic comparison of buprenorphine and methadone, non-pharmacologic interventions (such as counseling and social services) and contingencies were strictly controlled. Continued participation in this study was only contingent on an individual never missing more than 3 consecutive clinic visits.

The study was conducted under double-blind and double-dummy conditions (i.e., both oral and sublingual dosage forms were given to all participants). The three treatment groups were buprenorphine, 8 mg sublingual (53 participants), and methadone 20 (55 participants) and 60 mg oral (54 participants). Induction onto methadone 60 mg was accomplished by administering 20 mg initially, followed by daily increases of 10 mg until the 60 mg dosage level was achieved. For participants in the 20 mg group, 20 mg was given on the first day, followed by 30 mg for 4 days, 25 mg for 4 days, and then 20 mg daily. Induction onto buprenorphine was done by administering 2,4, and 8 mg over the

first three study days (8) and then continuing with 8 mg daily. The study consisted of a 119-day induction/maintenance phase, followed by a 56-day detoxification phase. Preliminary results from the first phase of the study are presented here.

For the analysis of urine samples for the presence of opiates, excluding methadone, data were analyzed from each Monday, Wednesday, and Friday sample with missing samples considered to be missing. All samples were initially screened by radioimmunoassay and then re- analyzed using enzyme-multiplied immunotechnique. Both methods used a cutoff value of 300 ng/ml. Over all the weeks of the study, buprenorphine treatment was associated with an average 63% of urine samples negative for opiates, methadone 60 mg with an average 51%, and methadone 20 mg with an average 36%.

Throughout the study, participants were asked every two weeks to report whether or not they experienced any of 14 adverse effects during the past week. These adverse effects were those which could be expected of the study medications, of illicitly administered opiates, or part of an opiate withdrawal syndrome. Self-reported adverse effects ranged from 37% to 100% for individual items within each week; however, no pattern of results was observed between treatment groups or across weeks.

Each day, participants completed a 22-item, yes/no, opiate withdrawal symptom checklist (Addiction Research Center Inventory subscale 459). From their responses, an average score for each participant was calculated by week. No differences were observed between treatment groups for self-reported opiate withdrawal symptoms across weeks.

DISCUSSION. Over the 17 weeks of the maintenance phase of the study, buprenorphine was both clinically and statistically better than methadone 20 mg, while methadone 60 mg and buprenorphine were not significantly different. Although the percentage of reported adverse effects appeared high, it could not be determined whether the effects were secondary to treatment medications, illicitly used substances, withdrawal symptomatology, or a combination of these factors. The lack of differences observed between treatment groups for self- reported opiate withdrawal symptoms across weeks could be interpreted to mean that there were no differences between treatments with respect to their ability to suppress opiate withdrawal symptomatology. However, it is more likely that participants who received methadone 20 mg had scores comparable to the other two groups because of increased usage of illicit opiates, as evidenced by the results of urine toxicology screens.

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AFFILIATION
 Addiction Research Center, National Institute on Drug Abuse
 Baltimore, Maryland 21224

Outpatient Comparison of Buprenorphine and Methadone Maintenance. II. Effects on Cocaine Usage, Retention Time in Study and Missed Clinic Visits

Paul J. Fudala, Rolley E. Johnson, and Jerome H. Jaffe

INTRODUCTION. This report is an extension of the study outlined in this volume (Johnson et al.) assessing the efficacy and safety of buprenorphine compared to methadone for the treatment of opiate dependence. When this study was begun, there were no published data which suggested that buprenorphine might be effective in decreasing cocaine self-administration in opiate addicts, While this study was in progress, though, two open-label trials by Kosten (1,2) and his colleagues gave preliminary evidence that less cocaine abuse was associated with buprenorphine than with methadone treatment of cocaine- abusing opiate addicts, Also, Mello and her colleagues (3) using rhesus monkeys, observed that buprenorphine produced a suppression of cocaine self-administration. However, no double-blind controlled investigation of the effects of buprenorphine on cocaine usage in humans has been reported.

METHODS. Study methods are reported in "Outpatient comparison of buprenorphine and methadone maintenance I," contained in this monograph. Participants were not initially stratified into treatment groups based on pre-study cocaine usage, however, prior to breaking the study blind, an analysis of participants was performed based on self-reported cocaine usage during the 14 days prior to their admission into the study. Participants were categorized into groups based on self-reported cocaine use of zero, 1 to 6, or 7 to 14 days; there were no significant differences between the groups. All participants, except one in the buprenorphine group, did report some prior use Of cocaine. For the analysis of urine samples for the presence of cocaine metabolite, data were analyzed from each Monday, Wednesday, and Friday sample with missing samples considered to be missing. All samples were initially screened by radioimmunoassay and then re-analyzed using enzyme-multiplied immunotechnique. Both methods used a cutoff value of 300 ng/ml.

RESULTS. The overall rate of cocaine-negative samples averaged approximately 43 percent over the first 17 weeks of the study, with no significant differences between groups observed for any week, and also, no pattern of results which suggested any treatment to be different from another.

Participants receiving buprenorphine or methadone 60mg had approximately equal retention rates, 40% and 37%, respectively, after the first 119 days of the study, while the retention rate of participants receiving methadone 20 mg was 20%, approximately one-half that of the other two groups. Differences in retention rates between the methadone 20 mg group and the other two groups began to become evident between about study day 40 and 50 with clearer differences between groups observable at about study day 75. When the survival curves of all three groups were compared through 119 days using Cox regression analysis, the differences between them were significant (p less than 0.05). The number of missed clinic visits for each subject was obtained and the percentage of missed visits through the first 119 study days was calculated. The overall percentage of missed clinic visits ranged from 12 to 15 % per group and these differences were not significant.

DISCUSSION. Results from this study did not support the hypothesis that buprenorphine is more effective than methadone in suppressing cocaine self-administration in opiate-dependent cocaine abusers. Buprenorphine 8 mg given sublingually and methadone 60 mg produced significantly greater retention rate when compared to methadone 20 mg through the maintenance phase of the study. Although the percentage of reported adverse effects appears high, we cannot determine whether the effects were secondary to treatment medications, illicitly used substances, withdrawal symptomatology, or a combination of these factors.

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AFFILIATION

Addiction Research Center
National Institute on Drug Abuse
Baltimore, Maryland 20857

Methadone Maintenance Outcome As A Function of Detoxification Fear: Preliminary Findings

Jesse B. Milby, Mary Gentile, Mary Kaye Sims,
A. Thomas McLellan, George Woody, and Neil Haas

Milby and colleagues identified "detoxification phobia" and researched its prevalence among methadone maintenance patients (Milby et al. 1979, 1980). They established the reliability and validity of the Detoxification Fear Survey Schedule (DFSS) (Milby et al., 1987), a screening instrument for detoxification fear, and established the concurrent validity of the fear phenomenon using psychophysiological measures (Raczynski et al., 1988). Methadone maintenance patients from three geographically and demographically distinct settings again revealed prevalence from 22-35% (Milby et al., 1986). Fear patients had higher scores, longer addiction histories, spent more of their lives addicted, and were older. Thus subgroups of patients experiencing detox fear have been reliably identified in different treatment populations. Detox fear patients have been proposed to have difficulty achieving a drug-free lifestyle, regardless of attaining other rehabilitation goals, and preliminary research has supported this contention. This study followed treated opioid addicts who were randomly selected in 1983-84 from 3 methadone maintenance populations.

METHOD

Subjects. Subjects from the original 1983-84 random sample of 271 drawn from 3 programs: Philadelphia (n=111), Sepulveda (n=100) VAMC's and UAB Birmingham (n=60) for whom charts were still available, served (Milby et al., 1986).

Procedure. Records were reviewed by two technicians trained by J.B.M. Each used operational definitions from a 5-year Birmingham follow-up study (Schumacher, Milby and Fishman, 1989) and were "blind" to each other's findings. An interview assessment and psychological test battery were completed on consenting subjects from the previous sample. Data were analyzed as a function of previously determined detoxification fear status. This study focuses on current treatment status and outcome variables from the chart review.

RESULTS

Current treatment status and initial outcome data from the 3 populations are presented in Table 1. next page.

TABLE 1

	PHILADELPHIA	SUPULVEDA	BIRMINGHAM
ORIG. SAMPLE	111	99	61
INTERVIEWED	111	43	25
CHART REVS.	34	70	58
DIED	11	11	4
DECLINED	16	7	10
NOT LOCATED	47	38	22
STILL IN TREATMENT	33	27	18

Of 105 patients for whom follow-up assessments were completed, 62% were currently in methadone maintenance and 50% reported abstinence. Average months on maintenance was 55.5 for non-fear patients (n=160, SD=34.8) versus 100.2 (n=65, SD=62.5) for those with detox fear (p$\geq$.000). The average detoxification attempts for non-fear patients was 2.3 (SD=2.3) vs. 1.6 (SD=2.2) for fear patients p$\geq$.013). Average successful detoxification from maintenance was 0.69 (SD=.91) for non-fear patients vs. 0.15 (SD=.40) for those with detox fear (p$\geq$.000).

DISCUSSION

These preliminary data suggest detox fear may be one of the important variables affecting methadone maintenance treatment outcome. Because detoxification is a final common path for addicts who aspire to a drug-free adjustment, detoxification can be a significant barrier for those having pathological fear of detoxification. Our previous prevalence studies of pathological detox fear suggest its presence in 22-35% (Milby et al., 1986). Its prevalence and these current results suggesting its negative impact on maintenance outcome, suggest outcome may be improved if this fear is identified and treated before detoxification is attempted. Treatment focused on detox fear patients in current methadone maintenance programs may have the additional benefit of freeing significant numbers of treatment slots occupied by patients who continue on maintenance because of their fear of detoxification.

AFFILIATION:

VAMC and University of Alabama at Birmingham

VAMC Philadelphia and University of Pennsylvania

VAMC Sepulveda and University of California at Los Angeles

Comparison of SCID and LEAD Standard Diagnoses for Drug and Psychiatric Disorders

K.J. Bryant, B.J. Rounsaville, and T.F. Babor

It is important to determine the adequacy of any new psychiatric diagnostic procedure. The Structured Clinical Interview for DSM-III-R (SCID) was recently developed by Spitzer, Williams and Gibbon to diagnose major psychiatric and drug dependence disorders. This clinical interview can be administered by a trained non-clinician and promises to provide an effective research tool for comparing varied samples with a wide range of disorders. The SCID must be compared to some known diagnostic standard to establish its criterion validity. Spitzer has proposed such a criterion against which structured clinical interviews can be compared. Called the "LEAD" standard this criterion is based on compound expert diagnosticians' pooled judgements.

The acronym "LEAD" stands for Longitudinal, Expert, and All Data. Spitzer proposes that the most valid expert diagnosis will be made through repeated opportunities to evaluate the patient (Longitudinal), through the combining of multiple viewpoints of several experts (Expert), and through access to Information about a patient which is as complete as possible, including observational, life history, and self-report data (All Data).

The present study compares separate and consensual diagnoses made by "LEAD" experts with diagnoses obtained from the SCID administered by a trained non-clinician interviewer. Subjects were selected from an Inpatient alcohol and drug treatment program and had multiple drug, alcohol and psychiatric diagnoses. This inpatient population provides a challenging test of diagnostic accuracy for both the SCID and LEAD experts.

The present research has three specific goals. The first is to establish the inter-rater reliability for two expert raters' drug and psychiatric diagnoses. The second goal is to combine these ratings using all available information and to obtain a criterion measure, the LEAD (Longitudinal, Expert, All Data) standard. The third goal is to compare the performance of a structured clinical interview based on DSM-III-R criteria (SCID) given by a trained research Interviewer to the expert-based LEAD standard.

In this presentation we will focus on the third goal: the comparison of the SCID diagnoses with the LEAD standard diagnoses. We will establish whether the SCID diagnoses are an adequate surrogate for expert diagnosticians' judgements within this sample and, thus, whether they provide a research tool that can be used by non-clinician research interviewers.

PATIENTS AND TREATMENT SETTING

The treatment setting is a 21day inpatient alcohol and drug treatment facility located at the University of Connecticut in Farmington. As part of an ongoing study, 29 patients (14 Male, 15 Female, Mean Age=32.5) received the full diagnostic battery and were independently interviewed by two expert clinicians. Patients were selected on the basis of gender, availabllity, assignment to primary clinician, ability to complete the interviews, and agreement to participate in a S-month follow-up. They received the diagnostic battery within the first week after admission and all procedures were completed before discharge.

SCID ADMINISTRATION, DIAGNOSTIC BATTERY

The research interviewers were trained In the administration of the SCID. The SCID was included in a battery of measures in which the patient was interviewed about drug use behaior and family history of psychiatric disorders. In addition, they completed self-report measures of drug use behavior. The SCID Interview required that individuals respond yes, no or probably to key items about drug use and psychiatric problems. These key items were followed by more specific items to establish a diagnosis.

Both Axis I and Axis II Personality Disorders were covered in this most recent 1989 version of the SCID. Diagnoses of substance dependence were based on the DSM-III-R nine criteria questions. An answer of yes to any of three of the eight criteria merited a diagnosis of substance dependence. Eight clusters of drug use disorders were covered: alcohol, cocaine, opiates, marijuana, stimulants, sedatives, hallucinogens and polysubstance drug use disorders.

For the purpose of these analyses, the presence or absence of substance use, whether abuse and dependence is the primary substance use definition of interest. For other psychiatric diagnoses the disorder is scored as present regardless of the prior substance use disorders.

LEAD EXPERTS

The three expert diagnosticians had had extensive experience with diagnosing and treating patients with substance use disorders. Each expert was the primary clinlcian on approximately 10 cases and the secondary diagnostician on 10 additional cases. For the

their initial ratings, the clinicians were instructed to make all DSM-III-R diagnoses independently from one another using their usual methods. After making initial diagnoses the two experts met to reconcile differences and all additional information (test outcomes, family history, etc) were provided except for SCID diagnoses. From this meeting consensual or "LEAD" diagnoses were agreed upon and reasons for differences recorded.

COMPARISON OF LEAD AND SCID RATES OF DIAGNOSES

LEAD experts made a total of 144 current and lifetime diagnoses of which 110 (78%) are current disorders. They made the same number of substance-related (55) as psychiatric (55) diagnoses within this sample of 29 individuals. This is approximately four diagnoses per patient ranging from two to nine diagnoses. In general, the LEAD experts characterize this group as equally drug and psychiatrically impaired.

The SCID yielded fewer total (lifetime + current) diagnoses (130) and current diagnoses (97), but the same proportion of total diagnoses to current diagnoses (78%). The SCID yielded the same number of current substance use disorders as the LEAD experts (55), although these were not the identical diagnoses. However, fewer current psychiatric diagnoses (42) were made using the SCID. This resulted in only 1.4 psychiatric diagnoses per patient in comparison with almost 2 for the LEAD experts. Overall the SCID characterized this patient group as more drug impaired than psychiatrically impaired.

DETECTION OF CURRENT DIAGNOSES

As previously stated, approximately 75% of all diagnoses are current diagnoses in this sample. Since detecting a current problem will influence the course of treatment that a clinician prescribes, failure to detect a current condition is a serious diagnostic error. The LEAD standard and SCID can be compared on the frequency with which 1) LEAD and SCID agree on a current diagnosis; 2) LEAD experts make a current diagnosis not made by the SCID; and 3) current diagnoses are made by the SCID, but not by the LEAD experts. Percent agreement can be calculated as the number of agreements over the total number of diagnoses.

Substance Use Disorders: There was agreement on 82% (50) of the 61 joint current LEAD and SCID diagnoses. Six current substance use diagnoses (10%) were made by the LEAD experts, but not by the SCID. Five current Substance Use diagnoses were made by the SCID, but not by the LEAD experts (8%).

Agreement ranged from 95% for cocaine to 50% for opiates. Agreement for other diagnoses fell in between (alcohol 88%, marijuana 82%, and sedatives 75%). Diagnoses for polysubstance (1) and stimulant (2) abuse or dependence were made by the LEAD

experts, but not by the SCID. A single consensual diagnosis was made for hullucinogens.

The SCID neither under- nor over-diagnosed drug and alcohol dependence disorders. The degree of disagreement between the LEAD experts and the SCID was less than that between the primary and secondary experts (approximately 70% agreement). The inability of the SCID to detect stimulant disorders was the only distinct problem when the SCID was compared with LEAD experts.

Psychiatric Diagnoses: LEAD and SCID psychiatric diagnoses agreed only 55% of the time (34 out of 62 diagnoses) for the nine psychiatric categories found within this sample. This is in contrast to the 82% agreement between the LEAD and SCID on substance use diagnoses. Notably, LEAD experts made 21 (32%) current psychiatric diagnoses that the SCID did not. The SCID made only seven (9%) current psychiatric diagnoses that the LEAD experts did not.

Closer examination of specific psychiatric diagnoses shows a wide range of agreement (77% to 25%). Agreement on the diagnosis of Major Depression (77%) and Antisocial Personality Disorder (70%) was substantially higher than for other diagnoses (Borderline 60%, Phobia-Simple 50%, Phobia-Social 38%, and General Anxiety 25%). No agreement was found for Dependent Personality, Panic Disorder with Agoraphobia or Simple Panic Disorder. However, there were few diagnoses of these conditions (1, 3 and 1, respectively). Combining categories may improve agreement.

CONCLUSIONS

When used by trained interviewers under research conditions, the SCID can provide a good substitute for LEAD expert judgements about a multiply-diagnosed inpatient sample. Diagnoses of substance use disorders made by either method are in excellent agreement. There is also good agreement between methods for the psychiatric diagnoses most prevalent within this sample-Depression and Antisocial Personality Disorder. Other diagnoses are not easily detected by the SCID.

Yale University School of Medicine

University of Connecticut Medical Center

Utility of the Cognitive Status Examination (CSE) For Detection of Neuropsychological Impairment in Substance Abuse Patients

R. Gillen, H. Kranzler, R. Kadden, and M. Wiedenman

INTRODUCTION

Cognitive deficits are prevalent among substance abuse patients and may be due to a combination of factors, including pre-existing problems, the direct cumulative toxic effect of alcohol and drugs, and factors secondary to the substance abuse, such as traumatic injury. This cognitive impairment may limit the effectiveness of rehabilitation efforts. There is, however, no consensus as to the best method for detection of these deficits. Full neuropsychological (NP) batteries are costly, time consuming and require highly trained personnel for interpretation. Though a number of brief, standardized mental status examinations are now available, these instruments have been criticized for a variety of reasons.

The Cognitive Status Exam (CSE; Barrett & Gleser, 1987) is a recently developed NP screening instrument which is relatively brief (20-30 minutes) and samples a wide range of skills including orientation, memory, language, visual-spatial, motor, and sensory skills. In a combined neurologic and psychiatric sample, the CSE correctly classified 85% of the subjects.

METHODS

We evaluated the ability of the CSE to detect NP impairment in a sample of substance abuse patients. Two hundred (102 females; 98 males) consecutive admissions to a 21-day substance abuse rehabilitation program participated. Their mean age was 38.5 years, and mean education was 11.9 years. Subjects were assigned to one of 4 groups based on DSM III-R diagnoses of abuse or dependence: alcohol only (28%), mixed alcohol and drug (42%), cocaine only (15%), and other drug only (15%). All subjects were classified clinically as having either no evidence of brain dysfunction (Unimpaired = 56%), mild dysfunction (Mild = 37%), or obvious impairment (Obviously Impaired = 7%). Co-morbid psychopathology was found in 21.5% of the sample.

All subjects were administered the CSE between the 7th and 10th day of hospitalization by either a neuropsychologist or a trained testing technician The reliability of scores between the two examiners based on 10 cases scored concurrently was extremely high (r = .98).

RESULTS

The range of CSE scores in this sample was 13 - 104, with mean (± SD) = 84.5 (± 13.1) and median = 87. An analysis of covariance (ANCOVA) compared total CSE score as a function of neurological classification, with both age and education being significant covariates (p < .001). Significant differences were found in total CSE score among the three groups (p < .05). The Unimpaired group performed significantly better than the Mild and Obviously Impaired groups (p < .001). An ANCOVA comparing CSE scores among the four substance use diagnostic categories showed no significant differences. Similarly, there was no significant difference between CSE scores for subjects with and without co-morbid DSM III-R Axis I psychopathology.

Based on a CSE cutoff score of 77, found optimal by Barrett and Gleser (1987), twenty five percent of the sample was identified as impaired. However, at this cutoff score the test had a sensitivity of only .36, suggesting that many patients with impairment escaped detection. Raising the cutoff score to 90 increased sensitivity to .69. In doing so, however, specificity declined to .42.

CONCLUSIONS

Although in this sample of patients the CSE was found to have low sensitivity, the criterion measure employed may not be the most appropriate. We are currently assessing the utility of the CSE using a full NP battery as criterion. The low sensitivity may, however, reflect the difficulty inherent in identification of the subtle cognitive impairment prevalent in substance abuse patients.

The promise of the CSE as a screening instrument for the detection of NP impairment in substance abuse patients remains uncertain. A significant proportion of the sample we studied was classified as impaired. However, the accuracy of this classification, true sensitivity of the CSE, and the relationship of this instrument to skills relevant to recovery from substance use disorders awaits further examination.

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Department of Psychiatry, University of Conn. School of Medicine

Use of Psychopathy Checklist With Opiate Addicts

John S. Cacciola, M. Rutherford, and A.I. Alterman

Psychopathy as measured by Hare's (1980) Psychopathy Checklist (PCL) is an alternative conceptualization of Antisocial Personality Disorder (APD). The PCL, a 20-item checklist, assesses Cleckley's notion of psychopathy using file and interview information. In addition to a global rating of psychopathy, two robust factors have been identified in the PCL: Factor 1, Psychopathic Personality Characteristics and Factor 2, Antisocial Lifestyle. Hare's work, however, has thus far been confined to male prisoners. This investigation is an attempt to expand the use of the PCL to opiate addicts.

Staff training was extensive. The first author was trained in a workshop conducted by Hare and his colleagues. He then trained three project staff members. Using 10 videotapes of prisoners with criminal file information (provided by Hare's group), interrater reliability of the project staff was established by comparing scores of individual raters against a consensus rating by members of Hare's group. Ten additional videotapes of PCL interviews with male methadone patients were made by project staff. Each of the four staff members administered at least two videotapes. Research technicians summarized the clinical charts of interviewed patients, and state criminal records were obtained. All staff independently scored each of the ten videotapes. Tapes were then rated by two of Hare's colleagues who provided a consensus score which was used to establish interviewer reliability on this sample. Following this training, the PCL was administered to new admissions of an outpatient methadone program as part of a larger research project. The PCL was also re-administered and independently scored by a different staff member one month later in order to evaluate test-retest reliability.

The Total score interrater reliabilities (Pearson r) of our group when compared to Hare's group were .83 for prisoner training tapes and .87 for methadone patient training tapes. Interrater reliabilities for the prisoner and methadone patients were .68 and .64 for Factor 1 and .65 and .83 for Factor 2. The one month test-retest reliabilities for the new methadone patients were .78 for Factor 1, .86 for Factor 2, and .95 for the Total PCL score. Near perfect correlations were obtained between PCL ratings based on interviews alone and ratings based on interview, file and RAP sheets for Factor 1, Factor 2 and Total score. This was true at baseline and one month.

Preliminary findings indicate that indeed the Psychopathy Checklist can be used reliably in a non-prison population, allowing one to examine important personality as well as behavioral correlates associated with the variable treatment outcome of APD substance abusers. Furthermore, the reliability of the PCL in different populations suggests its possible use as an alternative and more comprehensive means of assessing APD than the more limited behavioral criteria of the DSM-III-R.

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AUTHORS

John S. Cacciola, Ph.D., Megan J. Rutherford, Ph.D., and Arthur I. Alterman, Ph.D. are affiliated with the Veterans Administration Medical Center and the Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia PA 19104.

Problems Associated With the Assessment of Personality Disorders in Substance Abusers

Bernard Gaulier, Steve F. Butler, and Deborah L. Hailer

Prevalence of psychopathology in addicts tends to be quite high (Rounsaville, Weissman et al., 1982; Hesselbrock et al., 1985; Mirin et al., 1988). The effectiveness of substance abuse treatment appears to be mediated by patients' Axis I and Axis II psychopathology (e.g., McLellan et al., 1982; Rounsaville, Tierney et al., 1982; Kosten & Kleber, 1988). The investigation of personality disorders presupposes reliable and valid methods for diagnosis. Unfortunately, there is little consensus in the literature regarding Axis II assessment methodology (Reich, 1987). The present study was a pilot investigation to compare results of alternative assessment methods for Axis II disorders in a population of substance abusing women.

Sixteen females (mean age=31.4 years) with a primary Axis I diagnosis of substance abuse were recruited from an inpatient substance abuse ward and a perinatal addiction program. Subjects were assessed using two structured interviews: The Structured Clinical Interview for DSM-III (SCID and SCID II) and The Structured Interview for the DSM-III Personality Disorders-Revised (SIDP-R). Two self-report instruments were also used: the personality disorder scales of the MMPI (using MMPI-2); and the Millon Clinical Multiaxial Inventory (MCMI-II). In addition, subjects completed the Beck Depression Inventory (mean BDI=16.7), and the Shipley Institute of Living Scale (mean estimated IQ=90.9). All personality measures yielded a number of diagnoses. The number of subjects receiving an Axis II diagnosis ranged from 11 to 14 depending on the instrument used. The most frequently diagnosed personality disorders were antisocial, borderline, histrionic, self-defeating and dependent. Antisocial personality disorders overrepresented in the self-administered instruments (MMPI-2 and MCMI-II), probably because these did not differentiate whether the behaviors were present or not before age 15 (a necessary criterion for DSM-III-R diagnosis). Kappa values, comparing the extent of agreement between pairs of instruments, ranged from -.20 to .84. No pair of instruments agreed better than any other pair. For borderline and dependent diagnoses, pairs of

instruments consistently yielded higher kappa values than for other diagnoses.

These data indicate that female substance abusers have a high prevalence of personality disorders which deserve attention because of their potential impact on treatment outcome. Depending on the instrument used, however, different disorders were diagnosed for the same subjects. The four methods used in this study showed little agreement and, at this point, no single instrument or pair of instruments emerges as more useful or effective than any other. The limited extent of agreement may be due to a number. of factors: a) the sample is small, and the kappa values obtained thus represent over or underestimates; b) all the information obtained, whether through a self-administered questionnaire or a structured interview, constituted self-report and may not be reliable; c) subjects may have had difficulty completing four instruments which required endorsing a high number of negative traits, and may thus have responded differently on different instruments; and d) the construct validity of the DSM-III-R Axis II diagnoses may be low, thus making it difficult to assess the less "severe" disorders (it is notable that borderline personality disorder, one of the severe cluster B disorders, received the best agreement). It appears that further research is needed in order to develop reliable and valid methods for assessing personality disorders in this population.

References available upon request from the authors.

Affiliation

Medical College of Virginia
Division of Substance Abuse Medicine
Box 109, MCV Station
Richmond, Virginia 23298-0109

Symptom Clustering of Substance Dependence and Abuse: Examining Internal Validity of DSM-III, and-IIIR Symptom Criteria

Rumi K. Price, John E. Helzer, Linda B. Cottler, Lee N. Robins

In collaboration with the DSM-IV Work Group on Substance Use Disorders, the authors examined three questions about symptom clustering of substance abuse and dependence: 1) whether different classes of psychoactive substances may produce different sets of symptoms; 2) to access the adequacy of abuse and dependence; 3) to access the effects of demographic factors.   We re-analyzed the substance use sections of the St. Louis Epidemiologic Catchment Area Project, because the examination of symptom clustering of both DSM-III and III-R systems were feasible.   In this paper, latent-variable model techniques were employed to examine the three issues of symptom clustering simultaneously.

METHODS AND RESULTS

The second wave of St. Louis ECA interviews were conducted in 1982-3 with 3,004 respondents 18 years or over, who resided in the inner city, suburbs, rural and small towns of the St. Louis area; with 8.0% drawn from institutional settings. In the expanded NIMH Diagnostic Interview Schedule, all symptoms of DSM-III and a majority of the DSM-III-R symptoms are available for most classes of psychoactive substances listed in the criteria.

First, the symptom criteria of the two systems were examined in detail to "translate" the nomenclature to latent-variable models. The models were then empirically tested using PC-LISREL7 for each class of substances: alcohol, cigarette smoking, cannabis, amphetamine, sedatives, cocaine, opiates, hallucinogens, and for any illicit drug use. Age, gender, race, type of sample (household vs. institutional) were used as risk factors where the effects of these factors are estimated on each construct.

Some similar results emerged across most classes of substances:

1) None of the three models examined reached an acceptable level of fit.
2) The one-factor model of DSM-IIIR "Dependence" yielded a better fit than the three-factor models of DSM-III.
3) The three-factor overlapping-indicator model of DSM-III was a "bad" model--suggesting DSM-III's idea of overlapping abuse and dependence is empirically untenable.
4) A moderate to strong positive age effect was found on "Dependence" but not on "Pathological use" nor "Impairment".
5) A secular trend on symptom clustering was not found.
6) Effects of gender and race are substance-specific.

There are also findings specific to some substances but not for others:

Alcohol
1) The symptoms of DSM-III "Dependence Syndrome" cluster together better than the symptoms of other constructs.
2) The symptoms concerning aggression or recklessness while intoxicated (e.g., "physical fights") are not good indicators of alcoholism--perhaps they are indicators of underlying personality disorders?
3) Other less adequate indicators include: "rules to control drinking", "family objection", and "inflammation of pancreas".

Cigarette Smoking
1) Less adequate symptoms of withdrawal are: "headache", "drowsiness", "upset stomach".
2) Females reported a higher level of withdrawal.
3) Blacks reported a lower level of withdrawal.

Drugs
1) The symptom of emotional or psychological problems is the least adequate indicator of drug use disorders for most classes of drugs--this symptom appears to tap underlying psychopathology.
2) Blacks reported less symptoms overall for cannabis, amphetamine, sedatives, but not for cocaine nor opiates.
3) Females reported less symptoms overall for cocaine and opiates but not for cannabis, amphetamine nor sedatives.

AUTHORS

Rumi K. Price, Ph.D., M.P.E., Linda B. Cottler, Ph.D., M.P.H., Lee N. Robins, Ph.D. Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

John E. Helzer, M.D. Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT 05405

Cocaine Symptoms Are Predicted by Familial Psychopathology

Therese A. Kosten, Thomas FL Kosten, and Bruce J. Rounsaville

Cocaine use can elicit responses similar to anxiety disorders in non-anxious persons. Although these symptoms may be related to cocaine use frequency, not all cocaine users report them. Since alcohol responses are related to family history of alcoholism, we hypothesized that cocaine symptoms may be related to family history of anxiety. Alcoholism, and other psychiatric disorders, show familial aggregation suggesting that familial psychopathology may predict drug responses and vulnerability to substance dependence. We investigated the relationship of five cocaine symptoms to cocaine use frequency, and to proband and family psychopathology in a sample of cocaine using opiate addicts. Besides the hypothesized relationship of familial anxiety to cocaine symptoms, we also assessed the association of symptoms to familial depression and alcoholism.

METHOD

The subjects were 194 opiate abusers, drawn from a larger family study of substance abuse and psychopathology, who used cocaine at least once. They sought outpatient treatment for substance abuse during 1983-1985. All probands were white, 47% were male, and the mean (± S.E.M.) age was 28.3 ± 0.3 years.

Probands and 232 of their fist degree relatives (133 siblings and 99 parents) were interviewed directly using SADS-RDC. Psychiatric diagnoses of interest were directly using SADS-RDC. Psychiatric diagnoses of interest were substance abuse, depression (major or minor), anxiety disorders (phobia, panic disorder, or generalized anxiety), and alcoholism. We asked the probands about their cocaine use frequency and whether they had experienced any of these symptoms with cocaine: unconsciousness, violence, chest pains, paranoia, and insomnia.

RESULTS

There were 120 probands (62%) who were frequent cocaine users (at least 3 times a week). Frequent cocaine users, compared to all users, were more likely to report violence (27% vs 19%), chest pains (37% vs 29%). paranoia (59% vs 47%) and insomnia (69% vs 53%) (p's < 0.005). There was no association between frequent cocaine use and unconsciousness (13% vs 10%). Frequency of cocaine use was also not associated with proband or family history of

psychopathology. Due to the effect of cocaine use frequency on most symptom reports, we will report the effects of proband and family psychiatric diagnoses on cocaine symptoms for the frequent users only.

Proband psychiatric diagnoses were associated with some trends in reports of cocaine symptoms. Those with anxiety disorders tended to have less paranoia when using cocaine (42% vs 64%; $p < 0.1$) which may reflect a difference in baseline (nondrug) behavior. Proband depression tended to predict more violence (31% vs 15%; $p < 0.1$) and proband alcoholism tended to predict less unconsciousness (9% vs 20%; $p < 0.1$).

Family history of anxiety disorders was associated with greater reports of violence (24% vs 7%; $p < 0.01$), chest pains (53% vs 21%; $p < 0.001$). paranoia (68% vs 47%; $p < 0.01$). and insomnia (86% vs 60%; $p < 0.001$). Family history of depression predicted greater reports of chest pains (45% vs 19%; $p < 0.01$). Finally, familial alcoholism was associated with greater reports of violence (23% vs 7%; $p < 0.05$), but also predicted fewer reports of unconsciousness (0% vs 17%; $p < 0.001$).

Logistic regression (maximum likelihood) analyses were run for each cocaine symptom using the three proband and familial psychiatric disorders, cocaine frequency, and gender variables. Unconsciousness was significantly predicted by not having a family history of alcoholism ($p < 0.05$). Chest pains were significantly associated with cocaine use frequency ($p < 0.01$) and with family history of anxiety ($p < 0.05$). The symptom of paranoia was associated with familial anxiety ($p < 0.05$) and alcoholism ($p < 0.05$), and with cocaine frequency ($p < 0.01$). Violence was predicted by gender ($p < 0.05$); females were more likely to experience it. Finally, insomnia was predicted by cocaine use frequency ($p < 0.01$) and familial anxiety ($p < 0.005$).

DISCUSSION

Among cocaine using opiate addicts, cocaine use frequency predicts most cocaine symptoms, but familial psychopathology predicts specific symptom occurrences. Our results support our hypothesis that familial anxiety predicts certain symptoms, such as chest pains, paranoia, and insomnia. Another finding was that familial alcoholism predicted fewer unconsciousness responses. This latter effect extends the work of Schuckit in which familial alcoholism predicts decreased tendency to sway in response to alcohol. Thus, differential cocaine responses may be related to vulnerability to develop substance abuse which can be predicted by familial psychopathology.

AFFILIATION

Department of Psychiatry, Yale University School of Medicine.

Alterations in the Distribution of the Auditory P300 Evoked Response Potential: Similarities Between Ethanol and Divided Attention Task Performance

Scott E. Lukas, Jack H. Mendelson, Elena Kouri, Michelle Bolduc, and Leslie Amass

Event-related potentials (ERP) are sensitive electrophysiological correlates of CNS function. As such, they are frequently used to quantify the effects of various psychoactive drugs, especially ethanol. The P300 component of the ERP is a positive wave with a latency of 250-400 msec that is elicited when the subject has been instructed to attend to an infrequent stimulus (cf., Donchin et al., 1978). Evaluation of the P300 wave has gamed in popularity because it appears to be associated with cognitive rather than sensory processes. Patients with various psychiatric diseases associated with cognitive dysfunction such as dementia, depression, schizophrenia, and borderline personality disorder have been shown to have altered P300 waveforms (Polich et al., 1986; Pfefferbaum et al., 1984).

Acute ethanol administration alters P300 activity (Teo and Ferguson, 1986) and young boys with a positive family history (FHP) of alcoholism who have not previously received ethanol exhibit a lower P300 amplitude (Begleiter et al., 1984). The maximum amplitude of the P300 is found over the midline in the centro-patietal region of the brain regardless of the stimulus modality used. This, and the fact that its distribution is symmetrical and bilateral, suggests that the P300 originates from one site. This common origin further substantiates the association of the P300 wave with cognitive processes. Thus, the P300 wave appears to be a useful tool for measuring the acute effects of ethanol on the electrical activity of the brain associated with cognitive functioning. By studying the effects of ethanol on the origins and distribution of P300 waves, we can gain information about how ethanol alters cognitive processes. The present study was designed to determine the utility of using novel dipole localization algorithms to identify the origin of the auditory P300 EBP and to determine how ethanol and divided attention task performance alter P300 topography.

Subjects were divided into those with (FHP) and without (FHN) a family history of alcoholism to determine if differences existed between these groups. Adult male volunteers provided informed consent for their participation. They were prepared with 19 scalp electrodes for topographic electroencephalographic (EEG) and ERP recording. Each subject drank either placebo or 0.7 g/kg of beverage-grade ethyl alcohol using a programmed delivery system (Lukas et al., 1986). Each subject participated on two sessions, each separated by one week. The order of placebo/ethanol administration was randomized. Physiological and electrophysiological data were recorded for 2 hours after drinking. Auditory P300 EBPs were obtained a total of six times during each session (2 control and 4 post-

drinking) using an *oddball* paradigm both with and without a divided attention (e.g., story listening) task.

After ethanol administration, P300 latencies were delayed in the FHN subjects, but not in the FHP subjects. P300 amplitudes were similarly affected. This pattern mimicked the effects of the divided attention task. Source derivations of the P300 waves were also calculated using recently developed software. Ethanol caused the dipole source to move to a position inferior and posterior to its origin during control recordings. As with the other measures, the FHN subjects displayed greater changes in dipole location. Preliminary dipole analysis of EEG alpha activity revealed that it originates deep in the brain, perhaps in the thalamus. Ethanol also disrupted the apparent source of alpha activity. Since ERP data are typically composed of averages of many trials, it is likely that apparent changes in P300 latency really reflect increased variablility of the waveform's source. These data demonstrate that ethanol's effects on cognitive processing skills may be similar to those produced during divided attention.

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ACKNOWLEDGEMENTS

AFFILIATION

Alcohol and Drug Abuse Research Center
McLean Hospital/Harvard Medical School
115 Mill Street
Belmont, Massachusetts 02178

Nicotine Effects in EEG Spectral Data: Smokers vs. Non-smokers

Janet Brigham and Ronald I. Herning

Brain mapping is useful in studying patterns of brain activation produced by drugs. EEG studies with nicotine have been restricted to particular brain areas and generaLly have manipulated nicotine v abstinence. This study approached a fuller picture of nicotine's electrophysiological effects by studying EEG spectral data on 15 electrode sites in three cerebral lobes. Eyes-closed EEG was measured on male inpatient dependent smokers (n=8) after 2 days of smoking 24 cigarettes/day on a set schedule. Similar readings were taken from a matched control group (n=7) of outpatient nonsmokers. These data were spectralized into bands and topographically mapped. The band data were statistically analyzed.

Analysis of variance showed significant differences in lateralized spectral total and alpha power and in beta frequency across the frontal lobes. Compared to nonsmokers, smokers exhibited a decrease in total power at electrode sites Cz and F7, as well as a decrease in total power in the left frontal lobe. Smokers also showed alpha power suppression at sites F7, Fp1, and Fp2. Additionally, the left frontal lobe and, to a lesser extent, right frontal lobe had less alpha Rower in smokers. Beta frequency in the left frontal lobe was higher in smokers. The total power and alpha power differences between smokers and nonsmokers represented an exaggeration of patterns seen in nonsmokers.

The prefrontal cortex where these changes occur is involved in attention, perception, affect, and emotion. These findings suggest that future electmphysio1ogica.l investigations of nicotine should include inquiry into lateralization effects, with particular attention to how cognitive and affective functioning.

[1]Johns Hopkins University School of Medicine; [2]NIDA Addiction Research Center

Quantitative EEG Studies of Chronic THC Abuse: (1) Replications of Topographic Findings, (2) Within EEG Band Spectral Power Shifts, (3) Discriminant Function Analysis of Chronic Use vs Non-use

F. Struve, J. Straumanis, G. Patrick, and Y. Raz

In a pilot study (Clin. EEG, 20: 6-23, 1989) quantitative EEGs were obtained on 10 chronic THC users, 10 psychiatric patient non-users and 10 normal non-users. Chronic THC use was defined as 1 "joint" per day for > 1 year. However most THC users had smoked 3 to 15 joints/day for 5 to 15 years. All THC users were inpatients with no THC access and negative urines at EEG study. Using 21 scalp electrodes plus leads for monitoring ocular movements, 21 channels of monopolar (linked ear reference) EEG plus ocular potentials were recorded using a Cadwell Spectrum 32 instrument. Recordings were made with Ss reclined with eyes closed. All Ss were kept awake during EEG recording. Quantitative EEG measures were derived from 40 to 60 2½ second epochs of artifact free awake EEG activity. At all 21 scalp leads the quantitative values for Absolute and % Relative Power were obtained for each of the four traditional EEG frequency bands. Quantitative values for Coherence and Power Asymmetry were also obtained for 8 homologous lead pairs. When the THC group was contrasted with each non-user group, THC users had significant elevations of Absolute and Relative Power and Coherence of alpha over frontal cortex ("HYPERFRONTALITY OF ALPHA") as well as significant elevations of theta Coherence over frontal cortex. THC was also associated with lessor voltage increases of non-alpha frequencies over most cortical regions but without a frontal predominance (GENERALIZED POWER ENHANCEMENT).

A current replication study using new Ss and the same methodology contrasted 17 THC users with 21 patient non-users and 12 normal non-users. The replication was successful and all of the above findings were confirmed. In the replication study frontal delta Coherence was also elevated for THC users. In both studies intergroup differences in diagnosis and medication could not be controlled. Combining Ss from both studies we contrasted 13 medication free THC users with 13 medication free patient non-users (diagnosis uncontrolled) and 8 THC users matched for diagnosis with 8 patient non-users (medication uncontrolled). In both post hoc analyses the above THC effects were significant. In neither pilot, replication nor post hoc studies were gender or

608

age differences between groups significant.

All 80 Ss in the above studies were used in an effort to separate THC users from non-users using a discriminant function analysis. The dependent variable was daily use or non-use of THC. Candidate predictors were Absolute and Relative Power, Asymmetry and Coherence for all four EEG bands at all 21 electrode sites and transformations of these scores. The Discrimination Function analysis and its Jack-Knife replication were robust. Only two predictors- Z-scores for Absolute Power of alpha at F_3 (AF_3a) and at P_3 (AP_3a) were needed to generate the following successful discriminant a formula: D= (1.7416)(AF_3a) - (1.0048)(AP_3a).

		PREDICTED		JACK-KNIFE	
ACTUAL GROUP	N	THC	NO THC	THC	NO THC
THC USER	27	27	0	26	1
NON-USER	53	4	49	4	49
	CLASSIFICATION:	95% Correct		93.8% Correct	

Using both THC group Ss and current of former THC users not qualifying for inclusion in the study chronic THC group, we were able to identify 32 Ss who were confident in reporting long term duration of THC use and average number of joints used per day over long time periods. We found significant correlations between Discriminant Scores (as calculated above) and (1)Duration of THC use in years (r=0.44, p=.005), (2) Exposure defined as joints/day times duration (r=0.31, p=.038) and (3) Abstinence in years (r=-0.60, p<.001). When Partial Correlations controlling for abstinence effect were run, Discriminant Scores correlated with (1) Average Number of Joints/Day (r=0.39, p<.015), (2) THC Duration (r=0.43, p=.005), Exposure (r=0.43, p=.005) and (4) Logarithmic Transformation of Exposure (r-=0.65, p<.001)

Finally Ss (10 THC, 16 patient non-users, 9 normal non-user) with optical disc stored EEGs were used for a detailed spectral analysis. At each electrode site, Absolute Power values were obtained for each 1 Hz band from 1 to 25 Hz. For each 1 Hz band the excess Absolute Power of the THC group over that of a non-user group was expressed as a ratio:

$$\frac{(\overline{X} \text{ POWER THC GROUP}) - (\overline{X} \text{ POWER NON-USER GROUP})}{(\overline{X} \text{ POWER THC GROUP}) + (\overline{X} \text{ POWER NON-USER GROUP})} \times 100$$

Theoretically this ratio can range from -100% to +l00% with a zero value denoting no power differences between groups. THC is associated with power increase throughout the 1 to 25 Hz range but maximal THC effect involves the mid-theta to lower alpha range. Over frontal cortex THC users show maximal power increase over non-users in the 5-7 Hz range and to a lesser degree in the 8-10 Hz range. Over temporal-central-parietal cortex THC users are maximally deviant from non-users throughout the 5-10 Hz range with the largest THC effects seen in the 6-9 Hz range.

Neurophysiology Research Laboratory, Dept. Psychiatry, LSU School of Medicine, P.O. Box 33932, Shreveport, LA 71130-3932

Auditory Evoked Response Brain Potentials in Individuals at Risk for Alcoholism: Influence of Task Demands and Motivational Factors

Leslie Amass, Scott E. Lukas, and Jack H. Mendelson

Middle (N100, P200) and late (N200, P300) component evoked response potentials (ERPs) are electrophysiological correlates of brain function related to reception, encoding, processing and evaluating information. Individuals at risk for alcoholism have been characterized by low voltage P300 ERP amplitudes elicited by complex visual discriminations (Begleiter et al., 1984; O'Connor et al., 1986, 1987; Begleiter, 1990). Only one study has documented P300 ERP decrements in high-risk individuals with an auditory paradigm (Begleiter et al., 1987). Our laboratory has identified analogous auditory P300 ERP deficits in detoxified alcoholics and detoxified cocaine-dependent alcoholics (Amass et al., 1989). It is unclear if these anomalies represent a vulnerability marker for alcoholism.

This report discusses recent findings with individuals at high-risk for developing alcoholism. Five pairs of non-drug using individuals (25-38 yrs) with (FHP) and without (FHN) a positive family history of alcoholism were evaluated during two experiments designed to examine the influence of task demands and motivational factors on *auditory* evoked responses. ERPs were recorded at vertex with eyes open using an *oddball* paradigm consisting of a 1 kHz pure tone auditory stimulus. In the first condition, easy (A30 dB) and hard (A10 dB) task discriminations alone, and in the presence of a visual divided attention task, were utilized to measure stimulus-induced alterations in the amplitude and latency of middle- and late-component ERPs. In a second experiment, a monetary incentive component stressing accuracy was added to the tone counting task.

FHP subjects displayed aberrant N100 and P300 evoked responses. Significantly lower N100 ($p<0.1$) and P300 ($p<0.05$) amplitudes were observed in FHP subjects compared to FHN individuals, particularly for easy task discriminations. N100 amplitudes were reduced to difficult target stimuli in FHP subjects, but were not accompanied by the concomitant enhancement of the N100 wave to the non-target stimulus seen in FHN subjects ($p<0.05$). Increased task difficulty lowered P300 amplitude in FHN ($p<0.05$) but not FHP subjects There were no differences in ERP latency between groups. Target stimuli with high incentive value increased P200 amplitude during easy ($p<0.05$) and hard ($p<0.05$) task conditions only in the FHN subjects.

The results from these experiments indicate that FHP individuals adopt an undifferentiated mode of responding regardless of task demands or stimulus

values. The distinct abnormalities in the N100 and P300 evoked response suggest that PHP individuals are unable to modulate their attentional resources with changing task demands and fail to use appropriate encoding strategies during storage of relevant information. The encoding abnormalities present in PHP subjects represent an innate functional abnormality that may precede alcoholism and/or determine responses to acute ethanol intoxication. These deficits may reduce the ability to encode behavioral and physiologic cues of intoxication (a concept originally put forth by Lipscomb and Nathan, 1980), leading to increased tolerance and physical dependence following excessive ethanol consumption. Moreover, these neurophysiological procedures may be useful in classifying certain drug-dependent individuals as well as identifying those at risk for developing such problems.

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AFFILIATION

Alcohol and Drug Abuse Research Center
McLean Hospital/Harvard Medical School
115 Mill Street
Belmont, Massachusetts 02178

Cocaine Interaction With Sulpiride, Methysergide, Naloxone and Desipramine: Neurophysiological Effects of Mesolimbic and Neostriatal Neuronal Activity

P.M. Dougherty, J.-T. Qiao, R.C. Wiggins, and N. Dafny

The pharmacologic effects of cocaine (COC) are hypothesized to involve catecholamines and catecholamine-containing brain structures (1), especially sites containing dopamine (8). Target areas of the mesolimbic dopaminergic pathways which arise in the A 10 region, such as the nucleus accumbens (NAc) and medial prefrontal cortex (MPC), are suggested to mediate the reinforcing properties of COC (3,4,6). Rats will self-administer COC to the MPC, while in contrast microinjection of dopamine antagonists, such as sulpiride (SUL), to the MPC, will attenuate COC's reinforcing properties (4). The present study investigates the microiontophoretic effects of COC upon the neurons of two mesolimbic brain structures (the NAc and MPC), as well as a neostriatal site, the caudate nucleus (CN) which may participate in certain motor responses of COC (5). The effects of COC in the lateral thalamus were studied for control purposes. Finally, the interaction of COC with SUL, desipramine (DES), methysergide (METHY), and naloxone (NAL) within these brain areas were also investigated.

METHODS

Experiments were performed on Sprague-Dawley rats (250-350 g) anesthetized with urethane (1.25 g/kg, i.p.). A craniectomy was performed to allow stereotaxic placement of the microelectrodes within the target sites (7). Six barrels of a seven barrel micropipette were filled with the following solutions: 2 M NaCl plus saturated fast green dye (pH 5.0), 0.05 M cocaine hydrochloride (pH 4.8), 0.05 M desipramine hydrochloride (pH 4.7), and 0.1 M glutamic acid (pH 8.0). Other drugs used alternatively included: 0.2 M sulpiride hydrochloride (pH 5.6), 0.15 M naloxone hydrochloride (pH 5.0), and 0.02 M methysergide maleate (pH 5.4). Spotnateous neuron activity was recorded with the seventh barrel of the microelectrode which contained 2 M NaCl (20-30 MΩ). Each neuron was tested with 5 to 10 randomly ordered drug applications (separated by at least 5 min to eliminate interactions) as follows: drugs given alone for 60 s followed by 180 s post-drug recording; or drugs in combination. In each of the combination studies, DES or a receptor antagonist was administered for 30 s followed by COC application concomitantly for 60 s and 180 s post drug recordings. Fast green was ejected at the end of each experiment to mark the final electrode location. Spikes per

second and total spikes for each experimental segment were calculated. Drug effects were detected by analysis of variance testing between segments (Student Neuman Keuls test).

RESULTS AND DISCUSSION

Local application of COC affected the majority of the cells from the NAc, CN and MPC (93% overall), in a dose-related manner and with no significant differences between sites. The neurons of MPC were the most responsive (95%), the neurons of CN the next most responsive (92%), and cells of the NAc were the least responsive (86%). These results contract with those of the LT as only 1 Of 17 cells exhibited any effect following COC application. The primary effect observed for COC was suppression of firing in all these areas although excitatory effects were infrequently observed.

DES was primarily excitatory upon cells which were inhibited by COC in the CN, while DES produced inhibition similar to COC upon neurons of the MPC and NAc. When combined, the effects of COC and DES cancelled within the CN, but were additive within the other two areas. NAL and METHY given alone or in combination with COC failed to affect the discharges of any of the neurons studied. SUL given alone also did not affect baseline activity, however it prevented the effects of COC of the majority of the cells found in MPC, NAc and the CN.

The results demonstrate that direct application of COC upon neurons of the MPC, NAc, and CN induces a suppression in neuronal activity similar to that shown by other investigators (2,3,6). In addition, among the receptor antagonists studied, SUL alone reliably prevented COC activity in all three brain areas studied. Presently, the most successful treatments for COC withdrawal have involved the use of D2 receptor antagonists such as bromocriptine (9). Our results suggest SUL may also provide some usefulness. Finally, the plurality of effects observed for DES versus the activity of COC may support the observation of relatively poor efficacy of this drug for COC withdrawal treatment (10).

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AFFILIATIONS

University of Texas Medical School
    at Houston
Houston, Texas 77225

Changes in the Behavioral Effects of Cocaine by Magnesium in Squirrel Monkeys

Kathleen M. Kantak

INTRODUCTION

Over the past several years, my lab has been describing the psychomotor stimulant properties of magnesium and its interaction with stimulant drugs. Studies show a potentiation of apomorphine and amphetamine on motor related behaviors in mice and a potentiation of cocaine on mouse aggressive behavior by magnesium. In addition, the chronic disruptive effects of cocaine on aggression are attenuated by magnesium. One purpose behind continuing these types of experiments in primates stems from the observations in rats that magnesium will maintain self-administration after cocaine availability is discontinued over a 10 day period. These apparent substitution effects by magnesium are dose dependent and occur over a variety of fixed ratio and progressive ratio schedule conditions to maintain a constant level of magnesium Intake. These and other studies in rats and mice point to a need to examine the behavioral pharmacology of magnesium and cocaine interactions in primates to determine if there is a species generality to the effects of magnesium.

METHODS

Three male squirrel monkeys, ranging in weight from 860 to 885 gm, were exposed to a 5 component FI 3 min schedule of food reinforcement. Each component was at least 25 min in length and was initiated by a 10 min time out. Further schedule restrictions of a 10s time out following pellet delivery and a 1 min limited hold were imposed during each component. Cocaine was injected intramuscularly In a saline vehicle. $MgCl_2$ was injected subcutaneously in a distilled water vehicle. Saline was used for 0 dosing conditions. All injections were made during the initial 5 min of the first 10 min time out period. Drugs were given no more than twice weekly and sessions were 5 days per week.

RESULTS

Cocaine produced a characteristic inverted-U dose effect curve on FI 3 min responding. There were no differences

between saline injections and baseline over the 5 components. A dose of 0.03 mg/kg cocaine was ineffective in altering the rate of responding, and 0.1 mg/kg was minimally effective in increasing the rate of responding. Response rates were elevated after 25 min by 0.3 mg/kg cocaine. A dose of 1 mg/kg cocaine produced a biphasic effect with a response rate suppression at 25 min and a response rate facilitation at 75 min post-injection. Response rate suppression was maintained through the 125 min session by 3 mg/kg cocaine. There was little intrinsic action of $MgCl_2$ on responding for food under FI 3 min conditions. Response rates remained stable and at baseline levels following 30 mg/kg $MgCl_2$ Following 100 mg/kg $MgCl_2$ small reductions in responding were measured 100 and 125 min post-injection. The effects of cocaine at 25 min post-injection were not very pronounced, except with the higher doses of 1 and 3 mg/kg which suppressed responding. These dose effects of cocaine were essentially maintained following co-injection of 30 and 100 mg/kg $MgCl_2$ At 50 min post-injection. rate enhancement was obtained with 0.3 mg/kg cocaine, and rate suppression with 3 mg/kg cocaine. 100 mg/kg $MgCl_2$ which had little effects of its own, suppressed responding at all cocaine doses. The cocaine dose effect curve was flattened. 30 mg/kg $MgCl_2$ reduced responding associated with a rate enhancing dose of cocaine only. Thus, the reduction of cocaine's rate enhancing effects by $MgCl_2$ was dose dependent. This dose dependent reduction of cocaine's effects on responding by $MgCl_2$ continued to occur throughout the 125 min of testing. in addition to dose dependently blocking the rate enhancing effects of cocaine, $MgCl_2$ had some additional interesting effects that were observed at several time points. 30 mg/kg $MgCl_2$, which has no intrinsic action of its own, enhanced the rate of responding when administered with an ineffective dose of cocaine (0.03 to 0.1 mg/kg). The other interesting effect was shown by the ability of 30 mg/kg $MgCl_2$ to attenuate a rate suppressing dose of cocaine (1.0 to 3.0). These two effects were measured at different times and with different doses of cocaine in the three monkeys.

CDNCLUSIONS

A 100 mg/kg dose of $MgCl_2$ eliminated the stimulant properties of cocaine and flattened the dose effect curve for cocaine. A 30 mg/kg dose of $MgCl_2$ reduced responding associated with a rate enhancing dose of cocaine. Indicating that these effects were dose dependent. 30 mg/kg $MgCl_2$ also potentiated responding associated with an ineffective dose of cocaine and attenuated responding associated with a rate disrupting dose of cocaine. These findings are similar to those in rodents which showed a potentiation of an ineffective dose of acutely administered cocaine on aggressive behavior by acutely administered $MgCl_2$, and an attenuation of the chronic aggression disruptive effects of cocaine by acutely administered and chronically co-administered $MgCl_2$. $MgCl_2$ has an interesting and complex behavioral pharmacology in rodents and primates which suggests there may be a similar reaction with cocaine in humans.

Boston Univ. and The New England Regional Primate Res. Ctr.

Effects of Naltrexone on Cocaine Self-Administration by Rhesus Monkey

Nancy K. Mello, Jonathan B. Kamien,
Jack H. Mendelson, and Scott E. Lukas

Naltrexone, a long-acting mu opioid antagonist, effectively suppresses heroin self-administration in man (Meyer and Mirin, 1979; Mello et al., 1981). One recent clinical study reported that naltrexone treatment (100 to 150 mg 3 times per week) of opioid addicts also reduced cocaine-positive urines significantly in comparison to methadone (Kosten et al., 1989). These clinical data are not consistent with previous studies of opioid antagonist effects on stimulant self-administration in animal models. Most studies report that opioid antagonists do not suppress cocaine self-administration. In monkey, naloxone did not suppress cocaine-maintained responding (Woods and Schuster, 1971; Killian et al., 1978). In rat, naltrexone pre-treatment resulted in no change in cocaine-self-administration (Ettenberg et al., 1982) or increased cocaine self-administration (Carroll et al., 1986).

We studied the effects of daily treatment with naltrexone (0.32 to 3.20 mg./kg day) and saline on cocaine self-administration in rhesus monkeys. Cocaine (0.05 or 0.10 mg/kg/inj) and food (1 gm banana pellets) self-administration were maintained on an FR 4 (VR 16:S) schedule of reinforcement. Naltrexone, or an equal volume of saline control solution were infused slowly over 1 hr through 1 lumen of a double lumen intravenous catheter at the same time each day. Treatment with saline and each dose of naltrexone (0.32 and 3.20 mg/kg/day) were studied for 60 sessions over 15 consecutive days (Mello et al., 1990).

In contrast to previous studies with naloxone, the lower dose of naltrexone (0.32 mg/kg/day) significantly suppressed cocaine-maintained responding for the first 10 days of treatment (P < 0.05-0.01), but during days 11 to 15, cocaine self-administration did not differ significantly from saline treatment control levels. The higher dose of naltrexone (3.20 mg/kg/day) also suppressed cocaine-maintained responding significantly for the first 10 days of treatment (P < 0.05-0.01) but during days 11 through 15, cocaine-maintained responding did not differ significantly from base-line levels. Naltrexone's effects on cocaine self-administration were not dose-related. The low dose of naltrexone suppressed cocaine self-administration by an average of 28 percent over 15 days whereas the high dose of naltrexone suppressed cocaine-maintained responding by an average of 25 percent over 15 days.

Food self-administration decreased by 21 percent during 0.32 mg/kg/naltrexone administration. During 3.20 mg/kg/naltrexone administration, food self-

administration was only 3 percent below baseline levels. After termination of naltrexone treatment, cocaine and food self-administration remained at saline treatment base-line levels.

Naltrexone appears to be considerably less effective than buprenorphine in suppressing cocaine self-administration across the dose range studied. Buprenorphine (0.237-0.70 mg/kg/day) suppressed cocaine self-administration by an average of 72 to 93 percent in contrast to 25 to 28 percent suppression by naltrexone (Mello et al., 1989, 1990). Moreover, naltrexone did not consistently suppress cocaine self-administration in all subjects whereas buprenorphine did. These data suggest that buprenorphine's antagonist component contributes to, but does not solely account for its suppression of cocaine self-administration by rhesus monkey (Mello et al., 1989, 1990). Supported by: DA-00101, DA-00064, DA-00115, DA-04059 and DA-02519 from NIDA.

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AFFILIATION

Alcohol and Drug Abuse Research Center, Harvard Medical School-McLean Hospital, 115 Mill Street, Belmont, MA 02178

Buprenorphine Suppresses Cocaine Self-Administration by Rhesus Monkeys Over 1 to 4 Months of Daily Treatment

Jonathan B. Kamien, Nancy K. Mello, Jack H. Mendelson, and Scott E. Lukas

Buprenorphine, an opioid mixed agonist-antagonist, significantly suppressed cocaine self-administration by rhesus monkeys (Mello et al., 1989, 1990). Daily administration of 0.237, 0.40 and 0.70 mg/kg of buprenorphine suppressed cocaine self-administration by 72 to 93 percent (P < 0.01). Each buprenorphine dose was studied for 15 consecutive days (60 sessions) (Mello et al., 1989, 1990). Since clinical treatment with buprenorphine will involve long-term maintenance, it is important to determine if tolerance develops to buprenorphine's suppressive effects during chronic administration. The goal of this study was to evaluate the effects of long term daily buprenorphine treatment on cocaine self-administration by rhesus monkeys.

The effects of daily buprenorphine (0.32 mg/kg/day) or saline treatment on cocaine self-administration were compared over periods of 30 to 120 days. Buprenorphine (0.32 mg/kg/day) or an equal volume of saline was gradually infused over 1 hr through one side of a double lumen catheter at the same time each day. Cocaine (0.05 or 0.01 mg/kg/inj) and food (1 gm banana pellet) self-administration were maintained on an FR 4 (VR 16:S) schedule of reinforcement during 4 one hr sessions each day. Cocaine injections were limited to 20 per session or 80 per day. These studies are still ongoing, but data have been collected for 120 days in one subject, 60 days in two subjects and 30 days in another subject.

Our preliminary observations on 4 monkeys suggest that tolerance does not develop to the suppressive effects of daily buprenorphine treatment on cocaine self-administration. The introduction of buprenorphine usually suppressed cocaine self-administration by over 90 percent within 1 or 2 days. However, the degree of suppression of cocaine self-administration varied across subjects. Figure 1 shows that cocaine self-administration remained suppressed by 75 ± 11 percent during the first 30 days of treatment (N=4) and continued to be suppressed by 71 ± 19 percent during the next 30 days (N=3). Cocaine self-administration remained suppressed by 92 and 87 percent, respectively, during the third and fourth month of buprenorphine treatment (N=1). Food self-administration was initially suppressed but usually returned to base-line levels within 7 to 20 days.

Figure 1
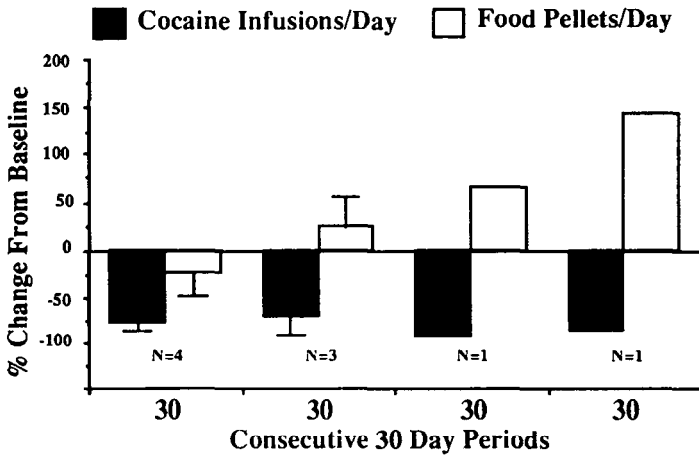
These data confirm and extend our previous findings that 15 consecutive days of buprenorphine treatment (0.237, 0.40 and 0.70 mg/kg/day) significantly suppressed cocaine self-administration by rhesus monkeys (Mello et al., 1989, 1990). Continuation of daily buprenorphine treatment for 1 to 4 months resulted in a persistent suppression of cocaine self-administration. It appears that tolerance does not develop to the effects of daily buprenorphine treatment (0.32 mg/kg/day) on cocaine-maintained responding over the dose-range and time period studied. Supported by Grants DA-00101, DA-00064, DA-00115, DA-04059 and DA-02519 from NIDA.

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AFFILIATION

Alcohol and Drug Abuse Research Center, Harvard Medical School-McLean Hospital, 115 Mill Street, Belmont, MA 02178

Cocaine vs. Food Choice in Rhesus Monkeys: Effects of Increasing the Response Cost for Cocaine

Michael A. Nader and William L. Woolverton

Research with cocaine has consistently demonstrated that it is a highly efficacious positive reinforcer. The purpose of the present study was to determine whether, with an alternative positive reinforcer available, the relative reinforcing efficacy of cocaine could be decreased by differentially increasing the number of responses necessary to receive an injection. Three rhesus monkeys, maintained at approximately 85% of their free-feeding body weights, were trained in a discrete-trials choice procedure to choose between various doses of intravenous cocaine (0.1-1.0 mg/kg) and various quantities of food (1-4 pellets; 1 g/pellet) in the presence of different colored lights (see Woolverton and Johanson, J. Exp. Anal. Behav. 41: 35-43, 1984 for details of method). Daily sessions terminated after 8 hrs or following the completion of 30 trials; trials were separated by a 10 min timeout. A particular dose of cocaine and quantity of food were available for at least 7 consecutive sessions. When choice was stable with a particular pair of reinforcers, the number of responses required to receive a cocaine injection was systematically increased by doubling the fixed-ratio (FR) value. When the amount of food available as the alternative to cocaine was held constant, and the number of responses necessary to receive cocaine or food were equivalent (i.e., FR 30), cocaine choice increased directly with dose. Increasing the number of responses required to receive a cocaine injection decreased cocaine choice. When the FR value was less than 240, increasing cocaine dose increased cocaine choice, resulting in a shift in the cocaine dose-response function to the right. However, when the FR was greater than 240 (i.e., 480 or 960), 100% cocaine choice could not be recovered with increases in dose, up to 1.0 mg/kg. To assess whether the effects of response cost were different when the alternative reinforcer was 4 pellets or 1 pellet, demand curves were constructed in a manner similar to those described by Hursh (J. Exp. Anal. Behav. 34: 219-238, 1980). The slope of the demand curve for cocaine was steeper when 4 pellets was the alternative reinforcer, suggesting that demand for cocaine was more elastic when the alternative reinforcer was of a larger magnitude. That is, when a larger amount of food was available, cocaine choice decreased more rapidly with increase in FR. These results demonstrate that cocaine self-administration can be decreased by increasing the response requirement for an injection and that this effect interacts with the magnitude of an alternative reinforcer to decrease choice. Thus, although cocaine is a highly efficacious reinforcer, its consumption (i.e., self-administration) is determined by cost factors similar to those that influence consumption of other commodities. (Supported by NIDA Grant DA-00250).

AFFILIATION: Departments of Psychiatry and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

Biological Evaluation of Compounds for Their Physical Dependence Potential and Abuse Liability.
XIV. Animal Testing Committee of the Committee on Problems of Drug Dependence, Inc. (1990)

Arthur E. Jacobson

One of the main purposes of the Committee on Problems of Drug Dependence (CPDD) continues to be the evaluation of drugs for their physical dependence potential and abuse liability (May and Jacobson 1989). These drugs are obtained from U.S. and foreign sources in pharmaceutical industries, universities, and governmental organizations. The Animal Testing Committee is responsible for methodological research and testing of drugs in the analgesic as well as the stimulant and depressant classes of compounds. The analgesic class of drugs was examined by researchers in the Department of Pharmacology and Toxicology of the Medical College of Virginia, Virginia Commonwealth University, Richmond, VA (under the direction of Drs. M. Aceto and L. Harris), and the Department of Pharmacology of the University of Michigan, Ann Arbor, MI (under the direction of Dr. J. Woods). The stimulants and depressants were examined in the Department of Psychiatry of the University of Chicago (UC), Chicago, IL (under the direction of Dr. W. Woolverton), the Department of Pharmacology of the University of Michigan CUM), Ann Arbor, MI (under the direction of Dr. G. Winger and C. France), and the Department of Pharmacology and Toxicology of the Medical College of Virginia (MCV), Virginia Commonwealth University, Richmond, VA (under the direction of Drs. G. Patrick and L. Harris).

The CPDD's Drug Evaluation Committee (Dr. T. Cicero, Chairman) is responsible for guiding the Animal Testing Committee (Dr. A. E. Jacobson, Chairman), and Human Testing Committee (Drs. N. K. Mello and M. W. Fischman, Cochairmen) and, at its annual meeting in April, in Chicago, the accomplishments of these Committees were discussed.

ANIMAL TESTING COMMITTEE - ANALGESICS

Statistics

Most of the 44 compounds which were released this year (5/1/89 -

4/30/90) were obtained from universities, domestic and foreign (33% and 22% of the total), and from governmental organizations (NIH and NIDA - 29%, and 9%, respectively). Remarkably few compounds (ca. 5% and 2% of the total) were obtained from pharmaceutical industry (domestic and foreign, respectively). The percentage of compounds from industry is as low, or lower, than at any time since 1981-1982. One consequence of the major source change of compounds from industry to the university and governmental institutions, is that compounds are obtained, generally, in lesser quantity; less than might be considered desirable. Several of the researchers from universities, unlike industrial groups, are interested only in our initial rodent studies and, perhaps, *in vitro* work.

Last year only 25 compounds were examined in one of the primary screens, the single dose suppression assay in monkeys (Aceto et al. 1989). This year, 30 compounds were evaluated in that assay. In the separate reports from UM (Woods et al. 1991) and MCV (Aceto et al. 1991), work is described on 28 compounds at UM, and 38 compounds at MCV. Twenty-one of these compounds were examined at both UM and MCV this year. The total number of compounds (45 [one compound was reexamined under a different NIH number]) is considerably lower than that of last year, when UM reported their work on 40 compounds and MCV on 51 compounds (with 26 compounds in common), for a total number of 65 compounds.

The announcements of the work of the testing facilities under the auspices of the CPDD to medicinal chemists, initiated by Dr. K. Rice (Chief, Laboratory of Medicinal Chemistry, NIDDK, NIH) through the American Chemical Society, resulted in inquiries from several groups in disparate places of which we were previously unaware (Italy, Yugoslavia, France, Canada, Australia, and universities in various states in this country, Georgia, Alabama, Pennsylvania, and Hawaii). The Drug Evaluation Committee, during its Chicago meeting in April, recommended that a brochure should be prepared with an outline of the work done by the Animal Testing Committee of the CPDD, and that the brochure should be widely circulated.

Type of Analgesics Evaluated

The evaluated analgesics have been categorized in six main groups, the 4,5-epoxymorphinans (fourteen compounds, listed in tables 1 - 3), 6,7-benzomorphans (table 4, seven compounds), phenylpiperidines and fentanyl-like compounds in table 5 (four compounds), phenylpiperidines related to haloperidol (table 6, four compounds), methadols (four compounds, table 7), and a group (eleven compounds) of miscellaneous structures (tables 8 and 9). The work which was accomplished with these compounds, both this year and during previous years at MCV and UM, is summarized in the various tables, and their molecular structures are depicted.

TABLE 1. 4,5-EPOXYMORPHINANS[a]

10365: NALMEFENE

10588: NOR-BINALTORPHIMINE (NOR-BNI)

10605: R = COCH₃
10606: R = COC₂H₅

10607
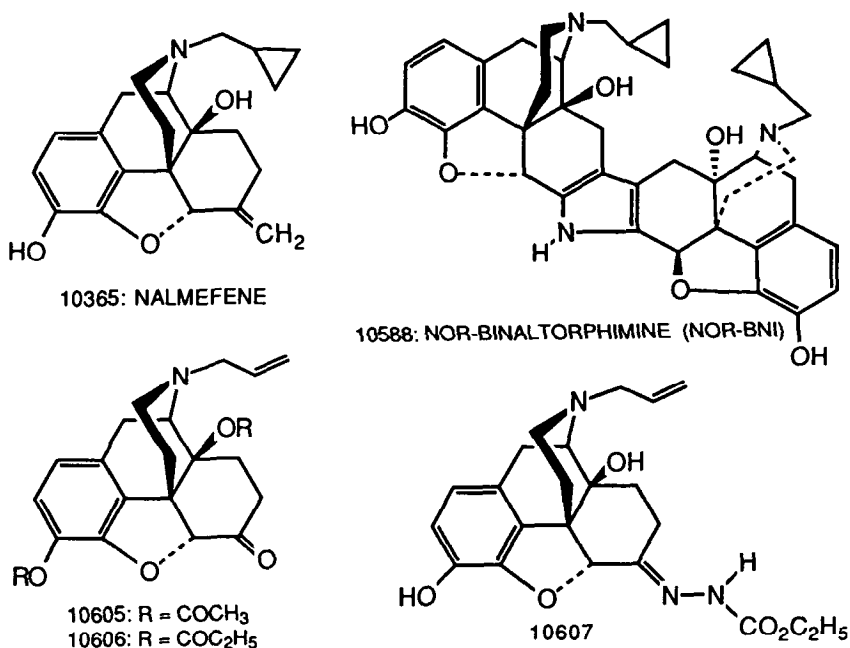
| NIH # | MOUSE ED50/AD50 | | | IN VITRO | | MONKEY |
	PPQ	TF	TFA	RBH (nM)	VD	SDS/PPTW
10365	I[b]	I[b,c]	0.001[b]	-	ANT(μ)	NS[b], PW[b,d]
10588	I	I	I	70.0	ANT(κ)	NS (2,8)[e]
10605	I	I	0.11	11.8[g]	ANT(μ,δ,κ) [g]	-
10606	I	I	0.8	59.2[g]	ANT(μ)[g]	-
10607	I	I	0.12	7.2[g]	1E-6(32)[A][g,h]	PW(0.5,2)[0.1N]

- -

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1986, 1985.
c) Tail flick assay - study of nalmefene antagonism of buprenorphine (1990).
d) Other assays - SA (NE), PPD (NE).
e) Exacerbates withdrawal at highest dose.
g) Previously reported - 1989.
h) Partial opioid agonist with significant $\mu$$\delta$,$\kappa$ antagonist activity.

624

TABLE 2. 4,5-EPOXYMORPHINANS (CONTINUED)[a]
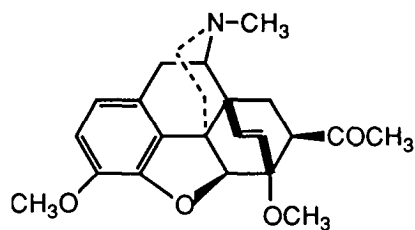
10620, 10632: (+)-THEVINONE
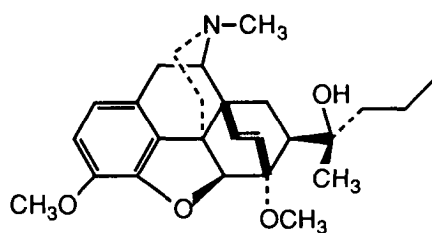
10621
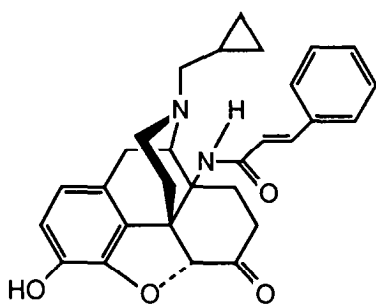
10630
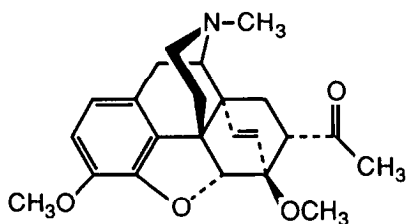
10631: (-)-THEVINONE

| NIH # | MOUSE ED50/AD50 | | | IN VITRO | | MONKEY |
	PPQ	TF	TFA	RBH	VD	SDS
10620 (10632)	I	I	I	>10 μM[b]	ANT(μ,**δ,κ**)	NS (1,4,16)
10621	I	I	I	>10 μM[b]	NE[b]	NS (4,16)
10630	I	I	1.3	0.73	ANT(μ,**δ,κ**)	NS (1,5)c
10631	2.0	8.3	I	1186	1.7E-8[d]	CS (8)

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1989.
c) Slow onset, long duration of action.
d) Not antagonized by μ,**δ,κ** antagonists (non-opioid agonist or antagonist.).

TABLE 3. 4,5-EPOXYMORPHINANS (CONTINUED)[a]

OH

CO_2CH_3

OH

10574

OH

6

HO

O

10575: 6α

10623: 6β

CO_2CH_3

N–CH_3

H

$CO_2C_2H_5$

§

H

N

HO

O

OH

O
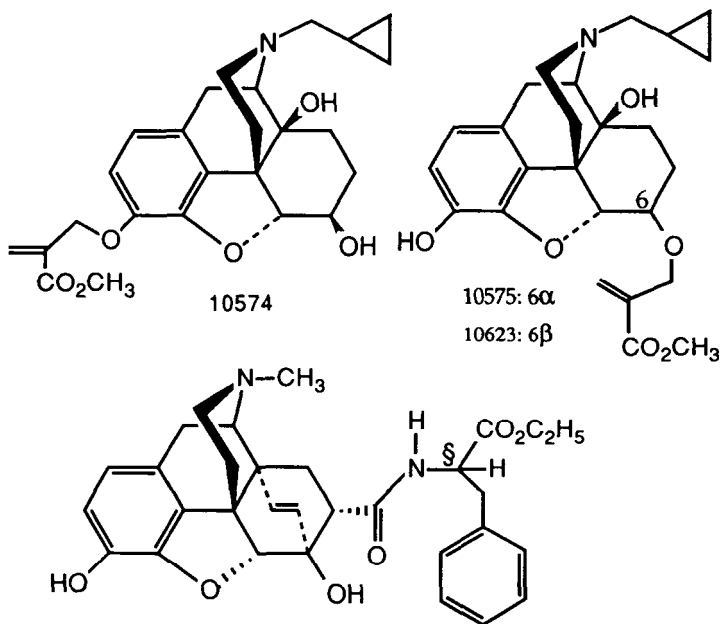
10635: § = L-PHENYLALANYL ETHYL ESTER
10636: § = D-PHENYLALANYL ETHYL ESTER

	MOUSE ED50/AD50				IN VITRO	MONKEY
NIH #	PPQ	TF	TFA	RBH (nM)	VD	SDS
10574	I	I	3.1	29.8	ANT(δ) [b]	NS (0.0075-0.03)
10575	I	I	0.07	3.1	ANT(μ,δ,κ̇)	NS (0.019,0.15)[c]
10623	I	I	0.03	4.4	ANT(μδ̇) [d]	NS (0.02-0.16)
10635	0.01	0.3	I	1.5	4.5E-9[e]	-
10636	0.4	0.9	I	1.4	2.1E-9[e]	-

- -

a) See text for explanation of column headings and abbreviations.
b) Insurmountable antagonist at μ and κ -opioid receptors.
c) PPt-W - Precipitated withdrawal [5 x naloxone].
d) Insurmountable antagonist at κ-opioid receptors.
e) Potent, selective agonist at μ receptors.

TABLE 4. 6,7-BENZOMORPHANS[a]
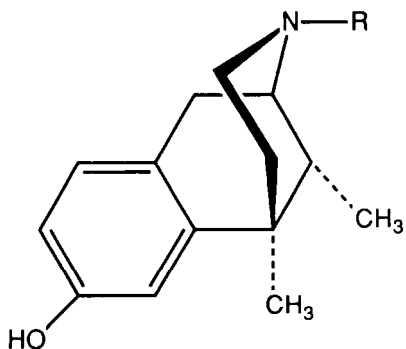
7589: R = *n*-BUTYL (±)

8209: R = *n*-HEXYL (±)

10626: R = *n*-HEXYL (+)

10627: R = *n*-HEXYL (-)

10648: R = BENZYL (±)

10649: R = BENZYL (-)

10650: R = BENZYL (+)

| NIH # | MOUSE ED50/AD50 | | | | | IN VITRO | MONKEY |
	HP	PPQ	TF	TFA	RBH	VD	SDS
7589	I[b]	9.7	I	1.4	101[c]	ANT(μ,δ,κ)[c,d]	NS[e](1,4)
8209	1.4[f]	0.07	2.1	I	358	5.43-7[g]	PS (2,8)
10626	-	1.0	20.4	I	>10 µM	ANT(κ)	NS (3,12)
10627	-	0.3	1.1	I	166	3E-7[h]	PS (1,5)
10648		-	I	I	-	-	NS (3,12)[e]
10649	-	13.4	I	20.3	485	ANT(μ,κ)[i]	NS[e]
10650	-	_j	_j	_j	>10 µM	ANT(μ)[k]	-

- -

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1972.
c) Previously reported - 1989.
d) May not be simple, competitive antagonist.
e) Exacerbated withdrawal.
f) Previously reported - 1977.
g) Agonist at µ and κ receptors.
h) Partial agonist at µ and κ receptors.
i) Non-competitive from Schild plots.
j) Preliminary data appear to be in accord with binding data.
k) Non-competitive at κ receptors.

TABLE 5. PHENYLPIPERIDINES AND FENTANYL-LIKE COMPOUNDS[a]

OCOCH$_3$

H$_3$C

OH

CH$_3$

N

CH$_3$

CH$_3$

10543

10604

OH

COC$_2$H$_5$

N

10651

N

O

O

N

N

N
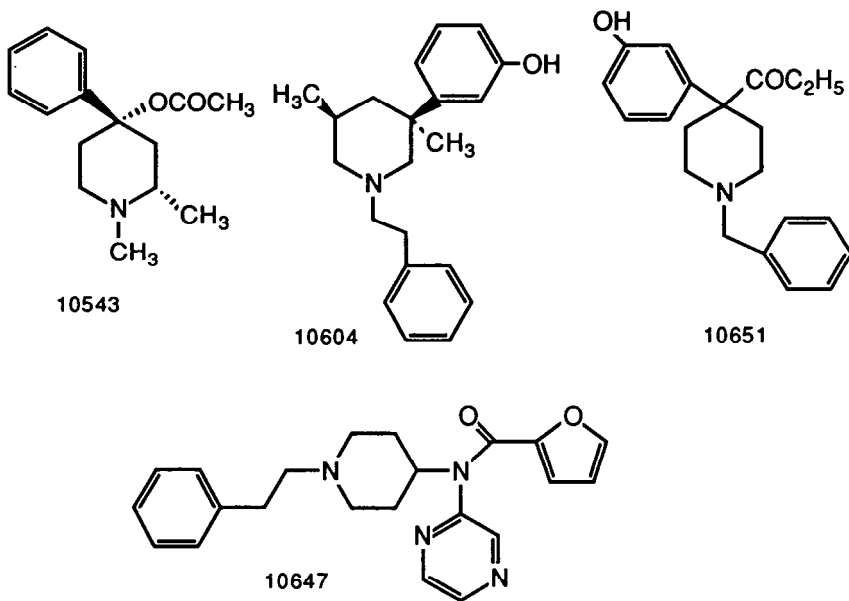
10647

	MOUSE	**ED50/AD50**		**IN VITRO**		**MONKEY**
NIH #	PPQ	TF	TFA	RBH	VD	SDS
10548	0.9[b]	15.2[b]	I[b]	76 μM	SEC	-
10604	4.7[d]	I[d]	I[d]	444	1.4E-7[e]	-
10647	0.08	0.4[f]	I	91	ANT(μ,δ,κ)[g]	NS (0.05-4)
10651	_h	_h	_h	1017	ANT(μ,δ,κ)	-

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1988.
c) EC50 not determinable. Effect blocked by naltrexone.
d) Previously reported - 1989.
e) Non-opioid (not antagonized by naltrexone).
f) Reversed by naloxone.
g) Other work (at UM, 1990) - SA, DD, analgesia and respiratory effects in monkeys.
h) Preliminary data appear to be in accord with binding data.

TABLE 6. PHENYLPIPERIDINES RELATED TO HALOPERIDOL[a]
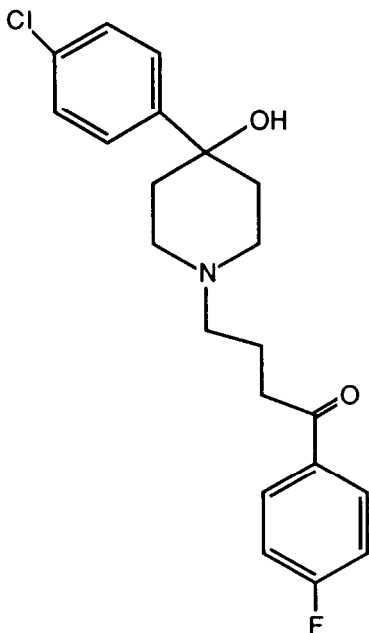
10625: HALOPERIDOL
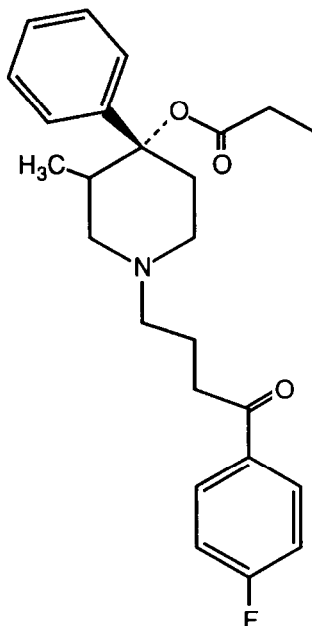
10639: 3*R*,4*S* (+)
10640: 3*S*,4*R* (-)
10641: (±)

| NIH # | MOUSE ED50/AD50 | | | IN VITRO | | MONKEY |
	PPQ	TF	TFA	RBH	VD	SDS
10625 (8032)	0.01[b]	14.6[b]	I [b]	>10 μM	ANT(μ,k)[c]	NS[b]
10639	0.1	0.56	I	-	-	CS (0.05,0.25)
10640	1.5	I	I	-	-	NS (0.5,0.25)
10641	0.11	0.77	I	-	-	CS (0.5,0.25)

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1989,1988,1963. Other Work - RI-SM (NS), RI-PPD; DD (at UM, 1990).
c) Agonist at 10^{-5} M, not reversed by naloxone.

629

TABLE 7. METHADOLS[a]

10652
(-)-α-ACETYL-N-NORMETHADOL

10653
(-)-α-N-ACETYL-N,N-DINORMETHADOL

10654
(-)-α-N-ACETYL-N-NORMETHADOL

10655
(-)-α-ACETYL-N,N-DINORMETHADOL
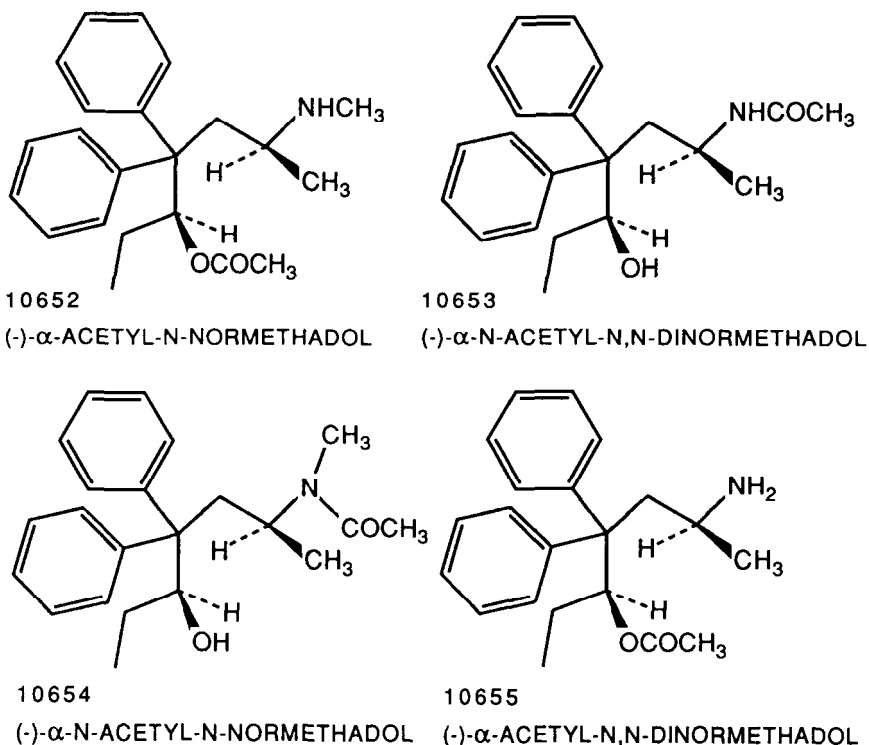
	MOUSE ED50/AD50			IN VITRO		MONKEY
NIH #	PPQ	TF	TFA	RBH	V D	SDS
10652	0.07	0.5	I	13.4	$8.2\text{E-}8^b$	CS (0.5)
10653	I	I	I	>6 μM	SE[c]	NS (3,12)
10654	I	I	I	>6 μM	SE[c]	NS (3,12)
10655	_d	_d	_d	22.3	$3.4\text{E-}7^e$	_d

- -

a) See text for explanation of column headings and abbreviations.

b) Potent, selective μ agonist.

c) Little or no opioid activity.

d) Preliminary data appear to be in accord with binding data.

e) μ Agonist; unusual response to naltrexone.

TABLE 8. MISCELLANEOUS[a]

O$_2$N ... N ... N ... H$_3$C N$^+$ Br$^-$ ... CH$_3$ ... OC$_2$H$_5$ ... 10520

10618: 1S,2R (-) - CIS
10619: 1R,2S (+) - CIS

O$_2$N ... N ... N ... H$_3$C N$^+$ I$^-$ CH$_3$ ... CH$_3$ ... OC$_2$H$_5$ ... 10521

10628: DITOLYLGUANIDINE (DTG)
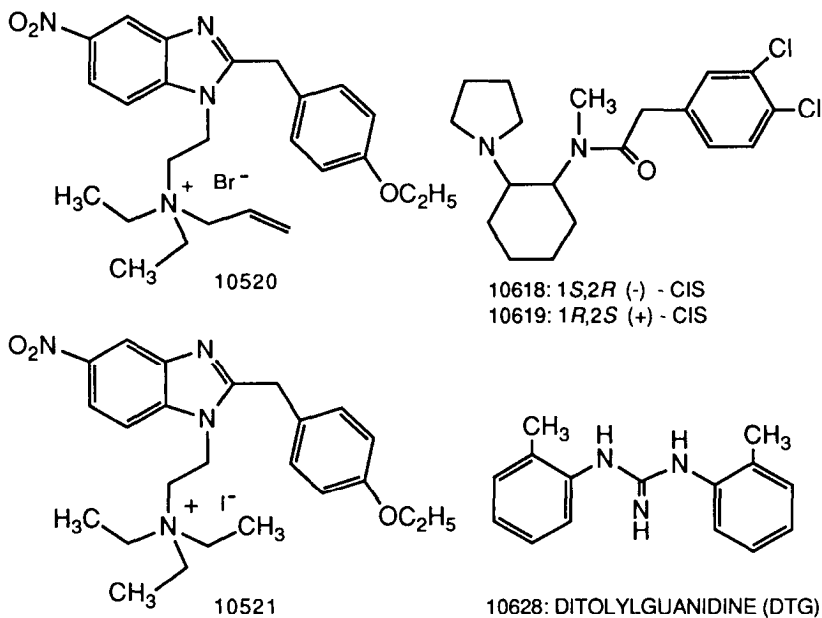
| | MOUSE ED50/AD50 | | | | IN VITRO | MONKEY |
NIH #	PPQ	TF	TFA	RBH	VD	SDS
10520	0.6	0.4	I	175	1.6E-7	CS (0.25,1)
10521	5.0	I	I	2800	5.83-6	-
10618	9.7	I	I	>10 μM	ANT(μ,κ)	NS (3.75,15)
10619	7.0	I	I	>10 μM	ANT(μ,κ)	NS[b](5,20)
10628	-	I	I	-	-	NSC, PW[d]

- -
a) See text for explanation of column headings and abbreviations.
b) May have exacerbated withdrawal.
c) Appeared to exacerbate withdrawal (8 mg/kg).
d) Did not precipitate complete withdrawal syndrome.

TABLE 9. MISCELLANEOUS (CONTINUED)[a]
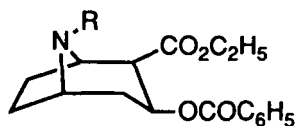
8211: R = CH$_3$ (COCAINE)
10664: R = H (NORCOCAINE)
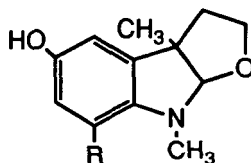
10633: R = H
10634: R = Br
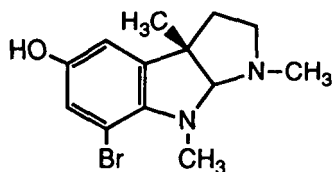
10642: (-)
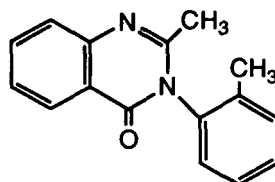
10643: METHAQUALONE

| NIH # | MOUSE ED50/AD50 | | | IN VITRO | | MONKEY |
	PPQ	TF	TFA	RBH (nM)	VD	SDS
08211	2.83[b]	I [b]	I [b]	-	-	PS[c]
10633	I	I	I	-	-	-
10633	I	I	I	-	-	-
10642	0.18	0.54	I	29.4	2.5E-8[d]	PS (0.5,2,8)
10643	-	-	-	-	-	NS (0.75-12)
10664	1.65	I	I	-	-	PS[e] (1,2)

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1986 (RI-PPD), 1987.
c) Morphine (0.6 mg/kg (s.c.) - no effect, alone) with cocaine (2 mg/kg i.v.) significantly suppressed morphine withdrawal.
d) Weak antagonist at μ,**δ, κ** receptors.
e) Attenuates abrupt morphine withdrawal.

ABBRREVIATIONS USED IN TABLES 1-9.

1) <u>MOUSE ED50 OR AD50</u>: <u>Antinoceptive Assays (sc injection)</u>

 Confidence limits for the ED50 and AD50 are listed in the MCV report (Aceto et al. 1991).
HP = hot plate; **N** = Nilsen; **PPQ** = phenylquinone; **TF** = tail flick; **TFA** = tail flick antagonism vs. morphine. These assays are performed at MCV, except for the HP and N (carried out at NIDDK, NIH).
I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

 For comparison: in mg/kg, sc in mice, morphine ED50 = 5.8 (5.7-5.9) in TF, 0.23 (0.20-0.25 in PPQ. Naltrexone AD50 = 0.007 (0.002-0.02) in TFA; Naloxone AD50 = 0.035 (0.01-0.093) in TFA.

2) <u>In Vitro Determination</u> (Data from UM, Woods et al. 1991))

   A) **RBH** = binding affinity, in the presence of 150mM NaCl, to rat or monkey cerebrum membrane preparations, in nM (parenthesized number, noted in previous reports, is the +sodium/-sodium [+Na/-Na] ratio). EC50 was determined by displacement of 0.5 nM [^{3}H]etorphine.
For comparison: morphine EC50 (from RBH) = 23.6 (1.69).
      NE = no effect.

<u>NOTE:</u> The present EC50 data cannot be directly compared with those from some previous reports (Jacobson 19.84, and preceding years) in which -Na values were quoted. However, the former numbers can be recalculated for comparison with those which are currently utilized through the use of the +Na/-Na ratio.

   B) **VD** = electrically stimulated mouse vas deferens EC50 values, rounded to one significant figure. Agonist activity is stated using "E" followed by a negative number: E = 10^{-x} M, where x = the negative number, thus: 1E-3 = 0.001 M (1 mM), 1E-6 = 1 μM, and 1E-9 = 1 nM (parenthesized numbers are maximum percent inhibition at EC50); [bracketed letters: **A** = antagonized by 10^{-7} M naltrexone; M = not antagonized by naltrexone; M = slight antagonism by naltrexone].
      SE = slight effect on twitch.
      NE = No significant agonist or antagonist effect.

      ANT = Antagonist activity. Parenthesized letters indicate μ,δ, and/or κ receptor antagonism. The antagonist effect may or may not be competitive (see the UM report (Woods et al. 1991) for these data).

Compounds which suppress the twitch and are not antagonized by naltrexone or UM 979 [NIH 8859, (-)-5,9α-dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan] are said to be non-opioid agonists (e.g., clonidine can suppress the twitch, but is not antagonized by naltrexone. It is a non-opioid agonist). (The effect of UM 979 is not noted in this report, but see the UM report (Woods et al. 1991) for these

homogenate assay and do not suppress the twitch in the VD may have narcotic antagonist properties. The opioid receptor at which the drug exerts its antagonist effect is determined by testing various concentrations of the drug to induce a blockade (antagonism) of the suppression of the twitch in the VD preparation caused by sufentanil (μ), DSLET (), or U50,488 () (for these data see Woods et al. 1991).

3) Monkey Colony Data (from MCV, Aceto et al. 1991; prior to 1988 from MCV or UM).

  A) **SDS** = single dose suppression
    NS = no suppression.
    CS = complete suppression
    PS = partial suppression. (Parenthesized numbers = dose range studied, in mg/kg; if CS, then dose at which CS was observed is noted in the parentheses). Potency comparison with morphine [M] may be stated, in brackets.

  B) NW or PPt-W = studies in non-withdrawn monkeys
    PW = precipitated withdrawal at dose levels: in mg/kg indicated in parentheses &/or comparison with naloxone [N], in brackets
    NP = no precipitation
    SP = slight precipitation.

4) Other Studies (OTHER):

  A)   **RI** = rat continuous infusion (data from MCV)

    a) **SM** = substitution for morphine
      NS = no substitution for morphine.
      CS = complete substitution.
      PS = partial substitution.

    b) **PPD** = primary physical dependence, in rats.

  B)   **ND** = non-dependent monkeys
    M-like = morphine-like effect.

  C)   **PPD** = primary physical dependence (in the rhesus monkey).

  D)   **SA** or **SI** = self-administration or self-injection (data from UM)
    NE = no effect.
    High = codeine-like.
    IN = intermediate between saline and codeine.
    SE = slight effect.

  E) **DD =** drug discrimination (data from UM)
    NE = no effect.
    CS = complete suppression.

Previous work on a compound is noted by year, the year listed in the monograph title (e.g. Problems of Drug Dependence 1986). Note that the monographs publication date may be one year <u>after</u> the titled year of the monograph. Previously published data are listed in the tables in the appropriate column, and the year in which the original work can be found is cited in the footnotes to the tables ("previously reported" work cited as "1983" indicates that the work was included in "Problems of Drug Dependence 1983", which was published in 1984. The work can be found in the Annual Report of either Aceto et al., or Woods et al.).

<u>NOTE</u>: Rounded numbers are used in the tables. For precise values, and details of the procedures, see the MCV and UM reports in these Proceedings (Aceto et al. 1991; Woods et al. 1991).

Observations About The Analgesics

The well-known nor-BNI (NIH 10588, table 1) was found to be a potent, noncompetitive, selective κ-opioid antagonist *in vitro*. It antagonized μ and δ receptors at much higher concentrations than were necessary for κ antagonism. Nor-BNI also displays narcotic antagonist activity *in vivo.*

It was interesting to note that NIH 10575 and 10623 (table 3) had fairly similar *in vitro* and *in vivo* activity, although they are stereochemically quite different at the C-6 position of the 4,5-epoxymorphinan structure. Both compounds are potent, non-selective, opioid antagonists. The effect of the stereochemical difference was observable in the *in vitro* work. NIH 10623, the C-6β compound, appeared to have insurmountable antagonist activity at the κ receptor.

The effect of **D** vs. **L** amino acids at C-7 of the 4,5-epoxymorphinan structure can be seen with NIH 10635 and 10636 in table 3, the former having an **L**-phenylalanyl residue and the latter the D-moiety. The **L**-amino acid containing compound, NIH 10635, was found to be somewhat more potent *in vivo,* especially in the PPQ assay for antinociceptive activity, and about the same or slightly less potent *in vitro.* In the electrically stimulated mouse vas deferens preparation, both compounds were potent selective agonists at the μ receptor.

Only three of the fourteen 4,5-epoxymorphinans seen in tables 1 - 3 appear to have agonist activity. Eight of the fourteen compounds had more, or less, opioid antagonist activity, *in vivo,* and the nor-BNI compound showed its antagonist activity *in vitro* and *in vivo.* Two compounds, NIH 10620 (or 10632) and 10621 (table 2) had, as expected, no *in vivo* or *in vitro* activity. These compounds are being synthesized

and examined at NIH by Dr. K. Rice and colleagues, and are the inactive enantiomers of the natural, (-), series of the 4,5-epoxymorphinans.

The N-substituted 6,7-benzomorphans (table 4) are being synthesized and examined by Dr. E. L. May at MCV, and will be the subject of a joint paper by MCV, UM, and NIH, next year. The (+) and (-) series from N-H to N-heptyl, at least, will be explored. The (+)-6,7-benzomorphans are of contemporary interest due to the discovery, in the past several years, that some of these compounds have PCP-like activity (Goldman et al. 1985), and (+)-pentazocine has been reported to be one of the most potent σ (non-opioid, non-dopaminergic binding sites distinct from PCP-binding sites) ligands (de Costa et al. 1989). NIH 10626, the (+)-N-hexyl derivative has, as would be predicted, much weaker activity as an antinociceptive *in vivo* than the (-)-relative (NIH 10627), but, surprisingly, the (+)-compound appears to have opioid antagonist activity, relatively selective for κ receptors, in the MVD assay. Also noteworthy is the N-benzyl compound in the (-)-series (NIH 10649) which displays narcotic antagonist activity *in vivo* and *in vitro,* and is a very weak antinociceptive in the PPQ assay.

A fentanyl-like compound, NIH 10647 in table 5, is of considerable interest. It is a potent agonist which does not suppress withdrawal in morphine-dependent monkeys (in the SDS assay), and appears to have antagonist activity *in vitro*. If this antagonist activity is substantiated, the NIH 10647 will be the first antagonist found in the fentanyl series. The compound appears to display μ opioid effects in drug discrimination, comparable to buprenorphine; its analgesic effects in the monkey appear, however, to be non-opioid.

Three new phenylpiperidines related to haloperidol were examined *this* year *in vivo,* continuing our investigation of potential neuroleptic-analgesics (table 6). The methadols in table 7 were examined for the National Institute on Drug Abuse. The methadol-like secondary amines (NIH 10652 and 10655) were potent *in vivo* and *in vitro,* and the two amides (NIH 10653 and 10654) were essentially inactive.

The etonitazene-derived quaternary amines, NIH 10520 and 10521 (table 8) were quite different *in vivo,* although they are structurally similar. One of them, the NIH 10520, does appear to cross the blood-brain barrier, which is unusual for quaternary amines. The two enantiomers in table 8, NIH 10618 and 10619, have little *in vivo* potency but appear to have μ and κ opioid antagonist activity *in vitro,* although they do not bind to opioid receptors. These compounds are known to have moderate, and selective, affinity for binding sites. In that regard, it is interesting to note that another σ ligand, DTG (table 9, NIH 10628), also appears to act as an opioid antagonist (SDS assay). The (-)-bromoeseroline (table 9, NIH 10642), like (-)-eseroline itself, was found to have good antinociceptive activity. NIH 10642 appeared to show some narcotic antagonist activity.

STIMULANTS AND DEPRESSANTS

Eight compounds were received for examination as stimulants or depressants this year. Several of these were examined at the request of the World Health Organization (WHO), and the work which was completed on four of them, CPDD 0028, 0030, 0031, and 0032, has been forwarded to the WHO and to the submitter of the drug, after perusal by the Drug Testing Committee of the CPDD. Work on at least one of the remaining drugs has been completed, but will not be released this year.
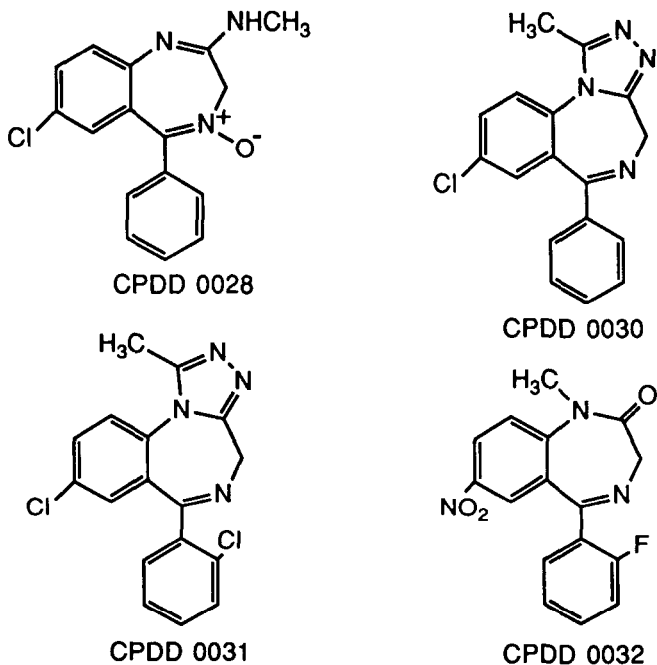
FIGURE 1. Structures of examined depressants

Chlordiazepoxide hydrochloride (CPDD 0028, Figure 1)

Chlordiazepoxide was found to produce pentobarbital-like effects on motor coordination and locomotor activity, and substituted partially for pentobarbital in dependent rats. Withdrawal from CPDD 0028 appeared to be mild. The rates of self-administration were greater than saline and slightly less than sodium methohexital. Orally-administered, the compound produced discriminative stimulus effects similar to those of pentobarbital in monkeys, and thus would be predicted from these experiments to have pentobarbital-like subjective effects in humans.

Alprazolam (CPDD 0030, Figure 1)

Alprazolam was found to produce dose-related effects that are characteristic of CNS depressant drugs. Its potency appeared to be at least 30 times that of pentobarbital and slightly greater than diazepam. Substitution of alprazolam suppresses signs of abstinence in pentobarbital-dependent rats, and mild signs of abstinence appeared following cessation of drug. Alprazolam appeared to be capable of causing barbiturate-like physical dependence. It also induced discriminative stimulus effects similar to those of pentobarbital in monkeys, and thus would be predicted from these experiments to have pentobarbital-like subjective effects in humans.

Triazolam (CPDD 0031, Figure 1)

Triazolam was found to induce discriminative stimulus effects similar to those of pentobarbital in monkeys, and thus would be predicted from these experiments to have pentobarbital-like subjective effects in humans.

Flunitrazepam (CPDD 0032, Figure 1)

Flunitrazepam was found to induce discriminative stimulus effects similar to those of pentobarbital in monkeys, and thus would be predicted from these experiments to have pentobarbital-like subjective effects in humans.

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May, E.L. and Jacobson, A.E. The Committee on Problems of Drug Dependence: A legacy of the National Academy of Sciences. A historical account. Drug and Alcohol Dependence 23(3):183-218, 1989. (See p. 207 for purposes and goals of the CPDD).

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AUTHOR

A. E. Jacobson, Ph.D., Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Dependence Studies of New Compounds in the Rhesus Monkey and Mouse (1990)

Mario D. Aceto, Edward R. Bowman, Louis S. Harris, and Everette L. May

All the compounds except cocaine, norcocaine, methaqualone, and the N-butyrophenone prodine-like compounds (NIH 10639, 10640, and 10641) were supplied by Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIADDK, NIH. The identities of all the compounds, except those indicated above, were unknown to us when they were originally submitted. These studies were conducted under the auspices of the Committee on Problems of Drug Dependence.

For the most part, the procedures described by Seevers and his colleagues (1936, 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3.0 mg/kg s.c. of morphine sulfate every 6 hr for at least 90 days before being used. This dose regimen was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single-dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2 1/2 hr after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hr after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test drug were noted. In both tests, a vehicle control and an appropriate positive control (naloxone hydrochloride, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 2 or 3 different treatments (doses) of a test compound were randomly allocated to the 4 or 5 monkeys of a group. Usually, 3 or 4 groups per compound were used. All drugs were given subcutaneously (1 ml/kg) and the vehicle was water except where indicated. The observer was "blind" with regard to the treatment given. A minimal 2-week washout and recuperation period between tests was allowed.

Three mouse tests were used in our laboratory to provide a preliminary estimate of the potency and profile of activity of each test compound. The tests were the tail-flick agonist (TF) and the morphine antagonist (TF vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in Table 1. In addition, Dr. Jacobson occasionally provided us with estimated starting doses. These doses were based on results obtained from the mouse-hot plate assay (HP) (Eddy and Leimbach, 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978). Reference data for this test are shown in Table 2.

SUMMARY OF COMPOUNDS TESTED

Compound NIH	Chemical Name or Generic Class	MOUSE				MONKEY	
		TF	TF vs M	PPQ	HP	SDS	PPt-W
7589A	6,7-Benzomorphan	+	+	+	-	+	-
8209	6,7-Benzomorphan	+	+	+	-	+	-
8211	Cocaine	-	-	-	-	+	-
10365	4,5α-Epoxymorphinan (Nalmephene)[a]	+	+	+	+	-	-
10520	N-Allyletonitazine bromide	+	+	+	+	+	-
10521	N-Ethyletonitazine iodide	+	+	+	+	-	-
10574	4,5x-Epoxymorphinan	+	+	+	-	+	-
10575	4,5x-Epoxymorphinan	+	+[b]	+		+	+
10588	Norbinaltorphimine	+	+	+	-	+	-
10605	Naloxone 3,14-diacetate	+	+	+	-	-	-
10606	Naloxone 3,14-dipropionate	+	+	+	-	-	-
10607	Naloxone ethoxycarbonylhydrozone	+	+	+	-	-	+
10618	Benzeneacetamide	+	+	+	-	-	-
10619	Benzeneacetamide	+	+	+	-	+	-
10620	(+)-Thevonine	+	+	+	-	+	-
10621, 10632	(+)-19-Propylthevinol	+	+	+	-	+	-
10623	4,5-Epoxymorphinan	+	+	+	-	+	-
10626	6,7-Benzomorphan	+	+	+	-	+	-
10627	6,7-Benzomorphan	+	+	+	-	+	-
10628	1,3-Diphenylguanidine	+	+	+	-	+	+
10630	14-Aminodihydromorphinone	+	+	+	-	+	-
10631	(-)-Thevinone	+	+	+	-	+	-
10633	Furanoindole	+	+	+	-	-	-

SUMMARY OF COMPOUNDS TESTED

| Compound | Chemical Name | MOUSE | | | | MONKEY | |
NIH	or Generic Class	TF	TF vs M	PPQ	HP	SDS	PPt-W
10634	Furanoindole	+	+	+	-	-	-
10635	6α,14α-Ethenisomorphinan	+	+	+	-	-	-
10636	6α,14α-Ethenisomorphinan	+	+	+	-	-	-
10639, I-389	4-Phenylpiperidine	+[c]	+	+	-	+	-
10640, I-489	4-Phenylpiperidine	+	+	+	-	+	-
10641, I-3481	4-Phenylpiperidine	+[d]	+	+	-	+	-
10642	Pyrroloindole	-	-	-	-	+	-
10643	Methaqualone	+	+	+	-	+	-
10647	4-Aminopiperidine	+	+	+	-	+	-
10648	6,7-Benzomorphan	+	+	+	-	+	-
10649	6,1-Benzomorphan	+	+	+	-	+	-
10652	N-Nor-LAAM	+	+	+	-	+	-
10653	N-Acetyl-N-N-dinormethadol	+	+	+	-	+	-
10654	N-Acetyl-N-normethadol	+	+	+	-	+	
10664	Norcocaine	+	+	+	-	+	-

[a] Special Study: Nalmephene or Naloxone vs Buprenorphine or Morphine
[b] Special Pretreatment Study
[c] Special Study: Naloxone vs ED50 in Tail-Flick Test
[d] Special Study: Naloxone vs ED80 in Tail-Flick Test

Table 1

Comparative Data-ED50, mg/kg s.c. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick Test	Tail-Flick Antagonist Test	Phenylquinone Test
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)
Cyclazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01 (0.005-0.03)
Nalorphine·HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)
Naloxone·HCl	None at 10.0	0.04 (0.01-0.09)	No Activity
Naltrexone·HCl	None at 10.0	0.007 (.002-0.02)	No Activity
Morphine Sulfate	5.8(5.7-5.9)	-----	0.23 (0.20-0.25)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time.

Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate Assay

	Hot Plate s.c./p.o.
Morphine Sulfate	0.98 (0.83-1.1) 8.3 (6.0-11.4)
Codeine Phosphate	6.8 (4.5-10.2) 13.5 (9.7-18.7)
Levorphanol Tartrate	0.2 (0.1-03) -
Mepetidine·HCl	5.3 (4.0-7.1) -
(-)-Metazocine·HBr	0.6 (0.5-0.9) 10.6 (8.6-14.1)
Dihydromorphinone·HCl	0.19 (0.15-0.25) 0.9 (0.7-1.2)
Nalorphine·HCl	9.9 (5.7-2.1) -
Cyclazocine	1.5 (1.1-2.1) -
Pentazocine	9.3 (6.7-12.8) -
Chlorpromazine·HCl	1.1 (0.9-1.5)

No dose response for naloxone and naltrexone.
Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive on the hot plate test.

<u>NIH 7589 A</u>   (±)-2- *n* -Butyl-2'-hydroxy-5,9 α-dimethyl-6,7-benzomorphan hydrobromide (N- *n* -Butyl-normetazocine   hydrobromide)
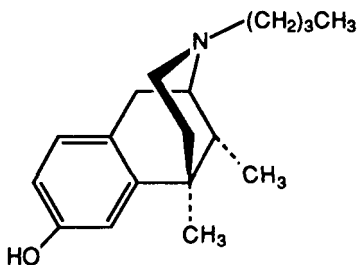
<u>MOUSE DATA</u>-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.08
2) TF vs. M - 1.4 (0.4 - 5.1)
3) PPQ - 9.7 (3.3 - 25.2)

[a]Clonic convulsions 1/6 mice

<u>MONKEY DATA</u>
   SDS

NIH 7589 did not substitute for morphine. Instead, it worsened withdrawal in a dose-related manner (fig.). At the higher dose, severe tremors and questionable myoclonic movements were noted. Vehicle consisted of phosphoric acid and water.
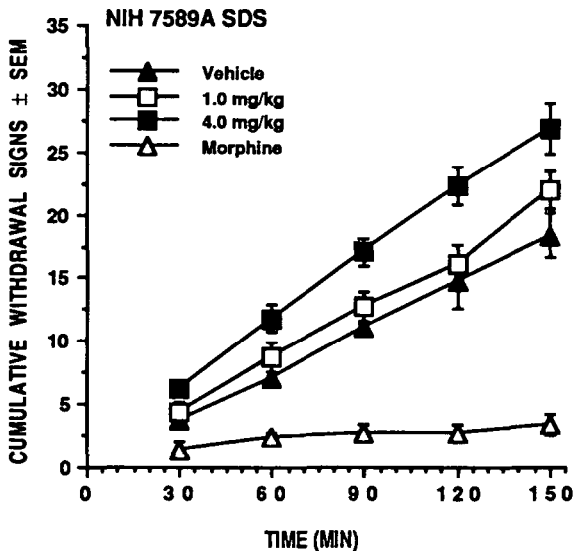
NIH 8209   (±)-2-*n*-Hexyl-2'-hydroxy-5,9a-dimethyl-6,7-benzomotphan hydrobromide (N-*n*-Hexyl-normetazocine hydrochloride)
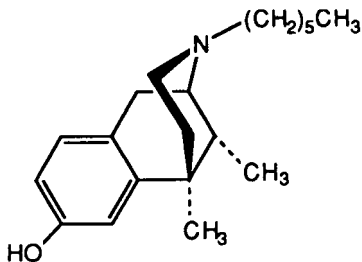
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 2.1 (1.2 - 3.6)
2) TF vs. M - Inactive at 1.0,
    10.0 and 30.0[a]
3) PPQ - 0.07 (0.02 - 0.26)
4) HP - 1.4 (1.3 - 1.6)

[a]Clonic convulsions 1/6 mice

MONKEY DATA
   (SDS)

NIH 8209 attenuated withdrawal and substituted partly for morphine (see plot of data). However, the attenuation was due primarily to a reduction in the incidence of retching and restlessness. In addition, 2 of 4 monkeys at the high dose also had relaxed abdominal muscles and did not vocalize when their abdomens were palpated. The drug's action was delayed by 30 min and had diminished by 2 1/2 hr. Partial substitution does not necessarily imply opioid activity.

645
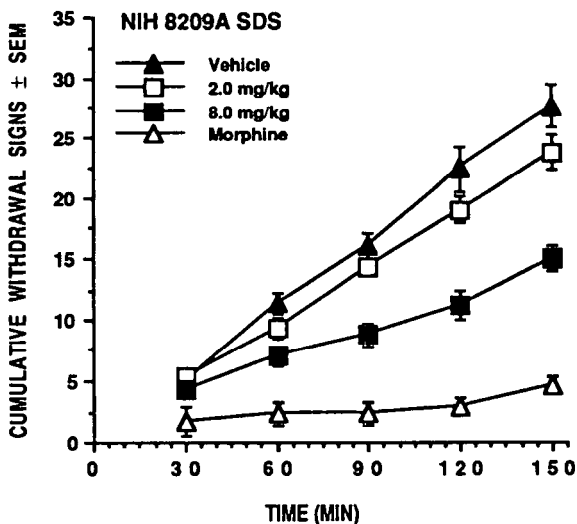
NIH 8211   Cocaine   hydrochloride
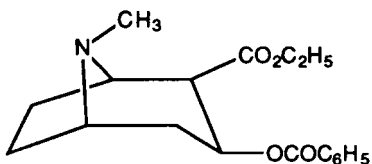
MONKEY DATA

SPECIAL   COCAINE-MORPHINE   INTERACTION   STUDY

Multiple drug abuse involving cocaine and heroin or methadone is a major
problem whose incidence is increasing. The consequences of multiple drug abuse
are many. They range from a higher risk of acquired immunodeficiency
syndrome (AIDS) to increased criminal activity. Further, the pharmacotherapy of
multiple drug abuse represents a formidable challenge. These studies were
conducted to provide additional insights regarding the phenomenon of
concomitant drug abuse.

The assay was initiated by the injection of a treatment regimen into a monkey of a
group of 4 or 5 that had not received morphine for 14-15 hr and showed definite
signs of withdrawal. Thus, each animal of a group was randomly allocated to
receive either sterile water, (1 ml/kg) or morphine at 0.3, 0.6 or 3.0 mg/kg s.c.
followed 10 minutes later by cocaine (2.0 mg/kg i.v) or sterile saline i.v. given as
a bolus dose of 3 ml in 40-60 s.

These results indicate that while cocaine per se significantly suppressed morphine
withdrawal, the combination of 0.6 mg/kg morphine and cocaine was much more
effective. Of course, morphine, at 3.0 mg/kg, completely suppressed withdrawal.
An illustration of data as the cumulative mean of withdrawal signs ± S.E.M. at
15, 30, 60, 90, 20 and 150 min is shown in the accompanying figure.

NIH 8211 MORPHINE-COCAINE INTERACTION STUDY
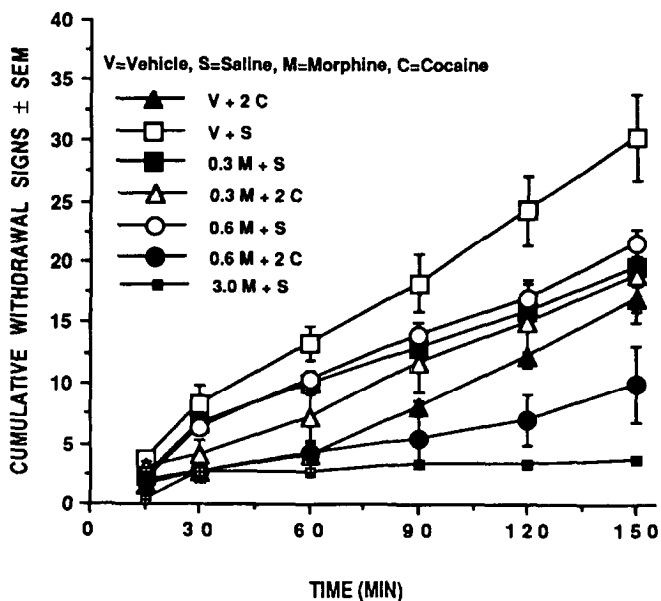
TIME (MIN)

<u>NIH 10365</u>   17-Cyclopropylmethyl-3,14 β-dihydroxy-4,5-epoxy-6-
methylenemorphinan hydrochloride (Nalmefene)
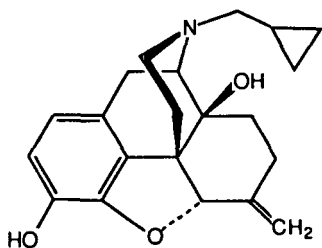
<u>MOUSE DATA</u>-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10 and
   30[a]
2) TF vs M - 0.001 (0.0002 -
   0.004)[a]
3) PPQ - Inactive at 1.0, 10 and
   30.0
4) HP-Inactive[a]

[a]Reported previously.

NIH 10365   17-Cyclopropylmethyl-3,14β–dihydroxy-4,5-epoxy-6-methylenemorphinan hydrochloride (Nalmefene) (continued)

Special Studies

NALMEFENE OR NALOXONE VS BUPRENORPHINE OR MORPHINE

Nalmefene·HCl given prior to ED_{80} of Buprenorphine·HCl in Tail-Flick AD_{50} = 0.02 (0.007 - 0.06)

Nalmefene·HCl given prior to ED_{80} of Morphine SO_4 - AD_{50} = 0.001 (0.0002 - 0.004)[a]

Naloxone·HCl given prior to ED_{80} of Buprenorphine·HCl - AD_{50} = 0.15 (0.06 -0.36

Naloxone·HCl given prior to ED_{80} of Morphine SO_4 = 0.035 (0.01 - 0.93)[a]

Nalmefene·HCl given after ED_{80} of Buprenorphine·HCl - 15% antagonism at 10.0 mg/kg

Naloxone·HCl given after of ED_{80} of Buprenorphine·HCl - 0% antagonism at 10.0 mg/kg

NIH 10520   N-Allyletonitazene bromide
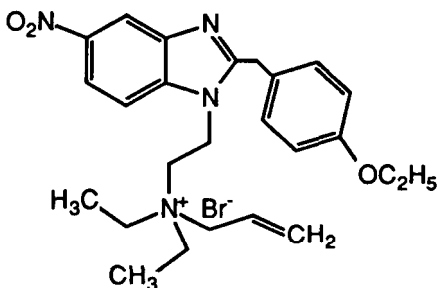
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1)   TF - 0.4 (0.2 - 1.0)
2)   TF vs M - Inactive at 1.0, 10.0 and 30.0
3)   PPQ - 0.6 (0.2 - 1.5)
4)   HP - 1.4 (1.0 - 1.9)

MONKEY DATA
    S D S

NIH 10520 substituted for morphine in a dose-related manner. Onset of action was prompt but duration of action was shorter (60-90 min) than that of morphine (see fig.). Some scratching and ataxia were noted.

NIH 10521   N-Ethyletonitazene   iodide
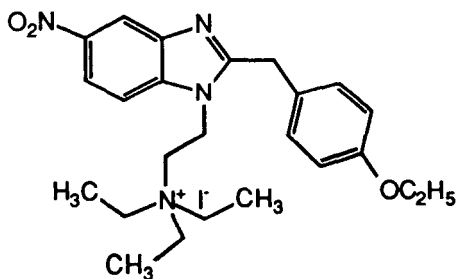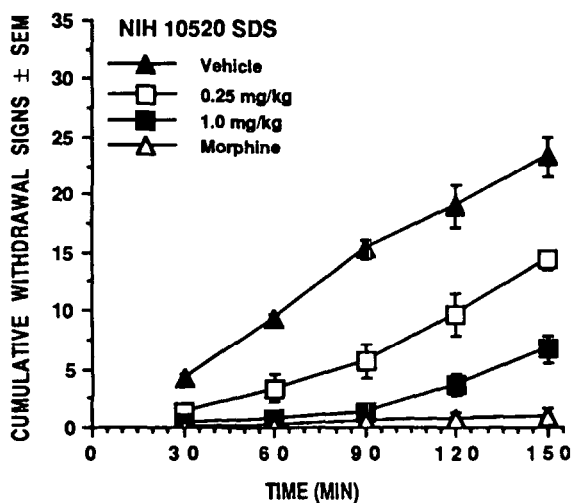
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0% at 1.0, 25% at 10.0
    and 24% at 30.0
2) TF vs M - Inactive at 1.0,
    10.0 and 30.0
3) PPQ - 5.0 (1.6 - 15.4)[a]
4) HP - Inactive at 25.0 and
    100.0

[a]Convulsions at 30.0; 3 of 6 mice
  died.

NIH 10574   4,5α-Epoxy-6β,14β-dihydroxy-3-(2 carbomethoxyallyloxy)-
17-cyclopropyhnethylmorphinan
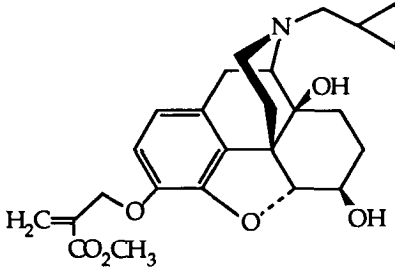
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 1% at 3.0; 31% at 10.0
    and 27% at 30[a]
2) TF vs M - 3.1 (1.1 - 8.8)[a]
3) PPQ - 20% at 1.0. Inactive at
    3.0, 10.0 and 30.0[a]

[a]Vehicle - Lactic acid and H_2O

MONKEY DATA
    SDS

The drug did not substitute for morphine at doses of 0.0075 and 0.03 mg/kg (see
fig.). One monkey at the higher dose retched frequently. Vehicle used consisted
of lactic acid and water.
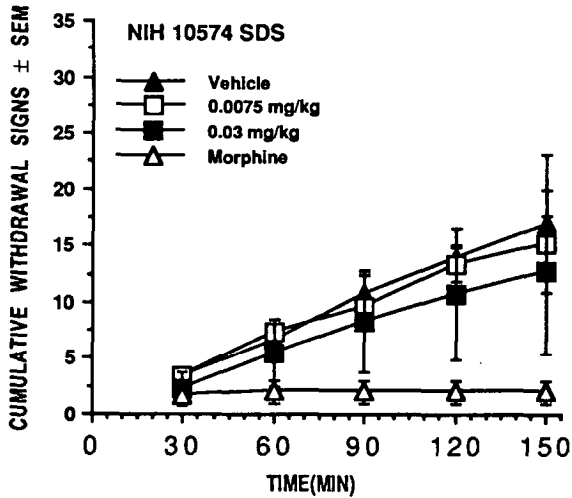
650

4,5α~Epoxy-3,14β~dihydroxy-6α~(2 carbomethoxyallyloxy)-17-cyclopropylmethylmorphinan
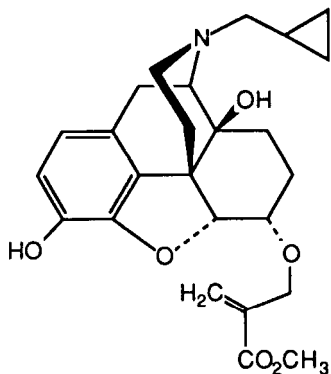
MOUSE DATA-ED50 OR AD50
(95% C.L. or mg/kg sc or % change)

1) TF - 1) Inactive at 1.0, 10.0 and 30.0
          2) Inactive at 0.1, 1.0 and 10.0
2) TF vs. M. - 1) 0% at 1.0, 0% at 10.0
                and 26% at 30[a]
              2) 0.07 (0.6 - 1.0)[b]
3) PPQ - Inactive at 0.1, 1.0, 3.0, 10.0
    and 30.0

[a]20 min drug pretreatment
[b]2 hr drug pretreatment

MONKEY DATA

A.   (SDS)

As shown in the fig. designated NIH 10575 SDS, this compound did not substitute for morphine. The drug exacerbated withdrawal at both doses.
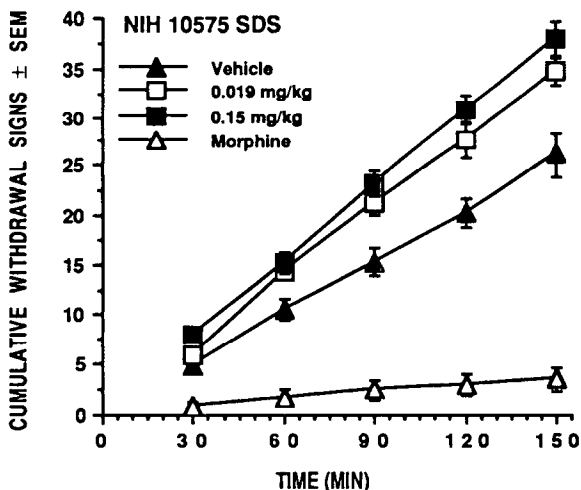
651

4,5α-Epoxy-3,14β–dihydroxy-6 α-(2 carbomethoxyallyloxy)-17-cyclopropylmethylmorphinan (continued)

B. (PPt-W)

NIH 10575 precipitated withdrawal (see NIH 10575 PPt-W fig.). The drug has a fast onset and its duration of action is longer than 2 1/2 hr. The drug is about 5 x more potent than naloxone, the reference standard.
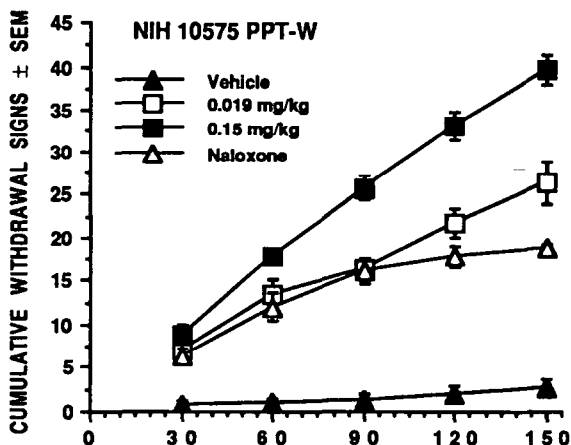
NIH 10588   Norbinaltoxphimine hydrochloride (Nor-BNI hydrochloride)
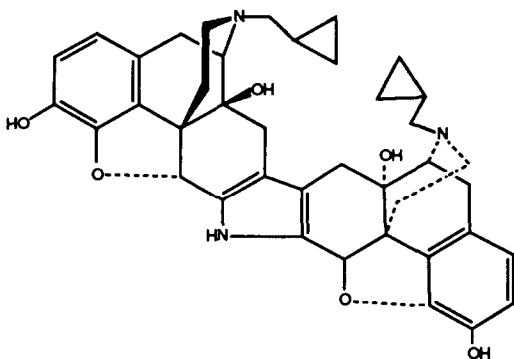
MOUSE DATA-ED OR AD50 (95% C.L. or mg/kg sc or %) change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0

MONKEY   DATA
   (SDS)

As shown in the fig., NIH 10588 did not substitute for morphine. Instead, the drug appeared to exacerbate withdrawal, especially at the higher dose. Frequent retching, vomiting, fighting, restlessness and vocalizing were noted. However, a full-blown withdrawal syndrome was not observed. Thus, these effects may be due to an inherent action and do not necessarily reflect mu antagonist properties.
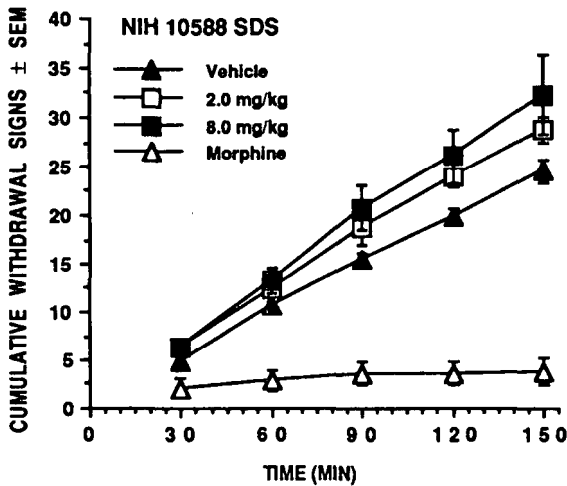
NIH 10605   Naloxone 3,14-diacetate
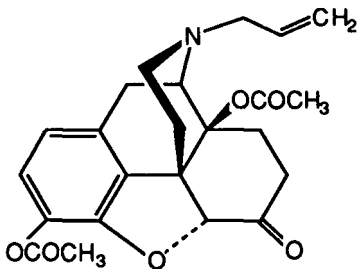
MOUSE DATA—ED OR AD50
(95% C.L. or mg/kg sc or %
change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs M - 0.11 (0.03 - 0.36)
3) PPQ - Inactive at 1.0, 10.0 and 30.0

NIH 10606   Naloxone 3,14-dipropionate maleate
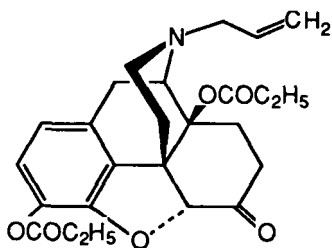
MOUSE DATA-ED OR AD50
(95% C.L. or mg/kg sc or %
change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs M - 0.8 (0.3 - 2.5)
3) PPQ - 1% at 1.0, 23% at 10.0
   and 3% at 30.0

NIH 10607   Naloxone ethoxycarbonylhydrazone
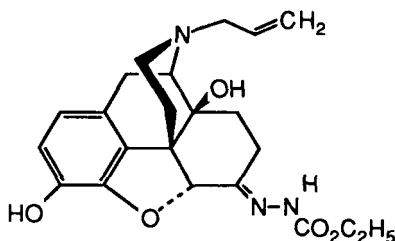
MOUSE DATA-ED OR AD50
(95% C.L. or mg/kg sc or %
change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs M - 0.12 (0.02 - 0.65)
3) PPQ - Inactive at 1.0, 10.0
   and 30.0

MONKEY DATA
    (PPt-W)

NIH 10607 precipitated withdrawal in morphine-dependent rhesus monkeys.
As shown in the graph, the effect was dose-related. Onset of action was
prompt, possibly even faster than that of naloxone, and offset was at least 1 hr
longer than naloxone. The drug appears to be about 1/10 as active as naloxone.
Severe tremors were noted in one monkey at the higher dose.
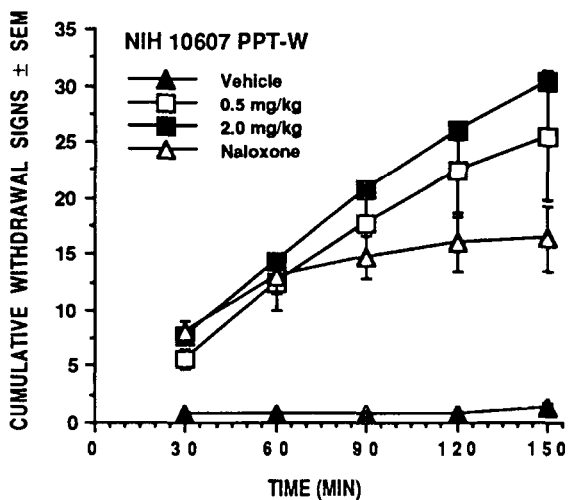
NIH 10618   1S,2R-(-)-cis,3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (-)-tartrate
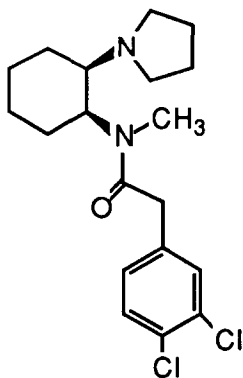
MOUSE DATA-ED OR AD50
(95% C.L. or mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and
    30.0
2) TF vs M - Inactive at 1.0, 10.0
    and 30.0
3) PPQ - 9.7 (2.3 - 32.3)

MONKEY DATA
    (SDS)

As shown in the fig., NIH 10618 neither substituted for morphine nor
exacerbated withdrawal in the dose range of 3.75 - 15.0 mg/kg.

NIH 10618   1*S*,2*R*-(-)-*cis*,3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (-)-tartrate (continued)
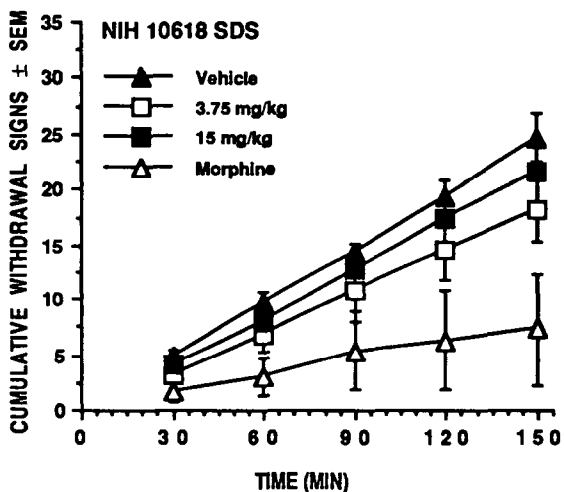
NIH 10619   1*R*,2*S*-(+)-*cis*,3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (+)-tartrate
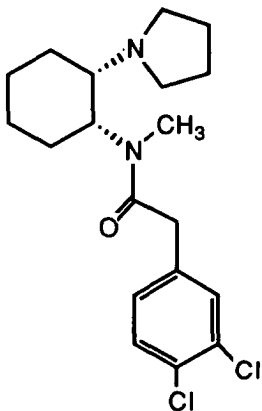
MOUSE DATA-ED OR AD50
(95% C.L. or mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 7.0 (2.3 - 21.3)

MONKEY DATA
    (SDS)

As illustrated in the accompanying graph, NIH 10619 did not substitute for morphine and may have exacerbated withdrawal. One animal receiving the highest

dose convulsed once and retched more frequently than vehicle controls during
the first half hr.
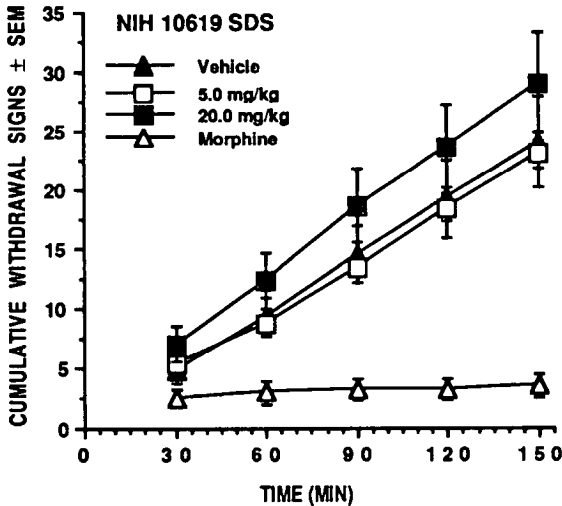
NIH 10620   (+)-Thevinone hydrochloride
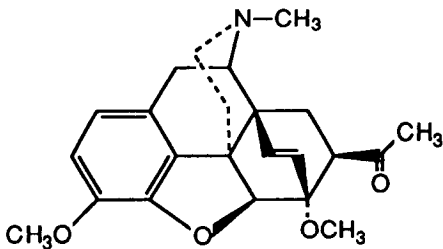
MOUSE DATA-ED OR AD50
(95% C.L. or mg/kg sc or %
change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs M - Inactive at 1.0,
   10.0 and 30.0
3) PPQ - 9% at 1.0, 14% at 3.0,
   29% at 10.0, 54% at 30.0 and
   69% at 60.0

MONKEY DATA
  (SDS)

NIH 10620 neither substituted for morphine nor exacerbated withdrawal (see
fig.). The apparent attenuation of the withdrawal syndrome at the high dose was
due primarily to a reduction in the signs designated retching and vomiting.

657

NIH 10620   (+)-Thevinone hydrochloride (continued)
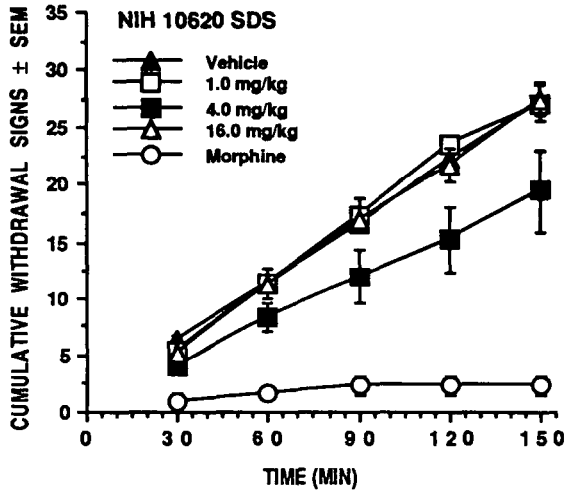
NIH 10621   (+)-19-Propylthevinol oxalate
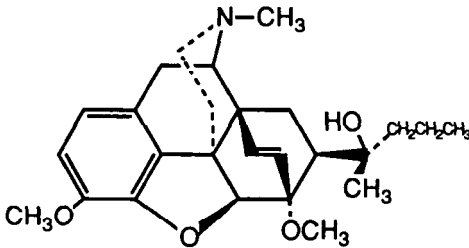
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg or %
change)

1) TF - Inactive at 1.0, 10.0
    and 30.0
2) TF vs M - Inactive at 1.0,
    10.0 and 30.0
3) PPQ - 0% at 1.0, 6% at
    10.0 and 22% at 30.0

MONKEY DATA
    SDS

NIH 10621 neither substituted for morphine nor exacerbated withdrawal at doses
4.0 and 16.0 mg/kg (see fig.). At the higher dose, one monkey retched
frequently during the first 30 min and from 61 - 90 min.
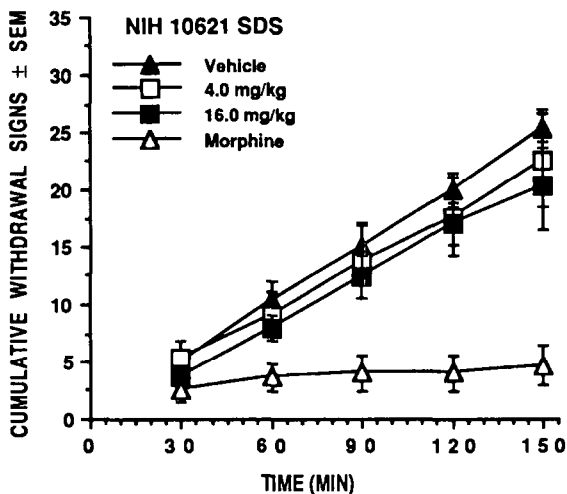
NIH 10623   4,5α-Epoxy-3,14β-dihydroxy-6β-(2-carbomethoxyallyloxy)-17-cyclopropylmethylmorphinan
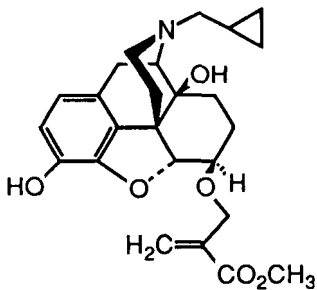
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
    30.0
2) TF vs M - 0.06 (0.03 - 0.12)
3) PPQ - 23% at 1.0, 0% at 10.0
    and 0% at 30.0

MONKEY DATA
    SDS

NIH 10623 did not substitute for morphine in the dose range 0.02 - 0.16 mg/kg
(see fig.). The drug appeared to exacerbate withdrawal; however, the increased
score was due primarily to an increase in the incidence of the signs designated
retching, tremors and wet-dog shakes.

NIH 10623   4,5α-Epoxy-3,14β-dihydroxy-6β-(2-carbometboxyallyloxy)-
17-cyclopropylmethylmorphinan   (continued)
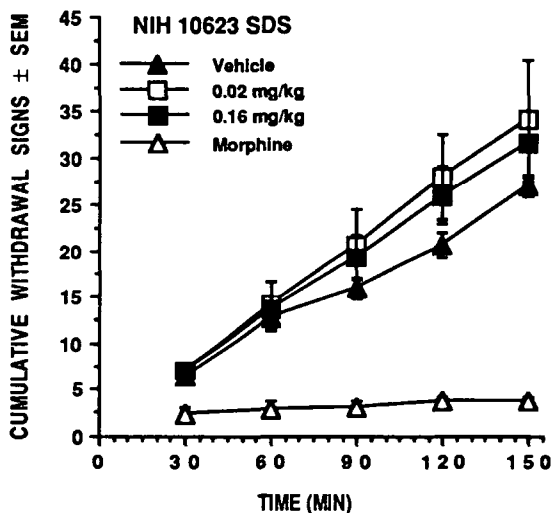
NIH 10626   (+)-2-*n*-Hexyl-2'-hydroxy-5,9α-dimethyl-6,7-benzomorphan
hydrochloride [((+)-N-*n*-Hexyl-normetazocine hydrochloride)]

See NIH 8209

MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 20.4 (12.9 - 32.2)
2) TF vs M - Inactive at 1.0,
    10.0 and 30.0
3) PPQ - 1.0 (0.4 - 2.8)

MONKEY DATA
    (SDS)

As shown in the fig., NIH 10626 neither substituted for morphine nor exacerbated withdrawal. At the higher dose, ataxia, slowing, and ptosis were noted. Also, two of the four monkeys at this dose showed a peculiar head tremor described as "bobbing". Vehicle consisted of lactic acid and water.

NIH 10626   (+)-2-*n*-Hexyl-2'-hydroxy-5,9α-dimethyl-6,7-benzomorphan hydrochloride [((+)-N-*n*-Hexyl-normetazocine hydrochloride)] (continued)
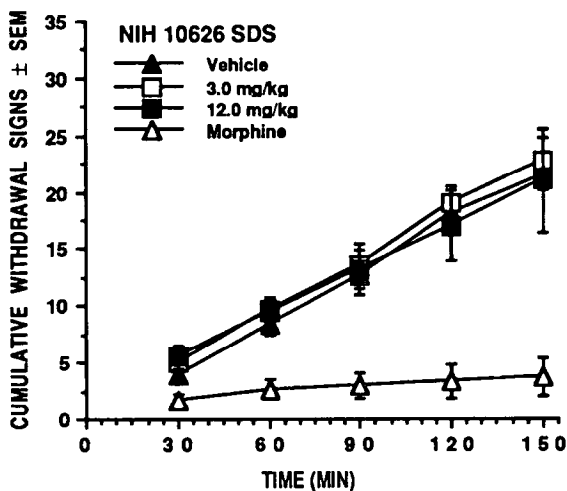
NIH 10627   (-)-2-*n*-Hexyl-2'-hydroxy-5,9 α-dimethyl-6,7-benzomorphan hydrochloride [((-)-N-*n*-Hexyl-normetazocine hydrochloride)]

SEE NIH 8209

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 1.1 (0.6 - 2.3)
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.7)

MONKEY DATA
    S D S

NIH 10627 substituted partly for morphine. It reduced the incidence of withdrawal signs designated retching, restlessness, rigid abdominal muscles, and vocalization when abdomen palpated. One monkey who received the high dose showed severe tremors. Partial substitution does not necessarily imply opioid activity.
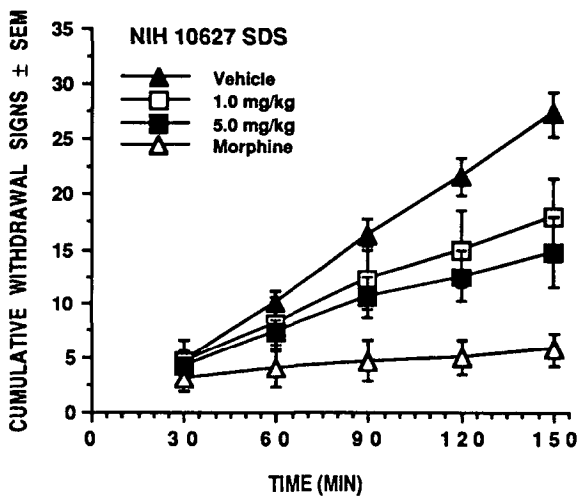
NIH 10628   1,3-Di(2-tolyl)guanidine   (DTG)
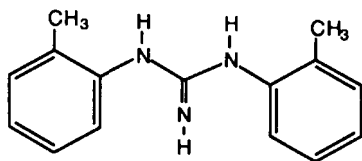
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 0.1, 10.0 and 30.0[a,b]

2) TF vs. M. - 14% at 1.0, 19% at 10.0 and 8% at 30.0[a,c]

3) PPQ - 1.23 (0.52 to 2.9)

[a]Vehicle - 40% DMSO aqueous solution plus lactic acid
[b]Lethal to 4 of 6 mice at 30.0
[c]Lethal to 5 of 6 mice at 30.0

MONKEY DATA

A.   (SDS)

DTG, a purported sigma agonist, was examined for possible interactions, in vivo, in mu (morphine)-dependent rhesus monkeys. The drug appeared to exacerbate withdrawal in withdrawn monkeys at 8.0 mg/kg (see fig.). This was due primarily to an increased incidence of retching and wet-dog shakes. The drug also

produced other signs not associated with withdrawal including ataxia, drowsiness, sagging, slowing, ptosis and prostration. Vehicle contained 20% Tween 80 and water.
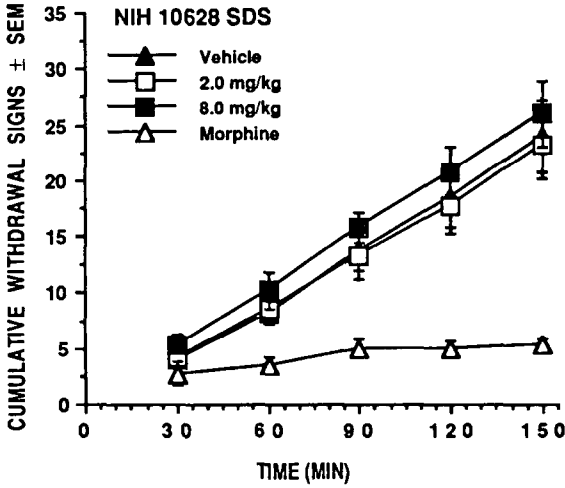
B.   (PPt-W)

In the precipitated withdrawal test, at the high dose, DTG appeared to precipitate withdrawal. However, this effect was due primarily to the signs designated retching and vomiting. The drug did not precipitate a full-blown withdrawal syndrome. As noted in the SDS study, the drug also produced ataxia, drowsiness, jaw sagging, slowing and prostration. Vehicle consisted of DMSO, lactic acid and water.
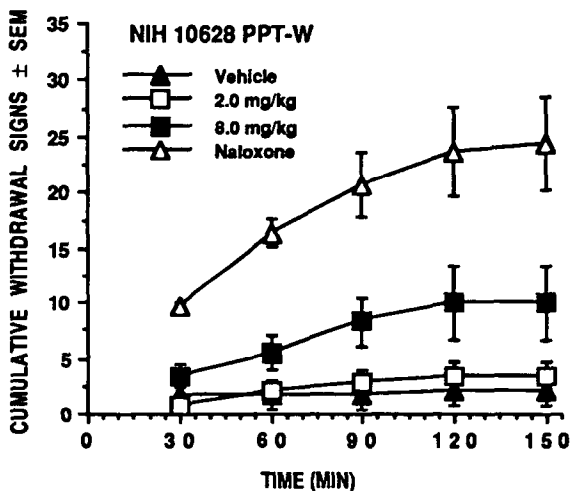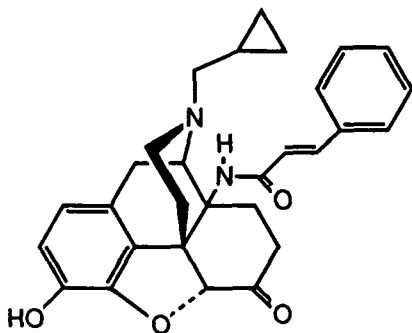
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs. M - 1.3 (0.5 - 3.8)
3) PPQ - 17% at 0.3, 37% at
   0.1, 66% at 0.3, 69% at 1.0,
   66% at 3.0, 46% at 10.0 and
   53% at 30.0

MONKEY DATA
    SDS

At doses of 1 and 5 mg/kg, NIH 10630 neither substituted for morphine nor
exacerbated withdrawal during the usual 2 1/2 hr observation period. However,
the monkeys receiving drug retched more often than vehicle controls. One
monkey receiving the higher dose was still in withdrawal 24 hr after receiving
drug as evidenced by rigid abdominal muscles and vocalization when abdomen
palpated. Apparently, onset of action was slow and duration long.
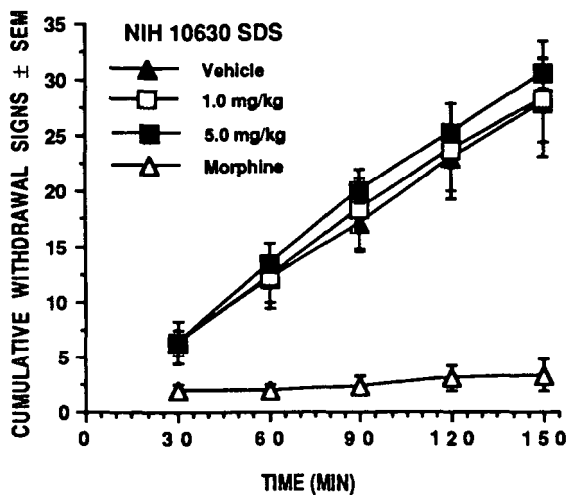
NIH 10631   (-)-Thevinone
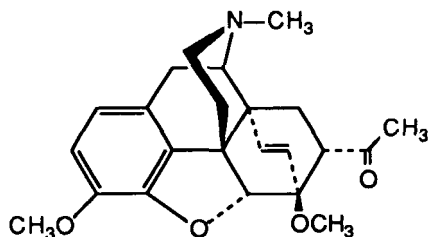
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 8.3 (2.7 - 27.6.0)
2) TF vs M - Inactive at 1.0.
    10.0 and 30.0.
3) PPQ - 2.0 (1.0 - 4.0)

MONKEY DATA
    S D S

As shown in the illustration, NIH 10631, completely suppressed withdrawal.
The effect was dose-related. One monkey at the higher dose and another at the
lower dose did not require an injection at noon. The drug acted promptly and its
duration of action was longer than that of morphine, the positive control. Potency
estimated as 1/2 - 1/3 that of morphine. Vehicle contained phosphoric acid and
water.
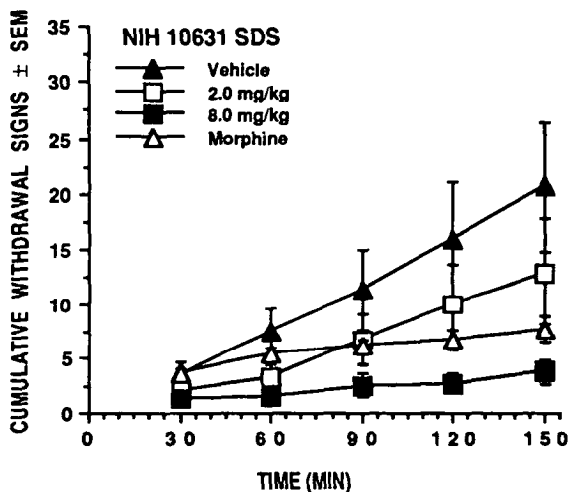
NIH 10633   (±)-Decarbamoylphysovenine
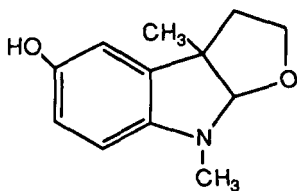
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs M - Inactive at 1.0,
   10.0 and 30.0.
3) PPQ -17% at 1.0, 37% at
   10.0 and 40% at 30.0

Vehicle - phosphoric acid and
H_2O

NIH 10634   (±)-7-Bromo-decarbamoylphysovenine
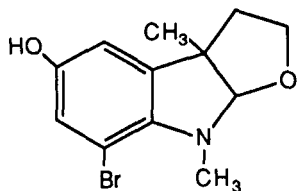
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
   30.0
2) TF vs M - Inactive at 1.0,
   10.0 and 30.0
3) PPQ - Inactive at 1.0, 10.0
   and 30.0

Note: Drug solution turned pink
upon standing for 1 1/2 hr, color
was dark red at 24 hr. These
solutions were not used.

NIH 10635   3,6-Dihydroxy-6α,,14α-ethenoisomorphinan-7 α-L-phenylalanyl-
ethyl ester hydrochloride

MOUSE DATA-ED50 OR
AD50 (95% C.L.) (mg/kg
or % change)

1) TF - 0.3 (0.1 - 0.9)
2) TF vs M - Inactive at
   1.0, 10.0 and 30.0
3) PPQ - 0.010 (0.004 -
   0.035)

NIH 10636   3,6-Dihydroxy-6,14-ethenoisomorphinan-7-D-phenylalanyl-
ethyl ester hydrochloride
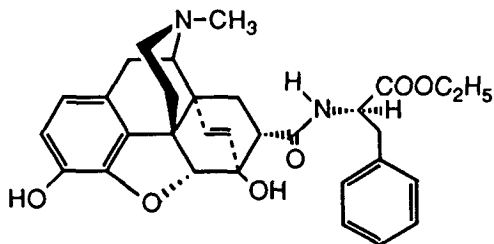
MOUSE DATA-ED50 OR
AD50 (95% C.L.) (mg/kg
or % change)

1) TF - 0.9 (0.4 - 2.0)
2) TF vs M - Inactive at
   1.0, 10.0 and 30.0
3) PPQ -0.4 (0.2 - 1.0)

667

NIH 10639, I-389   3R,4S-(+)-N-3-(p-Fluombenzoyl)propyl-3-methyl-4-phenyl-4-propionyloxypiperidine   fumarate
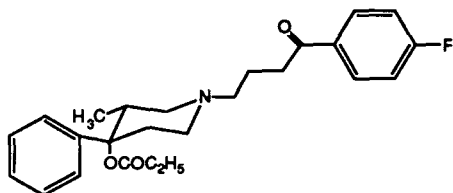
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.56 (0.25 - 1.28)
2)   TF vs M - Inactive at 1.3, 12.8 and 38.5[a]
3) PPQ - 0.1 (0.04 - 0.28)

Vehicle - Phosphoric acid and H$_2$O
[a]Mice reluctant to move

Special Study - Naloxone vs ED$_{80}$ of NIH 10639 in Tail-Flick Test = AD50 of 0.3 (0.01 - 0.06)

MONKEY   DATA
    SDS

NIH 10639 produced dose-related suppression of morphine withdrawal signs and substituted completely for morphine (see plot of data). In the one monkey receiving the higher dose, body and jaw sag, staring, ataxia, eyelid ptosis and slowing were noted. A challenge dose of 0.05 mg/kg of naloxone was given to this monkey. Naloxone effectively reversed these signs, implying opioid activity. At 0.25 mg/kg, similar signs were also noted. Onset of action was rapid and offset was about 2.5 hrs.
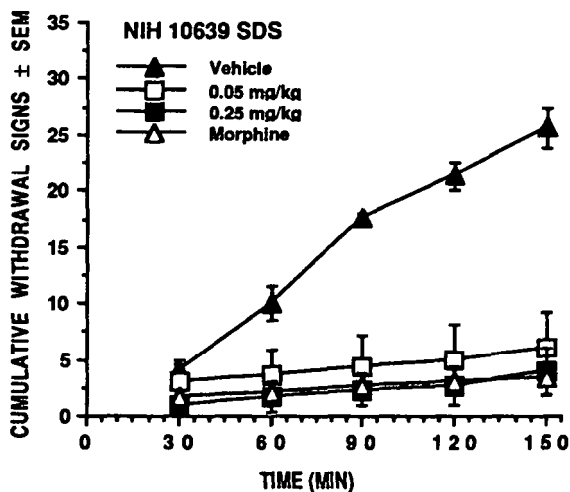
NIH 10640, I-489   3S,4R-(-)-N-3-(p-Fluorobenzoyl)propyl-3-methyl-4-phenyl-4-propionyloxypiperidine   fumarate

SEE NIH 10639

MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 1.0, 10.0 and 30.0
2) TF vs M - Inactive at 1.0.
    10.0 and 30.0
3) PPQ - 1.5 (0.6 - 3.8)

Vehicle - 10% DMSO,
phosphoric acid and H$_2$O

MONKEY DATA
    SDS

At doses of 0.5 and 2.5 mg/kg, 10640 neither substituted for morphine nor exacerbated withdrawal (see fig.). One monkey who received the higher dose appeared "cataleptic" at times. Note that vehicle consisted of DMSO, phosphoric acid and H$_2$O.

669
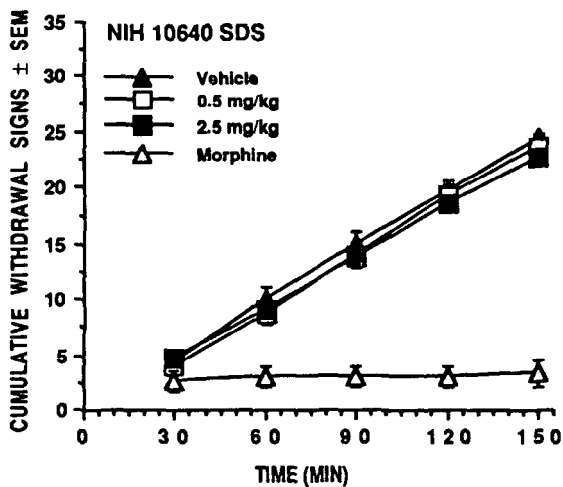
NIH 10641, I-3481 (±)-N-3-(*p*-Fluorobenzoyl)propyl-3-methyl-4-phenyl-4-propionyloxypiperidine fumarate

SEE NIH 10639

MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0.77 (0.36 - 1.65)
2) TF vs M - Inactive at 1.0,
   10.0 and 30.0
3) PPQ - 0.11 (0.03 - 0.38)

Vehicle - 10% DMSO,
phosphoric acid and H$_2$O

Special Study: Naloxone vs ED80 of NIH 10641 in Tail-Flick gave an AD50 of 0.04 (0.02 - 0.08)

MONKEY DATA
    S D S

Dose-related suppression of morphine withdrawal signs were noted. At the higher dose, namely, 0.25 mg/kg, complete suppression was observed (see fig.). While onset of action was rapid, offset of action was approximately one-half that of morphine. In addition, at the higher dose, ataxia, body and jaw sag and scratching were noted.
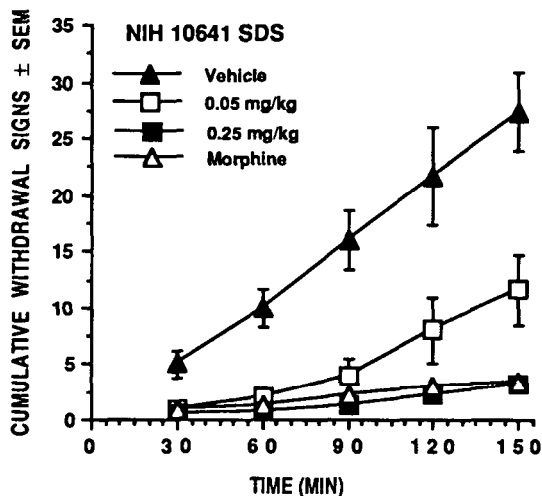
NIH 10642   (-)-Bromoeseroline   fumarate
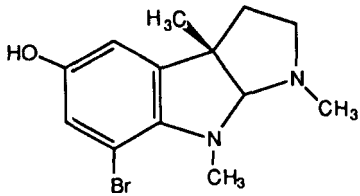
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0.54 (0.50 - 0.58)
2) TF vs M - Inactive at 1.0,
    10.0 and 30.0
3) PPQ - 0.18 (0.05 - 0.70)

MONKEY DATA
    SDS

NIH 10642 produced a dose-related suppression of withdrawal signs. However, two important signs designated vocalizes when abdomen palpated and rigid abdominal muscles were suppressed in only two of seven monkeys. The drug appeared to act promptly but the duration of action was approximately 1/2 that of morphine. Higher doses may show complete suppression.

671

NIH 1643   Methaqualone
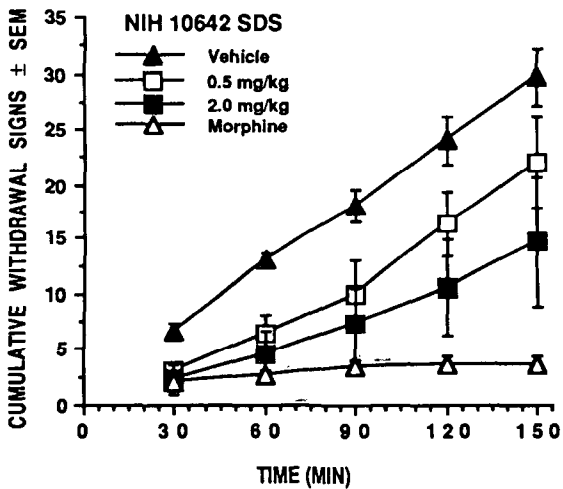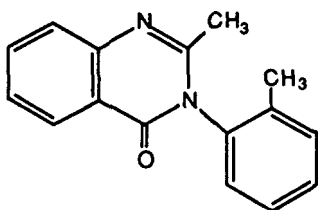
MONKEY DATA
    SDS

Anecdotal reports that many human abusers likened the effects of methaqualone to those of heroin (Goodman and Gilman, 6th ed.) prompted this study. As can be seen in the graph, methaqualone neither substituted for morphine nor exacerbated withdrawal. One monkey receiving 12 mg/kg (data not shown) was ataxic. Ingredients of vehicle were propylene glycol, benzyl alcohol, and water.
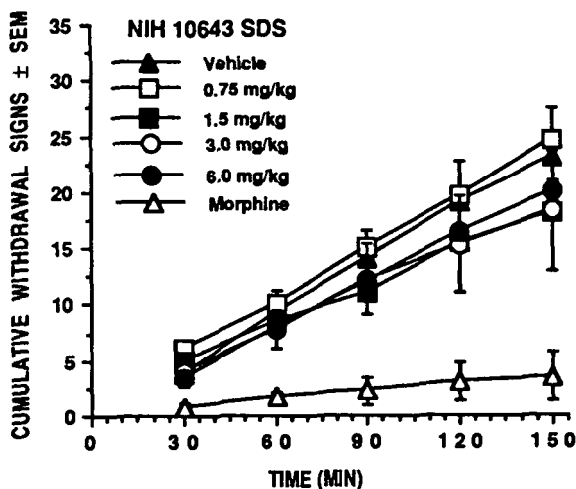
NIH 106147   1-(2-Phenylethyl)-4-(N-(2-pyrazyl)-2-furoylamido)piperidine
hydrochloride
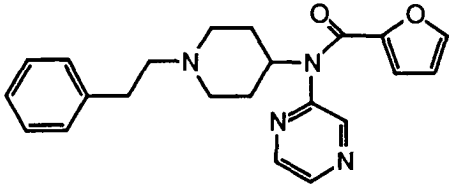
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0.4 (0.1 - 1.2)
2) TF vs. M - Inactive at 1.0 and
     10.0 and 30.0
3) PPQ - 0.08 (0.03 - 0.20)

MONKEY   DATA
     SDS

As shown in the fig. below, NIH 10647 neither substituted for morphine nor
exacerbated withdrawal. At the higher dose, some questionable catalepsy was
noted in one monkey and two of the animals may have developed relaxed skeletal
muscles and respiratory depression.
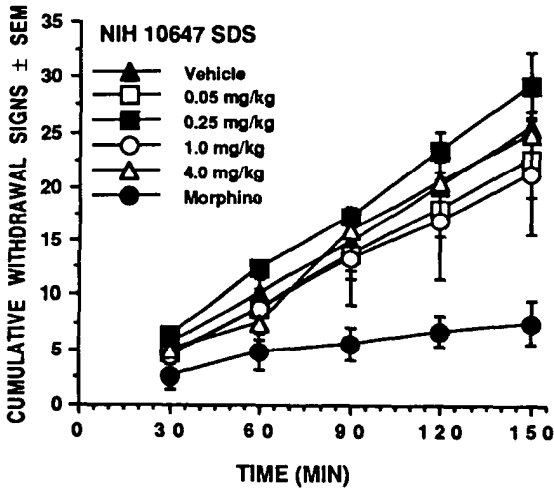
NIH 10648   (±-2-Benzyl-5,9 α·-dimethyl-2'-hydroxy-6,7-benzomorphan
hydrobromide [((±)-N-Benzylnormetazocine hydrobromide)]
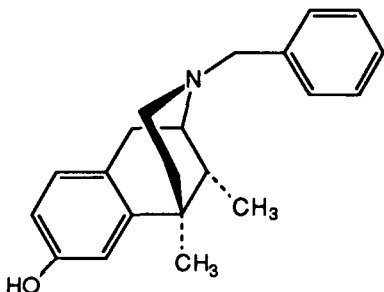
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, -10.0 and
   30.0
2) TF vs. M. - 26.0 (13.4 -
   50.4)
3) PPQ - Inactive at 1.0, 10.0
   and 30.0

Vehicle: Phosphoric acid and H_2O

MONKEY DATA
    SDS

As shown in the graph, NIH 10648 exacerbated withdrawal. The incidence of
the signs designated lying on side or abdomen, tremors, wet dogs, retching and
vomiting (especially) was higher than vehicle-treated controls. Body jerks were
also noted. However, the exacerbation did not appear dose related.
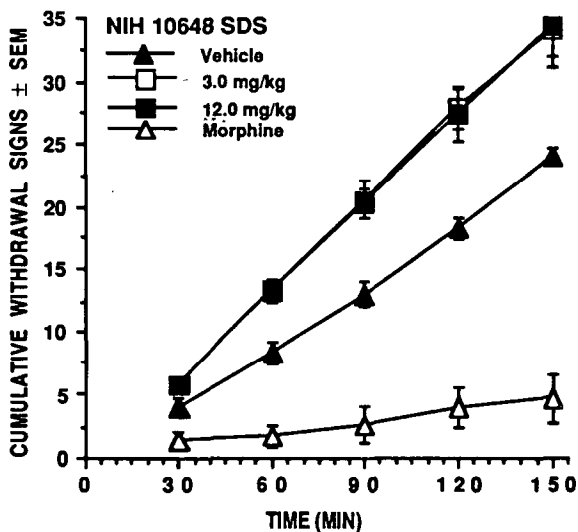
NIH 10649   (-)-2-Benzyl-5,9 α-dimethyl-2'-hydroxy-6,7-benzomorphan
hydrobromide   [((-)-N-Benzylnormetazocine   hydrobromide)]

SEE NIH 10648

MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
   30.0[a]
2) TF vs. M - 20.3 (8.3 - 49.6)[a]
3) PPQ - 13.4 (4.1 - 41.0)[a]

[a]Vehicle: Phosphoric acid and
H_2O

MONKEY DATA
    SDS

NIH 10649 did not substitute for morphine. Instead, the drug dose-dependently
exacerbated withdrawal. The drug acted promptly. One monkey given a dose of
15 mg/kg developed ataxia followed by a seizure (data not shown). This monkey
was given pentobarbital to terminate the seizure.
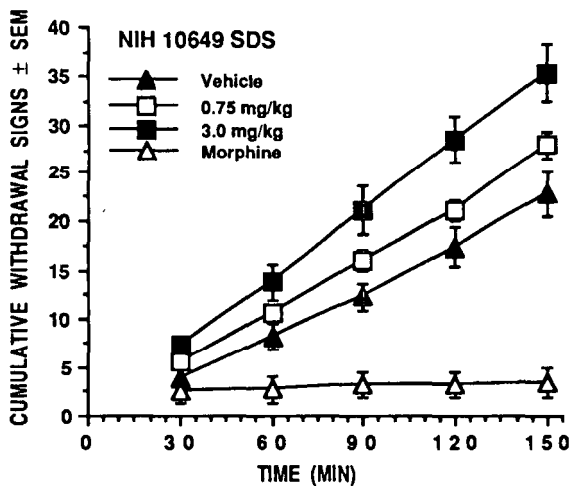
NIH 10562   (-)-α-Acetyl-N-normethadol hydrochloride (N-Nor-LAAM)
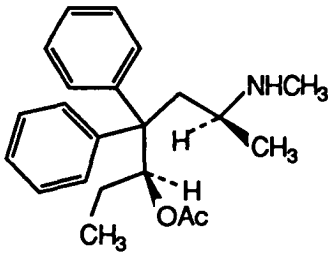
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0.5 (0.2 - 1.2)
2) TF vs. M - Inactive at 1.0,
     10.0 and 30.0
3) PPQ - 0.07 (0.03 - 0.17)

MONKEY DATA
   SDS

As shown below, NIH 10652 substituted completely for morphine at 0.5 mg/kg.
The drug acted promptly and duration of action at the high dose was
approximately 2 1/2 hr longer than that of morphine. Potency is estimated as 6 x
that of morphine. Two monkeys receiving the higher dose rubbed their faces on
the cage walls. This could indicate itching. The drug obviously has mu-agonist
properties.
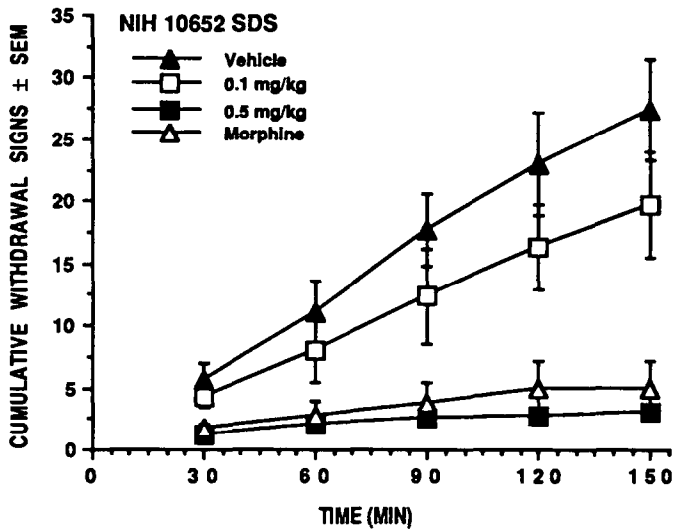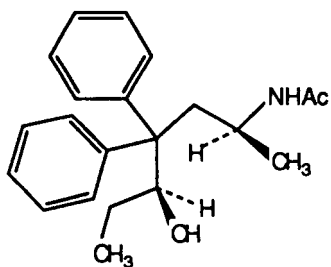
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and
    30.0[a]
2) TF vs. M - Inactive at 1.0,
    10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0
    and 30.0[a]

[a]Vehicle: 10% cyclodextrin
   aqueous solution + warming

MONKEY DATA
    SDS

At doses of 3 and 12 mg/kg, NIH 10653 neither substituted for morphine nor
exacerbated withdrawal. Tween 80, H_3PO_4, and H_2O were the ingredients of
this vehicle.
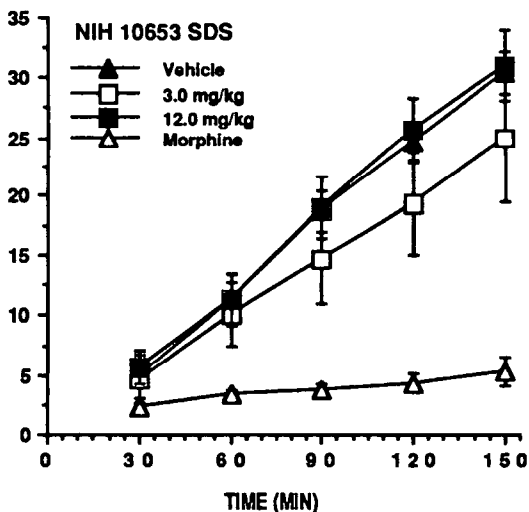
Conclusion: NIH 10653 lacks antinociceptive properties in mice and mu
               agonist/antagonist effects in monkeys.
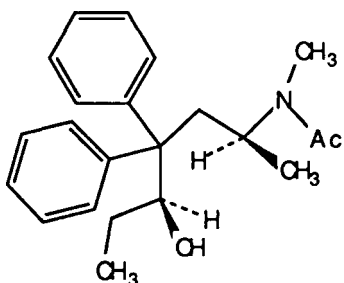
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 19% at 1.0, 26% at 10.0
    and 7% at 30.0[a,b]
2) TF vs. M - 0% at 1.0, 10.0
    and 26% at 30.0[a,b]
3) PPQ - Inactive at 1.0 and
    10.0, 29% at 30.0[a,b]

[a]Vehicle: Tween 80 + H_2O and
  warming
[b]Vehicle inactive in all tests

MONKEY DATA
    SDS

NIH 10654 did not substitute for morphine at 3 or 12 mg/kg. The drug may have
exacerbated withdrawal. However, the effect was not dose related. Also, vehicle
control values were low magnifying possible drug effects.
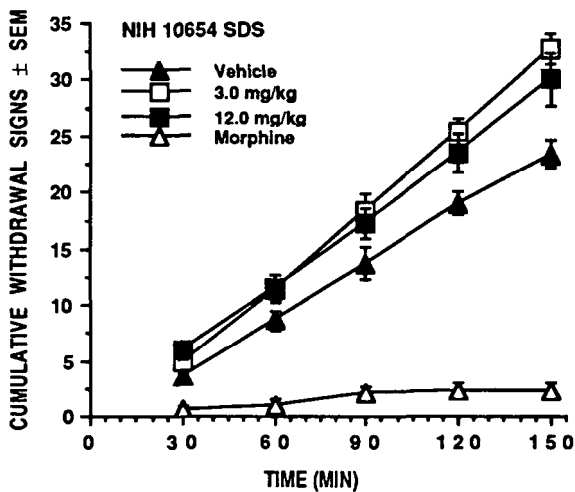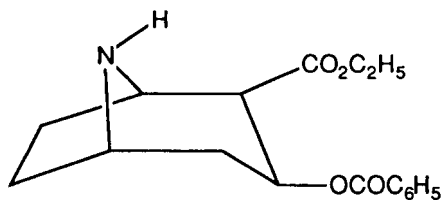
NIH 10664   Norcocaine

MONKEY DATA
   SDS

Kosten (1989) reported that cocaine attenuated opiate withdrawal in humans and rats. Recently we also demonstrated in our laboratory that cocaine attenuated morphine also demonstrated withdrawal in rhesus monkeys. Regarding possible mechanisms, the role of cocaine metabolites has been essentially ignored. We postulated that norcocaine, a metabolite of cocaine with central nervous system effects (Jones, 1984), played a significant role.

When norcocaine was given, it initially produced behavioral excitement which was not unlike that seen after cocaine. However, this effect dissipated in about 15-30 min after which the animal appeared normal.
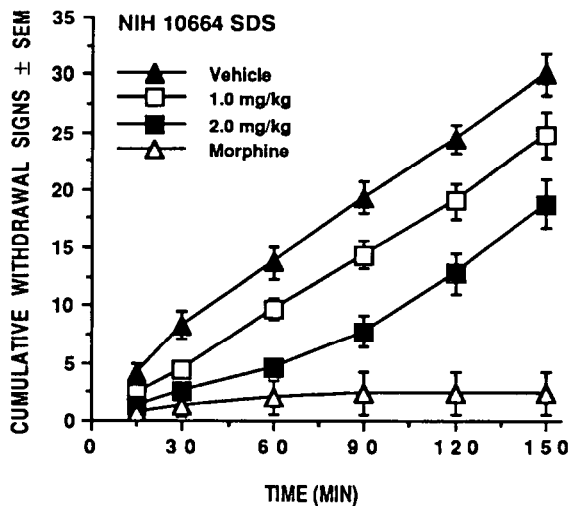
NIH 10664   Norcocaine

Norcocaine significantly and promptly attenuated, in a dose-dependent manner, the total number of withdrawal signs (see fig.). The action peaked at 90 min and waned during the rest of the testing period. The suppressive properties of norcocaine on individual withdrawal signs is shown in the table. It is obvious that norcocaine diminished the incidence of all the individual withdrawal signs except restlessness. It should be emphasized that norcocaine did not behave like a typical mu agonist in morphine-dependent monkeys (see results in the table).

Norcocaine appeared to have a biphasic effect regarding certain signs. At the low dose it was more effective in suppressing signs designated wet-dog shakes and retching than others namely, rigid abdominal muscles and vocalizes when abdomen palpated. At the high dose, the opposite was apparent for the signs retching whose incidence increased, and vocalizes when abdomen palpated or rigid abdominal muscles whose incidence decreased. We did not attempt to administer higher doses because severe tremors suggestive of impending convulsions during the initial excitement or rausch phase were observed

Summary

Norcocaine dose-dependently attenuates abrupt morphine withdrawal in rhesus monkeys. These results suggest a possible role for this metabolite in the interaction of cocaine with the opioid system.

TABLE 1

Comparison of the suppressive properties of norcocaine. morphine and vehicle at 90 min in withdrawn morphine-dependent monkeys

Treatment	Norcocaine		Vehicle	Morphine
Dose mg/kg i.v.	2.0	1.0	3 ml	3.0
No. of Animals	4	3	3	3
Withdrawal signs				
Lying on side or abdomen	1	2	-	-
Fighting	1	-	1	-
Avoids contact	-	-	-	1
Vocalizes				
Restless	7	9	10	3
Drowsy	-	-	-	-
Tremors	3	-	-	-
Wet-dog shakes	4	4	10	-
Retching	8	4	10	2
Vomiting	-			
Vocalizes when abdomen palpated	4	12	12	-
Abdominal muscles (rigid)	3	1 -	12	-
Total of Signs	31	41	55	6
Calculated P value	a,b	a,b		a

[a]Significantly different from vehicle control (p < 0.05, Mann-Whitney U Test).
[b]Significantly different from morphine control (p < 0.05, Mann-Whitney U Test).

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AFFILIATION

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613

Evaluation of New Compounds
for Opioid Activity (1990)

**James H. Woods, Fedor Medzihradsky, Charles B. Smith,
Gail D. Winger, and Charles P. France**

The evaluation of new compounds by the programs at the University
of Michigan and the Medical College of Virginia is coordinated by
Dr. Arthur E. Jacobson, Laboratory of Medicinal Chemistry,
NIDDK, National Institutes of Health, Bethesda, MD. The drugs,
which come originally from pharmaceutical companies, universites,
government laboratories, and international organizations are
submitted to Dr. Jacobson, who performs the MOUSE ANALGESIA
tests. Values obtained in these tests for some representative
opioid drugs are given in Table I.

At the UM and MCV laboratories, drug samples arrive from Dr.
Jacobson with only the following information: (1) an identifying
NIH number, (2) molecular weight, (3) solubility information and
 (4) a recommended starting dose. After the evaluation is
complete and the report submitted to Dr. Jacobson, the submitter
is requested to release the chemical structure to include with
the evaluation data in the ANNUAL REPORT. The submitter has up
to three years before release of the structure is required.
When the structure is released all of the data on the compound
are reported to the Committee.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the
discriminative stimulus effects of submitted drugs: one of these
groups discriminates the administration of the κ agonist
ethylketazocine (EKC); a second group discriminates the μ agonist
codeine; a third group is treated daily with morphine and
discriminates the opioid antagonist naltrexone.

The procedures used with the EKC-trained monkeys have been
described by Bertalmio et al. (1982). The monkeys are removed
from their home cages each day and seated in primate restraining
chairs. These chairs are placed in isolation chambers equipped
with two response levers, several stimulus lights and a cup to
receive Noyes, banana-flavored pellets. These monkeys are
required to make 100 consecutive responses on the correct one of

TABLE I

MOUSE ANALGESIA. Before submission to The University of Michigan, all compounds are evaluated for
analgesic activity by Dr. Arthur E. Jacobson. Shown below are comparative data (ED $\frac{50mg/kg}{mol/kg}$)
 (95% Confidence Interval) from Hot Plate" and Nilsen[d] assays.

Compound	HOT PLATE		NILSEN	
	(sc/mg/kg)	(oral,mg/kg)	(sc, mg/kg)	(oral, mg/kg)
NIH #	(sc. µmol/kg)	(oral, µmol/kg)	(sc. µmol/kg),	(oral, µmol/kg)
Morphine sulfate	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-l-7)	8.3 (6.0-11.4)
NIH 0001, 9929	2.9 (2.5-3.3)	18.9 (14.1-24.9)	3.9 (3.0-5.1)	24.9 (18.0-34.l)
Codeine phosphate	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
NIH 0002	17.1 (11.3-25.7)	34.0 (24.4-47.1)	18.6 (12.3-27.7)	37.0 (23.2-58.7)
Levorphanol tartrate	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
NIH 4590	0.5 (0.2-0.7)	-	0.5 (0.4-O-7)	6.2 (4.2-9.1)
Meperidine.HCl	5.3 (4.0-7.1)	-	-	-
NIH 5221	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
NIH 7569	1.9 (1.4-2.8)	34.1 (25.7-45.3)	1.6 (1.0-2.3)	83.6 (67.5-106.1)

TABLE I Continued

Dihydromorphinone.HCl NIH 0123	0.19 (0.15-0.25) ————————— 0.6 (0.5-0.8)	0.9 (0.7-1.2) ——————— 2.8 (2.2-3.7)	0.2 (0.15-0.3) ————————— 0.6 (0.5-0.9)	1.8 (1.5-2.1) ——————— 5.6 (4.7-6.5)
Nalorphine.HCl NIH 2105	9.9 (5.7-17.1) ————————— 28.4 (16.4-49.1)	- -	23.0 (16.2-32.7) ——————— 66.1 (46.6-94.0)	- -
Cyclazocine NIH 7981	1.5 (1.1-2.1) ————————— 5.5 (4.1-7.7)	- -	0.1 (0.07-0.16) ——————— 0.4 (0.3-0.6)	- -
Pentazocine NIH 7958	9.3 (6.7-12.8) ————————— 32.6 (23.5-44.9)	- -	6.5 (4.4-8.8) ——————— 22.8 (15.4-30.9)	- -
Naltrexone.HCl NIH 8503			No dose response	
Naloxone.HCl NIH 7890			No dose response	

No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.

Chloropromazine.HCl	1.1 (0.9-1.5) ————————— 3.2 (2.4-4.2)

a) Eddy and Leimbach (1953); b) Jacobson and May (1965); c) Atwell and Jacobson (1978); d) Perrine, Atwell, Tice, Jacobson and May (1972).

the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Each cycle lasts 15-min and consists of an initial 10-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are delivered before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six cycles are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the codeine procedure and the EKC procedure is that the codeine monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the codeine-trained monkeys are also done

685

using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily sessions consist of between two and six discrete, 15-min cycles with each cycle comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. Under these experimental conditions electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing five times consecutively (i.e., fixed-ratio 5) the lever appropriate for the solution administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug training days a single injection of naltrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (0-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (e.g., irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (e.g., alfentanil; France and Woods, 1989; France et al., 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone (i.e., precipitate withdrawal) are also studied for their ability to reverse responding on the naltrexone lever in morphine-abstinent (i.e., withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (<20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the

effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

The single-dose suppression (SDS) test determines the ability of a drug to suppress the signs of withdrawal in monkeys which have been made dependent by the chronic administration of morphine (3 mg/kg every six hours). Compounds suspected of having morphine-antagonist properties are tested for their ability to precipitate the withdrawal syndrome in nonwithdrawn (NW) morphine-dependent monkeys. Nondependent monkeys (Normals) are used to determine whether the acute effects of the test drug are reversible by nalorphine or naloxone. In a primary dependence (PDS) study, non-dependent monkeys receive the test drug every six hours for 30 days to determine whether withdrawal signs will appear when the animals are challenged with an antagonist or when drug administration is discontinued.

Details of these techniques have been presented in the ANNUAL REPORT to the Committee in 1963 (Minutes of the 25th Meeting) by Deneau and Seevers (1963) and by Villarreal (1973).

ANALGESIA IN RHESUS MONKEYS

The tail withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40°, 50°, or 55° C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40° C water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40° C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986), however, if a monkey fails to keep its tail in 40° C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40°, 50°, and 55° C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40° C water and less

687

than 5 seconds for 55° C water, monkeys receive the test compound. The test is identical to the pre-test, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a μ (e.g., alfentanil) or κ (e.g., U-50,488) opioid agonist.

RESPIRATORY FUNCTION IN RHESUS MONKEYS

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO_2 in air (France and Woods, 1990; Howell et al., 1988). Monkeys are restrained at the neck and waist while seated in a Plexiglas primate chair. Normal air or 5% CO_2 in air is delivered at a rate of 101/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a microprocessor, and are transformed according to known standards to frequency of respiration (f) in breaths/minute and to tidal volume (VT) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to CO_2. The last 3 minutes of exposure to CO_2 are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (e.g., alfentanil).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce a five-sec intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition is in effect, during which responses have no scheduled consequence and neither light

is illuminated. Each of the two daily sessions consist of 13
injections or 130 min, whichever occurs first. Other details of
the procedure and initial findings with a variety of narcotics
are given in previous reports (e.g., Woods, 1977; 1980).

Doses of the drugs are typically described in terms of mg/kg/
injection (inj). Duplicate observations of codeine (0.32
mg/kg/inj) and of saline are obtained for each monkey. A saline
substitution is conducted before and after the series of
observations on a test drug; the control rates of codeine-
reinforced responding are obtained by a random sampling of two
sessions interpolated between the drug-substitution sessions.
These data are represented in the following graphs with
individual symbols for each of the monkeys; each symbol is the
mean of duplicate observations for a given dose in each monkey.
The closed circles indicate the averaged data for observations on
the subset of monkeys used to study each drug under each of the
experimental conditions. In all cases, the rates of responding
given are those calculated during only the fixed-ratio portion of
each session.

DISPIACEMENT OF RADIOIABELED LIGAND BINDING

Details of the binding assay, based on the displacement of ^{3}H-
etorphine in rat brain membranes have been described previously
(Hedzihradsky et al., 1984). Briefly, aliquots of a membrane
preparation from rat cerebrum are incubated with ^{3}H-etorphine in
the presence of 150 mM NaCl, and in the presence of different
concentrations of the drug under investigation. Specific, i.e.,
opioid-receptor-related interaction of ^{3}H-etorphine is determined
as the difference in binding obtained in the absence and presence
of an appropriate excess of unlabeled etorphine. The potency of
the drugs in displacing the specific binding of ^{3}H-etorphine is
determined from log-probit plots of the data. See Table II for
representative results with different opioids.

To enhance the characterization of newly synthesized opiates, we
are now investigating their selectivity in binding to $\mu-$, $\boldsymbol{\delta}-$,
and $\boldsymbol{\kappa}\cdot$opioid receptors in membranes from monkey brain cortex.
Thus, we are now providing EC50 values of the tested compounds in
displacing the following radiolabeled opioid ligands:

        etorphine (nonselective, reflects opioid character),

        sufentanil or Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO); μ
        selective),

        (D-Pen^2-D-Pen^5]enkephalin (DPDPE; $\boldsymbol{\delta}$ selective),

        U-69,593 ($\boldsymbol{\kappa}$ selective).

Using the receptor-specific assays, we have described the
selectivity of various established opiates in brain membranes of
different species (Clark et al., 1988). The selection of monkey

TABLE II

EC50's of representative opioids for displacement of 0.5 nM ^{3}H-etorphine from rat brain membrane, and inhibition of the twitch of the mouse vas deferens preparation.

Compound	BINDING*	M V D
	EC50 (nM)	
DPDPE	---	5.52
U50,488	---	6.29
Fentanyl	36.2	37.1
DAMGO	23.9	81.3
Etorphine	0.37	0.0068
(-)Cyclazocine	0.53	11.9
Naltrexone	0.63	---
Bremazocine	1.42	0.29
UM 1071R*	1.55	---
Sufentanil	1.60	4.43
(-)SKF 10047	3.93	---
Ethylketazocine	6.60	11.6
Ketazocine	14.1	1.18
Morphine	23.6	395
DSLET	43.0	1.71
Dextrorphan	>6000	1010

* In the presence of 150 mM NaCl.

** 1R-SR-9R-2"R-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan hydrochloride

<u>brain</u> as the tissue for the selective binding assays strengthens the correlation between this <u>in</u> <u>vitro</u> assessment and the behavioral evaluation of the tested compounds. In the ANNUAL REPORT, the results of the selective binding assays are listed under "Binding in monkey brain cortex". See Table III for representative results with different opioids in rat and monkey brain.

Within our goal to enhance the molecular characterization of novel opioids (Medzihradsky, 1987) we have established a functional assay for receptor-effector interaction, reflecting receptor coupling to regulatory G protein. The method is based on the stimulation of brain GTPase by opioid agonists, a process blocked by antagonists (Clark and Medzihradsky, 1987). We are presently evaluating the quantitative responses of partial agonist-antagonists in this assay. Considering the variable efficacy of opioid receptor occupancy (Clark <u>et</u> <u>al</u>., 1989) the new assay provides a <u>functional</u> parameter in the characterization of novel opioids, distinguishing thereby between agonists and antagonists.

INHIBITION OF TWITCH IN ELECTRICALLY-STIMULATED MOUSE VAS DEFERENS PREPARATIONS.

The development of new, highly selective antagonists such as the irreversible mu receptor antagonist beta-funaltrexamine (beta-FNA) and the reversible delta receptor antagonist ICI-174864 have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse vas deferens preparation. Male, albino ICR mice, weighing between 25 and 30 g, are used. The mice are decapitated, the vasa deferentia removed, and 1.5 cm segments are suspended in organ baths which contain 30 ml of a modified Kreb's physiological buffer. The buffer contains the following (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; glucose, 11; $NaHCO_3$, 25; pargyline HCl, 0.3, tyrosine, 0.2; ascorbic acid, 0.1; and disodium edetate, 0.03. The buffer is saturated with 95% 02 - 5% CO_2 and kept at 37° C. The segments are attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments are stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage. See Table II for representative agonist potencies.

The following antagonists are studied: naltrexone HCl, ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and beta-FNA. Naltrexone and ICI-174864 are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. See Table IV for potencies of different competitive antagonists studied in relation to prototypic agonists. Beta-FNA is added to the organ baths after the initial equilibration period. Thirty min later, the beta-FNA is removed from the organ baths by repeated washings with fresh buffer. The tissues are washed three times

TABLE III

Inhibition of radiolabeled sufentanil, DPDPE and U69,593 binding in rat and monkey brain. In membranes from rat cerebrum and monkey brain cortex, the inhibition of specific equilibrium binding of 0.5 nM [^{3}H]sufentanil, 1.5 nM [^{3}H]DPDPE and 1.5 nM [^{3}H]U69,593 by five different concentrations of the listed compounds was investigated in the presence of 150 mM NaCl (modified from Clark et al., 1988).

Compound	[^{3}H] SUFENTANIL	EC50 (nM) [^{3}H]DPDPE	[^{3}H]U69,593
Rat cerebrum			
DAMGO	13.2	690	
Sufentanil	1.25	45.0	
Morphine	31.4	422	
β-FNA	6.99	43.9	
β-CNA	1.29	7.48	
Naloxone	6.37	14.3	
Etorphine	0.60	1.13	
Buprenorphine	1.07	1.12	
Bremazocine	1.79	1.12	
Superfit	576	16.5	
DSLET*	121	1.05	
ICI-174,864	58,900	59.0	
DPDPE	7,720	6.44	
U50,488	7,230	13,100	
U69,593	38,000	13,400	
Monkey cortex			
Sufentanil	1.18	81.1	>10,000
DPDPE	18,900	4.21	>10,000
U-69,593	10,700	17,000	8.41

* (D-Ser^2,Leu^5)-enkephalin-Thr^6

TABLE IV

Potencies of antagonists assessed in the mouse vas deferens

	pA$_2$ Values* determined with three agonists		
	Sufentanil (μ)	U50.488H (κ)	DSLET (δ')
Antagonist			
Naltrexone	8.76	7.74	7.41
Naloxone	7.99	6.90	7.35
Cyprodime**	7.41	6.15	5.98
Nalbuphine	7.23	6.31	5.76
Naltrindole	7.71	7.38	9.44
ICI-174,864	< 5.00	< 5.00	7.90

*The pA$_2$ value is the negative logarithm of the molar concentration of antagonist necessary to shift the agonist concentration-effect curve to the right by a factor of 2-fold.

**(-)-N-cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one

every 5 min for 30 min. Cumulative concentration-effect relationships for the various agonists are then determined 10 min after the last wash (i.e., 30 min after the beta-FNA was removed from the organ baths). EC50's are calculated by probit analysis, and pA$_2$ values are determined to assess relative potencies of antagonists. All drugs which are submitted for evaluation are studied in the following manner: 1) the submitted drug is tested on the vas deferens preparation in the absence and in the presence of naltrexone. The concentration of the unknown drug is varied from the lowest with activity to that which is maximally effective. 2) If the submitted drug inhibits the twitch, the ability of naltrexone to reverse the inhibition is determined. 3) The submitted drug is assessed for its ability to antagonize the actions of morphine on the vas deferens. 4) The drug is assessed for its ability to reverse the inhibition produced by a maximally effective concentration of morphine. 5) Finally, if the drug has opioid agonistic activity, studies are conducted to determine the receptor type upon which it acts. If it has antagonistic activity upon the vas deferens or upon any of the other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive) and the receptor selectivity is determined. For further details of the procedure see Smith (1986). Drugs studied in the preparation prior to 1987 were evaluated with the protocol reported in the 1985 Annual Report.

SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year, and the individual tests which were per-formed are shown in Table V. Also shown are dates of Reports to the Biological Coordinator, Dr. A.E. Jacobson, in which results are reported.

TABLE V

SUMMARY OF TESTS PERFORMED

NIH	CHEMICAL CLASS AND/OR GENERIC NAME	SA	MVD	BIND	DD	ANAL	RESP	REPORT*
8209A	Benzomorphan	−	+	+	−	−	−	10/24/90
10520	N-Allyletonitazene	−	+	+	−	−	−	06/12/87
10521	N-Ethyletonitazene	−	+	+	−	−	−	05/12/87
10543	Phenylpiperidine	−	+	+	−	−	−	10/07/88
10574	Morphinan	−	+	+	−	−	−	06/12/89
10575	Morphinan	−	+	+	−	−	−	10/17/88
10588	Norbinaltorphimine	−	+	+	−	−	−	06/12/89
10604	Phenylpiperidine	−	+	+	−	−	−	01/19/90
10618	Benzacetamide	−	+	+	−	−	−	03/30/89
10619	Benzacetamide	−	+	+	−	−	−	03/30/90
10623	Morphinan	−	+	+	−	−	−	12/06/89
10625	Haloperidol	−	+	+	+	−	−	12/06/89
10626	Benzomorphan	−	+	+	−	−	−	12/06/90
10627	Benzomorphan	−	+	+	−	−	−	12/06/89
10630	Morphinone	−	+	+	−	−	−	12/01/89
10631	Thevinone	−	+	+	−	−	−	12/06/89
10632	Thevinone	−	+	+	−	−	−	10/22/89
10635	Peptide-alkaloid	−	+	+	−	−	−	12/06/89
10636	Peptide-alkaloid	−	+	+	−	−	−	12/06/89
10637	Phenylpiperidine	−	+	+	−	−	−	12/06/89
10642	Bromoeseroline	−	+	+	−	−	−	12/06/89
10647	Phenethylpiperidine	+	+	+	−	+	+	12/06/89, 04/13/90
10649	Benzomorphan	−	+	+	−	−	−	12/06/89
10650	Benzomorphan	−	+	+	−	−	−	12/06/89
10651	Phenylpiperidine	−	+	+	−	−	−	12/06/89
10652	Methadol	−	+	+	−	−	−	01/03/89
10653	Methadol	−	+	+	−	−	−	01/03/89
10654	Methadol	−	+	+	−	−	−	01/03/89
10655	Methadol	−	+	+	−	−	−	01/03/89

* Date report was submitted to CPDD Biological Coordinator.

(+)-2-n-Hexyl-2'-hydroxy-5,9 -dimethyl-6.7-benzomor-
phan hydrochoride (N-n-Hexyl-normetazocine hydrochloride)
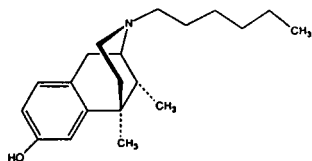
MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 1.4 (1.3-1.6)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 358 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.44×10^{-7}	100%
After naltrexone	1.22×10^{-5}	100%
After nor-binaltorphimine	2.52×10^{-6}	100%
After ICI-174,864	5.90×10^{-7}	100%

SUMMARY

NIH 8209 was active, but not very potent, in both assays. Its
agonist actions in the vas deferens appeared to be sensitive to
antagonists selective for μ and κ receptors.

NIH 10520   N-Allyletonitazene bromide
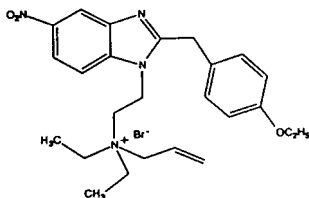
MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 1.4 (1.0-1.9)

   ... (continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 175 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.64×10^{-7}	97.7%
After naltrexone	1.07×10^{-5}	47.9%
After ICI-174,864	3.75×10^{-7}	92.2%
After $\boldsymbol{\beta}$-funaltrexamine	1.39×10^{-6}	48.0%

NIH 10520 neither altered responses to nor reversed the in-
hibitory effects of sufentanil.

   SUMMARY

NIH 10520 had significant opioid activity in both preparations.
The selective antagonism by narcotic antagonists in the mouse vas
deferens suggests that the compound has μ-receptor opioid
activity in this preparation.

NIH 10521   N-Ethyletonitazene iodide
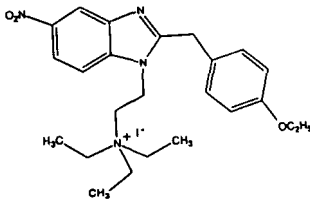
DISPLACEMENT OF SPECIFIC ^{3}H-
ETORPHINE BINDING

EC50 of 2800 nM in the presence
of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.76×10^{-6}	71.7%
With ICI-174,864	3.30×10^{-6}	88.9%

Naltrexone and pretreatment of the vas deferens with ß-funaltre-

697

NIH 10521   N-Ethyletonitazene iodide

   ...(continued)

xamine virtually abolished responses to NIH 10521. NIH 10521
(10^{-7} M) didn't reverse the inhibitory effects of sufentanil.

SUMMARY

NIH 10521 appears to have significant opioid activity in both
preparations, although its potency is quite low.

NIH 10543   (-)-α-4-Acetoxy-1,2-dimethyl-4-phenylpiperidine
hydrochloride
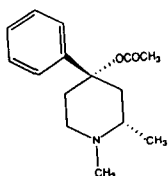
MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 33% at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 76 μM (22% inhibition at 6 μM) in the presence of 150 mM
NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10543 was studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-6} M to 10^{-4} M. Concentrations between 10^{-6} M and 10^{-4} M
caused a slight inhibition of the twitch. The EC50 for this drug
could not be determined, but the maximum response was a 100%
inhibition of the twitch (n=3). Naltrexone did not antagonize
the inhibitor actions of NIH 10543. In the presence of
naltrexone, 10^{-7} M, the EC50 for this drug was 2.25 x 10^{-5} M, and
the maximum response was a 31.8% inhibition of the twitch (n=3).
This inhibitory action was completely blocked by naltrexone, 10^{-7}
M. NIH 10543 in a concentration of 3 x 10^{-6} M did not antagonize
the actions of sufentanil (a μ agonist), DSLET (a δ agonist) or
U50,488H (a κ agonist).

SUMMARY

Taken together, the results from the two preparations suggest
that NIH 10543 might have opioid actions only at high concentra-
tions.

4,5α-Epoxy-6β,14β-dihydroxy-3-(2-carbomethoxyal-lyloxy)-17-cyclopropylmethylmorphinan
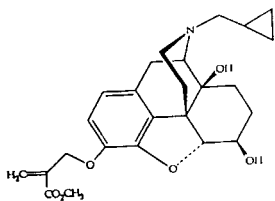
DISPLACEMENT OF SPECIFIC
^{3}H-ETORPHINE BINDING

EC50 of 29.8 nM in the
presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10574 was submitted as a glue-like substance which was
dissolved in IN HCl. NIH 10574 was studied upon the isolated,
electrically stimulated mouse vas deferens preparation in
concentrations which ranged from 10^{-8} M to 3 x 10^{-4} M. This
drug caused a slight inhibition of the twitch. In control
experiments the maximum response was an 11.9 ± 5.0% inhibition of
the twitch which occurred at a concentration of 10^{-5} M. EC50's
could not be computed. In the presence of naltrexone, 10^{-7} M,
the magnitude of the inhibition at the 10^{-5} M concentration was
slightly greater (21.7 ± 3.2% inhibition). NIH 10574 acted as a
potent opioid antagonist. This drug in concentrations of 10^{-8} M
to 10^{-6} M caused parallel shifts to the right in the DSLET (a δ
agonist) concentration-effect curve. The pA$_2$ value against
DSLET was 7.96 ± 0.44 (λ = 0.87, n = 10). Against U50,488H NIH
10574 acted as an unsurmountable (noncompetitive) antagonist in
that it caused decreases in the maximum response with no change
in EC50 and at a concentration of 10^{-7} M completed abolished all
responses to U50,488H. The interaction with sufentanil (a μ
agonist) was typical of that seen between an unsurmountable
antagonist and an agonist with for which there are spare
receptors. At low concentrations NIH-10574 caused parallel
shifts to the right in the sufentanil concentration-effect curves
and at higher concentrations decreased the maximum responses. A
pA$_2$ value calculated for this drug against sufentanil was 8.58 ±
0.50 (λ = 1.18, n = 8).

SUMMARY

NIH 10574 had significant opioid activity in both preparations.
In the vas deferens, the compound exerted antagonist actions
against each of the selective agonists.

NIH 10575   4,5α-Epoxy-3,14β-dihydroxy-6α-(2-carbomethoxyal-lyloxy)-17-cyclopropylmethylmorphinan
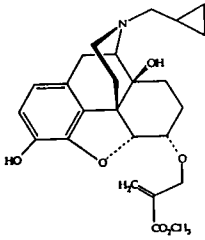
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of 3.08 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10575 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	7.34 ± 0.51	1.18
DSLET	7.31 ± 0.68	1.85
U-50,488	7.04 ± 0.44	1.19

Concentrations of NIH 10575 higher than 3 x 10^{-6} M completely suppressed responses to the three agonists.

SUMMARY

NIH 10575 was somewhat less potent than naltrexone in the binding assay. In the vas deferens preparation, it was less potent than naltrexone at μ receptors and differed from naltrexone in its selectivity for the three types of opioid receptor.

NIH 10588   Norbinaltorphimine hydrochloride (nor-BNI hydrochlo-ride)
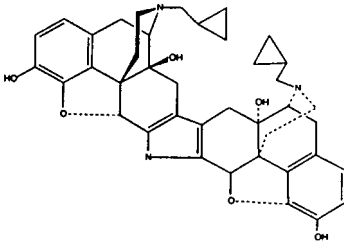
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of 70.0 nM in presence pf 150 mM NaCl.

Norbinaltorphimine hydrochloride (nor-BNI hydrochloride)

... (continued)

MOUSE VAS DEFERENS PREPARATION

NIH 10558 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 3×10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	7.46 ± 0.27	0.77
DSLET	7.72 ± 0.10	1.14

At very low concentrations, NIH 10588 caused shifts to the right in the U50,488H (a κ receptor agonist) concentration-effect curve and markedly decreased the maximum response to this agonist. In the presence of various concentrations of NIH 10588 EC50's and maximum responses for U50,488H were as follows: control EC50, 3.91×10^{-8} M ± 0.58 (n = 12); 10^{-10} M NIH-10588, 6.92×10^{-8} M ± 1.46 (n = 3, a 1.8-fold shift), $85.6 \pm 1.1\%$ inhibition; 10^{-9} M NIH-10588, 2.17×10^{-7} M ± 0.41 (n = 3, a 5.6-fold shift), $57.5 \pm 6.8\%$ inhibition; 10^{-8} M NIH-10588, 3.06×10^{-7} M ± 2.44 (n = 4, a 7.8-fold shift), $29.9 \pm 4.4\%$ inhibition.

SUMMARY

NIH 10588 had interesting actions in the mouse vas deferens; it has a unique profile of activity. This drug was a highly selective, noncompetitive antagonist at κ opioid receptors.

NIH 10604   3,5-Dimethyl-3(3-hydroxyphenyl)-1-phenylethylpiperidine hydrochloride
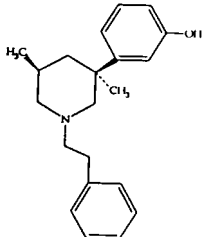
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 444 nM in the presence of NaCl.

NIH 10604   3,5-Dimethyl-3(3-hydroxyphenyl)-1-phenylethylpiperi-
dine hydrochloride

   ...(continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.35×10^{-7}	66.8%
After naltrexone	1.47×10^{-7}	66.4%

NIH 10604 failed to antagonize the inhibitory effects of
sufentanil, DSLET, or U50,488.

SUMMARY

Although NIH 10604 had significant, but low, potency in disp-
lacing etorphine, it failed to have significant opioid agonist or
antagonist activity in the mouse vas deferens. It did inhibit
the vas deferens partially by a non-opioid mechanism.

NIH 10618   1S,2R-(-)-cis-3,4-Dichloro-N-methyl-N-[2-(1-pyrroli-
dinyl)cyclohexyl]-benzeneacetamide (-)-tartrate
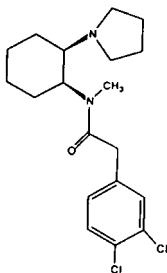
DISPLACEMENT OF SPECIFIC ^{3}H-
ETORPHINE BINDING

EC50 of >10,000 nM (9.4%
inhibition at 6 μM) in presence
of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10618 studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-9} M to 3×10^{-5} M. No concentration of this drug
inhibited the contractions of the vas deferens and it was
evaluated as an antagonist. pA_2 values against the following
agonists were:

Agonist	pA_2 values	Slope
Sufentanil	7.00 ± 0.51	1.42
U50,488	6.86 ± 0.55	1.55

NIH 10618   1S,2R-(-)-cis-3,4-Dichloro-N-methyl-N-[2-(1-pyrroli-
dinyl)cyclohexyl]-benzeneacetamide (-)-tartrate

    ... (continued)

NIH 10618 did not antagonist the actions of DSLET in concentra-
tions in up to 10^{-5} M.

SUMMARY

NIH 10618 was an extremely interesting compound that appeared to
act as an antagonist in the vas deferens preparation, but had no
significant affinity for the etorphine binding site.

NIH 10619   1R,2S-(+)-cis-3,4-Dichloro-N-methyl-N-[2-(1-pyrroli-
dinyl)cyclohexyl]-benzeneacetamide (+)-tartrate
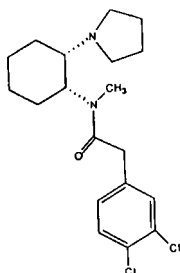
DISPLACEMENT OF SPECIFIC ^{3}H-
ETORPHINE BINDING

EC50 of >10,000 nM (9.9% inhibi-
tion at 6 μM) in presence of 150
mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10619 studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-9} M to 3 x 10^{-5} M. No concentration of this drug
inhibited the contractions of the vas deferens and it was
evaluated as an antagonist. pA_2 values against the following
agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.28 ± 0.52	1.31
U50,488	5.29 ± 0.93	2.34

NIH 10619 did not antagonize the actions of DSLET in concentra-
tions in up to 10^{-5} M.

SUMMARY

NIH 10619, like NIH 10618, acted as an antagonist upon the mouse
vas deferens without any significant affinity for the central
binding site. This was an unusual finding; see NIH 10618.

4,5α-Epoxy-3,14β-dihydroxy-6β-(2-carbomethoxyal-lyloxy)-17-cyclopropylmethylmorphinan
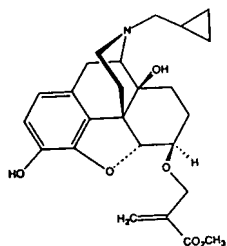
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 4.36 nM in presence of 150 mM NaCl.

MOUSE VAS DEFEEENS PREPARATION

NIH 10623 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3×10^{-5} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	8.98 ± 0.39	1.36
DSLET	8.32 ± 0.24	0.68

Concentrations of NIH 10623 between 10^{-8} and 10^{-6} decreased the maximum responses to U50,488 and caused slight shifts to the right in the concentration-effect curves. At a concentration of 10^{-6} M, NIH 10623 completely blocked all responses to U50,488.

SUMMARY

NIH 10623 was a potent compound in both preparations. It had antagonist actions against each prototypic agonist in the vas deferens. There was a suggestion that its actions were insur-mountable at the κ receptor in this preparation.

NIH 10625 (8032) Haloperidol
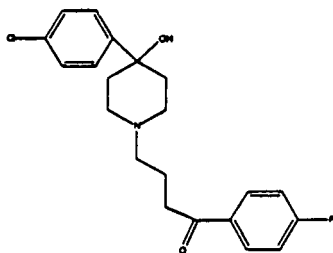
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of >10,000 nM (39.3% inhibition at 6 μM) in presence of 150 mM NaCl.

704

... (continued)

MOUSE VAS DEFERENS PREPARATION

NIH 10625 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-5} M. Only the 10^{-5} M concentration of this drug inhibited the twitch. This inhibition was complete and was not blocked or reversed by naltrexone. NIH 10625 acted as an opioid antagonist. This drug caused shifts to the right in the concentration-effect curves for sufentanil (a μ agonist) and U50,488 (a κ agonist) but not for DSLET (a δ agonist). pA_2 values were as follows: against sufentanil, 5.98 ± 0.19 (λ = 0.79, n = 6) and against U50,488, 6.44 ± 0.12 (λ = 0.65, n = 10). For the purpose of comparison, pA_2 values for naltrexone are as follows: against sufentanil, 8.76 ± 0.34; against DSLET, 7.41 ± 0.12; and against U50,488, 7.74 ± 0.12.

DRUG DISCRIMINATION IN RHESUS MONKEYS

The discriminative stimulus effects of NIH 10625 were studied in three morphine-treated (3.2 mg/kg/day) rhesus monkeys discrimi-nating between 0.01 mg/kg of naltrexone and saline. Under these conditions monkeys generalize completely to naltrexone at a cumulative dose of 0.01 mg/kg (triangles, left panel). Up to a dose that eliminated responding (0.1 mg/kg), NIH 10625 failed to substitute for naltrexone in any of the monkeys (circles) and did not produce directly observable signs of withdrawal. Up to a dose that eliminated responding, NIH 10625 failed to reverse responding on the naltrexone lever in morphine-abstinent (withdrawn) monkeys (circles). Under these conditions the opioid agonist alfentanil reversed naltrexone lever responding at a cumulative dose of 0.032 mg/kg (triangles, right panel).

705

NIH 10625 (8032) Haloperidol
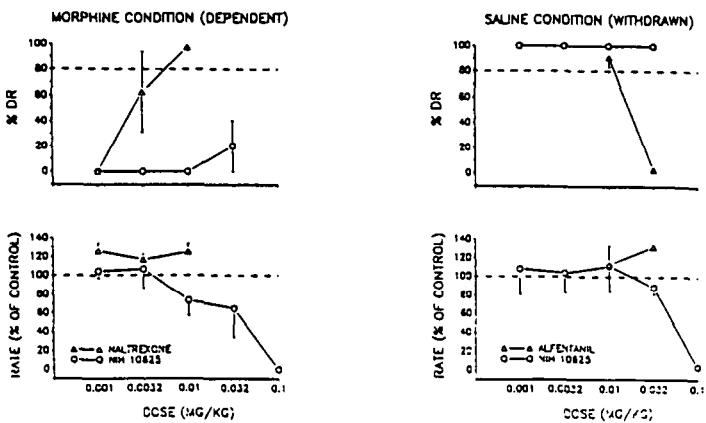
... (continued)

SUMMARY

NIH 10625 was not potent in either preparation, but there was
antagonist activity at both μ and κ receptors in the vas
deferens. It had no opioid agonist or antagonist activity in
morphine-treated rhesus monkeys discriminating naltrexone:

NIH 10626   (+)-2-n-Hexyl-2'-hydroxy-5,9 α-dimethyl-6,7-benzomor-
phan hydrochloride ((+)-N-n-Hexyl-normetazocine hydrochloride)
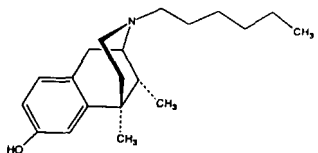
DISPLACEMENT OF SPECIFIC
^{3}H-ETORPHINE BINDING

EC50 of >10,000 nM (38.4%
inhibition at 6 μM) in
presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10626 studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-9} M to 3×10^{-5} M. No concentration of this drug
inhibited the contractions of the vas deferens and it was
evaluated as an antagonist. pA_2 values against the following
agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.24 ± 0.66	1.71
DSLET	5.65 ± 0.95	2.37
U50,488	7.42 ± 0.37	1.08

NIH 10626   (+)-2-n-Hexyl-2'-hydroxy-5,9 α-dimethyl-6,7-benzomor-
phan hydrochloride ((+)-N-n-Hexyl-normetazocine hydrochloride)

   ...(continued)

SUMMARY

NIH 10626 was not potent in either assay though it appeared more
potent in the vas deferens. In this preparation, it was an
antagonist, but its antagonist actions were not simply competi-
tive due to the deviation of slopes of the Schild plots for its
interaction with sufentanil and DSLET.

─────────────

NIH 10627   (-)-2-n-Hexyl-2'-hydroxy-5,9 α-dimethyl-6,7-benzomor-
phan hydrochloride ((-)-N-n-Hexyl-normetazocine hydrochloride)
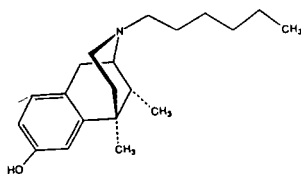
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of 166 nM in presence
of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.08×10^{-7}	80.7%
After naltrexone	2.38×10^{-6}	84.1%
After ICI-174,864	2.39×10^{-7}	87.1%
After nor-binaltorphimine	8.86×10^{-7}	64.5%

SUMMARY

NIH 10627 was active in both preparations; it was less potent
than morphine. Its actions in the vas deferens were complex,
suggesting actions at more than one receptor. It appeared to be
a partial agonist in the mouse vas deferens preparation with
actions at both μ and κ receptors.

707

NIH 10630   14β-Cinnamoylamino-7,8-dihydro-N-cyclopropylmethyl-
normorphinone mesylate
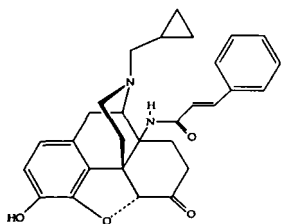
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of 0.73 nM in presence
of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10630 studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-10} M to 3 x 10^{-5} M. No concentration of this drug
inhibited the contractions of the vas deferens and it was
evaluated as an antagonist. pA_2 values against the following
agonists were:

Agonist	pA_2 values	Slope
Sufentanil	9.56 ± 0.48	1.13
DSLET	9.17 ± 0.42	1.25

Concentrations of NIH 10630 which antagonized the actions of
DSLET and sufentanil, caused a shift (noncompetitive) to the
right and downward in the concentration-effect curves for
U50,488.

SUMMARY

NIH 10630 was a very potent compound in both preparations. It
had potent, nonselective antagonist actions in the vas deferens.

NIH 10631   (-)-Thevinone
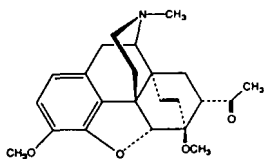
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of 1186 nM in the
presence of 150 mM NaCl.

NIH 10631   (-)-Thevinone

   ...(continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.66×10^{-8}	63.8%
After naltrexone	2.05×10^{-8}	52.5%
After ICI 174,864	4.78×10^{-8}	55.9%
After nor-binaltorphimine	3.46×10^{-9}	64.8%

SUMMARY

NIH 10631 displaced etorphine in the binding assay at high
concentrations. It was a partial agonist in the mouse vas
deferens preparation and appeared to act at δ opioid receptors.

NIH 10632   (+)-Thevinone (NIH 10620 in MCV report)
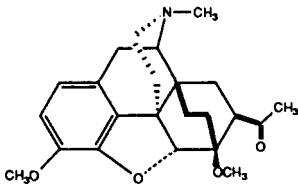
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of >10,000 nM (0%
inhibition at 6 μM) in the
presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.45×10^{-8}	17.9%
After naltrexone	2.41×10^{-8}	12.0%

Concentrations of NIH 10632 up to 10^{-5} M did not shift appreci-
ably the concentration-effect curves for sufentanil (a μ
agonist), DSLET (a δ agonist) or U50,488H (a κ agonist).

SUMMARY

NIH 10632 was devoid of significant opioid activity in either
preparation.

NIH 10635   3,6-Dihydroxy-6α,14α-ethenoisomorphinan-7α-L-phenylalanyl ethyl ester hydrochloride
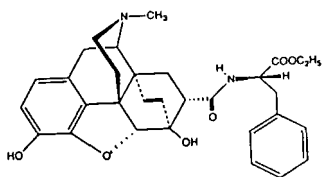
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.51 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.51×10^{-9}	97.7%
After naltrexone	8.52×10^{-8}	94.4%

Neither ICI-174864 (a δ antagonist) nor nor-binaltorphimine (a κ antagonist) significantly shifted the NIH 10635 concentration-effect curve.

SUMMARY

NIH 10635 was potent in both preparations; its actions in the vas deferens appear to be mediated specifically by the μ receptor.

NIH 10636   3,6-Dihydroxy-6α-14α-ethenoisomorphinan-7α-D-phenylalanyl ethyl ester hydrochloride
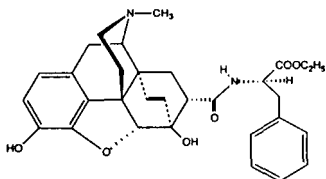
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.36 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.13×10^{-9}	100%
After naltrexone	6.61×10^{-8}	100%

Neither ICI-174864 (a δ antagonist) nor nor-binaltorphimine (a κ antagonist) significantly shifted the NIH 10636 concentration-effect curve.

NIH 10636   3,6-Dihydroxy-6α-14α-ethenoisomorphinan-7α-D-phenylalanyl ethyl ester hydrochloride

   ... (continued)

SUMMARY

NIH 10636 was quite potent in both preparations; its action in the vas deferens were as a selective μ opioid agonist.

NIH 10637   trans-3-Hydroxy-4-anilino-N-phenethylpiperidine
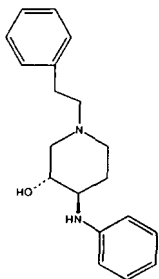
DISPLACEMENT OF SPECIFIC
^{3}H-ETORPHINE BINDING

EC50 of >10,000 nM (8.2% inhibition at 6 μM) in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.17 x 10^{-8}	50.5%
After naltrexone	3.10 x 10^{-8}	37.8%
After ICI-174,864	6.64 x 10^{-8}	46.4%
After nor-binaltorphimine	8.93 x 10^{-9}	49.4%

NIH 10637 was a very weak antagonist at μ and κ opioid receptors. It caused shifts to the right in the concentration-effect curve for sufentanil (a μ agonist) and caused shifts to the right and downward in the U50,488 (a κ agonist) concentration-effect curve. NIH 10637 did not antagonize the actions of DSLET (a δ agonist). Due to an insufficient supply of drug, pA_2 values were not determined.

SUMMARY

NIH 10637 had very little opioid activity in either preparation. There was a suggestion of antagonist activity in the vas deferens preparation at high concentrations.
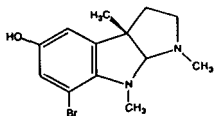
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 29.4 nM in presence of 150 mM NaCl.

MOUSE VAS DEFEEENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.47×10^{-8}	54.8%
After naltrexone	3.81×10^{-8}	22.8%
After ICI 174864	1.86×10^{-8}	47.0%
After nor-binaltorphimine	3.58×10^{-8}	54.9%

NIH 10642 was a weak antagonist at μ, κ, and δ opioid receptors. It caused shifts to the right in the concentration-effect curves for sufentanil (a μ agonist) and DSLET (a δ agonist), and caused shifts to the right and downward in the U50,488 (a κ agonist) concentration-effect curve. pA$_2$ values were as follows:

Agonist	pA$_2$ values	Slope
Sufentanil	6.39 ± 0.28	0.8
U50,488	5.78 ± 0.65	1.7

NIH 10642 was a very weak antagonist of DSLET and caused a 14-fold shift to the right in the concentration-effect curve at a concentration of 10^{-5} M.

SUMMARY

NIH 10642 was a potent opioid in both preparations. Its actions in the vas deferens were complex; its agonist actions were μ-receptor mediated, but it had antagonist actions at the κ and δ receptors in this preparation as well.

<u>NIH 10647</u>   1-(2-Phenylethyl)4-(N-(2-pyrazyl)-2-furoylamido)-
piperidine hydrochloride
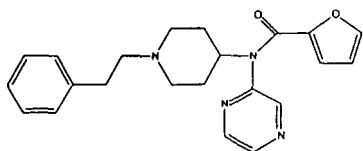
DISPLACEMENT OF SPECIFIC
^{3}H-ETORPHINE BINDING

EC50 91.0 nM in the
presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10647 was studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch
at any concentration and was evaluated as an opioid antagonist.
pA_2 values for NIH 10647 were as follows:

Agonist	pA_2 values	Slope
Sufentanil	6.84 ± 0.42	1.24
DSLET	6.45 ± 0.35	1.05
U-50,488	5.80 ± 0.41	1.21

DRUG DISCRIMINATION IN RHESUS MONKEYS

In two monkeys discriminating between 0.0032 mg/kg of the
agonist ethylketocyclazocine and saline, NIH 10647 produced only
vehicle-appropriate responding up to a dose, 3.2 mg/kg, that
eliminated lever pressing (triangles, Figure 1). NIH 10647
substituted completely for alfentanil in one monkey and partially
for alfentanil in a second monkey discriminating between saline
and 0.0056 mg/kg of alfentanil (squares, Figure 1). In morphine-
treated monkeys discriminating between naltrexone and saline, NIH
10647 substituted completely for naltrexone in all three subjects
at a dose of 1.0 mg/kg (circles, Figure 1).

NIH 10647   1-(2-Phenylethyl)4-(N-(2-pyrazyl)-2-furoylamido)-
piperidine hydrochloride

... (continued)
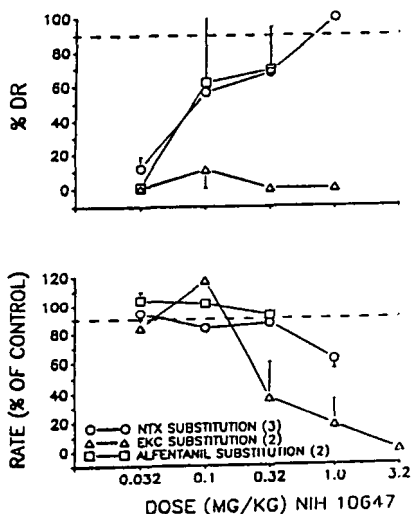
ANALGESIC EFFECTS IN RHESUS MONKEYS

The latency for monkeys to remove their tails from a thermos
containing 50° or 55° C water was determined every 30 minutes (1
cycle) with increasing doses of NIH 10647 being administered over
consecutive cycles. Under control conditions monkeys removed
their tails within two seconds upon tail immersion in 50° or 55°
C water. NIH 10647 increased in a dose-related manner the
latency for monkeys to remove their tails from warm water (upper
panel, Figure 2) and produced a maximum effect (20 second
latency) at a cumulative dose of 10.0 mg/kg. In Figure 2 the
tail withdrawal latencies are expressed a percentage of the
maximum possible effect and plotted as a function of dose. This
effect of NIH 10647 on tail withdrawal latency was not altered by
pretreatment with either 0.1 mg/kg of naltrexone or 0.1 mg/kg of
quadazocine (data not shown); these doses of antagonists are
sufficient to antagonize the effects of opioid μ agonists under
these conditions. In a separate study the time course of
analgesic effects was studied in two subjects that received a
single s.c. injection of the maximally-effective dose of NIH
10647, 10.0 mg/kg (Figure 2, lower panel). This dose of NIH
10647 produced a maximum effect for 50° C within 15 minutes and
for 55° C within 30 minutes. The analgesic effects of 10.0 mg/kg
of NIH 10647 were markedly diminished 60 minutes after injection
(see 55° C) and were no longer evident 105 minutes after drug
administration.

714

NIH 10647   1-(2-Phenylethyl)4-(N-(2-pyrazyl)-2-furoylamido)-
piperidine hydrochloride

... (continued)
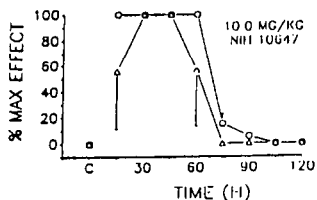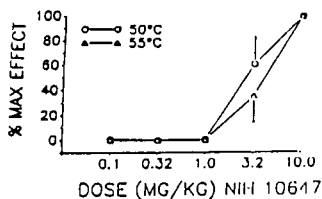
RESPIRATORY EFFECTS IN RHESUS MONKEYS

NIH 10647 was studied for its effects on ventilatory frequency
(f) and volume (VT) in two monkeys while breathing air and while
breathing 5% CO_2 in air. When administered alone, doses of NIH
10647 larger than 1.0 mg/kg had moderate effects on ventilatory
function, decreasing both f and VT in less than 70% of control
for monkeys breathing air and for monkeys breathing 5% CO_2. When
administered as a pretreatment to alfentanil, a single injection
of 1.0 or 10.0 mg/kg of NIH 10647 decreased f and VT to 60-70% of
control for monkeys breathing air and for monkeys breathing 5%
CO_2; subsequent administration of alfentanil did not produce
further decrease in respiratory function up to doses 18-32 times
larger than doses that produce apnea under control conditions.

In summary, as a discriminative stimulus NIH 10647 appeared to
have μ opioid effects: substitution for a μ but not a κ agonist
and substitution for naltrexone. A similar profile of discrimi-
native stimulus effects occurs with buprenorphine. Second,
although it is not clear whether the effects of NIH 10647 in
decreasing f and VT are opioid receptor mediated, doses of NIH
10647 that have respiratory depressant effects clearly attenuated
the effects of alfentanil. A similar profile of effects on
respiratory function occurs with buprenorphine and with nal-
buphine. Together, these results suggest NIH 10647 is a low
efficacy μ agonist. Finally, NIH 10647 has analgesic effects at
large doses, however, these effects do not appear to be mediated
by μ opioid receptors.

715

1-(2-Phenylethyl)4-(N-(2-pyrazyl)-2-furoylamido)-piperidine hydrochloride

   ... (continued)

SELF-ADMINISTRATION IN RHESUS MONKEYS

Doses of NIH 10647 were substituted in single, 130 min test sessions; each test session was separated by at least three sessions in which either codeine or saline was delivered contingently on lever press responses. Doses of 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg/inj NIH 10647 were evaluated; each dose was evaluated twice in each monkey with the exception of 0.1 midk/inj, which was tested only once in monkey 615P, and 0.001 mg/kg/inj which was not tested in monkey 615P.

NIH 10647 maintained rates of responding as high or nearly as high as those maintained by codeine in two of the three monkeys. In the third monkey, rates intermediate between those maintained by saline and those maintained by 0.32 mg/kg/inj codeine were maintained by 0.01 mg/kg/inj NIH 10647. This dose maintained the highest average rate in the three monkeys.

The figure below is a graphic representation of the data described. The data from individual monkeys are indicated by each animal's identification number and the accompanying open symbols (an average of two observations at each point, except where noted above). The closed circles are averages of these individual data. The closed squares at the COD and SAL points are historical averages of data obtained in a group of 20 monkeys under conditions of 0.32 mg/kg/inj codeine and of saline self-administration. The topmost dashed lines are ± 3 standard errors of the mean of the codeine grand average; the bottommost dashed line is + 3 standard errors of the mean of the saline grand average.
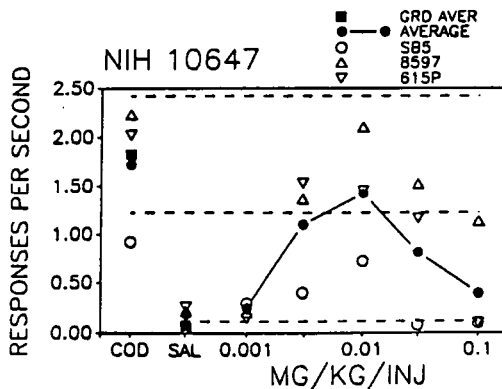
1-(2-Phenylethyl)4-(N-(2-pyrazyl)-2-furoylamido)-piperidine hydrochloride

... (continued)

SUMMARY

NIH 10647 was less potent than naltrexone in both preparations. In the vas deferens, its antagonist actions were found with each of the prototypic agonists. Its in vivo actions resemble that of a μ agonist with low efficacy; this generalization is being evaluated further at present. However, its actions cannot be accounted for soley with this hypothesis. Its efficacy as an analgesic is unusually large and apparently non-opioid in the monkey. This is a very unusual and interesting compound.

NIH 10649   (-)-2-Benzyl-5,9 **x**-dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide ((-)-N-Benzylnormetazocine hydrobromide)
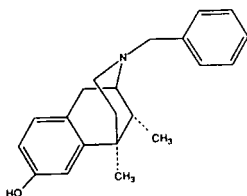
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 485 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10649 acted as an unusual opioid antagonist in the vas deferens preparation. It caused parallel shifts to the right in the concentration-effect curves for sufentanil (a μ agonist), caused shifts to the right and downward in the concentration-effect curves for U50,488 (a **κ** agonist) and had very little effect upon responses to DSLET (a **δ** agonist). pA_2 values were as follows: against sufentanil, 6.30 ± 0.26 (λ = 0.75, n = 6) and against U50,488, 6.01 ± 0.11 (λ = 0.75, n = 6). NIH 10649 did not antagonize DSLET at a concentration of 10^{-6} M and caused only a 2.8-fold shift to the right in the DSLET concentration-effect curve at a concentration of 10^{-5} M. For the purpose of comparison, pA_2 values for naltrexone are as follows: against sufentanil, 8.76 ± 0.34; against DSLET, 7.41 ± 0.12; and against U50,488, 7.74 ± 0.12.

NIH 10649   (-)-2-Benzyl-5,9 α-dimethyl-2'-hydroxy-6,7-benzomor-phan hydrobromide ((-)-N-Benzylnormetazocine hydrobromide)

   .... (continued)

SUMMARY

NIH 10649 was not potent in either of the preparations. It had antagonist actions that were not simply competitive.

NIH 10650   (+)-2-Benzyl-5,9 α-dimethyl-2'-hydroxy-6,7-benzomor-phan hydrobromide ((+)-N-Benzylnormetazocine hydrobromide)
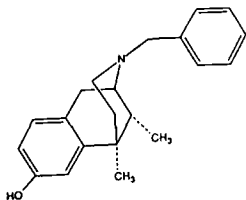
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 >10,000 nM (36.7% inhibition at 6 μM) in the presence of 150 ml4 NaCl.

MOUSE VAS DEFERENS PREPARATION

This drug did not inhibit the twitch at any concentration. This drug caused parallel shifts to the right in the concentration-effect curves for sufentanil (a μ agonist), but caused shifts to the right and downward in the concentration-effect curves for U50,488 (a κ agonist). It did not block responses to DSLET (a δ agonist) at concentrations up to 10^{-5} M. The pA$_2$ value against sufentanil was 5.62 ± 0.40 (λ = 1.14, n = 6). pA$_2$ values were not calculated for its antagonism of U50,488 because the antagonism was either irreversible or noncompetitive, but significant antagonism occurred at a concentration of 10^{-5} M.

SUMMARY

NIH 10650 failed to have significant affinity for the etorphine binding site, but was a weak antagonist in the vas deferens preparation.

NIH 10651   1-Benzyl-4-m-hydroxyphenyl-4-ketoethylpiperidine hydrochloride (N-Benzylnorketobemidone hydrochloride)
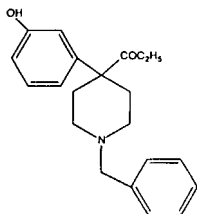
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 1017 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10651 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3×10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
DSLET	6.62 ± 0.66	1.82
U50,488	5.88 ± 0.98	2.40

NIH 10651 at a concentration of 3×10^{-6} M caused a 3.7-fold shift to the right in the sufentanil concentration-effect curve, and a concentration of 10^{-5} M caused a 15.5-fold shift to the right and a 64.7% decrease in the maximum response.

SUMMARY

NIH 10651 was not potent in either preparation; its actions in the vas deferens were complex.

NIH 10652   (-)-**α**·-Acetyl-N-normethadol hydrochloride
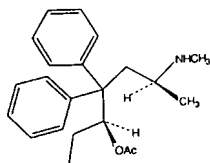
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 13.4 nM in the presence of 150 mM NaCl.

<u>NIH 10652</u>   (-)-**α**-Acetyl-N-normethadol hydrochloride

   ... (continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.17×10^{-8}	99.8%
After naltrexone	1.51×10^{-6}	100%

Neither ICI-174,864 (a **δ** antagonist) nor nor-binaltorphimine (a **κ** antagonist) significantly shifted the NIH 10652 concentration-effect curve.

SUMMARY

NIH 10652 had significant opioid activity in both preparations; it was a μ agonist in the vas deferens.

<u>NIH 10653</u>   (-)-**α**-N-Acetyl-N,N-dinormethadol
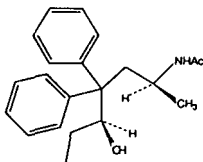
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10,000 nM (0% inhibition at 6 μM) in the presence of 150 nM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10653 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3×10^{-5} M. The first time that this drug was studied, concentrations between 10^{-9} and $3 \times 10^{-}$ M caused an inhibition of the twitch. In subsequent experiments, it caused only a slight inhibition of the twitch at 10^{-6} M and 3×10^{-6} M, and although it markedly inhibited the twitch at 10^{-5} M and 3×10^{-5} M, the inhibition was followed immediately by a marked increase in twitch magnitude. It was unclear whether these responses were affected by naltrexone, 10^{-7} M. EC50's and maximum responses could not be determined. In a concentration of 10^{-6} M, NIH 10653 did not alter responses to sufentanil, DSLET or U50,488H. Thus, based on the present experiments one cannot say whether this drug has activity as an opioid agonist, although it appears to be devoid of activity as an opioid antagonist.

720

NIH 10653   (-)-**α**·-N-Acetyl-N,N-dinormethadol

    ... (continued)

SUMMARY

NIH 10653 had insignificant affinity for the etorphine binding
site and did not appear to have opioid activity in the vas
deferens.

────────────────

NIH 10654   (-)-**α**-N-Acetyl-N-normethadol
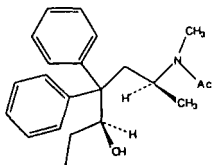
DISPLACEMENT OF SPECIFIC
[3]H-ETORPHINE BINDING

EC50 of >10,000 nM (3.8%
inhibition at 6 μM) in the
presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10654 was studied upon the isolated, electrically stimulated
mouse vas deferens preparation in concentrations which ranged
from 10^{-9} M to 3 x 10^{-5} M. This drug caused no inhibition of the
twitch at 10^{-6} M and 3 x 10^{-6} M and, although it markedly
inhibited the twitch at 10^{-5} M and 3 x 10^{-5} M, the inhibition was
followed immediately by a marked increase in twitch magnitude.
It was unclear whether these responses were affected by naltre-
xone, 10^{-7} M. EC50's and maximum responses could not be
determined. In a concentration of 10^{-6} M, NIH 10654 did not
alter responses to sufentanil, DSLET or U50,488H. Thus, based on
the present experiments one cannot say whether this drug has
activity as an opioid agonist, although it appears to be devoid
of activity as an opioid antagonist. The actions of NIH 10654
are very similar to those of NIH 10653.

SUMMARY

NIH 10654 had insignificant affinity for the etorphine binding
site and did not appear to have opioid activity in the vas
deferens.

NIH 10655   (-)-α-Acetyl-N,N-dinormethadol hydrochloride
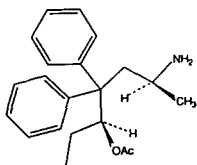
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 22.3 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.43×10^{-7}	97.7%
After naltrexone	could not be determined	9.6%

Neither ICI-174,864 (a **δ** antagonist) nor nor-binaltorphimine (a **κ** antagonist) significantly shifted the NIH 10636 concentration-effect curve.

SUMMARY

NIH 10655 appeared to be a μ agonist on the mouse vas deferens and had affinity for opioid receptors in rat brain membranes.

722

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AFFILIATION: The Drug Abuse Basic Research Program, Departments of Pharmacology, Psychology, and Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-0626.

SUBJECT INDEX

*In order to simplify the Index, the subject subheadings along with page numbers
can be found under both the chemical name and the NIH number.*

Phenmetrazine
　　clinical trial for treatment of cocaine dependence, 512-513
Phenobarbital
　　mouse analgesia in the hot-plate assay, 643, 684
　　self-administration in baboons, 329
1-(2-Phenylethyl)-4-(N-(2-pyrazyl)-2-furoylamido)piperidine hydrochloride (NIH 10647)
　　biological evaluation of physical-dependence potential and abuse liability, 628
　　dependence evaluation in rhesus monkeys, 673
　　displacement of specific ^{3}H-etorphine binding, 713
　　drug discrimination in rhesus monkeys, 713-714
　　inhibition of electrically stimulated mouse vas deferens preparations, 713
　　mouse analgesia, 673
　　monkey analgesia, 714-715
　　respiratory effects in rhesus monkeys, 715
　　self-administration in rhesus monkeys, 716
5-Phenylmorphans
　　probes for narcotic receptor mediated phenomena, 388-389
PLO17 ([MePhe^3, D-Pro^4]morphiceptin)
　　immunomodulatory activity of, 82-88
PMMA
　　See N-Methyl-1-(4-methoxyphenyl)-2-aminopropane
Polymerase chain reaction
　　testing in i.v. drug users in methadone treatment, 492-493
Positron emission topography
　　evaluation of cocaine, ethanol and marijuana effects on cerebral blood flow and glucose metabolism, 47-53
　　mapping the metabolic correlates of drug induced euphoria, 54-60
　　measurement of brain ethanol concentrations in humans, 68-74
　　effects of ethanol, diazepam and amphetamine on regional cerebral metabolic rates for glucose, 61-67
Prazosin
　　clinical trial for treatment of cocaine dependence, 512-513
Pravadoline
　　ligands at the cannabinoid binding site, 300-301
　　profile of pharmacological activity, 425-426
Pregnancy
　　opiate and cocaine binding to placenta from drug abusing mothers, 278-279
　　substance abuse patterns during, 280-281
Procaine
　　dopaminergic potency in the nucleus accumbens, 355-356
Proenkephalin
　　regulation of gene and transcription factors *in vivo,* 123-129
　　tissue-specific splicing of mRNA, 116-122
Prodynorphin
　　gene expression in neuronal and endocrine cell types, 109-115
(+)-19-Propylthevinol oxalate (NIH 10621)
　　biological evaluation of physical-dependence potential and abuse liability, 625
　　dependence evaluation in rhesus monkeys, 658-659
　　mouse analgesia, 658
Psychiatric disorders
　　history of problems, state of affairs, 203-212
Psychopathy Checklist
　　use with opiate addicts, 597-598

AUTHOR INDEX

757

759

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GPO Stock #017-024-01302-l $6
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