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Editorial

It is a pleasure to introduce this Special Issue of *Alcohol.*

As I am sure you will see from the number and quality of manuscripts published in this issue, Professor Gessa has done a tremendous job in organizing this material. I would also like to acknowledge the excellent work of the authors of each manuscript. I am very impressed with the quality of all the manuscripts that were submitted.

I believe this Special Issue of *Alcohol* presents a unique opportunity for the members of the scientific community who are involved in all facets of research on alcohol and drug abuse. The manuscripts included in this issue cover essentially all aspects of gamma-hydroxybutyric acid, from the basic chemistry of this compound to its clinical use. The manuscripts reflect the excellent job that was done in matching authors' expertise with the topic of the respective manuscripts by those authors. In many manuscripts, new research data are presented as well. Again, Professor Gessa has done an excellent job of organizing this issue to cover all aspects of gamma-hydroxybutyric acid, and I congratulate him for all his efforts.

Finally, I would like to emphasize that, per the editorial policy for *Alcohol*, all manuscripts in this issue were peer-reviewed by experts in the respective field, as well as by Professor Gessa. This peer-review is intended to ensure high-quality material in *Alcohol.* On the basis of the manuscripts that are included in this issue, this policy has been successful.

In closing, it has been a pleasure for me and the Managing Editor to work with Professor Gessa, and we look forward to working with him in the future.

Thomas R. Jerrells, PhD
Editor-in-Chief

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Preface to the Special Issue

Gian Luigi Gessa

"Bernard B. Brodie" Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124 Cagliari, Italy

The 8th Congress of the Italian Society on Biological Psychiatry, held in Naples September 29 through October 3, 1998, hosted a symposium titled "Gamma-Hydroxybutyric Acid (GHB): A Neurotransmitter, a Medicine, a Drug of Abuse". The present special issue forms the proceedings of the above symposium, with the most relevant findings obtained in the field of GHB research.

Both basic and clinical data presented at the meeting and included here support the symposium title:

1. Gamma-hydroxybutyric acid is an endogenous constituent of the mammalian brain, where it functions as a neurotransmitter or a neuromodulator.
2. Gamma-hydroxybutyric acid exerts alcohol-like effects in both animal models of alcoholism and human alcoholics.
3. Some of the psychotropic effects of GHB may confer abuse liability to the drug, the subject of which has generated a widespread debate on the drug's usefulness in the therapy for alcoholism and narcolepsy.

Over the past 20 years, Maitre's laboratory has made a great effort to investigate carefully the brain GHB neurotransmitter system. The existence of brain mechanisms for GHB synthesis, release, and reuptake, as well as of specific binding sites, has been demonstrated. Possible physiological functions of the GHB system include a regulatory role of some gamma-aminobutyric acid and dopamine mechanisms in the brain.

Results of pharmacological studies from the University of Cagliari have demonstrated the ability of GHB to suppress (1) the intensity of alcohol withdrawal signs in rats made physically dependent on alcohol and (2) voluntary alcohol intake in alcohol-preferring rats. Further animal study results, suggesting that GHB and alcohol share several pharmacological effects, led to the hypothesis that GHB may exert its antialcohol effects by mimicking the actions of alcohol in the central nervous system.

These animal data prompted clinical studies in alcoholic patients. The present issue includes articles from research groups headed by Addolorato, Gallimberti, and Moncini. They refer to some limited double-blind and more extended open study findings demonstrating the effectiveness of GHB in (1) rapidly suppressing signs and symptoms of alcohol withdrawal syndrome and (2) reducing craving for alcohol, consumption of alcoholic beverages, and relapses in alcoholics. For instance, Gallimberti and colleagues referred to results of their initial double-blind placebo-controlled trial showing that the acute administration of 50 mg/kg GHB, a nonhypnotic dose in alcoholics, induced an immediate and significant reduction of alcohol withdrawal score in alcoholic patients. Results of a subsequent double-blind survey from the same research group showed that daily administration of 50 mg/kg GHB, at the end of the 90-day treatment period and in comparison with findings in the placebo-treated group, led to significant reductions of alcohol craving score (∼60%) and number of daily drinks (∼50%), as well as to a threefold increase in the number of days of abstinence. Addolorato and coworkers also found that an improved treatment outcome, in terms of prolonged abstinence, could be reached by appropriate tritiation of the daily dose of GHB. Data obtained from the retrospective study by Carpanini and Beghè with more than 700 alcoholics under treatment with GHB indicate that the drug is generally safe, well tolerated, and devoid of major adverse side effects.

The rapid onset of GHB action as well as patients' reports on the subjective feelings perceived after GHB ingestion are consistent with the hypothesis that GHB may represent for alcoholism the analogue of methadone hydrochloride for heroin addiction. The replacement hypothesis for the mechanism of the antialcohol effects of GHB intrinsically supports the possibility that GHB, like methadone, can produce positive reinforcing properties in laboratory animals and be abused by human beings. Fattore and colleagues reviewed studies, mostly conducted in their laboratory, the results of which demonstrate that GHB (1) induces conditioned place preference (a reliable experimental paradigm for investigating the positive reinforcing properties of drugs of abuse) in rats and (2) is self-administered, both orally and intravenously, by mice and rats. In keeping with these data, Galloway and coworkers describe the illicit use of GHB and its abuse potential among nonalcoholic individuals, a growing and worrying phenomenon, particularly in Anglo-Saxon countries, which in 1991 induced the U.S. Food and Drug Administration to issue an advisory warning on the unsafe and illicit intake of GHB.

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However, abuse liability in alcoholic patients taking GHB for control of alcohol consumption and craving seems to be a less serious phenomenon, limited to a small portion of patients. Indeed, according to reports by Addolorato and Gallimberti, the percentage of alcoholic patients maintained with GHB who self-increased the dose of GHB recommended by the physician varied between 10 and 15. No case of GHB abuse has ever been reported among inpatients receiving GHB for the treatment of alcohol withdrawal syndrome.

The need for effective pharmacotherapies for alcoholism requires a careful evaluation of any possible novel treatment that research work may propose. The positive features of GHB treatment (i.e., reduction of craving for alcohol, alcohol consumption, frequency of relapse, and withdrawal symptoms) should militate against the possibility of banning GHB from pharmacopoeia. On the basis of postmarketing surveillance, the percentage of alcoholic patients abusing GHB may be further reduced by stricter medical surveillance and assignment of the medication to a responsible caregiver of the patient. We agree that more studies, possibly recruiting a larger number of patients, are needed to investigate further both aspects of GHB pharmacology: therapeutic effectiveness, and potential abuse liability. The present issue of the journal may offer a first, comprehensive insight into present knowledge on GHB.

Acknowledgments

We are most grateful to Robert D. Myers and Thomas R. Jerrells, former and present Editors-in-Chief of the journal, for having accepted inclusion of this material in *Alcohol*. We hope that the wide dissemination that this renowned journal will provide may stimulate further investigations on substitution therapies for alcoholism without an a priori demonization of this possible strategy.

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Gamma-hydroxybutyric acid Efficacy, potential abuse, and dependence in the treatment of alcohol addiction

Giovanni Addolorato[a,b,*], Fabio Caputo[b], Esmeralda Capristo[a], G. Francesco Stefanini[c], Giovanni Gasbarrini[a]

[a]*Institute of Internal Medicine, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168 Rome, Italy*
[b]*G. Fontana Center for the Study and Treatment of Alcohol Addiction, Università di Bologna, Bologna 40100, Italy*
[c]*Department of Internal Medicine, Ospedale di Faenza, Faenza 48100, Italy*

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Abstract

The main objective in alcoholism therapy is to achieve and maintain abstinence and to prevent relapse. Pharmacotherapy may be necessary in treating persons who are not helped by group or psychosocial support alone. Among the substances experimented with in the past few years, gamma-hydroxybutyric acid has been effective in preventing alcohol withdrawal syndrome and in inducing a reduction in craving and an increase in the abstinence rate in treated alcoholics, in view of the alcohol-mimicking effects of the drug on the central nervous system. However, a possible development of craving for the drug and the risk of abuse and physical dependence have been reported in subjects who used gamma-hydroxybutyric acid for different reasons, including alcoholism therapy. The present review updates the existing differences in drug abuse behavior, side effects, and poisoning in the use of gamma-hydroxybutyric acid in a treatment alcoholism program and in self nonclinical illicit use.

Keywords: Gamma-hydroxybutyric acid; Alcoholism; Efficacy; Abuse; Dependence; Side effects; Withdrawal; Clinical use; Illicit use

1. Introduction

Alcoholism is a metabolic disease presenting the clinical features of craving, loss of control, "obsessional thinking," tolerance, and physical dependence (Gianoulakis, 1996). The first step of the treatment is to achieve the remission of the acute symptoms and of the withdrawal syndrome. After the patient has undergone detoxification, the main objective is to maintain abstinence or, alternatively, to reduce alcohol consumption and to prevent relapse and drop-out. With this aim, a psychological approach (psychoeducational, family, group intervention) and counseling are essential components of therapy, although effective only for some of these patients.

Alcoholics Anonymous has demonstrated efficacy for only a small percentage (5%–15%) of alcohol-dependent patients in the United States (Erickson, 1996; Erickson & O'Neill, 1995;), and short- and long-term interventions in an outpatient setting seem to increase the percentage of abstinent patients (7%–39%) [for review, see Dall'Aglio et al. (1997) and Edwards & Rollnick (1997)]. However, the number of patients who do not succeed in maintaining abstinence with psychological support alone is still high. These findings suggest that pharmacotherapy may be necessary in treating alcoholics who are not helped by present 12-step or other psychosocial therapies (Erickson, 1996).

Craving and obsessional thinking are thus important aspects to be considered in these patients. In particular, craving is a strong desire for alcohol that also appears after a long period of abstinence (especially in consequence of the "first drink" or of "alcohol use-related situations"), and it refers to a state of mind that originates from the subcortical area of the brain (thus it is not easily defined or quantified). Obsessional thinking refers to a mental state in which alcoholics, especially during the initial stage of treatment, have a constant internal dialogue about whether to maintain abstinence or to drink (Carter et al., 1997). Serotonin has been hypothesized to play a major role in obsessional thinking about alcohol or in the craving for it (Carter et al., 1997; Kranzler & Anton, 1994), but other neurotransmitters such as dopamine, acetylcholine, and opioids may be implicated (Poldrugo & Addolorato, 1999).

The concomitant use of pharmacotherapy and psychological approaches could produce an additive effect in the

* Corresponding author. Tel.: +39-6-3015-4334; fax: +39-6-3550-2775.
E-mail address: ecapristo@pelagus.it (E. Capristo).

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control of the compulsive desire for alcohol and in maintaining alcohol abstinence; in particular, while psychosocial approaches may increase cerebrocortical inhibitory control mechanisms, pharmacological agents, acting on the aforementioned neurotransmitters, may decrease the subcortical brain drive mechanisms of obsessional thinking and craving (Carter et al., 1997).

Among the latest drugs tested against this background, gamma-hydroxybutyric acid (GHB) has proved to be particularly effective.

2. Brief considerations of the efficacy of gamma-hydroxybutyric acid in alcoholism therapy

Gamma-hydroxybutyric acid, a metabolite of gamma-aminobutyric acid (GABA), is a short-chain, four-carbon fatty acid with neurotransmitter and neuromodulatory functions (Vayer et al., 1987) and is present in different concentrations in various mammalian cerebral areas, in particular in the hypothalamus and basal ganglia (Snead & Moreley, 1981). The concentrations naturally present in the brain suggest a physiological role for this compound (Poldrugo & Addolorato, 1999). Although GHB has been termed a "GABA agonist" (Meldrum, 1981) and the effects of GHB have been hypothesized to be related to its GABAergic action (Anden et al., 1973), this compound has also been shown to interfere with the brain activity of dopamine, serotonin, acetylcholine, and opioids (Gessa et al., 1968, 2000; Snead & Bearden, 1980; Spano & Przegalinski, 1973; Roth et al., 1980).

Evidence of a relation between the action of ethanol and GHB has appeared since the 1960s [for a review, see (Poldrugo & Addolorato, 1999)]. In the past 10 years, the findings on the effectiveness of GHB both in inhibiting the voluntary ethanol consumption in Sardinian ethanol-preferring rats and in suppressing ethanol withdrawal syndrome in alcohol-dependent animals [for a review, see Gessa et al. (2000)] has led some researchers to investigate the possible use of GHB in the clinical treatment of alcohol addiction.

In human beings, the efficacy of nonhypnotic doses of GHB administered orally to suppress alcohol withdrawal syndrome was first reported by Gallimberti and coworkers (Gallimberti et al., 1989). In a subsequent controlled double-blind study, these investigators showed the efficacy of GHB at a dose of 50 mg/kg (divided into three daily doses for 3 months) in also increasing the number of abstinent days and reducing both the number of daily drinks consumed by alcoholics and their alcohol craving (Gallimberti et al., 1992). These effects were presumed to be related to GHB's interference with the activity of brain dopamine, serotonin, acetylcholine, opioids, and GABA (Gallimberti et al., 1992; Gessa et al., 1968; Snead & Bearden, 1980; Spano & Przegalinski, 1973).

These studies, the results of which were later replicated by others (Di Bello et al., 1995; Zolesi et al., 1994), although performed in the short term with absence of data by follow-up investigation, indicated that GHB could be a new potential drug that could be used in the multidisciplinary therapy of alcohol dependence. In this regard, in a study performed to evaluate the effect of the introduction of GHB in routine clinical practice in an alcoholism treatment program including psychosocial support, Cibin and colleagues (personal communication) showed that the drug was able to increase the permanence of the treatment significantly, which is considered a success in itself in alcoholism therapy.

A subsequent study evaluated the usefulness, tolerability, and safety of GHB activity in alcoholics in prolonged outpatient weaning, considering the abstinence from alcohol and craving extent as measures of outcome in a 6-month period of drug administration (Addolorato et al., 1995) and in a 1-year drug-free follow-up period (Addolorato et al., 1996). The drug proved to be effective in reducing craving and in improving the abstinence rate, as indirectly confirmed by the decrease in relapse in the 6 months and 1 year after GHB discontinuation; moreover, the drug was also manageable in regard to general safety (3), as confirmed by the reduction in indices of liver damage (due to alcohol intake reduction or cessation or both) during the treatment. Vertigo, increased sleepiness, and fatigue were reported as transitory side effects by about 30% of patients, which resolved after 2 to 3 weeks of GHB intake; no other side effects, including anabolic effect (Gerra et al., 1994; Takahara et al., 1977), were found. The lack of GHB-related anabolic effects during long-term administration of the drug in alcoholics has also been recently confirmed in a longitudinal study (Addolorato et al., 1999b).

In most of the studies, the rate of "nonresponders" to GHB therapy is about 30% to 40% of alcoholics treated; these patients often reported a temporary reduction in craving not sufficient to control their desire for alcohol (Addolorato et al., 1998a). However, it should be stressed that, in a majority of studies, the drug (50 mg/kg) was divided into three daily administrations, although there seems to be no "scientific background" for this division, because the half-life of GHB is relatively short (Ferrara et al., 1992) and this limitation could be one of the reasons for failure in regard to total abstinence from alcohol. Recently, patients who did not respond to the conventional fractioning of GHB were shown to benefit from greater fractioning of the same dose (50 mg/kg) of the drug. In particular, the administration of GHB six times a day caused abstinence from alcohol in a great percentage of nonresponders to the administration of GHB three times a day (Addolorato et al., 1998a, 1998c); the increased division of the administration of the drug seems to be capable of inducing a significant reduction in craving if the intervals between the doses are not greater than 4 h (Addolorato et al., 1998c). These findings could indeed be related to the short half-life of the drug or to the fact that increasing the number of daily GHB administrations raises the compliance of the patients.

Despite all these encouraging preclinical and clinical studies, GHB is not yet fully considered a potentially drug

useful in alcoholism therapy. A recent review on the treatment of the alcohol problem published in one of the most important medical journals provides extensive data on the medications either approved or not yet approved by the U.S. Food and Drug Administration, but there is no mention of GHB either in the management of alcohol withdrawal syndrome or in the prevention of relapse (O'Connor & Schottenfeld, 1998); see also (Addolorato et al., 1998b).

3. Craving for gamma-hydroxybutyric acid and risk of abuse and dependence in alcoholics

A possible development of craving for the drug during its use at a low dose as therapy for alcoholism was first reported by our group in the aforementioned multicentric study (Addolorato et al., 1996, 1997b) and resulted in a number of subjects who abused the drug by six to seven times the recommended dose. Gamma-hydroxybutyric acid abuse in treated alcoholics was subsequently confirmed by other investigations; in particular, Galloway and colleagues reported a series of eight cases of drug abuse; for seven of the patients, the initial GHB use/abuse was referred for several reasons and, for one patient, it was referred for alcoholism therapy (Galloway et al., 1997). It should, however, be pointed out that the nonclinical self-administration reported in nonalcoholics was done to obtain euphoria, hypnotic effects, an increase in libido, and anabolic effects. In contrast, in our experience, the drug abuse was partly due to a search for its psychotropic effects and partly related to the lack or temporary reduction in craving reported by the patients as being insufficient to control their longing for alcohol; the subjects under treatment therefore repeated the administration of the drug several times to control the craving. This could be related to the short half-life of GHB, and this observation is indirectly supported by the fact that no case of GHB abuse was reported with the greater fractioning of the drug (Addolorato et al., 1998c).

In regard to the side effects of GHB abuse, our patients described vertigo, a slowing down in reflexes, difficulty in driving, and somnolence; no anabolic effects (Addolorato et al., 1999b) or case of coma, which occurred when GHB was abused for other reasons (Louagie et al., 1997; Takahara et al., 1977; Thomas et al., 1997), was found.

When drug abuse was immediately reported by a family member and the administration was stopped or the correct dosages were restored, patients reported only mild anxiety and insomnia, which disappeared in about 1 week (Addolorato et al., 1997b) and no real physical GHB dependence was found.

However, we recently observed a case of GHB dependence, followed by withdrawal syndrome on discontinuation of the drug, in a treated alcoholic (Addolorato et al., 1999a). Briefly, a 36-year-old alcoholic woman with a 10-year history of alcohol abuse was included in a treatment program, consisting of psychological support counseling, a self-help group, and 50 mg/kg/day of GHB administered orally. Total abstinence from alcohol was obtained in a few days without signs of alcohol withdrawal syndrome and, after 5 months of complete abstinence, GHB was discontinued; no drug withdrawal syndrome or side effects due to drug suspension were reported. After 5 years of complete abstinence, the patient requested another cycle of GHB for a sudden craving for alcohol, and the GHB was administered again. This time the patient secretly increased the dose and drank as much as 3/4 of a bottle (about 18 g GHB) every day for 4 months, searching for its euphoric and anxiolitic effects. Interestingly, she described her craving for GHB to be identical with that experienced years earlier for alcohol but now with a clear preference for GHB. On discontinuation of the drug, the patient manifested a withdrawal syndrome, consisting of high anxiety levels, tremor, sweating, nausea without vomiting, and tachycardia. Total regression was obtained within 2 h with diazepam, 20 mg, administered orally. The patient took diazepam for another 6 days and, after its suspension, the symptoms did not reoccur.

4. Abuse of gamma-hydroxybutyric acid in nonalcoholics

In the spring of 1990, GHB appeared in the United States on the commercial market as a health food product to promote "natural sleep," weight loss, and muscular development touted as a replacement for L-tryptophan, which the U.S. Food and Drug Administration had taken off the market the preceding year. By November 1990, nine states had reported 57 cases of GHB poisoning and related illnesses such as seizures and comas (Chin et al., 1992; U.S. Food and Drug Administration, 1991), and the U.S. Food and Drug Administration issued a ban that removed GHB from the market. Despite the ban, GHB continued to be illegally produced and sold in widely varying degrees of purity (Meldrum, 1981), and a recent report from the Poison Control Centers in New York and Texas stated that, from August 1995 to September 1996, there were a further 69 cases of acute poisonings (Carter et al., 1997).

The nonclinical self-administration of GHB is linked to different reasons; the observation that GHB increases the release of growth hormone (Gerra et al., 1994; Takahara et al., 1977) contributed to its inappropriate use by bodybuilders, although the efficacy of GHB in promoting muscle development is not widely documented (Luby et al., 1992). Gamma-hydroxybutyric acid has also been used as a diet aid (Tunnicliff, 1997), to treat insomnia (Chin et al., 1992), and above all as a euphoriant. Gamma-hydroxybutyric acid's euphoria-inducing effect has made it popular as a recreational drug (Tunnicliff, 1997) in the United States and in the United Kingdom, where it is sold clandestinely mostly on the street (Louagie et al., 1997), at "rave parties" (Anonymous, 1994), and in nightclubs (Thomas et al., 1997), under the names "liquid ecstasy," "liquid X," "Georgia Home Boy," "Grievous Bodily Harm," "Soap," "Cherry Menth," "Easy Lay," "G-Riffick," and "Salty Water," among others (Marwick, 1997).

In the past few years, many case reports (Galloway et al., 1997; Li et al., 1998; Louagie et al., 1997; Thomas et al., 1997) have shown several acute side effects in nonalcoholic subjects who took this drug at high doses and without medi-

cal supervision. The adverse effects are described with doses ranging from 2.5 g to 30 g (Chin et al., 1992; Dyer, 1991; Dyer et al., 1990; Luby et al., 1992), taken mostly in a single administration alone or with other recreational drugs. The most common symptoms detected are dizziness, nausea, vomiting, weakness, tonic-clonic seizure activity, loss of peripheral vision, confusion, agitation, hallucination, delirium, amnesia, hypotonia, and anesthesia; one case of a Wernicke-Korsakoff syndrome, with cognitive and oculomotor disorders and ataxia, has also been recently reported in a patient who chronically abused GHB for a period of 1 month (Friedman et al., 1996). Doses higher than 10 to 20 g can decrease cardiac output and produce severe respiratory depression, seizurelike activity, and coma (Dyer, 1991; Li et al., 1998; Louagie et al., 1997; Thomas et al., 1997). Coma and respiratory depression may be potentiated by the concomitant use of alcohol (Louagie et al., 1997). These side effects appear within 15 min, and acute symptoms seem to remit within about 2 to 96 h. The severity and the duration of side effects depend on the dose or the association with other drugs or both. Benzodiazepines and opiate antagonists are not effective for GHB overdose. Although there is some evidence that physostigmine may reverse sedation induced by GHB alone (Li et al., 1998), at present, the treatment of overdose symptoms is aimed mostly at protecting the respiratory tract. However, although some subjects require mechanical ventilation and intensive care (U.S. Food and Drug Administration, 1991; Li et al., 1998), in others the GHB overdose surprisingly resolves without treatment, as recently shown in a 23-year-old woman who, having been taken to a hospital emergency department in a coma induced by a combination of GHB, alcohol, and marijuana, suddenly woke up after about 45 min and was able to walk out of the hospital after another 15 min (Louagie et al., 1997).

Finally, in addition to GHB acute abuse and related acute toxicity, a case of possible physical dependence due to chronic recreational use at a dose of 25 g/day of the drug for 2 years has been reported (Galloway et al., 1994). The chronic intake of GHB was related to feelings of relaxation, increased libido, and striking euphoria; on drug discontinuation, the subject showed insomnia, tremor, and anxiety for 12 days that resolved without sequelae.

The increasing number of reports regarding the abuse of GHB and the possible risk of dependence have led the U.S. Drug Enforcement Agency to consider that it be classified as a schedule I drug: “drugs that do not have currently accepted medical use in the United States, have a high abuse potential, and are not proven to be safe under medical supervision.” At present, although GHB manifacture and sale is prohibited under the Food, Drug, and Cosmetic Act, its possession is not illegal under federal law (Carter et al., 1997).

5. Conclusions and comments

The studies available at present indicate that GHB is a possible substance to be used in alcoholism therapy in view of the alcohol-mimicking effects of the drug on the central nervous system (Agabio et al., 1998; Colombo et al., 1995a, 1995b, 1998), with a rationale similar to that for using methadone in heroin addiction (Colombo et al., 1995a). Obviously, cases of craving for GHB with consequent abuse of the drug and possible dependence may occur during treatment (Addolorato et al., 1996, 1997b, 1999a). On one hand, these observations in alcoholics could support the aforementioned similarity between GHB and alcohol and outline its potential efficacy in alcoholism management, but, on the other habd, they suggest the necessity that it be used under strict medical surveillance in a multidisciplinary treatment including a supportive psycosocial program and the cooperation of a family member to obtain prompt reports of abuse in an initial phase (Addolorato et al., 1997b, 1999a; Poldrugo & Addolorato, 1999). In fact, it should be pointed out that the immediate suspension of the drug in these patients does not give rise to serious symptoms in most cases (Addolorato et al., 1997b) and, in the event of GHB dependence, the administration of a low dose of benzodiazepines would seem to be sufficient to achieve total regression of the withdrawal syndrome in a short time, at least if recognized early (Addolorato et al., 1999a). Moreover, because GHB abuse could be partly related to the short half-life of the drug, it is mandatory not to increase the dose (e.g., in nonresponder patients), taking into account the likelihood that this current practice among some practitioners in alcoholism therapy may induce physical dependence, whereas a greater fractioning of the same dose of the drug may reduce the craving and increase the efficacy without increasing the cost and thus reduce the risk of drug abuse (Addolorato et al., 1998a, 1998c). Increasing the dose would in any case seem to be of no use, because both the oral absorption and the elimination of GHB are fast processes and no accumulation occurs in the plasma (Ferrara et al., 1992). In the near future, a slow-release form of GHB is expected, with prolonged action that could increase the percentage of therapeutic success (Addolorato et al., 1998c).

Regarding GHB abuse in other conditions, this risk seems to be higher in some countries in which the use of GHB is increasing, not for alcoholism therapy but for its euphoric and anabolic effects, such as in the United Kingdom where the drug is not controlled under the Misuse of Drugs Act and is thus freely available in the club scene (Thomas et al., 1997), and in the United States (Tunnicliff, 1997). In this case, the danger of GHB acute toxicity, overdose, and physical dependence may be greater than what is seen in clinical administration, because the GHB utilized is synthesized in underground uncontrolled laboratories and the GHB concentration in the bottle sold clandestinely can greatly vary from a dose of 3 g to a toxic concentration of 20 g (Louagie et al., 1997; Shapiro, 1994). Moreover, whereas in alcoholics under treatment the abuse of the drug is only due partly to the increase in the prescribed dose and partly to the tendency to self-administer the drug several time a day (Addolorato et al., 1998c), the abuse of GHB as a

recreational substance mainly occurs acutely in a single self-administration. These observations may account for the differences in severity both of the side effects related to GHB abuse and of the symptoms that appear on abrupt suspension of the administration seen in subjects who use GHB for alcoholism therapy (Addolorato et al., 1996, 1997a, 1997b, 1999b; Gallimberti et al., 1989, 1992) and in others who take the "street version" (Anonymous, 1994; Carter et al., 1997; Chin et al., 1992; Galloway et al., 1994; Li et al., 1998; Louagie et al., 1997; Marwick, 1997; Thomas et al., 1997).

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ALCOHOL

Safety and tolerability of gamma-hydroxybutyric acid in the treatment of alcohol-dependent patients

Franco Beghè, Maria Teresa Carpanini*

Clinical and Pharmacological Research Unit "Piertomaso Tessitore" and Pharmaco-surveillance Department , Laboratorio Farmaceutico C.T., Via Dante Alighieri 71, 18038 Sanremo, Italy

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Abstract

Gamma-hydroxybutyric acid (GHB) has been in clinical use in Italy since 1991 for treatment of alcohol dependence. Results of phase III and phase IV studies have shown that the drug is effective and well tolerated in the treatment of alcohol withdrawal syndrome and in reducing alcohol consumption and alcohol craving. Pharmacosurveillance indicates that abuse of gamma-hydroxybutyric acid is a limited phenomenon in clinical settings when the drug is dispensed under strict medical surveillance and entrusted to a referring familiar member of the patient.

Keywords: Abuse potential; Alcohol; Alcoholism; Gamma-hydroxybutyric acid

Gamma-hydroxybutyric acid (GHB), a metabolite of gamma-aminobutyric acid and a putative neurotransmitter or neuromodulator (Maitre, 1997), has been proposed as an effective drug in the treatment of alcohol withdrawal syndrome and in the control of alcohol consumption and craving (Gallimberti et al., 1989, 1992). More recently, an open study (Di Bello et al., 1995) and two comparative studies of clomethiazole (Nimmerrichter et al., 1997) and oxazepam (Ceccanti et al., 1995) have further demonstrated that GHB is effective for the suppression of withdrawal symptoms in alcoholics. Addolorato and colleagues (1996, 1998) have confirmed the efficacy of GHB in improving the rate of abstinence in the medium-term treatment of alcohol-dependent patients.

Preclinical data strongly support the hypothesis of a close similarity of the pharmacological profile of GHB and alcohol. Gamma-hydroxybutyric acid mimics alcohol in different central actions, and drug discrimination studies in rats have shown a symmetrical generalization between alcohol and GHB in which GHB substitutes for the discriminative stimulus effects of alcohol and vice versa (Gessa et al., 2000). Consequently, it has been suggested that GHB may represent a substitution therapy for alcohol dependence, similar to methadone for heroin addiction.

Therefore, it is not surprising that GHB has rewarding properties inducing self-administration and conditioned place preference in rats (Fattore et al., 2000) and is abused by human beings (Centers for Disease Control, 1991; Chin et al., 1992; Dyer, 1991; Food and Drug Administration, 1991; Friedman et al., 1996; Galloway et al., 1994, 1997; Krawczeniuk, 1993; Luby et al., 1992; Marwick, 1997; Stell & Ryan, 1996; Stephens & Baselt, 1994). Abuse of GHB, particularly when illicitly promoted, seems to be due to the production of a "high" or euphoria (Centers for Disease Control, 1991; Food and Drug Administration, 1991). Acute poisonings, with vomiting, drowsiness, hypotonia, vertigo, loss of consciousness, tremor, myoclonus, hypotension, bradycardia, and/or respiratory depression or arrest have been reported (Centers for Disease Control, 1991; Food and Drug Administration, 1991). Their severity and duration depend on the dose of GHB (2 g to more than 30 g) and/or the frequent concurrent use of other central nervous system depressants, such as alcohol and other psychoactive drugs. The sustained use of high doses of GHB may induce physical dependence and withdrawal syndrome; the latter has been observed in eight cases and was characterized by tremors, insomnia, and anxiety and was resolved without sequelae (Galloway et al., 1997).

The present report is aimed at surveying the safety and tolerability of GHB in the treatment of alcohol withdrawal syndrome and in medium-term treatment of alcoholism during phase III and phase IV studies (Addolorato et al., 1996, 1998; Avanzi et al., 1996; Ceccanti et al., 1995, 1996; Cibin & Zavan, 1995; Di Bello et al., 1995; Gallimberti et al., 1989, 1992; Manzato et al., 1995; Montesano et al., 1997; Mosti & Zurla, 1995; Nimmerrichter et al., 1997; Streppa-

* Corresponding author. Tel.: +39-184-5924337; fax: +39-184-504507.

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rola et al., 1995; Vendramin & Bertuola, 1995; Zolesi et al., 1995; data on file, Laboratorio Farmaceutico C.T., Sanremo, Italy). The data reported here are based on published literature and on data from a pharmacosurveillance file (data on file, Laboratorio Farmaceutico C.T. S.r.l. Sanremo, Italy).

Results of a survey of phase III and IV studies indicate that GHB has been used in 368 alcoholic inpatients who were successfully treated for alcohol withdrawal syndrome with a dosage of 50–150 mg/kg/day for 7.09 ± 3.53 days. Abuse was never shown and withdrawal signs were not observed after GHB discontinuation. In 16.03% of the patients a transient and mild vertigo, particularly after the first administration of the drug, has been observed. Diarrhea (5 patients), rhinitis (3 patients), nausea (2 patients), headache (2 patients), dry mouth (1 patient), and seizures (1 patient) have been also reported. In a single case (seizures) treatment was discontinued (Nimmerrichter et al., 1997).

Results of the same survey indicate that GHB has been administered as a maintenance treatment to 732 outpatients considered as alcohol-dependent with a dosage of 50–100 mg/kg/day in three or more oral doses, for 132.2 ± 57.9 days. The dosage was made according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R criteria, age range 18–73 years, male:female ratio = 2.5:1). These patients were also attending a supportive psycosocial program, and the administration of the drug was entrusted to a family member.

A percentage of these patients (varying according to the different reports from 2.6 to 10.1%) showed craving for the drug and increased their dosage (up to 6–7 times the recommended dose). When the correct dosages were restored, patients complained of mild anxiety and insomnia, which disappeared in about one week (Addolorato et al., 1996). Conversely, no drug withdrawal syndrome was reported by the patients who had shown adherence to designated GHB dosage when treatment was discontinued (Addolorato et al., 1996).

Transient and mild vertigo and/or drowsiness, particularly at the beginning of the treatment, have been observed in 21.7% of patients. Six patients (0.81 %) complained of headache (3 patients), myalgia (2 patients), and insomnia (1 patient). The adverse reactions spontaneously disappeared and never induced treatment withdrawal.

Recently, a greater fractioning of the same dose of GHB (into six daily doses vs. three conventional ones) has been shown to increase the abstinence rate and to reduce the incidence of abuse: no abuse was reported among the 119 patients who received the treatment (Addolorato et al., 1998).

A double-blind, multicentric study is ongoing to confirm GHB capacity in improving the rate of abstinence: 288 patients have been treated with three different doses of the drug (25 mg, 50 mg, and 100 mg/kg) or placebo for 6 weeks. A severe procedure of drug accountability has been adopted and abuse has never been observed. Results of this study will be available soon.

Laboratorio Farmaceutico C.T. (Sanremo, Italy), the pharmaceutical company marketing the drug gamma-hydroxybutyric acid (Alcover®), has sold more than 500,000 preparations of the drug since 1991, and nearly 20,000 patients have probably experienced it. In Italy, the drug can be used for the treatment of alcoholism under the supervision of a physician, and it has been assigned to Schedule H, implying its distribution, free of charge, by hospital pharmacy. Gamma-hydroxybutyric acid is also dispensed on payment by public pharmacies. A written prescription is required.

With regard to GHB poisoning, whereas in nonalcoholic subjects the drug acts synergistically with alcohol and other central depressant drugs to produce respiratory depression, the cross tolerance to the sedative effect of alcohol and GHB observed in rats (Gessa et al., 2000) suggests that poisoning in alcoholics should be a limited phenomenon. A single case of fatal intoxication due to contemporary intravenous use of heroin has been reported in Italy (Ferrara et al., 1995).

In conclusion, our data suggest that when GHB is used under strict medical surveillance in a multidisciplinary strategy, and its administration at home is entrusted to a referring family member, abuse is a limitable phenomenon, and the drug is safe. A greater fractioning of the daily dosage seems to further reduce the incidence of abuse (Addolorato et al., 1998).

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ALCOHOL

Design and structure-activity relationship analysis of ligands of gamma-hydroxybutyric acid receptors

Jean-Jacques Bourguignon*, Martine Schmitt, Bruno Didier

Laboratoire de Pharmacochimie de la Communication Cellulaire, ERS 655 du CNRS, Faculté de Pharmacie, 74 route du Rhin, B.P. 21, Illkirch, 67401, France

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Abstract

With the use of [^{3}H]gamma-hydroxybutyric acid, binding experiments allowed the screening of new compounds as ligands of gamma-hydroxybutyric acid receptors. Starting from the acid-alcohol gamma-hydroxybutyric acid structure, structure–activity relation analysis and lead optimization highlighted gamma-hydroxybutyric acid derivatives with significantly increased affinities, when compared with the affinity of gamma-hydroxybutyric acid. Further pharmacological studies with the use of gamma-hydroxybutyric acid derivatives allowed the characterization of the first competitive antagonist acting at gamma-hydroxybutyric acid receptors (NCS 382).

Keywords: Gamma-hydroxybutyric acid; Derivatives; Lactone; Alcohol; Drug design; Crotonic acids; Ligands; Receptors; Antagonist

1. Introduction

Gamma-hydroxybutyric acid (GHB) is a short-chain fatty acid identified in the central nervous system and kidney as a reductive catabolite of gamma-aminobutyric acid (GABA) (Laborit, 1964). This compound is reported to possess original pharmacological properties (Carter & Snead, 1977; Vayer et al., 1987)—in particular, hypnotic effects (Roth & Suhr, 1970) as a result of paradoxical sleep induction (Godbout & Pivik, 1982). Additionally, a beneficial effect in ethanol-suppressing withdrawal syndrome in rats (Colombo et al., 1995b; Gessa et al., 2000) and men (Addolorato et al., 2000; Gallimberti et al., 2000; Moncini et al., in press) was described for GHB.

For a better characterization of the physiological role of GHB (Maitre, 1997), particularly as a neuromodulator in the central nervous system, a large program of synthesis of GHB derivatives was undertaken. The objective was twofold:

1. To afford pharmacological tools for efficient in vitro characterization of the different GHB-specific targets (enzymes, receptors, carriers).
2. To prepare these compounds with increased central bioavailability.

It is known that, after intraventricular administration of [^{14}C]GHB, more than 50% of GHB is eliminated in about 5 min (Doberty et al., 1975b). In addition, GHB, like other carboxylic acid derivatives (e.g., dipropylacetate, aspirin), probably binds to serum albumins by competing with endogenous fatty acids for specific binding sites on these circulating proteins. Thus relatively high doses of GHB may be needed to allow nonbound hydrophilic GHB to penetrate the blood–brain barrier.

Different strategies can be developed to mask the carboxylic acid moiety of GHB temporarily and other GHB derivatives, particularly by means of GHB prodrugs such as amides (structure **6** in Fig. 1).

Different groups have postulated that the nonacidic butane diol (structure **1** in Fig. 1) (Colombo et al., 1990; Maxwell & Roth, 1971; Roth & Giarman, 1968; Sprince et al., 1966) or gamma-butyrolactone (GBL, structure **2** in Fig. 1) (Arena et al., 1980; Roth & Giarman, 1966b; Snead, 1982) constitute efficient GHB bioprecursors with potentially increased in vivo efficacies. They have been identified as endogenous substances in rat brain at concentrations of about 1/10 of those of GHB (Doberty et al., 1975a). In both cases, specific enzymes—lactonase (Roth et al., 1966a; Fishbein & Bessmann, 1966) and dehydrogenase (Snead et al., 1989), respectively—may be catalysts in their biotransformation into GHB.

Gamma-butyrolactone and GHB produced effects in animals suggested to be similar to petit mal absence seizures in human beings—see references cited in Flunk et al. (1982a, 1982b) and Depaulis et al. (1988). The effects of both GBL and GHB are prevented by trimethadione and ethosuximide,

* Corresponding author. Tel.: +33-0388-676808; fax: +33-0388-674794.

E-mail address: jjb@pharma.u-strasbg.fr (J.-J. Bourguignon).

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which are effective in the treatment of petit mal absence seizures.

With GHB structural equivalents (GHB bioprecursors or prodrugs) taken into consideration, known pharmacological agents having some structural similarities with GHB or GBL are listed in Fig. 1.

When the differently substituted GBLs (structure **3**) were considered, the beta-substituted compounds proved to be potent convulsants, producing seizures very distinct from those produced by unsubstituted GBL (structure **2**) (Flunk et al., 1982a). The alpha-substituted compounds possessed substantial anticonvulsant activity very similar to that of the antiabsence antiepileptic drugs (Flunk et al., 1982b). However, the authors concluded that the mechanism of action of these substituted lactones in the central nervous system does not involve the GHB system, even if they may produce, after lactone ring opening, the corresponding substituted GHB derivatives (Levine et al., 1985). In a similar manner, a series of gamma-benzoylbutyrolactones (structure **3**; $R_1 = R_2 = H$, $R_3 = COPh$) were reported to present dopamine-modulating properties, but different from those observed with GBL (Cignarella et al., 1995). Interestingly, in another work dealing with the effects of GHB-related compounds on spontaneous generalized nonconvulsive seizures (epileptic rats presenting spontaneous spike-and-wave discharges), GHB and GBL increased the duration of the spike-and-wave discharges in a dose-dependent manner, whereas gamma-crotonolactone (GCL, or unsaturated GBL, structure **4**) suppressed the spike-and-wave discharges in epileptic rats (Depaulis et al., 1988). In a similar manner, a series of GHB amides (structure **5**) were reported to have presented anticonvulsant activity ranging from 100 to 300 mg/kg in the maximal electroshock seizure screening. However, the mechanism of action of these compounds is not clearly elucidated (Malawski et al., 1997).

Specific GHB binding studies allow the charactetization of two populations of GHB binding sites. The high-affinity binding site shows a K_D value of 95 nM with a low capacity (B_{max} = 0.56 pmol/mg of protein), whereas the lower-affinity binding site (K_D = 16 μM) presents a higher capacity (B_{max} = 46 pmol/mg of protein) (Benavides et al., 1982).

Further pharmacological studies of GHBergic function are needed to find potent ligands of GHB receptors with either agonistic or antagonistic properties. Binding experiments on rat brain homogenate with the use of [^{3}H]GHB constituted an efficient screening method for a first selection of new compounds with significant affinity for GHB receptors (Benavides et al., 1982). However, because of the existence of a low-affinity GHB binding sites with high B_{max}, a relatively high IC_{50} value was found for GHB (IC_{50} = 6.6 μM) when [^{3}H]GHB was used. The compounds listed in

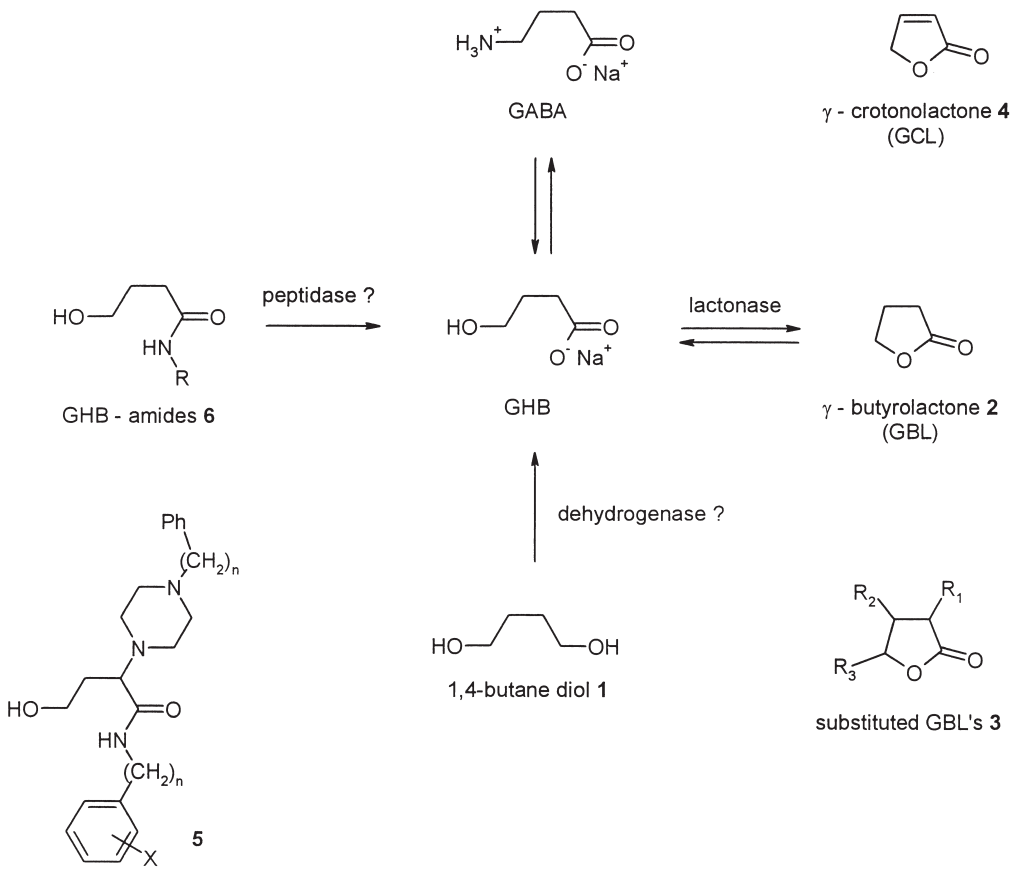

Fig. 1. Gamma-hydroxybutyric acid bioprecursors, prodrugs, and structurally related compounds.

Table 1
Evaluation of the affinity of a series of gamma-hydroxybutyric acid derivatives for gamma-hydroxybutyric acid receptors

		% Inhibition		
Nº	Name	at 10 μM	at 1 μM	IC_{50}, μM
GHB derivatives				
	GHB	55	35	6.6
9a	α - Me	24		
9b	α - Ph	ns		
9c	α - Me	59		
9d	β - Ph	ns		
9e	γ - Me	63	12	
16	γ - Ph	56	13	6.8
17	γ - CH_2Ph			2.3
17a	γ - CH_2Ph (R)			1.8
17b	γ - CH_2Ph (S)			25.0
17c	γ - CH_2-(p - Cl - Ph)			1.5
17d	γ - CH_2-(p - OM - Ph) (NCS 435)			0.1
18	γ - $(CH_2)_2$Ph			14.0
8a		ns		
8b		53	22	
10		ns		
11		ns		
12		ns		
14		ns		
15cis			9	
15 trans			26	
Conformationally constrained GHB derivatives				
13	T-HCA	64	44	2.9
19	γ - Ph T-HCA	70	39	3.3
19a	γ - p - CF_3 - Ph T-HCA	80	40	1.2
19b	γ - p - Cl -Ph T-HCA	58	31	3.9
19c	γ - o -Cl -Ph T-HCA	86	60	2.4
20a	$n = 1$ cis	14	2	
20b	$n = 2$ trans	36	11	
20c	$n = 3$ cis + trans (NCS 399)	83		1.1
20d	$n = 3$ cis (NCS 400)	46		
20e	$n = 3$, trans (NCS 401)	91		
21a	$n = 1$	ns		
21b	$n = 2$	58	31	
21c	$n = 3$ (NCS 382)	84	38	8.0

Gamma-aminobutyric acid, 1,4 butane diol, gamma-butyrolactone: no significant binding at 10 μM.

Table 1 were first tested at a concentration of 10 μM for their ability to inhibit [^{3}H]GHB binding (25 nM) on rat brain membrane preparation (Benavides et al., 1982). If at 10 μM the compound inhibited [^{3}H]GHB binding by more than 50%, compounds were studied at a concentration of 1 μM, and then dose–inhibition curves were generated with eight drug concentrations in triplicate incubations. The IC_{50} values were determined for the most active compounds.

With the structure of GHB as a starting point, different approaches proved to be efficient in drug design and were considered for this purpose (see Figs. 2 and 5):

1. Homology. The distance between the carboxylate anion of GHB and the OH (H-bond donor) is crucial. Thus the inferior (structure **8a** in Fig. 3; $n = 0$) and superior (structure **8b**; $n = 2$) homologues were prepared and tested.
2. Topology in the vicinity of the receptor. To explore the possible substitutions in the alpha, beta, or gamma position of GHB; differently substituted GHB derivatives were prepared (structures **16–18**).
3. Isosteric replacements. Other chemical functions can mimic both the carboxylate and the OH alcohol. A first set of isosteres was tested as GHB ligands (see structures **10–12** in Fig. 3).
4. Conformational restriction. Gamma-hydroxybutyric acid, like GABA, has a freely rotating C-4 chain in its structure, which confers great flexibility on the molecule. Thus GHB can adopt various conformations (folded, semi-extended, fully extended) (Allan & Johnson, 1983). Knowledge of the active conformation of GHB (conformation of GHB bound to the receptor) may be helpful in the design of new potent GHB receptor ligands. Different modes of rigidification have been undertaken. A similar study was developed earlier to search for GABA receptor ligands (Allan & Johnson, 1983; Breckenridge et al., 1981; Johnston et al., 1979), as illustrated in Fig. 3. These

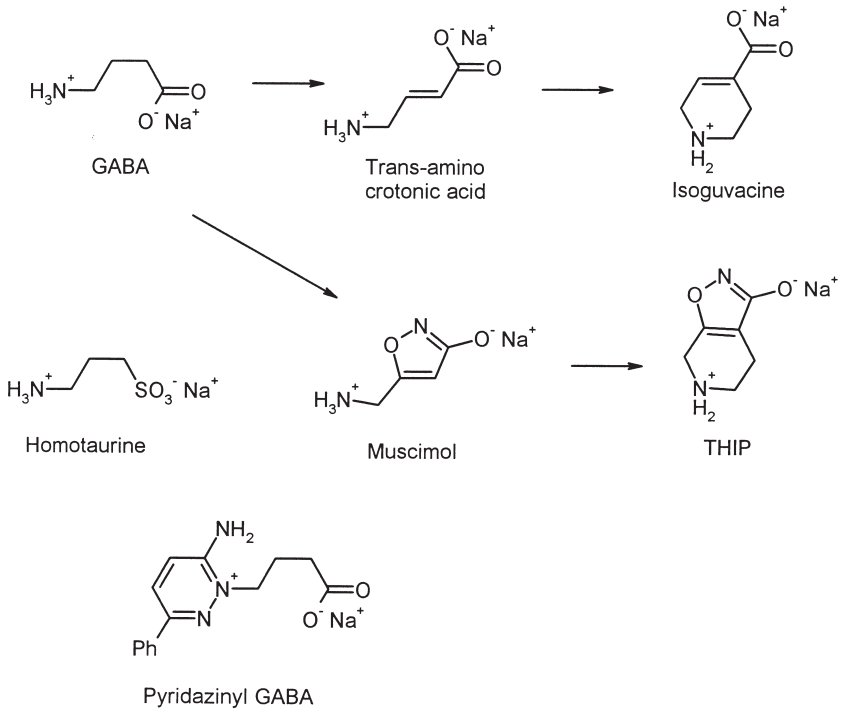

Fig. 2. Design of ligands of gamma-aminobutyric acid (GABA) receptor.

works highlighted particularly interesting GABA receptor ligands, including agonists (homotaurine, isoguvacine, muscimol, 4,5,6,7-tetrahydroisoxazolo[4,5-c] pyridin-3-ol), antagonists (pyridazinyl-GABA) (Wermuth & Biziere, 1986), and corresponding isosteres) (Melikian et al., 1992).

2. Homology

As observed for GABA (Allan & Johnson, 1983; Galli et al., 1980), shortening the GHB chain (structure **8a** in Fig. 3; $n = 1$) was detrimental for binding, whereas increasing its length (structure **8b**; $n = 2$) afforded a compound with a

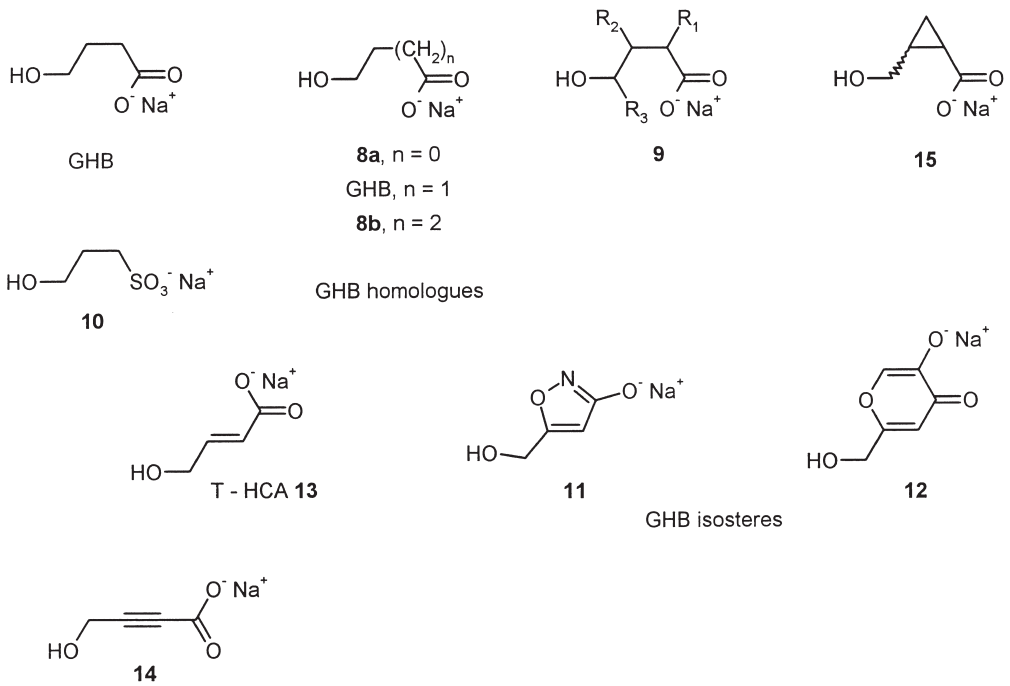

Fig. 3. A first exploration of structural modifications in the structure of gamma-hydroxybutyric acid.

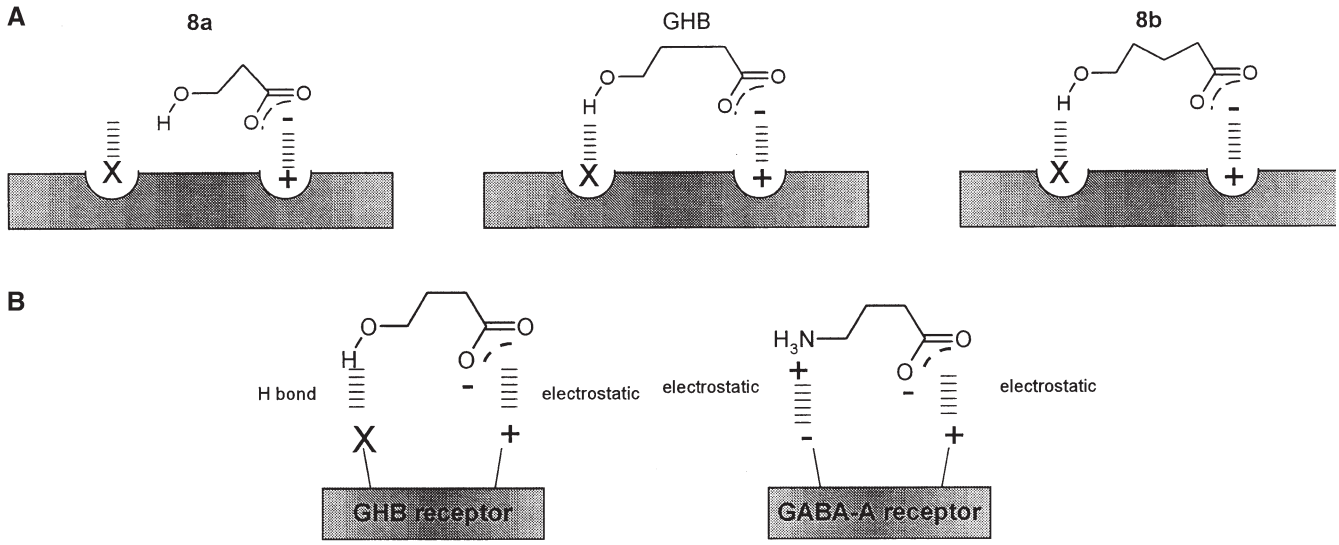

Fig. 4. Schematic representations of ligand–receptor interactions for both gamma-hydroxybutyric acid and gamma-aminobutyric acid.

similar affinity (compare structures GHB and **8b** in Fig. 4A). Figure 4a illustrates the following observations:

1. When *n* is too small, the molecule is too short and only one of its polar ends can establish an interaction with the complementary site of the receptor. The molecule is poorly active (structure **8a**).
2. When *n* is large enough, a good interaction can be established with the complementary sites of the receptor (optimal interaction with GHB).
3. When *n* is too large, if the molecule is flexible and if steric hindrance is low (examples are GABA and GHB), a satisfactory fit with the receptor remains possible (structure **8b**).

3. Isosteric replacements

Historically, GHB was considered by Laborit to be a GABA isostere (Laborit, 1964). In accord with the isostery rules, the OH alcohol function of GHB might be efficiently replaced by the amino group of GABA. However, GABA does not bind to GHB receptors, and GHB cannot compete with [^{3}H]GABA for $GABA_A$ receptors (Benavides et al., 1982). It is interesting to note that some authors hypothesized that GHB may bind to $GABA_B$ receptors, but with a very low affinity (Bernasconi et al., 1992).

If the nature of specific interactions in both $GABA_A$ and GHB receptor binding is considered, these data are not surprising. At physiological pH, GABA exists as a zwitterionic structure and interacts with its receptor through two probable electrostatic interactions, whereas GHB presents a specific H bond instead of an electrostatic interaction (involvement of the alcohol group, Fig. 4B).

Among the other isosteric replacements known to be efficient in other systems (Allan & Johnson, 1983), we prepared and tested the sulfonic acid GHB analogue (structure **10** in Fig. 3), the muscimol hydroxyl derivative (structure **11**), and the kojic acid (structure **12**). The isoxazol ring presents acid properties, which can be compared with those of carboxylic acids (p*K*a = 4.78) (Krogsgaard-Larsen et al., 1975). Surprisingly, however, all these compounds were found to be inactive in binding experiments.

4. Conformational restriction

The easiest way to build conformational flexibility restriction in both the GABA structure and the GHB structure consists in the introduction of a double bond in the C4 chain. The insaturation forces the four C1–C4 carbon atoms of GHB in the same plane, and the E-configuration of the double bond can only mimic semi-extended or extended conformations of GHB.

Among the first set of GHB semi-rigid analogues, we prepared trans gamma-hydroxy crotonic acid (T-HCA, structure **13**), which proved to have a good affinity for GHB receptors. In addition, this synthetic compound was identified as a naturally occurring substance in kidney and brain (Vayer et al., 1985). In rat brain, the concentration of T-HCA is about 10 times as high as that of GHB. Thus T-HCA interferes with GHB transport and binding in brain membranes and may represent an intermediate of GHB catabolism in the brain.

In a comparison of K_D and B_{max} values of both GHB and T-HCA, the latter compound may bind to specific GHB receptor subclasses (Hechler et al., 1990), in relation to the typical extended or semi-extended conformations that it is allowed to adopt. The availability (Schmitt et al., 1988) and the use of [^{3}H]T-HCA in binding experiments may constitute an efficient tool for the characterization of a first GHB receptor subclass.

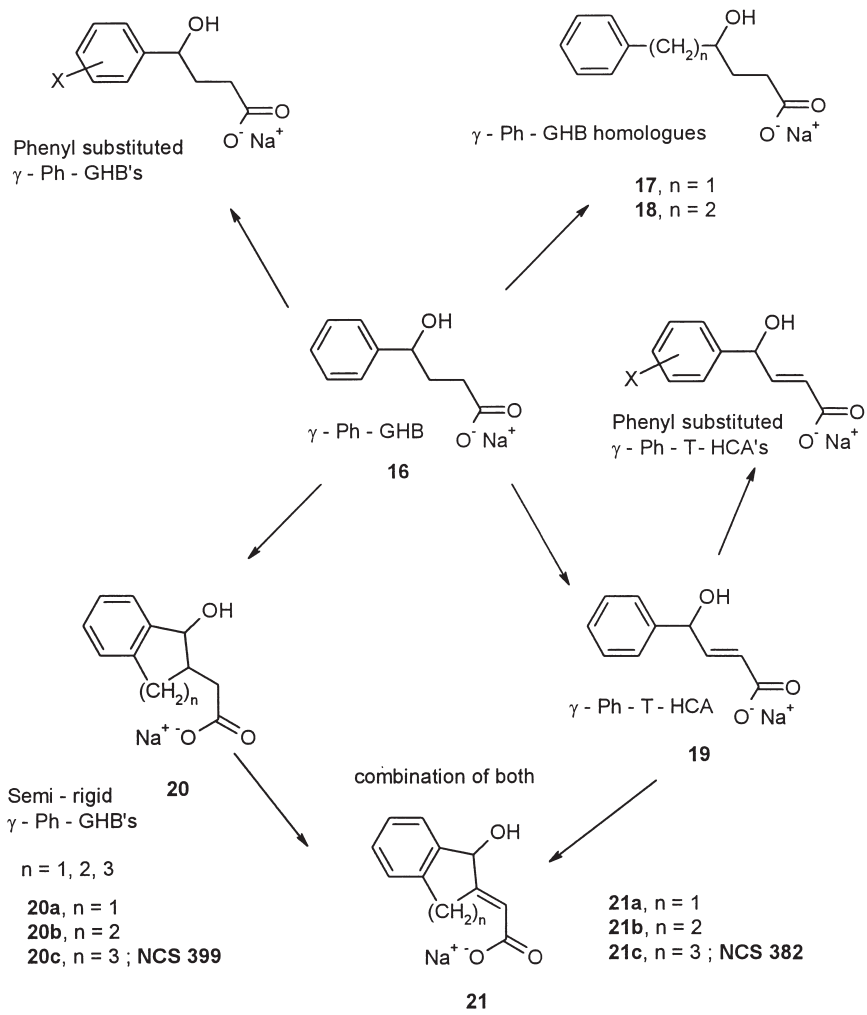

Fig. 5. Further structural modifications starting from gamma-phenyl-gamma-hydroxybutyric acid (structure **16**).

The replacement of the double bond by a triple bond (structure **14** in Fig. 3) significantly modified the geometry of GHB and led to an inactive compound.

Other modes of conformational restriction also were considered by bridging alpha and beta positions (cyclopropyl derivatives, structure **15** in Fig. 3 (Arena et al., 1980) and beta and gamma positions (see structures **20** and **21** in Fig. 5), but in all cases stereochemical considerations rendered the analysis more complex (existence of mixtures of diastereoisomers for compounds **15** and **20**).

5. Substituent effects

The introduction of novel substituents and functional groups at different positions is an efficient strategy in drug design for increasing the affinity of a given ligand toward its specific target. Thus systematic exploration at different positions of GHB was undertaken with the use of small (methyl) or large (phenyl) groups (see Table 1). No substitution was allowed in the alpha position, and only small substituents were tolerated in the beta position. However, the presence of a methyl or a phenyl group in the gamma position afforded compounds with still-significant affinity.

Thus we focused our attention on these gamma-substituted GHB derivatives. In particular, gamma-phenyl GHB (structure **16**) was selected as a lead compound for further structural modifications. With compound **16** as a starting point, aromatic substitutions and conformational restriction were achieved as reported in Fig. 5.

A first analysis of substituent effects on the aromatic ring of gamma-phenyl GHB is reported in Table 1. It is well known that specific substituents in the ortho, meta, or para position of a phenyl ring present in the structure of a given ligand may increase its potency by beneficial combination of electronic, geometric, and lipophilic effects.

In accord with a strategic scheme proposed by Topliss (Craig, 1980) as a practical guide for rapidly choosing the most beneficial aromatic substituent, we prepared *p*-chloro and *p*-methoxy derivatives. When compared with gamma-phenyl GHB, these compounds present a similar affinity, whereas ortho substitution was detrimental for binding.

In another part of the work, the gamma-phenyl GHB su-

perior homologues (structures **17** and **18** in Fig. 5; $n = 1$ and 2, respectively) were prepared and tested.

Table 1 clearly shows that the gamma-benzyl derivative (structure **17**) presented a slightly better affinity than gamma-phenyl GHB (structure **16**). However, a further increase in the distance between the phenyl ring and the alcohol led to a significant decrease in affinity (compare compounds **16**, **17,** and **18**) within this novel series of GHB derivatives (gamma-benzyl GHBs, structure **17**). Clear-cut beneficial aromatic substituent effects were observed. In particular, the para-methoxy derivative **17d** (NCS 435) was found to be about 20 times as potent as the unsubstituted **17**. This 1–2 order-of-magnitude increase in affinity may correspond to the establishment of a novel H-bond interaction involving the lone pairs of the methoxy group.

The chiral gamma-benzyl GHB (structure **17**) was initially tested as a mixture of enantiomers. More recently, both *R* (structure **17a**) and *S* (structure **17b**) enantiomers were prepared separately and tested for their capacity to inhibit [^{3}H]GHB binding. Data in Table 1 clearly show that the active stereoisomer is *R*, whereas the other enantiomer, *S*, is significantly (more than 10 times) less active. This result constitutes the first characterization of stereochemical behavior dealing with the GHB receptor binding processes. In addition, the displacement curve for NCS-435 shown in Fig. 6 supports the hypothesis that NCS-435 binds to a unique population of GHB receptors ($R = 0.998$).

To restrict the conformational flexibility of compound **16**, a homologous series of benzocycloalkanol acetic acids (structure **20** in Fig. 5) was prepared as semi-rigid GHB derivatives. Because they possess two asymmetric centers, the mixtures of cis and trans enantiomers were prepared separately and tested.

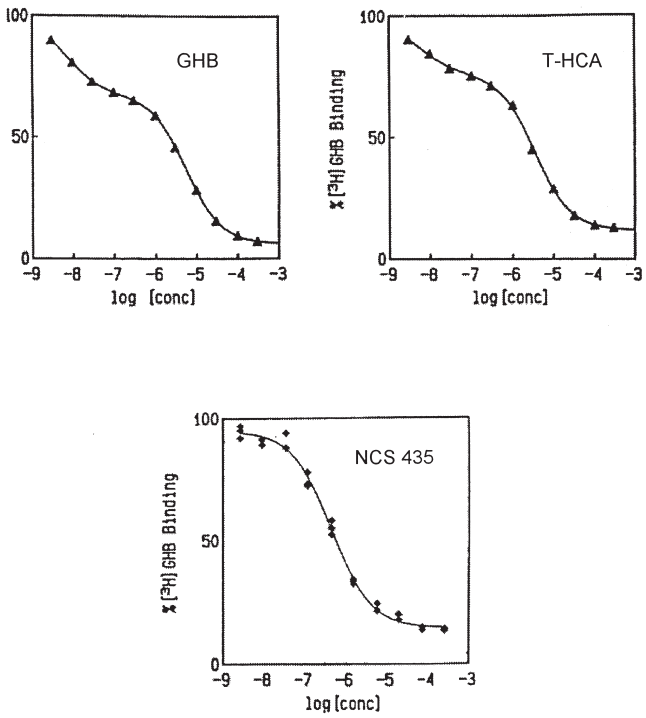

Fig. 6. Displacement curves of [^{3}H]gamma-hydroxybutyric acid by gamma-hydroxybutyric acid and gamma-hydroxybutyric acid analogues [4]. Three separate experiments were performed in triplicate at each concentration (variation <5%, statistical fitting with GraphPad program).

Among these homologues, the superior homologue (NCS 399, structure **20c**; $n = 3$) was found to be the most potent, particularly the trans isomer (compare structures **20d** and **20e**).

The gamma-phenyl T-HCA series (structure **19**) resulted from the combination of two beneficial structural modifications: (1) the introduction of a double bond in the GHB skeleton (T-HCA), and (2) the introduction of an aromatic ring at the gamma-position of GHB.

As described earlier for other GHB derivatives, a preliminary exploration of substituent effects at the aromatic ring of gamma-phenyl T-HCAs (structure **19**) was undertaken (Table 1) and led to a different structure–activity relation analysis, compared with data obtained within the gamma-phenyl GHB series (structure **17**). The most beneficial effect of chlorine was observed not in the para but in the ortho position (compare structures **19b** and **19c**). Some representatives of these gamma-phenyl T-HCAs were selected for further in vivo pharmacological experiments (see the next section).

A series of interesting semi-rigid analogues of T-HCA also was prepared in our laboratory (structure **21**). Formally, they combine in the same molecule:

1. The beneficial effect of an aromatic ring close to the alcohol (compound **16**).
2. The combination of two modes of restriction of conformational flexibility (double bond in compounds **19** and bridge between the aromatic ring and beta position in compounds **20**).

The homologous series of compound **21** ($n = 1$–3) was considered here. As found in the GHB series (structure **20**), the most active compound (NCS 382, structure **21c**; $n = 3$) presented specific conformational behavior as a result of its high degree of rigidity. In addition, this compound presented original antagonistic properties (Maitre et al., 1990).

6. Pharmacological properties of gamma-hydroxybutyric acid analogues

In accord with the data in the literature, agonists and antagonists at the GHB receptor are expected to modulate dopaminergic firing in the brain, particularly in the striatum (Hechler et al., 1993). GHB has been reported to induce both in vivo and in vitro accumulation of dopamine by inhibition of its depolarization-induced release. Several T-HCA derivatives (compounds **13** and **19**) behaved like GHB and showed similar inhibition in dopamine release. Their efficacy may be correlated with their affinity for GHB receptors (Hechler et al., 1993). Interestingly, the semi-rigid T-HCA derivative NCS 382 did not show any effect in dopamine release, but a pretreatment of animals with NCS 382 (intra-

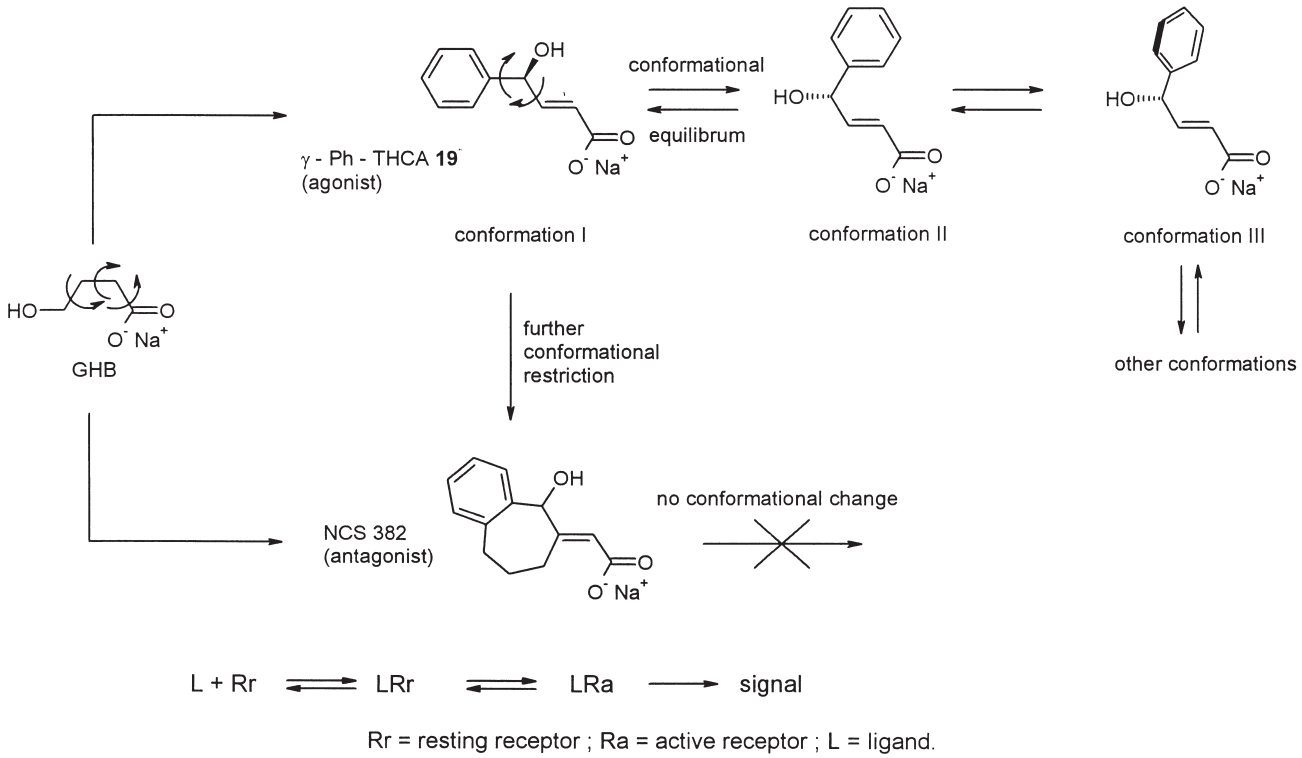

Fig. 7. Structural modification of a gamma-hydroxybutyric acid agonist, leading to an antagonist.

peritoneal administration) prevented the expected effects of GHB after local administration (Maitre et al., 1990). Thus, in this experiment, NCS 382 behaved as a GHB antagonist. The antagonistic character of this compound was further confirmed by different groups who used various models depicting specific actions of GHB. Gamma-hydroxybutyric acid induces an increase in cGMP in the rat hippocampus. NCS 382 had no effect in this model but fully abolished GHB effects when administered 1 hour before the GHB injection (Maitre et al., 1990). Behavioral GHB properties were also blocked by a pretreatment of NCS-382. Thus the neuroleptic-like (Hechler et al., 1993), electrophysiological (Godbout et al., 1995), or sedative (Schmidt et al., 1991) discriminative stimulus effects (Colombo et al., 1995a) of GHB were efficiently antagonized by NCS 382.

Because the structure of NCS 382 strongly resembles that of GHB, a direct competition with GHB on specific GHB binding sites can be postulated. From a molecular point of view, it is interesting to consider how GHB and GHB receptor agonists (i.e., gamma-phenyl T-HCA, compound **19**) led to an antagonist (NCS 382) (Fig. 7).

The semi-rigid gamma-phenyl T-HCA (structure **19**) still presents some rotating bonds (C-beta–C-gamma, C-gamma–phenyl), which allows it to adopt various energetically possible conformations (i.e., conformations I, II, III, and others). It is not the case for the rigid antagonist NCS 382. Bridging the aromatic onto the beta position suppressed (C-beta–C-gamma) or strongly decreased (C-gamma–phenyl) the rotating flexibility of the GHB molecule, keeping the carboxylate, the alcohol, and the aromatic ring in a specific tridimensional configuration. Thus the conformation of the semi-rigid NCS 382 determined by x-ray crystallography may characterize the active conformation of GHB.

Furthermore, it is well established that a typical ligand L in the presence of a resting receptor R_r yields a complex LR_r. The induction of conformational change driving the resting receptor (R_r) into an active state (R_a) requires some minimal flexibility of the ligand L to adapt to this conformational event (as in gamma-phenyl T-HCA). If the compound is too rigid and bulky (as is NCS 382), the receptor is kept in the resting state and the ligand behaves as an antagonist. Other similar examples supporting this hypothesis are available in the literature on different targets and are in good agreement with the theory of Ariëns (Ariëns et al., 1979).

7. Conclusion

Binding experiments with the use of [^{3}H]GHB constituted an efficient screening method for putative ligands of GHB receptors. The rational design starting from the structure of GHB yielded different series of GHB derivatives. Among them, the gamma-benzyl GHB derivative (structure **17** in Fig. 5) showed significantly increased affinity, compared with that of GHB. In particular, NCS 435 (para-methoxy derivative **17d**) was found to be about 50 times as potent as GHB.

Another series of alpha-beta unsaturated GHB (T-HCA) and derivatives (structures **19** and **21**, respectively) was par-

ticularly promising. The most rigid compound within this series (NCS 382) proved on various models to act as a competitive antagonist at the GHB receptors.

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Characterization of the discriminative stimulus effects of gamma-hydroxybutyric acid as a means for unraveling the neurochemical basis of gamma-hydroxybutyric acid actions and its similarities to those of ethanol

Giancarlo Colombo[a,*], Roberta Agabio[b], Mauro A.M. Carai[b,c], Carla Lobina[b,c], Marialaura Pani[b], Roberta Reali[c], Gian Luigi Gessa[a,b,c]

[a]*C. N. R. Center for Neuropharmacology and* [b]*Bernard B. Brodie Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124, Cagliari, Italy*
[c]*Neuroscienze S.c.a. r.l., Via Palabanda 9, I-09123 Cagliari, Italy*

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Abstract

The present paper reviews the drug discrimination studies, both from the literature and from this laboratory, conducted to investigate the pharmacological profile of the discriminative stimulus effects of gamma-hydroxybutyric acid. Collectively, the results of these studies suggest that: (1) the discriminative stimulus effects of gamma-hydroxybutyric acid are composed of different cues, each one being the effect of gamma-hydroxybutyric acid on a specific receptor system; (2) the proportion of each component cue varies as the training dose of gamma-hydroxybutyric acid is increased; (3) the gamma-aminobutyric acid B-mediated cue is a major ingredient of the mixed stimulus of gamma-hydroxybutyric acid, but it is more prominent at high training doses than at low training doses of gamma-hydroxybutyric acid; and (4) positive modulation of the gamma-aminobutyric acid A receptor is a relevant part of the discriminative stimulus effects of low gamma-hydroxybutyric acid doses. Finally, data indicating symmetrical generalization between the discriminative stimulus effects of a specific range of doses of gamma-hydroxybutyric acid and those of ethanol are discussed in regard to their further support of the hypothesis that gamma-hydroxybutyric acid may exert its antialcohol effects through a substitution mechanism.

Keywords: Gamma-hydroxybutyric acid; Discriminative stimulus effects; Compound stimulus; $GABA_A$- and $GABA_B$-mediated component; Ethanol; Symmetrical generalization; Substitution mechanism

1. The drug discrimination procedure

Several lines of evidence, collected in the past 30 years, indicate the suitability and sensitivity of drug discrimination procedures in investigating discriminative stimulus effects (i.e., the effects, taken together, perceived after drug administration) of psychoactive drugs in laboratory animals (Glennon et al., 1991; Overton, 1987; Samele et al., 1991; Stolerman et al., 1995) and their highly predictive validity as the animal correlate of human subjective effects (Kamien et al., 1993).

In drug discrimination procedures, laboratory animals are trained to recognize the interoceptive cues of a given dose of a certain drug (training drug) and associate these cues with a specific behavior (usually motivated by food reinforcement in food-restricted animals). Namely, animals are trained to carry out a particular task (e.g., pressing a lever in a two-lever operant procedure or running an arm of a T-maze) each time at which they detect the effects of the training drug (drug condition) and behave differentially (e.g., pressing the second lever or running the opposite arm) when those effects are absent (control or nondrug condition) (Fig. 1). Thus, animals learn to associate the internal state induced by the training drug with an observable and measurable behavior; in other words, the response behavior is the means by which the animal "self-reports" whether it feels "drugged" or not. The well-demonstrated sensitivity of the drug discrimination procedure suggests that the animal is "self-reporting" the specific effects of the dose of the drug that it has been trained to discriminate (Goudie & Leathley, 1993).

When animals have been successfully trained to discriminate the training drug, substitution and blockade tests can be conducted. In substitution tests, administration of a second drug (testing drug) in place of the training drug, which still produces the training drug-associated behavior, is indicative of the similarity of the discriminative stimulus effects of the testing drug to those of the training drug (Fig.

* Corresponding author. Tel.: +39-070-675-8422; fax: +39-070-657-237.
E-mail address: colomb@unica.it (G. Colombo).
[1]Present affiliation: Neuroscienze S.c.ar.l., Cagliari, Italy.

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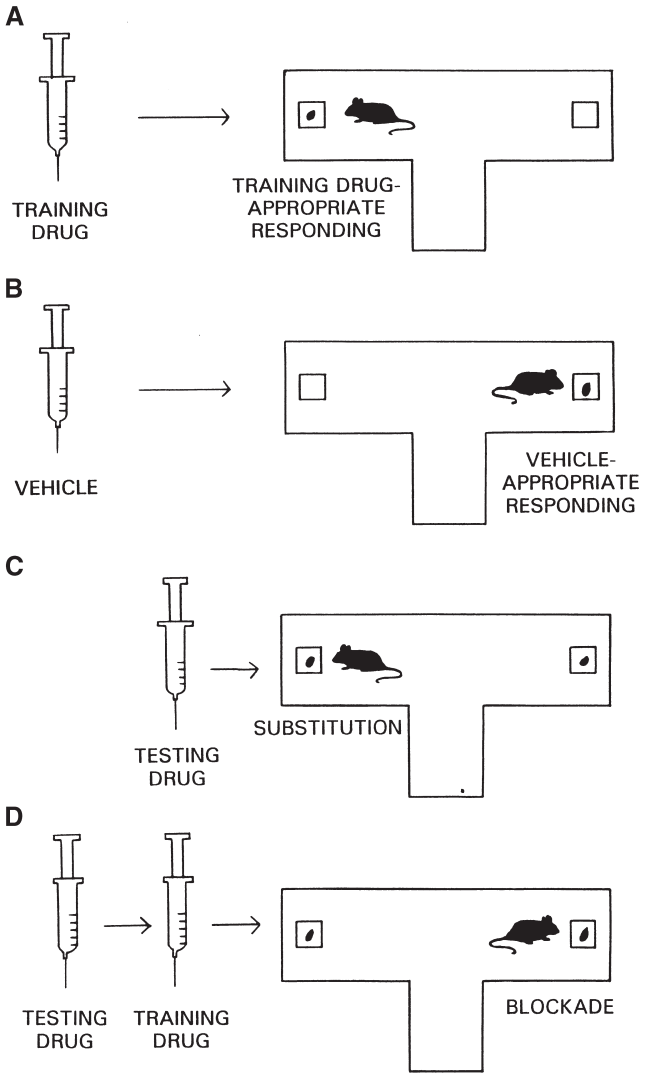

Fig. 1. T-maze, food-reinforced drug discrimination technique used in the authors' laboratory (Colombo et al., 1996). The apparatus is made of black Plexiglas and consists of a central stem (start point) and two opposite runways (right and left arm). A sliding door divides the start point from the arms. A recessed food cup is placed at the far end (goal area) of each arm, and sunflower seeds are used as reinforcements to motivate responding of food-deprived rats. Examples of training sessions under (A) the drug condition and (B) the vehicle condition. Each daily session consists of 10 consecutive trials (runs). Rats are trained to run (A) the drug-appropriate arm (the left arm for half the rats and the right arm for the other half) after the administration of the training drug and (B) the vehicle-appropriate arm (the opposite arm) after the administration of drug vehicle. Only the selection of the condition-appropriate arm is food reinforced. Sessions under drug condition and vehicle condition are alternated daily. Five consecutive correct training sessions define the criterion for acquisition of the discrimination. When rats have been trained to criterion, (C) substitution tests and (D) blockade tests are performed. In substitution (or generalization) tests, the degree of similarity between the discriminative stimulus effects of the training drug and those of different doses of the testing drug is assessed. Average selection of the drug-appropriate arm higher than 80% after administration of the testing drug is indicative of complete substitution for the discriminative stimulus effects of the training drug. Blockade tests are aimed at evaluating the ability of the testing drug to interfere in the perception of the discriminative stimulus effects of the training drug. Average selection of the drug-appropriate arm lower than 20% after combination of the testing drug indicates complete blockade of the discriminative stimulus effects of the training drug. In test sessions, the choice of each arm is food reinforced.

1). In blockade tests, a combination of both testing and training drugs, resulting in the selection of the non-drug-associated behavior, accounts for a reduced perception of the discriminative stimulus effects of the training drug (Fig. 1). The results of a large number of studies have shown that (1) drugs acting in a similar manner at a specific class of receptors possess similar discriminative stimulus effects and (2) antagonists at a specific receptor block the discriminative stimulus effects of receptor agonists (Colpaert, 1986; Goudie & Leathley, 1993; Holtzman, 1990; Overton, 1982). Thus, when the mechanism of action of the testing drug is known, the results of the substitution and blockade tests may provide relevant information on the neural substrates mediating the discriminative stimulus effects of the training drug.

2. The mixed discriminative stimulus effects of gamma-hydroxybutyric acid

The ability of gamma-hydroxybutyric acid (GHB) in controlling discriminative responding in laboratory animals was demonstrated for the first time by Dr. Jerrold C. Winter almost 20 years ago (Winter, 1981). However, few studies followed the initial work by Winter (1981); as a consequence, the pharmacological profile of the discriminative stimulus effects of GHB is poorly investigated to date.

The results of the study by Winter (1981) suggested that (1) the discriminative stimulus effects of GHB are composed of different cues, each one being the effect of GHB on a specific neurotransmitter system; (2) the gamma-aminobutyric acid (GABA)-mediated cue is a major ingredient of the mixed stimulus of GHB; and (3) although to a minor extent, an opioid as well as a serotonergic component are also comprised. These results, at least those concerning the GABA component, have been subsequently confirmed by findings from a study undertaken in this laboratory (Colombo et al., 1998).

2.1. Gamma-aminobutyric acid-mediated component

The study by Winter (1981) employed female CFN-strain rats trained to discriminate 200 mg/kg GHB, administered intraperitoneally, from saline in a water-reinforced, two-lever operant procedure. Muscimol and chlordiazepoxide, agonist and positive modulator at the $GABA_A$ receptor, respectively, partly substituted for GHB; indeed, administration of 1 mg/kg muscimol (intraperitoneally) and 20 mg/kg chlordiazepoxide (intraperitoneally) resulted in 56% and 80% mean selection of the GHB-associated lever, respectively. Furthermore, the $GABA_A$ receptor antagonist bicuculline partly antagonized the GHB cue, as indicated by the reduction, to approximately 50%, of mean GHB-appropriate responding after the i.p. injection of 2 mg/kg bicuculline. In addition, the $GABA_B$ receptor agonist baclofen partly substituted for GHB, eliciting approximately 70% of GHB-appropriate responding at doses of 3 and 6 mg/kg (i.p.) (Winter, 1981).

In the study undertaken in this laboratory (Colombo et al., 1998), male Long-Evans rats were trained to discriminate either a low (300 mg/kg, i.g.) or high (700 mg/kg, i.g.) dose of GHB from water, with the use of a T-maze, food-reinforced drug discrimination procedure, which had been set up and validated earlier (Colombo et al., 1996) (Fig. 1). Two training doses of GHB were used because GHB has been described to exert dose-dependent, biphasic behavioral effects, with low doses producing motor stimulation and high doses causing sedation and anesthesia (Maitre, 1997); thus, we hypothesized that the GHB cue may vary both quantitatively and qualitatively as the dose of GHB is increased.

In this study, baclofen (1–10 mg/kg, i.p.) dose dependently substituted for both 300 and 700 mg/kg GHB (Fig. 2). Interestingly enough, although complete substitution (defined as an average of 80% or more of total session entries in the "GHB-appropriate arm") was observed in both rat groups, baclofen showed a higher potency in substituting for 700 than for 300 mg/kg GHB, as demonstrated by the leftward shift of the substitution curve.

The $GABA_A$-positive modulator diazepam (1–4 mg/kg, i.p.) substituted for GHB with a decreased potency as the training dose of GHB was increased (Fig. 3); indeed, diazepam partly substituted for the low GHB training dose (producing approximately 70% selection of the "GHB-appropriate arm" at the dose of 4 mg/kg), whereas it completely failed to elicit GHB-associated responding in the high GHB training dose.

The results of the substitution tests with baclofen and diazepam in this study (Colombo et al., 1998) suggest that (1) the discriminative stimulus effects of GHB, besides varying quantitatively (i.e., intensity of the stimulus), also vary qualitatively (different proportion of the component cues) as the training dose of GHB is increased; (2) the $GABA_B$-mediated cue is a prominent component of the discriminative stimulus effects of GHB, although being more salient at the dose of 700 than at the dose of 300 mg/kg GHB; and (3) positive modulation of the $GABA_A$ receptor is a relevant part of the interoceptive stimuli produced by 300 mg/kg GHB, whereas it is apparently not relevant in the mediation of the discriminative stimulus effects of 700 mg/kg GHB.

These results are consistent with data obtained from drug mixture studies indicating that, as the ratio of one component is increased, the contribution of its cue to the discriminative stimuli of the mixture increases while the cue of the other component declines (Garcha & Stolerman, 1989; Mariathasan et al., 1991; Mariathesen & Stolerman, 1993; Stolerman & Mariathasan, 1990; Stolerman et al., 1987).

To define the contribution of the $GABA_B$ component to the discriminative stimulus effects of GHB more precisely, the study conducted in our laboratory (Colombo et al., 1998) also evaluated the efficacy of the $GABA_B$ receptor antagonist CGP 35348 in attenuating the GHB interoceptive cue. CGP 35348 (25–100 mg/kg, i.p.) was administered 15 min before GHB; it partly (i.e., average of total session entries in the "GHB-appropriate arm" was between 20% and 40%) and completely (i.e., average of 20% or less of total session entries in the "GHB-appropriate arm") blocked the discriminative stimulus effects of 300 and 700 mg/kg GHB, respectively (Figure 4). Indeed, a combination of the highest CGP 35348 dose tested and the training doses of GHB resulted in 33.6% and 15.2% average selection of the "GHB-appropriate arm."

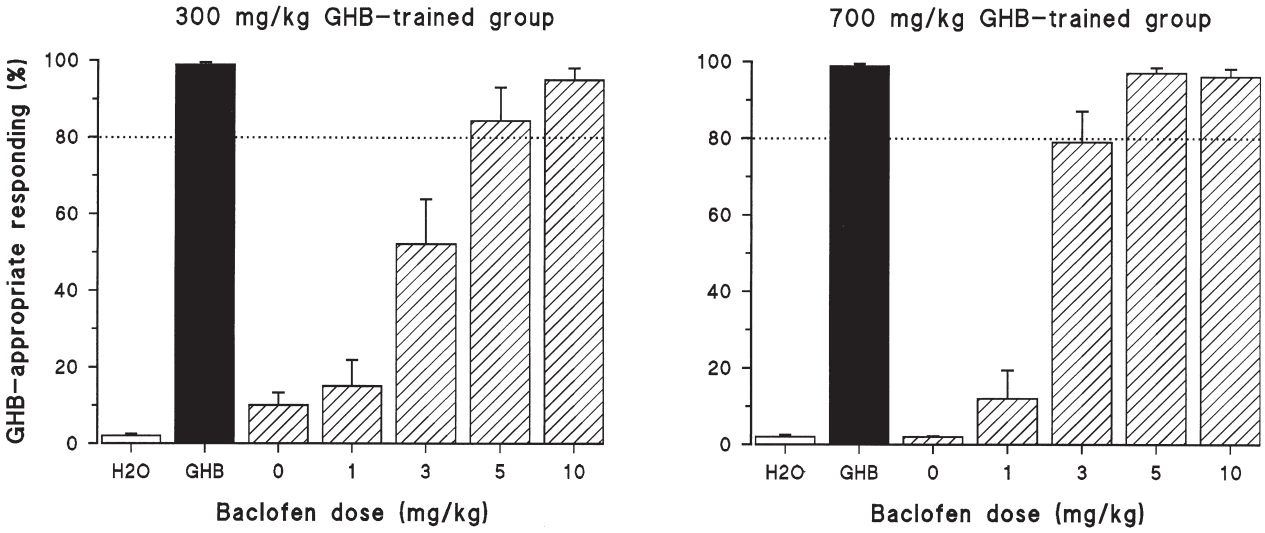

Fig. 2. Average percentage of entries into the "gamma-hydroxybutyric acid (GHB)-appropriate arm" after the administration of different doses of baclofen (i.p.) in rats trained to discriminate either 300 mg/kg (left panel) or 700 mg/kg (right panel) gamma-hydroxybutyric acid (i.g.) from water in a T-maze, food-reinforced drug discrimination procedure. Gamma-hydroxybutyric acid and water (H_2O) bars represent the average percentage of entries into the gamma-hydroxybutyric acid-appropriate arm during training sessions with gamma-butyric acid and water, respectively. The dashed line indicates the limit (80%) of gamma-butyric acid-appropriate responding for complete substitution of the discriminative stimulus effects of GHB. Each bar is the mean ± S.E.M. of two determinations per rat in seven rats of the 300 mg/kg gamma-hydroxybutyric acid training group and five rats of the 700 mg/kg gamma-hydroxybutyric acid training group.

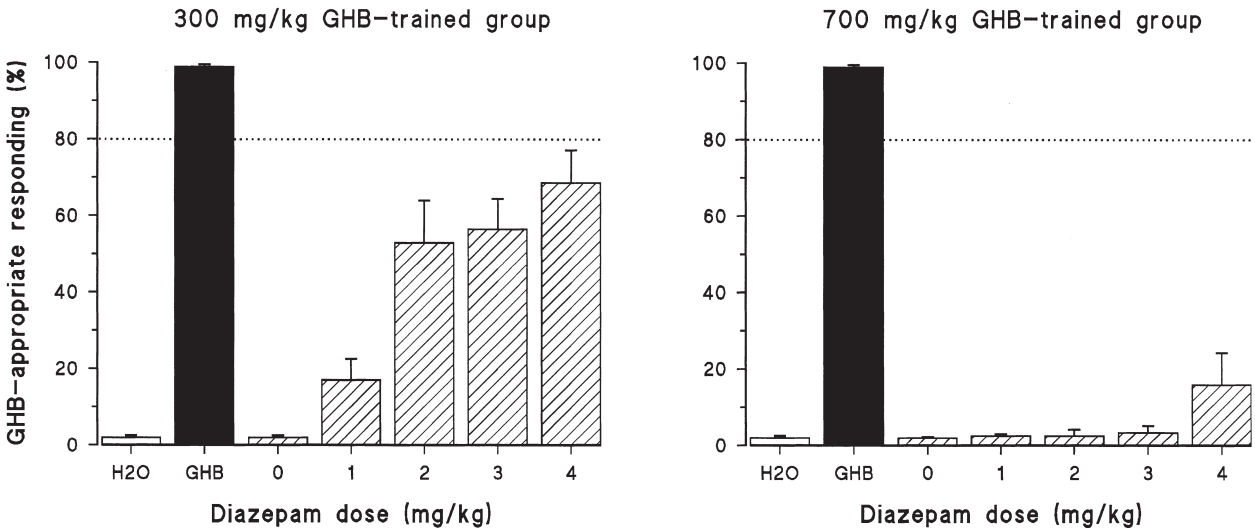

Fig. 3. Average percentage of entries into the "gamma-hydroxybutyric acid (GHB)-appropriate arm" after the administration of different doses of diazepam (i.p.) in rats trained to discriminate either 300 mg/kg (left panel) or 700 mg/kg (right panel) gamma-hydroxybutyric acid (i.g.) from water in a T-maze, food-reinforced drug discrimination procedure. Gamma-hydroxybutyric acid and water (H_2O) bars represent the average percentage of entries into the gamma-hydroxybutyric acid-appropriate arm during training sessions with gamma-hydroxybutyric acid and water, respectively. The dashed line indicates the limit (80%) of gamma-hydroxybutyric acid-appropriate responding for complete substitution of the discriminative stimulus effects of gamma-hydroxybutyric acid. Each bar is the mean ± S.E.M. of two determinations per rat in seven rats of the 300 mg/kg gamma-hydroxybutyric acid training group and six rats of the 700 mg/kg gamma-hydroxybutyric acid training group.

Again, data obtained from drug discrimination studies on the interoceptive cues produced by drug mixtures can provide a key to understanding these results. Complete blockade of the discriminative stimulus effects of a drug mixture has been demonstrated to occur only when the stimuli produced by each component are blocked. Indeed, blockade of one component resulted at most in partial attenuation of the discriminative stimulus effects of the mixture, and such attenuation was proportional to the prominence of the component cue (Stolerman et al., 1987; White & Stolerman, 1994). According to the hypothesis on the multiple-component profile of the discriminative stimulus effects formulated by Winter (1981), the complete blockade of the discrimination of 700 mg/kg GHB by CGP 35348 is suggestive of the prominence of the $GABA_B$ component of the GHB cue at this drug dose, and such prominence tends to overshadow the perception of the other components. In other words, the rats trained to discriminate 700 mg/kg GHB from water based

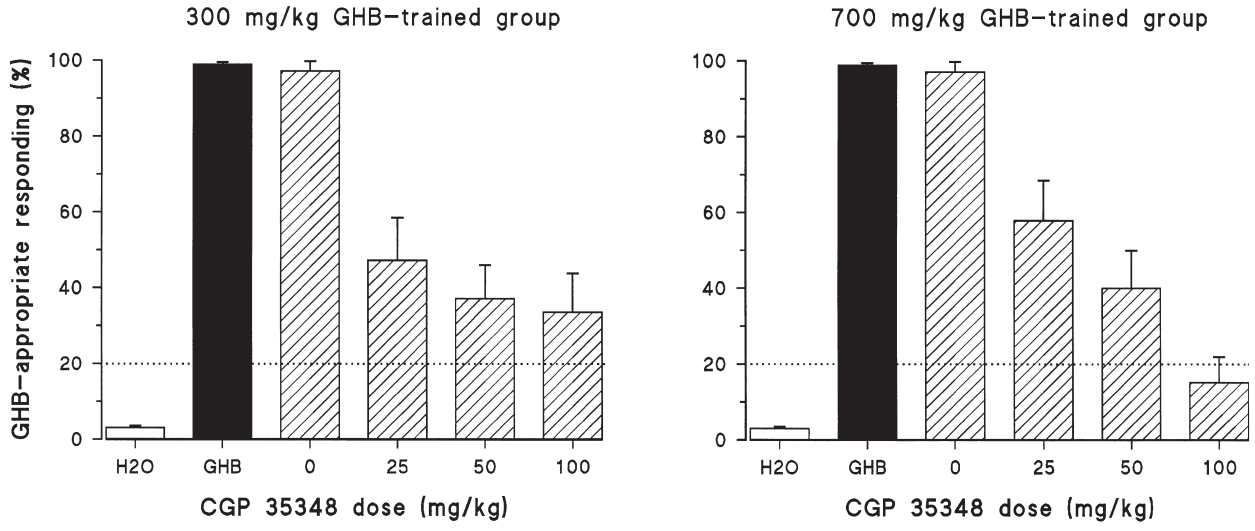

Fig. 4. Average percentage of entries into the "gamma-hydroxybutyric acid (GHB)-appropriate arm" after the administration of different doses of CGP 35348 (i.p.), given 15 min before the gamma-hydroxybutyric acid training dose, in rats trained to discriminate either 300 mg/kg (left panel) or 700 mg/kg (right panel) gamma-hydroxybutyric acid (i.g.) from water in a T-maze, food-reinforced drug discrimination procedure. Gamma-hydroxybutyric acid and water (H_2O) bars represent the average percentage of entries into the gamma-hydroxybutyric acid-appropriate arm during training sessions with gamma-hydroxybutyric acid and water, respectively. The dashed line indicates the limit (20%) of gamma-hydroxybutyric acid-appropriate responding for complete blockade of the discriminative stimulus effects of gamma-hydroxybutyric acid. Each bar is the mean ± S.E.M. of two determinations in each of the seven rats of both gamma-hydroxybutyric acid training groups.

the GHB discrimination predominantly on the $GABA_B$-mediated cues; when these stimuli were blocked and other possible components were disclosed, rats were unable to recognize the GHB cues and selected the water-associated arm of the maze. At the lower GHB training dose (300 mg/kg), CGP 35348 pretreatment resulted at most in partial blockade of the discriminative stimulus effects of GHB, consistently with the somewhat lower potency of baclofen in substituting for 300 mg/kg GHB. These results suggest that the discrimination of 300 mg/kg GHB is only partly based on the $GABA_B$ component. Data obtained from the study by Winter (1981) and those obtained from this laboratory (Colombo et al., 1998) suggest that the $GABA_A$ component may play a distinctive role in the mediation of the internal cues of a low GHB dose. Indeed, (1) the benzodiazepines chlordiazepoxide (Winter, 1981) and diazepam (Colombo et al., 1998) partly substituted for two approximately equivalent doses of GHB [200 mg/kg, i.p., in the study by Winter (1981); 300 mg/kg, i.g., in the study by this group (Colombo et al., 1998)], and interestingly (2) the $GABA_A$ receptor antagonist bicuculline partly blocked the discriminative stimulus effects of 200 mg/kg GHB (i.p.) (Winter, 1981).

The combined data obtained from the studies by Winter (1981) and this laboratory (Colombo et al., 1998), suggesting a robust involvement of the $GABA_B$ receptor in the mediation of the internal stimuli of GHB, are in close agreement with those of electrophysiological, biochemical, and behavioral investigations. The results of these investigations indicate that (1) baclofen and GHB have several pharmacological properties in common, including sedative effects, the induction of generalized absence seizures, and an increase in dopamine and serotonin metabolism in the rat striatum and mesolimbic system (Czuczwar et al., 1984; Da Prada & Keller, 1976; Engberg & Nissbrandt, 1993; Ito et al., 1995; Nissbrandt & Engberg, 1996; Waldmeier & Fehr, 1978; Williams et al., 1995; Xie & Smart, 1992), and that (2) some of these effects are antagonized by $GABA_B$ blockers (Engberg & Nissbrandt, 1993; Ito et al., 1995; Nissbrandt & Engberg, 1996; Snead, 1996; Williams et al., 1995; Xie & Smart, 1992).

The neurochemical basis of the substitution of diazepam and baclofen for the interoceptive stimuli of GHB does not seem to be a direct interaction of GHB with $GABA_A$ and $GABA_B$ receptors, because GHB has been shown to possess a very weak affinity for both receptors (Bernasconi et al., 1992; Olsen et al., 1981). It is more likely that the GABA-like effects of GHB are due to the conversion of GHB into GABA, which, in turn, binds to both $GABA_A$ and $GABA_B$ receptors (Hechler et al., 1997). The prominence of the $GABA_B$ component in the discriminative stimulus effects of GHB observed in the present study is consistent with the higher sensitivity of $GABA_B$ receptors to GABA, with respect to $GABA_A$ receptors (Hechler et al., 1997). Alternatively, the $GABA_B$-like effects of GHB may be secondary to activation of a GHB recognition site related to, although separate from, a $GABA_B$ receptor, forming a presynaptic $GABA_B$/GHB receptor complex that regulates neurotransmitter release (Snead, 1996).

2.2. Opioid-mediated component

In the study by Winter (1981), morphine elicited intermediate responding (54% and 60% mean GHB-appropriate responding at doses of 3 and 6 mg/kg, i.p.) in rats trained to discriminate 200 mg/kg GHB, i.p., from saline. Furthermore, morphine-induced partial substitution of the GHB cue was completely blocked by pretreatment with the opioid receptor antagonist naloxone (administered intraperitoneally at a dose of 0.4 mg/kg). However, naloxone, administered per se up to a dose of 10 mg/kg, failed to alter the GHB cue. These data suggest that an opioidergic component may be present, although its contribution to the overall stimulus control of this dose of GHB appears to be rather modest.

2.3. Serotonin-mediated component

Winter (1981) also reported that administration of the serotonin agonist lysergic acid diethylamide resulted in intermediate responding (maximal mean GHB-appropriate responding being 45% at a dose of 0.1 mg/kg, i.p.) in rats trained to discriminate 200 mg/kg GHB, i.p., from saline. This result suggests that the compound stimulus produced by this dose of GHB may also be associated, although minimally, with stimulation of the serotonin system.

2.4. Other studies

In the pioneering study by Winter (1981), neither the direct dopamine agonist apomorphine nor the indirect dopamine agonist *d*-amphetamine elicited GHB-like behavior. Furthermore, the uncompetitive *N*-methyl-d-aspartate (NMDA) receptor antagonists phenciclidine (Winter, 1981) and dizocilpine (Colombo et al., 1998) did not substitute for both low and high doses of GHB. These results suggest that (1) stimulation of the dopaminergic system and (2) antagonism of the NMDA-mediated neurotransmission, at least by blockade of the binding site within the cation channel associated with the receptor complex, do not contribute to the perception of the GHB stimuli. This laboratory has reported that the administration of the cannabinoid receptor agonist WIN 55,212-2 failed to substitute for both 300 and 700 mg/kg GHB, suggesting that activation of this receptor does not produce discriminative stimulus effects similar to those elicited by GHB (Colombo et al., 1998).

Finally, the results of an earlier investigation undertaken by this laboratory demonstrated that the discriminative stimulus effects of both 300 and 700 mg/kg GHB, administered intragastrically, were completely blocked by the i.p. administration of the GHB receptor antagonist NCS-382 (Colombo et al., 1995a), suggesting that the interoceptive cues of GHB were also mediated by the stimulation of GHB receptors. However, reevaluation of these data may be necessary, because the results of a subsequent study showed that NCS-382

dramatically reduced ethanol absorption from the gastrointestinal system and, consequently, ethanol discrimination (Colombo et al., 1999). Thus, further studies are needed to verify whether the reported attenuation of the discrimination of orally administered GHB by NCS-382 (Colombo et al., 1995a) might have been due to a reducing effect of NCS-382 on GHB absorption in the gastrointestinal tract.

To date, only a handful of studies have investigated the ability of GHB to substitute for the discriminative stimulus effects of other drugs. Chronologically, the first was an investigation undertaken in this laboratory (Colombo et al., 1995b). The results of this investigation, described in detail in Section 2, indicated that a low dose of GHB (300 mg/kg, i.g.) completely substituted for 1 g/kg (i.g.) ethanol.

The results of a recent study by Beardsley and colleagues (Beardsley et al., 1996) demonstrated that GHB (tested at doses as high as 300 mg/kg, i.p) failed to substitute for heroin and phencyclidine in rats trained to discriminate 0.3 mg/kg heroin (s.c.) and 2 mg/kg phencyclidine (i.p.), respectively, from vehicle. These data complement those indicating that stimulation of the opioid receptor (Winter, 1981) and antagonism at the NMDA receptor (Colombo et al., 1998; Winter, 1981) are modest or even absent components of the GHB cue. In the same paper, GHB (again, tested up to 300 mg/kg, i.p.) was also reported to be unable to antagonize the discriminative stimulus effects of cocaine in rats trained to discriminate 10 mg/kg cocaine (i.p.) from saline (Beardsley et al., 1996).

A more recent study used rhesus monkeys trained to discriminate either *d*-amphetamine (0.56 or 1 mg/kg, i.g.), pentobarbital (10 mg/kg, i.g.), or the benzodiazepine triazolam (0.1 mg/kg, s.c.) from saline (Woolverton et al., 1999). Doses of GHB varying from 1 to 178 mg/kg, administered both subcutaneously and intragastrically, were tested for substitution of the training conditions. GHB induced a maximum of approximately 50% *d*-amphetamine-appropriate responding in three of four monkeys trained to discriminate *d*-amphetamine from saline. Each dose of GHB elicited 0% responding in the pentobarbital-associated lever in pentobarbital-trained subjects. Finally, GHB elicited triazolam-like discriminative stimulus effects in only one of three monkeys trained to discriminate triazolam from saline. The same study (Woolverton et al., 1999) also investigated the capability of GHB to substitute for the benzodiazepine receptor antagonist flumazenil (0.32 mg/kg, s.c.) in diazepam-maintained rhesus monkeys, an experimental condition that mimics a negative $GABA_A$ modulation. No dose of GHB produced any substitution for the training condition.

Finally, a meeting report by Sannerud and Gauvin (1997) described the results of a study investigating the substitutability of GHB (0.1–320 mg/kg, i.p.) for the benzodiazepine chlordiazepoxide, as well as the anxiogenic and convulsant agent pentylenetetrazole, in rats trained to discriminate 3 mg/kg chlordiazepoxide (i.p.), 15 mg/kg pentylenetetrazole (i.p.), and saline under a three-choice discrimination task. Doses of GHB at the high end of the dose range elicited exclusively pentylenetetrazole-appropriate responding, whereas midrange doses of GHB engendered chlordiazepoxide-appropriate responding. The latter data are in agreement with those suggesting the existence of a $GABA_A$ component in the mediation of the discriminative stimulus effects of low to moderate doses of GHB (Colombo et al., 1998; Winter, 1981).

3. Cross-substitution between the discriminative stimulus effects of gamma-hydroxybutyric acid and ethanol

Recent clinical studies, reviewed in great detail in other articles in this issue (Addolorato et al., 2000; Gallimberti et al., 2000; Moncini et al., 2000), have featured GHB as an effective agent in the pharmacotherapy of alcohol dependence. Several lines of evidence, demonstrating that GHB and ethanol have a number of biochemical, electrophysiological, and pharmacological similarities, suggest that GHB exerts its effects on ethanol dependence by mimicking ethanol actions in the central nervous system (Gessa et al., 2000). In other words, GHB efficacy in controlling alcohol craving, consumption, and withdrawal syndrome would be exerted through a substitution mechanism, similar to that underlying methadone use in heroin addiction.

Two drug discrimination studies conducted in this laboratory further support the substitution hypothesis (Agabio et al., 1995; Colombo et al., 1995b). In these studies, Long-Evans rats were trained to discriminate either ethanol (1, 1.5, or 2 g/kg, i.g.) or GHB (300 or 700 mg/kg, i.g.) from water in a T-maze food-reinforced procedure. The results of these investigations indicated that GHB and ethanol cross-substituted; however, this symmetrical generalization occurred only at the doses of 300 mg/kg GHB and 1 g/kg ethanol, because no other dose of either drug substituted for the training doses of the other drug. These results suggest that, within narrow dose ranges, GHB and ethanol elicit discriminative stimulus effects that are perceived as being similar by the rats.

Rather interestingly, GHB possesses the maximal efficacy in reducing voluntary ethanol intake in selectively bred alcohol-preferring (P) rats (June et al., 1995) and Sardinian alcohol-preferring (sP) rats (Agabio et al., 1998; Gessa et al., 2000) at doses ranging from 200 to 400 mg/kg. The results of previous studies aimed at determining the daily drinking pattern of both P rats (Murphy et al., 1986; Waller et al., 1982) and sP rats (Agabio et al., 1996) showed that most of their drinking occurred in distinct binges, each one being about 1 g/kg ethanol. Thus, the suppressing effects of GHB on voluntary ethanol intake and the amount of ethanol usually consumed by P and sP rats in each drinking episode are obtained at doses similar to those that cross-substituted in the present study.

Collectively, these data suggest that the reducing effects of GHB on voluntary ethanol intake in P and sP rats are due,

at least in part, to the substitution of GHB for ethanol-reinforcing effects, rendering further ethanol ingestion superfluous.

4. Similarities and differences between the "frames" of the compound stimulus of gamma-hydroxybutyric acid and ethanol

The two features of the discriminative stimulus effects of GHB (namely, a multiple-component frame and the dose-dependent quality of the stimulus) (Colombo et al., 1998; Winter, 1981) have also been identified in the discriminative stimulus profile of ethanol (Grant & Colombo, 1993a, 1993b, 1993c). First, at least three receptor systems ($GABA_A$, NMDA, and 5-HT_1 subtype of the serotonin receptor) seem to take part in the mediation of the discriminative stimulus of ethanol (Grant & Colombo, 1993a, 1993b, 1993c). Second, the results of substitution tests with $GABA_A$-positive modulators, NMDA antagonists, and serotonin agonists suggest that (1) the $GABA_A$-mediated component is more prominent at low doses of ethanol (Grant & Colombo, 1993a) and (2) discrimination of high doses of ethanol are predominantly based on the NMDA component (Grant & Colombo, 1993b), whereas (3) the serotonin contribution is apparent at doses of ethanol in the 1–1.5 g/kg range (Grant & Colombo, 1993c).

Generally, drug discrimination studies with ethanol have resulted in asymmetrical generalizations: benzodiazepines, barbiturates, NMDA antagonists, and certain serotonin agonists have substituted for ethanol, but ethanol has not consistently substituted for these drugs (Colombo et al., 1997). Grant (1994) and Barry (1991) independently and elegantly proposed that the lack of symmetrical generalization might be due to (1) the ability of a single component cue (a highly specific cue, conceivable as a one-pitch sound or a simple geometric figure, such as a triangle) to be recognized as a part of the mixture (a stimulus with greater generality, such as a five-pitch sound or a complex figure in which a triangle is just a component of its boundary) and (2) in contrast, the inability of the compound stimulus to be perceived as similar to the single, specific component.

The symmetrical generalization between GHB and ethanol (Agabio et al., 1995; Colombo et al., 1995b) was therefore unique and predictive of a similarity, at least at those particular doses, in the pharmacological profile of their discriminative stimulus effects. In this light, the results of the studies on the "frame" of the discriminative stimulus effects of GHB and ethanol are somewhat puzzling and not fully reconciliable at present.

A first evidence in favor of this hypothesis is the presence of a $GABA_A$-mediated cue in the discriminative stimulus effects of both drugs, because some positive modulators at the $GABA_A$ receptor complex have been reported to substitute for doses of 300 mg/kg GHB (Colombo et al., 1998) or for a dose similar to that used in the study by Winter (1981) and 1 g/kg ethanol (Grant & Colombo, 1993a). Second, a serotonergic component has been identified in the interoceptive cues of GHB and ethanol, as demonstrated by the partial substitution of lysergic acid diethylamide for GHB (200 mg/kg) (Winter, 1981) and partial or complete substitution of different 5-HT_1 receptor agonists (namely, trifluoromethylphenylpiperazine, CGS 12066B, RU 24969, and mCPP) for ethanol (1 g/kg, i.g.) (Grant & Colombo, 1993c; Grant et al., 1997).

Two discrepancies weigh against this hypothesis. First, baclofen substituted for 200 (Winter, 1981) and 300 (Colombo et al., 1998) mg/kg GHB but completely failed to substitute for 1 g/kg ethanol, i.p. (Shelton & Balster, 1994), indicating that $GABA_B$-mediated neurotransmission has a role in the perception of the discriminative stimulus effects of a low dose of GHB but not of ethanol. Second, antagonism of the NMDA receptor is part of the discriminative stimulus effects of 1 g/kg ethanol but not 300 mg/kg GHB, as shown by the substitution of dizocilpine and phencyclidine for ethanol (Grant & Colombo, 1993b) but not for GHB (Colombo et al., 1998; Winter, 1981).

Further studies are needed to clarify the neural circuitry mediating the ethanol-like part of the discriminative stimulus effects of GHB.

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ALCOHOL

Gamma-hydroxybutyric acid
An evaluation of its rewarding properties in rats and mice

L. Fattore, M.C. Martellotta, G. Cossu, W. Fratta*

B.B. Brodie Department of Neuroscience, University of Cagliari, Via Porcell n. 4, 09124-I, Cagliari, Italy

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Abstract

Gamma-hydroxybutyric acid, an endogenous compound present in mammalian brain and supposed to be a neurotransmitter or neuromodulator, has been shown to affect several aspects of dependence from some drugs of abuse. It has been successfully used in clinical practice to alleviate both alcohol and opiate withdrawal symptoms. The aim of this study was to investigate whether gamma-hydroxybutyric acid possesses rewarding properties by means of conditioned place preference and intravenous self-administration paradigms. In the present study, gamma-hydroxybutyric acid induced conditioned place preference in rats, was intravenously self-administered by drug-naive mice, and altered cocaine intravenous self-administration in rats. Although to date the physiological role of this compound still remains unclear, there is no doubt that gamma-hydroxybutyric acid, in addition to its proved effect on alcohol and opiate dependence, possesses reinforcing properties of its own and may interfere with the neurochemical events in the rewarding effects produced by psychostimulant drugs. Our investigation points out the abuse liability of this drug, suggesting the use of particular precaution in handling gamma-hydroxybutyric acid as a clinically useful drug.

Keywords: Gamma-hydroxybutyric acid (GBH); Conditioned place preference (CPP); Intravenous self-administration (IVSA); Reinforcing properties; Cocaine; NCS-382; Dependence; Reward; Rats; Mice

1. Introduction

Gamma-hydroxybutyric acid (GHB) is the transaminated and reduced metabolite resulting from degradation of gamma-aminobutyric acid (GABA) within the mammalian brain (Roth, 1970; Rumigny et al., 1980). Furthermore, it is widely distributed in extraneuronal tissues such as kidney, heart, and skeletal muscle (Nelson et al., 1981). The discoveries of the compound's biosynthetic system (Cash et al., 1979) and concentration in the synaptosomal fraction (Maitre et al., 1983b), mechanisms of release (Maitre et al., 1983a, 1990), transport (Benavides et al., 1982a), turnover (Vayer et al., 1988), and inactivation suggest a role for GHB as neuromodulator or neurotransmitter in the mammalian brain (Vayer et al., 1987). The existence of specific high-affinity binding sites for GHB in both rat and human brain supports this hypothesis. With the use of quantitative autoradiography, these sites, though heterogeneously distributed in the brain tissue, have been observed to be mainly located in a few restricted areas, particularly in the striatum and hippocampus (Hechler et al., 1989). Although little is known about the possible physiological role of GBH, several pharmacological effects have been observed after GHB administration in both animals and human beings.

Gamma-hydroxybutyric acid was originally presented by Laborit and colleagues as an useful agent in anesthesia (Laborit et al., 1962) and was subsequently used in the treatment of narcolepsy (Broughton & Mamelak, 1979). More recently, a role for GHB in drug dependence was hypothesized on the basis of its efficacy, in nonhypnotic doses, in decreasing alcohol craving (Gallimberti et al., 1992) and suppressing the withdrawal syndrome in both alcohol (Gallimberti et al., 1989) and heroin (Gallimberti et al., 1993) addicts.

Further support for an action of GHB on the neural systems mediating drug reinforcement came from warnings from the U.S. Food and Drug Administration (U.S. Food and Drug Administration, 1991) and the National Institute on Drug Abuse, suggesting the potential abuse liability of GHB, which is described as an unapproved drug easily available on the black market and often taken with alcohol or other drugs to produce a "high" (Frederick et al., 1994; Galloway et al., 1997). An increasing number of anecdotal reports, particulary from the United States and the United Kingdom, have indicated a growing popularity of GHB as a recreational drug (Stell & Ryan, 1996).

Preclinical evidence further supports this possibility. Results of studies in laboratory animals have shown that, when

* Corresponding author. Tel.: +39-70-6758421; or +39-70-6758418; fax: +39-70-657237.

E-mail address: wfratta@unica.it (W. Fratta).

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GHB is administered to animals as a drug, several neurochemical and behavioral modifications are induced. A selective increase in striatal dopamine levels in rats occurs (Gessa et al., 1966), striatal tyrosine hydroxylase activity increases (Morgenroth et al., 1976), and the spontaneous firing of dopaminergic neurons in the substantia nigra is modified (Roth et al., 1980); in the hippocampus, it is associated with an increase in cGMP levels and inositol phosphate turnover (Vayer & Maitre, 1989).

Gamma-hydroxybutyric acid reduces alcohol intake in genetically selected alcohol-preferring rats, alleviates the severity of the withdrawal syndrome in ethanol-dependent rats, substitutes for ethanol in the drug-discrimination paradigm, and is orally self-administered by rats (Colombo et al., 1995a). All these data are coherent with the hypothesis that GHB could interact with the rewarding processes in the mammalian brain.

The aim of the present study was to evaluate whether GHB possesses reinforcing properties and therefore is characterized by abuse liability. To this purpose, we examined the ability of GHB to induce conditioned place preference (CPP) in rats. This method has proved to be a reliable animal model of drug-induced reward (Schechter & Calcagnetti, 1993), extensively used to assess the affective properties of a large variety of drugs of abuse, such as cocaine (Mucha et al., 1982; Snead, 1994), amphetamine (Reicher & Holman, 1977; Sherman et al., 1980), heroin (Bozarth & Wise, 1981), and morphine (Katz & Gormezano, 1979).

Then, we evaluated the possibility of intravenous self-administration (IVSA) of GHB in drug-naive mice. This model, which has proved to assess the reinforcing properties of "classical" drugs of abuse such as morphine (Kuzmin et al., 1992, 1996b, 1997), cocaine (Kuzmin et al., 1992, 1996a, 1997), nicotine (Martellotta et al., 1995), and cannabinoids (Martellotta et al., 1998), has the great advantage of allowing a rapid screening of drugs in a large number of animals.

Finally, we analyzed the effect of acute pretreatment with GHB on cocaine IVSA in rats, by using the nose poke as operandum. Cocaine IVSA in rodents has been regarded as a useful animal analogue for studying cocaine dependence in human beings (Koob, 1992). Pharmacological treatments have been shown to affect the acute reinforcing properties of cocaine, both in reducing cocaine-seeking behavior in animals (Hubner & Koob, 1990; Pulvirenti & Koob, 1994) and in achieving effective therapies in human beings (Withiers et al., 1995). These observations prompted us to conduct a study aimed at the investigation of the effects of systemically administered GHB in rats self-administering cocaine intravenously to establish whether pretreatment with GHB could modify the acute reinforcing properties of cocaine.

2. Methods

In all the following experiments, animals had free access to food and water and were maintained on a 12-h reversed light–dark cycle (dark from 9:00 A.M. to 9:00 P.M.). Ambient temperature (22 ± 1°C) and humidity (60%) were constant. All procedures had been approved by the local Animal Care Committee for animal use in research.

2.1. Conditioned place preference

Two groups of adult male Sprague-Dawley rats (Harlan-Nossan, Milan, Italy) weighing from 180 to 200 g at the beginning of the conditioning sessions were used in CPP experiments. Animals were housed six per cage and handled daily for approximately 10 min in the first week after arrival. Experimental procedures started in the second week and took place at the same time each day in the dark phase of the cycle (between 9:00 A.M. and 2:00 P.M.). A third group of animals was used for locomotor-activity experimentation.

Gamma-hydroxybutyrate (sodium salt; Sigma, Italy) was dissolved in tap water at a volume of 5 ml/kg and was administered to rats at doses of 87.5, 175, and 350 mg/kg by intragastric probe (Pediatric feeding tube, Medico Plast, Germany), 30 min before placing them in the appropriate compartment. The gamma-hydroxybutyric acid administration interval was chosen to ensure that the peak drug plasma concentration coincided with the conditioning interval (Lettieri & Fung, 1979). The same conditions at different doses (175, 350, or 700 mg/kg) were maintained for the motor-activity experiment.

Cocaine hydrochloride (Sigma, Italy) was dissolved in 0.9% saline solution (volume of injection: 5 ml/kg) and injected intraperitoneally immediately before placing rats in the appropriate compartment.

All injections occurred in the home cage room. In all experiments, solutions were freshly prepared before use; all doses refer to the salt.

The CPP apparatus consisted of eight rectangular plastic shuttle boxes (30 × 60 × 30 cm), each divided by a guillotine door into two distinct compartments of equal size, containing different visual and tactile cues. Visual cues were present in the walls, which were either brown (B) or black and white striped (S); tactile cues were present in the floor, being either grid (G) or chequered (C). All these cues—producing four possible combinations: BC, BG, SC, SG—were present in the compartments in a counterbalanced order. The experimental room was sound attenuated and dimly lit. With the use of two videocameras, rats were filmed during the preconditioning and test phases, when the guillotine doors were raised, to calculate time spent on each side. An animal was considered to be in a particular compartment only when all four paws were in that area. Motor activity was measured by placing the animals individually in motility cages (Omnitech Digiscan Animal Activity Monitor, Columbus, OH, USA). Each cage had two sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart and 2 cm above the cage floor. Motor activity was defined as the horizontal activity counts.

The first CPP experiment consisted of three consecutive phases: preconditioning (phase I), conditioning (phase II), and postconditioning, or test (phase III). The total time re-

quired was 24 days. Before the experiment started, each animal was randomly assigned to one of five different treatment groups ($n = 8$/group). In phase I, 3 days were dedicated to accustoming the animals to CPP boxes and determining their initial side preferences. The guillotine doors were raised, and each rat was placed in one of the compartments (the start box) according to a counterbalanced order (i.e., ensuring representation of all different cues in equal number in the start compartments) and allowed to explore both sides for 15 min/day. Time spent by each animal in the two compartments was recorded on the third day to calculate the spontaneous preference of each animal for either of the two sides.

For the subsequent phases of the experiment, the compartment where the rat spent the most time was considered to be the preferred side, whereas the other was the nonpreferred side. It should be stressed that the animal's unconditioned preference was not restricted to one specific cue combination. More specifically, BC was preferred by 22.5% of animals, BG by 20%, SC by 30%, and SG by 27.5%. Conditioning training (phase II) lasted 20 days and consisted of 10 alternated presentations of drugs (GHB or cocaine) and vehicle (tap water or saline). Cocaine was used to provide a positive control for the method.

On odd-numbered training days, drug-treated rats were confined to their previously nonpreferred side for 30 min. On even-numbered training days, each animal was given the vehicle, either tap water or saline, respectively 30 min before or immediately before being confined in the opposite, initially preferred, side for the same length of time. A control group received tap water intragastrically at a volume of 5 ml/kg in both compartments and, after 30 min, animals were placed in the shuttle box for a further 30 min. On the test day (phase III), animals did not receive any treatment. Rats were placed in the start compartment with the guillotine doors raised and with free access to both sides. The amount of time spent by the drug-free animal in each side during a 15-min period was again recorded. The difference in time spent in the nonpreferred compartment between postconditioning and preconditioning tests, expressed as difference scores (Δt_1), was considered the critical measurement for evaluation of preference induced by the drug: a positive difference was considered an index of reward.

In the second CPP experiment, all treatment groups ($n = 10$/group) were randomly assigned to one of the four possible compartments, again in a counterbalanced order. Phase I was conducted exactly as in the first experiment, and the time was measured to ensure that no strong spontaneous preference was expressed by the rats. In this second experiment, the vehicle was simply associated with the start box and the drug with the nonstart box in a conditioning period of 10 + 10 days of alternating presentations as in the previous experiment. Control animals received tap water intragastrically in both sides. The difference in time (Δt_2) between drug- and vehicle-paired compartments on test day was considered a measurement of preference for each rat.

In the locomotor-activity experiment, rats were pretreated with GHB, as described for CPP experiments. Thirty minutes after treatment, they were individually put into the motility cages, and horizontal activity was scored for 60 min. All data were collected every 10 min and processed with the Digiscan Analyzer.

The significance of individual differences (Δt) was analyzed by using Student's paired t-test. One-way analysis of variance, followed by post hoc Newman-Keuls, was performed for between-group comparisons.

2.2. Intravenous self-administration in drug-naive mice

Male CD1 mice (Harlan Nossan, Milan, Italy) weighing between 25 and 28 g were used. On arrival, animals were housed six per cage and acclimatized to laboratory conditions for at least 1 week before use.

Gamma-hydroxybutyrate was freshly dissolved in heparinized (1%) saline in a volume of 1 μl/kg/injection at the beginning of the experiments. NCS-382, kindly donated by Dr. M. Maitre, was freshly dissolved in saline solution and injected intraperitoneally in a volume of 1 ml/kg or in heparinized (1%) saline for the IVSA tests.

As previously described (Kuzmin et al., 1992, 1996a, 1996b; Martellotta et al., 1994, 1995), mice were tested in pairs in identical test cages. Each test cage had a frontal hole through which an infrared detector activated a cumulative recorder (Coulbourn Instruments, Basile, Italy) and operated a syringe pump (Life Science Instruments, CA, USA) to deliver solution contingent on a nose-poke response (NPR). A rear vertical chink was made on the opposite wall through which the tail was extended outside the box and taped to a horizontal surface, allowing access to the lateral tail veins with a 27G winged needle connected to the syringe through Teflon tubing. Each nose poke of an active mouse resulted in a contingent injection of 1.0 μl of either saline or the drug dissolved in saline, both to the active and a yoked passive mouse. Nose-pokes of the yoked control were counted but had no programmed consequences.

Mice were first placed in the test cage for 10 min (pretest) during which the tail was taped but no needle was inserted. Pairs of animals were selected on the basis of approximately equal levels of nose poking during the pretest and randomly allocated to the different experimental groups. After this time, needles were inserted in lateral tail veins, and intravenous injections were made contingent on each nose-poke response of the active mouse. Self-administration sessions lasted 30 min; each mouse was used only once.

As a gradual measurement of the reinforcing effect of the drug solution ("R" criterion), the logarithm of the ratio of the cumulative number of nose-poke responses between the active and the passive mice during the 30-min period of self-infusion minus the logarithm of the ratio of nose-poke responses during the pretest was used (Kuzmin et al., 1997). Logarithms were used to normalize distribution of data. Significance was determined by means of one-way analysis of variance followed by Newman-Keuls test.

2.3. Intravenous self-administration in rats

Male Long Evans rats (Harlan-Nossan, Milan, Italy) weighing between 300 and 350 g at the start of the experiments were used in the intravenous cocaine self-administration experiments. After the surgical insertion of a catheter, animals were individually housed.

Cocaine hydrochloride (Sigma, Italy) was dissolved in sterile saline solution at the dose of 0.5 mg/kg/injection. Gamma-hydroxybutyrate was dissolved in tap water in a volume of 5 ml/kg and administered at doses of 175, 350, and 700 mg/kg by intragastric route 30 min before starting the cocaine self-administration session.

The IVSA apparatus consisted of eight Plexiglas cages, 30 × 30 × 30 cm. Two holes, provided with fotobeam detectors, were made 2 cm above the floor, 15 cm apart. Nose poking in one of the holes (defined as active) switched on the infusion pump, injecting the cocaine solution into the animal's venous system. Nose poking in the other hole (defined as passive) had no effect on the pump. The assessment of self-administration schedules and the collection of data were programmed through PC software (Ecos, Italy).

Animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and implanted with silastic catheters inserted into the right external jugular vein, as previously described (Caine et al., 1993; Martellotta et al., 1994). The catheter was passed under the skin and exited in the mid-scapular region. Free passage of liquid through the catheters was checked before the start of each self-administration session with a solution of heparinized saline (100 IU/ml).

Five days after surgery, each rat was allowed access to cocaine at a dose of 0.5 mg/kg/injection under a continuous reinforcement schedule with the time-out corresponding to the infusion time (∼5 s), during 3-h daily sessions. Only rats that developed a stable pattern of cocaine intake with a range of less than 10% over three consecutive baseline sessions were selected for the study. Each dose was tested, once for each animal, in a random order, and a minimum of three no-pretreatment days separated each test day. Because not all animals completed the entire set of experiments (owing to catheter blockages), data were computed for independent rather than correlated samples. Each treatment, however, included a minimum of six subjects.

The number of injections earned in the 180-min session was recorded, and statistical analysis of the data was computed by using a one-way analysis of variance. Individual means comparisons were made by using the Newman-Keuls post hoc test. Nose-poking activity in the passive hole was virtually absent after a stable baseline of drug intake had been reached and throughout the experiment, so this factor was not computed in the analysis of data.

3. Results

3.1. Conditioned place preference

Fig. 1 shows the results of the effect of different doses of GHB on conditioned place preference. The mean times (in seconds) ± SEM spent by rats in the nonpreferred compartment before conditioning (i.e., baseline) were as follows: 281.85 ± 35 for vehicle group; 329.67 ± 26 for GHB 87.5; 211.78 ± 39 for GHB 175; 198.95 ± 32 for GHB 350; and 247.11 ± 29 for cocaine.

Compared with the finding in the preconditioning phase, on test day, all GHB- and cocaine-treated rats spent significantly more time on the nonpreferred side, showing a significant shift in preference toward the environment that had been associated with the drug. On the other hand, rats treated with water in both compartments (controls) exhibited no significant shift in preference. Between-group comparison revealed a significant difference with respect to the control group for each treatment, with the exception of the lowest GHB dose.

The results of the second experiment are summarized in Fig. 2, illustrating how animals injected with GHB developed a preference for the drug-paired side. The mean times (in seconds) ± SEM spent by rats in the nonstart compartment before conditioning (pretest) were as follows: 445 ± 14 for vehicle group; 467 ± 10 for GHB 87.5; 461 ± 8 for GHB 175; and 447 ± 23 for GHB 350. Statistical analyses revealed a significant difference between drug- and vehicle-paired compartments on test day only for drug-treated rats. Post hoc comparisons revealed that each of the two extreme doses was significant with respect to the central one, according to a bell-shaped curve. The saline-treated group did not exhibit any significant preference for either compartment. Furthermore, it should be noted that GHB induced CPP in 100% of treated animals.

Pretreatment with GHB at doses of 175, 350, and 700 mg/kg did not significantly affect spontaneous motor activity in any direction, as shown in Table 1.

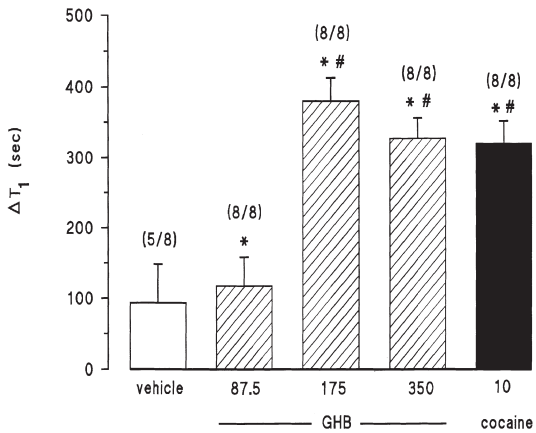

Fig. 1. Each bar represents the mean ± SEM (n = 8 rats/group) of differences in time spent in the nonpreferred (drug-paired) environment between postconditioning and preconditioning test sessions. Doses are expressed in milligrams per kilogram. Numbers in parentheses: number of animals that shifted positively from nonpreferred side/total number of animals in the group. *$p < 0.01$, Student's t-test for paired data (preconditioning and postconditioning). For comparison between groups: analysis of variance [$F(4,35) = 9.98$, $p < 0.01$] followed by Newman-Keuls test: #$p < 0.01$ vs. control group.

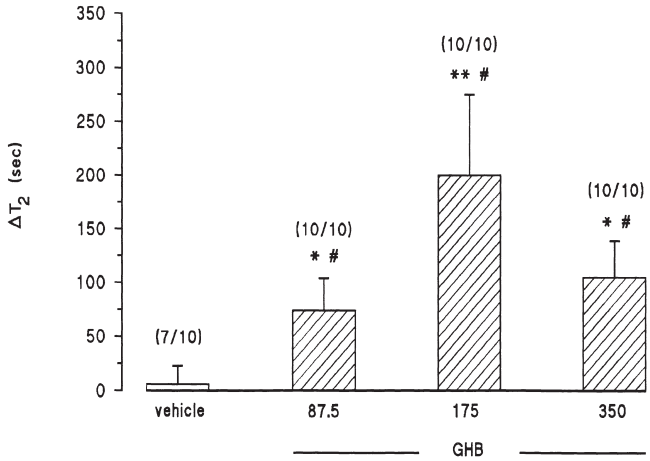

Fig. 2. Each bar represents the mean ± SEM (n = 10 rats/group) of differences observed between time spent in the drug-paired environment and time spent in the vehicle-paired environment. Doses are expressed in milligrams per kilogram. Numbers in parentheses: number of animals that showed preference for drug-paired side/total number of animals in the group. *p < 0.05, **p < 0.01, Student's t-test for paired data (drug- and vehicle-paired environment). For comparison between groups: analysis of variance [$F(3,36) = 4.51$, $p < 0.01$] followed by Newman-Keuls test: #$p <$ 0.01 vs. control group.

3.2. Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice

As shown in Fig. 3 (top), no statistically significant difference was observed in the mean number of NPRs from active and passive mice when vehicle injections were contingent on NPRs. Therefore, at these conditions, "R" did not differ from 0. Increasing concentrations of GHB significantly influenced self-administration in active mice, whereas no differences were observed in NPRs of passive yoked mice. Gamma-hydroxybutyric acid influenced "R" in a concentration-dependent manner; post hoc comparisons revealed that the lowest concentration tested, 0.01 mg/kg/injection, failed to significantly modify "R." Concentrations of 0.05 and 0.1 mg/kg/injection significantly increased "R" and were therefore considered to possess reinforcing properties. The highest GHB concentration tested (0.5 mg/kg/injection) failed to modify "R". Results obtained in the present experiment demonstrated that the reinforcing effect of GHB in drug-naive mice is concentration dependent, according to a bell-shaped curve.

Table 1
Spontaneous locomotor activity in GHB treated rats

Time	0	175	350	700
0′–30′	7007 ± 769.7	9267 ± 473.5	8297 ± 754.8	6564 ± 875.8
0′–60′	9439 ± 725	14057 ± 1132	11324 ± 777	12697 ± 1768

Values represent mean ± SEM (n = 6 rats/group) of cumulative locomotor activity counts over 30 and 60 min of observation. Doses are expressed as mg/kg (IG). ANOVA [$F(3, 20) = 2.82$; n.s.].

To verify whether the GHB reinforcing effect was specifically mediated by an interaction at the GHB receptor level, mice were pretreated with the specific GHB receptor antagonist NCS-382 at a dose of 12.5 mg/kg. This dose was chosen on the basis of separate experiments aimed at verifying whether NCS-382 would have evident side effects, particularly motor side effects that could aspecifically interfere with GHB reinforcing effects. Results obtained from these experiments showed that, only at doses of 50 mg/kg or more, i.p., was NCS-382 able to decrease spontaneous motor activity in this strain of mice significantly (data not shown). As shown in Fig. 3 (bottom), pretreatment with NCS-382 was able to antagonize GHB (0.1 mg/kg/injection) self-administration.

In a separate set of experiments (Fig. 4) aimed at defining whether NCS-382 could induce either reinforcing or ad-

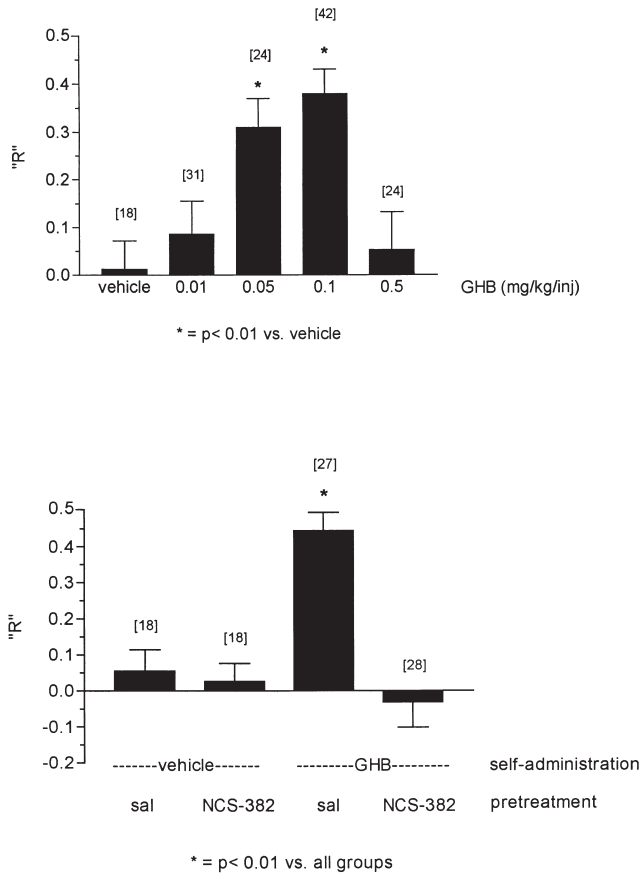

Fig. 3. Concentration-dependent gamma-hydroxybutyric acid (GHB) self-administration and antagonism of gamma-hydroxybutyric acid self-administration by NCS-382 in drug-naive mice. (Top) Each bar represents R ± SEM for each gamma-hydroxybutyric acid concentration. Number of pairs are indicated in parentheses. Analysis of variance [$F(4,134) = 7.73$, $p <$ 0.01] followed by Newman-Keuls test: *$p < 0.01$ vs. control group. (Bottom) Each bar represents R ± SEM for each group self-administering either vehicle or gamma-hydroxybutyric acid (0.1 mg/kg/injection). Mice were pretreated either with saline or NCS-382 (12.5 mg/kg, intraperitoneally) 10 min before the intravenous self-administration test. Number of pairs are indicated in parentheses. Analysis of variance [$F(3,87) = 13$, $p <$ 0.01] followed by Newman-Keuls test: *$p < 0.01$ vs. control group.

verse effects, we observed that, when NCS-382 (concentrations ranging from 0.1 to 0.5 mg/kg/injection) was made available contingent on NPRs, no significant differences were observed between NPRs of active and passive yoked mice.

3.3. Intravenous cocaine self-administration in rats

Animals pretreated with GHB reduced their cocaine intake with respect to basal values in a dose-dependent manner. The lowest dose of GHB tested was ineffective on basal cocaine intake, whereas doses of 350 and 700 mg/kg (intragastrically) were able to decrease the cocaine intake significantly with respect to the control group and with respect to each other, as revealed by post hoc comparisons (Fig. 5).

Fig. 6 shows the cocaine self-administration patterns of a representative rat during 3-h sessions. The animal strongly decreased its requests for cocaine in a constant manner during the entire session when pretreated with GHB at the dose of 700 mg/kg, whereas the lower dose (350 mg/kg) produced its effect mainly in the first part of the self-administration session. In neither case was there a total interruption of self-administration behavior. We observed no sedation or hypnotic episodes in GHB-pretreated rats during or after the self-administration sessions.

Animals that self-administer drugs at stable levels tend to adjust the dose during the session by modifying the response frequency (Koob, 1993). A decrease in the rate of responding usually occurs when the unit dose of the reinforcer is increased and vice versa. Therefore, the effect of GHB on the cocaine self-administration pattern could be considered similar to that of an increase in the cocaine unit dose.

4. Discussion

The present results show that GHB induces CPP in rats, is intravenously self-administered by drug-naive mice, and decreases intravenous cocaine self-administration in rats.

As shown in the first CPP experiment, the lowest dose tested did not produce CPP, whereas the subsequently higher dose elicited a maximal positive effect. This “step up” dose–effect relation, often observed in CPP experiments conducted with psychostimulants, has not yet been sufficiently clarified (Carr et al., 1989; Snead, 1994). In the second experiment, however, we found a significant bell-shaped dose–response curve for GHB-induced CPP. In this case, even the lowest dose was seen to induce preference. This dose–response curve is rather similar to that observed with other drugs of abuse (Bardo et al., 1995).

These results strongly confirm the hypothesis that GHB possesses rewarding properties and dispels all doubts concerning a possible aspecific effect caused by the lack of a clear dose–effect relation in the first experiment. Apart from the warning from the U.S. Food and Drug Administration concerning the potential abuse liability of GHB, possible rewarding effects of GHB were suggested by Colombo and colleagues, who found that GHB is preferred over water by rats in a free-choice drinking paradigm (Colombo et al., 1995a). Furthermore, the results demonstrate that the dose of GHB needed to induce CPP is in the same range as that used for oral self-administration.

In contrast with drugs of abuse such as opioids, cocaine, and amphetamines, in GHB-induced CPP, a greater number of trials are required before preference is observed. In a preliminary pilot experiment in which animals were conditioned for 5 + 5 trials, we failed to demonstrate GHB-induced CPP (data not shown). This apparently slow action of GHB might be caused by some unpleasant effect of the drug that subsequently disappears either because of the development of tolerance or because rewarding effects overcome the distaste. This slow action, however, is not unique to GHB. Similarly, alcohol, which has many features in common with GHB (Fadda et al., 1983; Gallimberti et al., 1992), needs a high number of conditioning trials (Bozarth, 1990). A further example of a slow-acting drug is fluoxetine

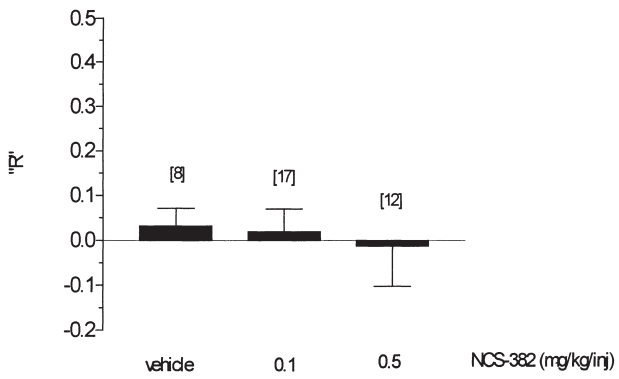

Fig. 4. NCS-382 self-administration in drug-naive mice. Each bar represents R ± SEM for each NCS-382 concentration. Number of pairs are indicated in parentheses., Analysis of variance [F(2,34) = 0.08, n.s.].

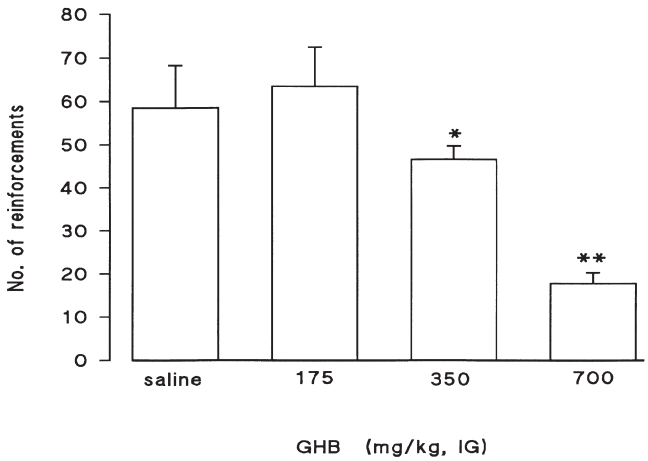

Fig. 5. Effect of intragastric gamma-hydroxybutyric acid (GHB) pretreatment on cocaine self-administration (0.5 mg/kg/injection) in rats with nose poking as operant paradigm. Each bar represents mean ± SEM of the cumulative number of reinforcements of six animals. Analysis of variance [$F(3,20) = 120.31$, $p < 0.01$] followed by Newman-Keuls test: *$p < 0.05$ and **$p < 0.01$ vs. control group.

hydrochloride, which has been shown to require 10 + 10 trials to induce CPP in rats (Collu et al., 1997).

It has also been shown that GHB pretreatment does not affect spontaneous locomotor activity in rats in any direction, therefore excluding any possible confounding variable deriving from locomotor-activating or sedative action of the drug.

Subsequently, we demonstrated that GHB is intravenously self-administered by drug-naive mice according to a concentration-dependent bell-shaped curve. Such a response to GHB is not qualitatively dissimilar to response obtained with this particular model of self-administration with the use of "classical" drugs of abuse such as cocaine, morphine, and nicotine (Kuzmin et al., 1992; Martellotta et al., 1995), suggesting that GHB is able to induce positive reinforcing effects in drug-naive mice. Gamma-hydroxybutyric acid reinforcing effects are completely antagonized by pretreatment with the specific GHB receptor antagonist NCS-382, indicating the GHB receptor as the primary target for GHB reinforcing effects.

Finally, the results of cocaine IVSA experiments in rats showed that GHB pretreatment significantly reduces the cocaine intake with respect to basal values in rats self-administering the unit dose of 0.5 mg/kg/injection. By causing a decrease in cocaine intake, GHB pretreatment seems to mimic the effect of changes in the unit dose of the reinforcer: a decreased rate of responding usually follows an increase in cocaine unit dose and vice versa. This effect led us to suppose a synergistic action of GHB on the reinforcing properties of cocaine.

All our findings are in agreement with a number of observations suggesting that GHB may interfere with the brain systems responsible for the expression of the acute reinforcing properties of drugs of abuse and for the expression of the neuroadaptative changes of the dependence process. Indeed, other findings have shown that GHB both reduces ethanol intake in alcohol-preferring rats and alleviates the withdrawal syndrome in ethanol-dependent rats and is self-administered by rodents (Colombo et al., 1995a). Moreover, GHB has been reported to ameliorate symptoms of the alcohol withdrawal syndrome (Gallimberti et al., 1989) and opiate abstinence in human beings (Gallimberti et al., 1993).

It is important to note that there are clinical reports of GHB use in combination with psychostimulants, such as methamphetamine (Frederick et al., 1994), and GHB interactions with stimulants have been detected, even with controversial results, by Beardsley and colleagues (Beardsley et al., 1996). These findings may lead to speculation that the effects of GHB may be mediated by a neurochemical substrate common to alcohol, opiates, and cocaine.

The intimate mechanism through which GHB affects de-

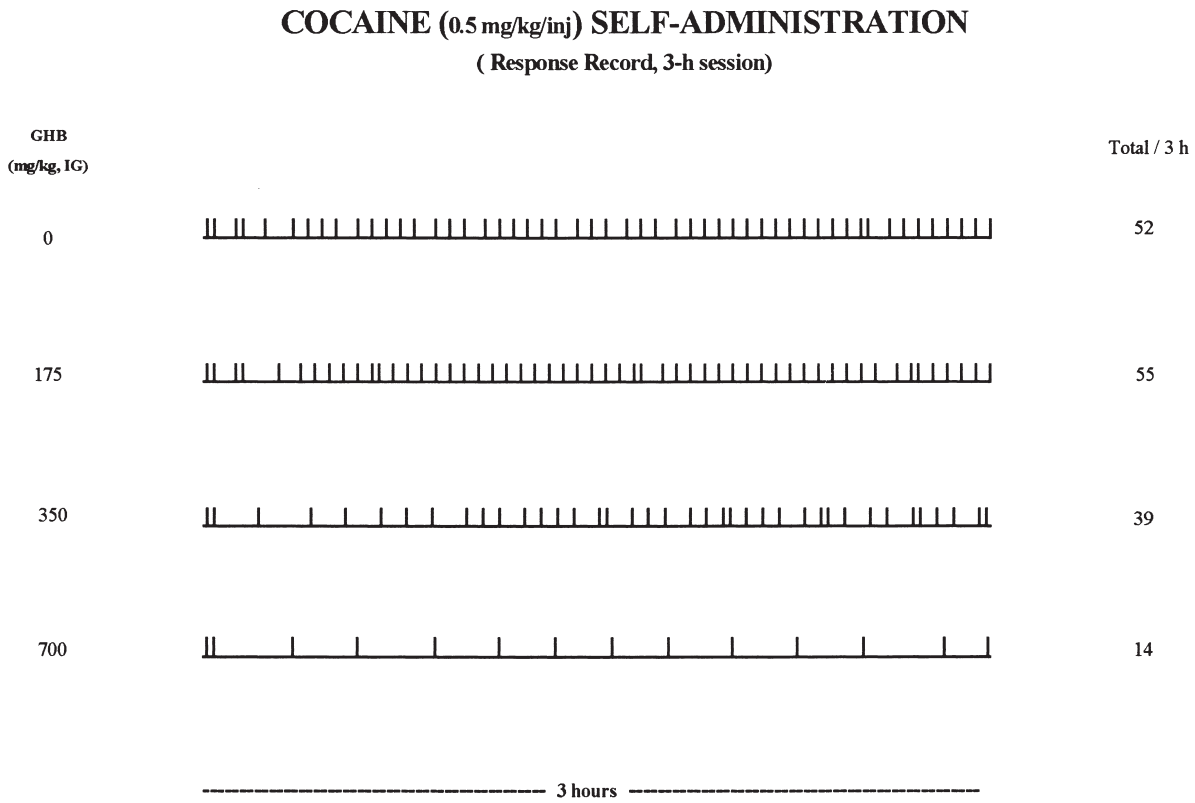

Fig. 6. Cocaine self-administration pattern for a representative rat after intragastric acute pretreatment with gamma-hydroxybutyric acid (GHB). Each event record represents a separate session and each mark represents an intravenous infusion of cocaine (0.5 mg/kg/injection). Injections were delivered at equal time intervals and, as the doses of gamma-hydroxybutyric acid were increased, the regular pattern of responding was maintained, though with longer interreinforcement intervals.

pendence on several abused drugs is still unknown. Lacking further experimental evidence to establish a mechanistic relation for our results, we can only raise a hypothesis.

The brain dopamine system has been suggested as a critical neurochemical component of the neural circuity mediating the reinforcing properties of some drugs of abuse (Koob, 1992). An activation of the dopaminergic system by GHB has been well documented in several reports. Low doses of GHB stimulate the firing rate of dopaminergic neurons in the substantia nigra (Broughton & Mamelak, 1979; Diana et al., 1991) and increase dopamine release from the caudate nucleus of cats and rats (Cheramy et al., 1977; Hechler et al., 1991). After an initial attenuation of dopamine levels (Gessa et al., 1966), GHB was shown to induce an enhancement of tyrosine hydroxylase activity and a stimulation of dopamine release (Morgenroth et al., 1976; Spano et al., 1971). Finally, Maitre and colleagues (Maitre et al., 1990) found that the GHB receptor antagonist NCS-382 is able to inhibit GHB-induced stimulation of dopamine release, demonstrating the specificity of this action.

Because an activation of dopaminergic transmission is considered to play a primary role in the rewarding effect of drugs of abuse (Di Chiara & Imperato, 1988), the hypothesis that GHB, similarly to opioids, cocaine, nicotine, and, in particular, alcohol (Imperato & Di Chiara, 1986), may exert its rewarding effect through a stimulation of dopaminergic transmission cannot be ruled out. However, direct evidence to support this hypothesis has yet to be provided.

Although the literature concerning GHB–dopamine interaction is controversial (Feigenbaum & Howard, 1996a), GHB has been shown to interact with specific receptors localized mainly in the dopaminergic structures of the brain (Benavides et al., 1982b) and participate in the regulation of dopamine synthesis (Morgenroth et al., 1976) and release (Gessa et al., 1966; Martellotta et al., 1994). Besides, the importance of experimental conditions and route of administration in evaluating the effect of GHB on dopamine release has been underscored (Howard & Feigenbaum, 1997). Given this evidence, modulation of the brain dopamine system, possibly within areas of the limbic forebrain, could present a candidate mechanism that explains the effect of GHB on cocaine reinforcement.

Otherwise, GHB interacts with other neurotransmitter systems, including GABA, opioids (Cash, 1994), acetylcholine (Sethy et al., 1976), and serotonin (Maitre, 1997), and the possibility that GHB may interfere with these mechanisms cannot be ignored.

Many effects of GHB, such as cross-tolerance against ethanol (Colombo et al., 1995b), a decrease in alcohol withdrawal symptoms (Gallimberti et al., 1989), and abuse in human beings (U.S. Food and Drug Administration, 1991; Frederick et al., 1994; Galloway et al., 1997), are similar to those induced by barbiturates and benzodiazepines, which primarily interact with GABA receptors, as discussed by Nissbrandt and Engberg (1996). However, the possibility that the GHB effect on alcohol intake could be due to an interaction at the $GABA_A$ level has been ruled out (Biggio et al., 1992). In this regard, there is a growing literature suggesting a relation between GHB and $GABA_B$-mediated mechanisms (Feigenbaum & Howard, 1996b), although at present this interaction seems limited to an involvement in the pathogenesis of experimental absence seizures (Snead, 1996). On the other hand, there is evidence that GHB and $GABA_B$ binding sites are completely different in their regional distribution, apart from layers I–III of the rat's cerebral cortex (Snead, 1994).

A GHB-induced accumulation of Met-enkephalin in the striatum has been reported (Gobaille et al., 1994), possibly through a nigrostriatal, dopamine-mediated mechanism. This potentiation of the endogenous opioid system could explain some effect of GHB on drug craving and addiction. Gamma-hydroxybutyric acid also increases the serotonin turnover in the striatum and mesolimbic areas (Waldmeier & Fehr, 1978), possibly by an increase in the availability of tryptophan in brain. Connected to this effect and leading to an accumulation of tryptophan catabolites, GHB might influence glutamatergic activity, in particular by a modulation of *N*-methyl-D-aspartate receptors (Stone, 1993). Even in this case, however, a relation with the dopaminergic system has been suggested(Maitre, 1997).

In conclusion, irrespective of the mechanisms, the present results further support the hypothesis of an abuse liability of GHB, confirming its involvement in drug-abuse mechanisms. They also provide evidence of the possibility that GHB serves as a cocaine substitute and, consequently, might be a useful pharmacologic agent to be used in the treatment of cocaine addicts, in addition to its current use for alcoholics.

Acknowledgments

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Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence

Luigi Gallimberti[a,*], Maurizio Raffaele Spella[b], Carlo Alberto Soncini[b], Gian Luigi Gessa[c]

[a]*Department of Medical and Surgical Sciences, Addictive Medicine Unit, University of Padua, Via Giustiniani 2, I-35100, Padua, Italy*
[b]*Addictive Behavior Medical Center, Via san Mattia 16, I-35121, Padua, Italy*
[c]*Bernard B. Brodie Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124, Cagliari, Italy*

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Abstract

We briefly review two double-blind, placebo-controlled surveys conducted in this laboratory with the aim of evaluating the efficacy of gamma-hydroxybutyric acid in the treatment of alcohol withdrawal syndrome as well as alcohol craving and consumption in alcoholics. In the first study, acute administration of 50 mg/kg gamma-hydroxybutyric acid, a nonhypnotic dose in alcoholic patients, resulted in a rapid and significant reduction of the severity score of alcohol withdrawal signs and symptoms that lasted as long as 7 hours. In the second study, treatment with 50 mg/kg/day gamma-hydroxybutyric acid for 3 consecutive months (1) reduced the number of daily drinks by approximately 50%, (2) increased the days of abstinence approximately threefold, and (3) reduced the alcohol craving score by up to 60%. These results feature gamma-hydroxybutyric acid as an effective agent for the treatment of alcohol dependence. Data on the effect of gamma-hydroxybutyric acid on opiate withdrawal syndrome also are reviewed. Administration of 25 mg/kg induced a marked reduction of opiate withdrawal score in both heroin- and methadone-dependent subjects. Finally, we report the cases of adverse reactions to and abuse of gamma-hydroxybutyric acid revealed in a retrospective analysis of patients recruited in this laboratory over a 10-year period.

Keywords: Gamma-hydroxybutyric acid; Alcohol withdrawal syndrome; Alcohol craving and consumption; Opiate withdrawal syndrome; Alcoholics; Heroin addicted

1. Introduction

Gamma-hydroxybutyricacid (GHB) was synthetized in the late 1950s by Henri Laborit with the intent of discovering a gamma-aminobutyric acid (GABA) analogue capable of crossing the blood–brain barrier (Laborit et al., 1960a). Gamma-hydroxybutyric acid accomplished the aim of penetrating the blood–brain barrier but exerted a number of central effects not mediated by the GABA receptors. So it was that this drug started down a road that, after more than 40 years, still seems to be far from the end.

Gamma-hydroxybutyric acid was initially used as a general anesthetic (Laborit et al., 1960b) and hypnoinducer (Laborit & Weber, 1965). Moreover, the anxiolytic (De Couedic & Voisse, 1964) and antidepressive (Rinaldi et al., 1967) properties of GHB were soon recognized. Gamma-hydroxybutyric acid was also tested for the treatment of (1) schizophrenia (Levy et al., 1983; Schuls et al., 1981; Tanaka et al., 1966), (2) acute alcohol intoxication (Benda et al., 1960; Langlois et al., 1960), and (3) opiate (De Couedic & Voisse, 1964) and barbiturate (Appia, 1967) dependence. Because of its ability to stimulate REM sleep, GHB was also used to "shorten" the psychoanalytical approach in psychotherapy (Appia, 1967).

In the late 1970s, in view of the proved hypnoinducing effect of GHB, Broughton and Mamelak (1979) tested its efficacy in the treatment of narcolepsia. Administration of GHB restored the cytoarchitectonics of narcoleptic sleep (characterized by scarce and fragmented REM and reduced percentage of slow-wave deep sleep). Subsequently, Snead and colleagues (Snead, 1977, 1978, 1990; Snead & Bearden, 1980; Snead & Liu, 1993; Snead et al., 1980) showed that, in rhesus monkeys, GHB was capable of evoking a clinical picture resembling that of petit mal epilepsy in human beings. Further studies in the same laboratory featured GHB-induced absence seizures as a suitable experimental model for testing potentially effective antagonists for petit mal epilepsy (Snead, 1992). According to this hypothesis, the antiepileptic agent valproate sodium was reported to normalize the GHB-induced petit mal picture in monkeys. Interestingly, valproate sodium is a drug routinely used in the treat-

* Corresponding author. Tel.: +39-49-662026; fax: +39-49-8780483.
E-mail address: l.gallimberti@libero.it (L. Gallimberti).

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ment of alcohol withdrawal syndrome (Rosenthal et al., 1998) and is capable of enhancing brain levels of GHB (Snead & Bearden, 1980).

In the 1980s, data obtained by our group (reviewed herein) provided evidence that GHB is an effective treatment in alcohol dependence. More recent data suggest that GHB may be effective in opiate dependence. Owing to its low toxicity (Palatini et al., 1993), rapid metabolism (Laborit, 1964), and antioxidant properties (Mamelak, 1977), GHB is currently being reevaluated as an anesthetic agent in cases of (1) compromised renal and hepatic functionality, (2) cerebral edema, and (3) ischemia, as well as in (4) hypovolemic states (Kleinschmidt & Mertzlufft, 1995).

2. Gamma-hydroxybutyric acid and alcohol dependence

Different lines of experimental evidence have demonstrated the effectiveness of GHB in animal models of alcoholism. Briefly, (1) the acute administration of GHB to rats rendered physically dependent on alcohol reduced the intensity of alcohol withdrawal signs; and (2) nonsedative doses of GHB, administered acutely, produced a dose-dependent reduction (as much as 70% compared with findings in saline-treated controls) of voluntary alcohol intake in selectively bred alcohol-preferring (P) and Sardinian alcohol-preferring (sP) rats (Gessa et al., 2000). Furthermore, (1) cross-tolerance to the motor-impairing effects of GHB and alcohol has been observed, suggesting the presence of common adaptive changes in neural substrates to chronic alcohol and GHB; (2) low to moderate doses of alcohol significantly reduced voluntary GHB intake in GHB-consuming Sardinian alcohol-preferring rats; and (3) both alcohol and GHB possess similar discriminative stimulus effects (i.e., the animal correlate of human subjective feelings elicited by a psychoactive drug), as suggested by the symmetrical generalization between the two drugs (Gessa et al., 2000). On the basis of these results, we suggested that GHB exerts its reducing effects on antialcohol effects by mimicking alcohol actions in the central nervous system.

The aforedescribed results in preclinical experiments prompted us to evaluate the efficacy of GHB in reducing alcohol withdrawal symptoms as well as alcohol craving and consumption in alcoholics. The first randomized double-blind survey (Gallimberti et al., 1989) recruited 23 patients, who met the *Diagnostic and Statistical Manual of Mental Disorders* DSM III-R criteria for alcohol withdrawal syndrome. Patients were divided into two groups and treated with 50 mg/kg/day GHB ($n = 11$) and placebo ($n = 12$). Gamma-hydroxybutyric acid and placebo were given orally as a syrup. Six withdrawal signs and symptoms (namely: tremors, sweating, nausea, anxiety, depression, and restlessness) were evaluated in each patient 30 min before and 1, 2, 3, 5, and 7 h after GHB administration. Each symptom was scored on a 4-point scale (0 to 3, paralleling the increased severity); therefore, the individual score ranged from 0 to 18 points.

As shown in Table 1, the mean score of alcohol withdrawal syndrome in the placebo group increased significantly over the 7-h observation period. In contrast, GHB administration resulted in a decrease in alcohol withdrawal score, which had been apparent within the first hour of observation. Slight and transient vertigo was the only side effect described by GHB-treated patients. No patient reported somnolence after the administration of GHB. These results were later confirmed by findings from an open study by Moncini and colleagues (Moncini et al., 2000).

A second study carried out in our laboratory assessed the efficacy of GHB on craving for alcohol as well as consumption of alcoholic beverages (Gallimberti et al., 1992). This survey included 82 patients with a history of alcoholism, according to DSM III-R criteria, of 5 years or more. Patients were divided into two groups and received, under a randomized double-blind experimental design, 50 mg/kg/day GHB and placebo for a period of 3 months. Gamma-hydroxybutyric acid and placebo were administered orally (in form of syrup) three times per day. Patients underwent the initial interview and the first drug administration under a day-hospital regimen; they were subsequently monitored as outpatients and examined on the first three days from 8:00 A.M. to 6:00 P.M. and thereafter at weekly intervals. Craving for alcohol was defined as the overwhelming preoccupation and urge for alcohol. The intensity of craving was measured by a questionnaire based on that conceived by Stunkard and Messick (1983) for the evaluation of dietary restraint, disinhibition, and hunger. The questionnaire contained 11 yes-or-no questions, for which each "yes" yielded one point. Alcoholuria as well as the alcohol consumption self-reported

Table 1
Effect of gamma-hydroxybutyric acid (GHB) (50 mg/kg, per os) on alcohol withdrawal syndrome in alcoholics

	Total score					
Treatment group (no. of patients)	30 min before treatment	After treatment (h) 1	2	3	5	7
GHB (11)	12.6 ± 6.1	7.2 ± 3.9*	4.2 ± 3.1**	2.1 ± 1.6**	1.5 ± 1.7**	2.6 ± 1.3**
Control (12)	11.8 ± 5.7	11.8 ± 4.7***	11.3 ± 3.5***	12.6 ± 9.2***	13.6 ± 6.5***	14.7 ± 4.3***

Values are means ± SD.
*$p < 0.05$
**$p < 0.01$ (Pratt's test for comparison of scores before and after treatment).
***$p < 0.05$ (Mann-Withney test for comparison of control and gamma-hydroxybutyric acid [GHB] groups).

and reported by relatives were used to assess alcohol intake. Finally, a once-monthly examination of the main clinical and hematochemical parameters was performed.

Among the 82 patients who were enrolled in the study, 71 (36 and 35 in the GHB and placebo group, respectively) completed the course. At the commencement of treatment, no difference in severity of alcoholism and alcohol intake was observed between GHB and placebo groups. In the 3-month treatment period, no reduction was revealed regarding the number of daily drinks and days of abstinence in the placebo group (Table 2). In contrast, GHB-treated patients reduced the number of drinks that they consumed daily by approximately 50% and increased the days of abstinence approximately threefold (Table 2). As shown in Table 3, GHB significantly reduced the alcohol craving score. This effect was already apparent in the first month and persisted throughout the entire treatment period. Placebo treatment produced only a modest reduction in craving score in the first month (Table 3).

The results of this study were confirmed by findings from an open study conducted by Addolorato and colleagues, in which 77% of subjects remained abstinent throughout a 6-month treatment with 50 mg/kg/day GHB (Addolorato et al., 2000), and by results from a double-blind survey carried out by Moncini and coworkers, reporting a reduction in relapse rate and alcohol craving score in patients receiving 50 mg/kg/day GHB (Moncini et al., 2000). More recently, results from a study by Addolorato and colleagues demonstrated that therapy with GHB can be improved by a greater fractioning of the daily dose of drug (Addolorato et al., 2000). Indeed, alcoholic patients who did not respond to the conventional fractioning of GHB daily dose (three administrations per day) benefited, in regard to an increased number of abstinence days and a reduction in the alcohol craving score, from a greater fractioning (six times per day) of the same daily dose of GHB.

3. Gamma-hydroxybutyric acid and heroin dependence

The first observation on the efficacy of GHB in the treatment of opiate dependence dates to 1964, when De Couedic and Voisse reported "at least two cases in which GHB seemed to have replaced morphine-like drugs ... by placating the state of need typical of the drug addict" (DeCouedic & Voisse, 1964). By using GHB in the management of alcohol withdrawal syndrome in a number of alcoholics who concomitantly abused heroin, we observed that GHB suppressed not only the alcohol withdrawal symptoms, but also the heroin withdrawal symptoms. Therefore, we performed a double-blind, placebo-controlled trial to verify whether GHB was effective in suppressing the withdrawal syndrome in heroin- and methadone-dependent subjects.

In this study (Gallimberti et al., 1993), 22 patients were heroin addicts, with a 3–6 year history of heroin use, and 19 subjects had been undergoing a maintenance treatment with methadone (30–60 mg/day for at least 6 months before the start of the survey). All patients had expressed their interest in discontinuing opiate consumption. They were hospitalized for 8 days. On admission, patients underwent a full medical and psychiatric examination and tests were performed to detect opiate metabolites, amphetamine, cocaine, benzodiazepines, barbiturates, cannabinoids, and alcohol. Withdrawal symptoms were assessed by using the scale described by Gold and associates (Gold et al., 1978). Briefly, 21 items (i.e., signs and symptoms associated with opiate withdrawal syndrome such as craving, anxiety and restlessness, tremors, yawning, goose flesh) were rated as present (score 1) or absent (score 0). Gamma-hydroxybutyric acid (25 mg/kg) and placebo were administered orally every 2 to 6 hours for 8 consecutive days.

All patients showed an increase in withdrawal score during the 3 hours immediately before treatment. Table 4 shows the effect of the first administration of GHB and placebo. Gamma-hydroxybutyric acid induced a marked reduction in withdrawal score in both heroin- and methadone-dependent subjects. The gamma-hydroxybutyric acid effect had a rapid onset (it was apparent within 15 min after drug administration) but short duration (at the 3-h observation interval, withdrawal score tended to increase). When the withdrawal items were evaluated singly, GHB was found to be

Table 2
Effect of gamma-hydroxybutyric acid (GHB) (50 mg/kg/day, per os) on alcohol consumption in alcoholics

	During the 3-month treatment period		
Response	Placebo	GHB	*p*
Daily drinks (mean ± SEM)	9.3 ± 0.7	4.7 ± 0.4	<0.01
% of abstinent days (mean ± SEM)	8.4 ± 1.6	25.9 ± 3.1	<0.001

Each value is the mean ± SEM from 35-placebo and 36 gamma-hydroxybutyric acid (GHB)-treated subjects. Values of alcohol intake before treatment were based on a single interview, whereas those during treatment were obtained by weekly interviews (means ± SEM). Therefore, the statistical significance of the results was calculated by comparing the values of GHB versus placebo, during the 3-month treatment period (Student's *t*-test).

Table 3
Effect of gamma-hydroxybutyric acid (GHB) (50 mg/kg/day, per os) on alcohol craving

	Craving score	
Month of treatment	Placebo	GHB
Before treatment	8.5 ± 0.3	8.9 ± 0.5
1st month	5.1 ± 0.6**	2.1 ± 0.1**
2nd month	7.5 ± 0.4	3.3 ± 0.4*,**
3rd month	7.6 ± 0.3	3.1 ± 0.6*,**

Data are the means ± SEM obtained by averaging the scores of the 4 weekly interviews during each month. Baseline scores were those of the first visit before treatment (maximum score 11). *$p < 0.001$ with respect to placebo value, **$p < 0.001$ with respect to basal value by Student's *t*-test.

In consideration of multiple comparisons, to protect against false-positive results, level of significance was fixed as follows $n = 0.05/9 = 0.0055$; therefore, values of $p > 0.0054$ were considered statistically not significant.

effective in reducing all signs and symptoms with the exception of diarrhea and insomnia. All patients reported relief from subjective distress. On subsequent days, the withdrawal score for the GHB-treated patients remained constantly reduced in comparison with that for the placebo-treated subjects. On the eighth day, GHB administration was interrupted and patients were observed for a period of 5 to 6 hours. Subsequently, patients received an intravenous injection of naloxone (0.4 mg); no withdrawal signs and symptoms were recorded. The results of this study suggest that GHB is effective in suppressing opiate withdrawal in human beings.

The preceding results were later confirmed in a study carried out by our group (Gallimberti et al., 1994), in which GHB (given orally at a dose of 300 mg/kg/day, eight times per day) alleviated opiate withdrawal symptoms in two opiate addicts who had abruptly interrupted long-term methadone treatment. The antiwithdrawal effect of GHB (50–150 mg/day/day) in opiate addicts was also confirmed by Fischer and coworkers (Fischer et al., 1995). In contrast, minimal effects of GHB on naloxone-precipitated opiate withdrawal were described by Rosen and colleagues (Rosen et al., 1996). In this study, opiate-dependent inpatients maintained on the opioid levorphanol were given a challenge oral dose of GHB (15 or 30 mg/kg) or placebo followed 1 hour later by an intravenous injection of naloxone (0.4 mg/70 kg). With the exception of an attenuation of "hot/cold" feelings in both GHB-treated groups, no differences in multiple withdrawal measures were recorded among groups. The different timing of GHB administration—postwithdrawal in our studies, before naloxone in the study by Rosen and colleagues (Rosen et al., 1996)—and the reversal of putative opioid receptor-mediated, antiwithdrawal effects of GHB were hypothesized to account for these discrepancies (Rosen et al., 1996). Further studies are therefore necessary to give a definitive answer on the efficacy of GHB in the treatment of opiate withdrawal syndrome.

Interestingly, a double-blind survey by Gerra and colleagues investigated the effect of the combination of GHB (1750 mg/day, administered orally three times per day) plus naltrexone (50 mg/day), in comparison with placebo plus naltrexone, on craving for heroin in heroin addicts (Gerra et al., 1994). The addition of GHB resulted in a significant reduction in the heroin-craving score and in the number of drop-outs. Finally, the consumption of other addictive drugs (namely, benzodiazepines, cannabis, cocaine, and alcohol) also was reduced.

4. Gamma-hydroxybutyric acid and adverse reactions

In the past 10-year period of GHB use in the treatment of dependence on alcohol and different psychoactive substances, we have observed a number of adverse reactions, largely induced by an inadequate use of the drug. These reactions include vertigo, nausea, vomiting, asthenia, epileptic-like tonic-clonic seizures, confusion, agitation, hallucinations, respiratory insufficiency, and loss of consciousness. These reactions, often occurring after combined intake of GHB and other substances such as alcohol, amphetamine, and heroin, are similar to those observed by other investigators (Center for Disease Control, 1991; Chin et al., 1992; Dyer, 1991; Einspuch & Clark, 1992; Ferrara et al., 1995; Galloway et al., 2000; James, 1996; Luby et al., 1992; Ross, 1995; Sanguineti et al., 1997).

The present retrospective assessment regards 195 patients meeting the DSM III-R criteria for dependence from psychoactive substances. All patients were included in an integrated treatment program (pharmacological and psychosocial). In some cases, GHB was administered at high doses (as much as 300 mg/kg/day), the overall daily dose was fractioned in three to eight administrations, and treatment duration varied from a few weeks to 10 years. Each event was either observed by the clinician or reported by the patient and classified as follows: slight (no complication, no treatment), medium (some complication, no treatment), and serious (life endangering, death or permanent lesions). Each group was divided into three subgroups on the basis of the type of dependence: 138 patients were alcohol dependents, 23 were opiate dependents, 34 were polydrug dependents.

Slight adverse reactions were observed in 159 patients (corresponding to 81.5%): vertigo ($n = 113$), vomiting ($n = 41$), and nausea ($n = 23$). Moderate adverse reactions were monitored in 38 subjects (15.4%): general anesthesia (lasting from 30 min to 30 h) associated with bradypnea ($n =$

Table 4
Effect of gamma-hydroxybutyric acid (GHB) (25 mg/kg, per os) on opiate withdrawal syndrome in heroin-dependent and methadone-maintained patients

Drug Withdrawn (no. of subjects)	Treatment	Before treatment	Total withdrawal score (Min after treatment)					
			15	30	60	90	120	180
Heroin (14)	GHB	15.1 ± 1.5	4.8 ± 0.9[a]	2.5 ± 1.9[a]	2.1 ± 0.8[b]	1.6 ± 1.2	1.3 ± 0.1[b]	5.1 ± 1.9[b]
Heroin (8)	Placebo	16.7 ± 1.0	15.3 ± 1.8[c]	13.7 ± 2.1[c]	18.1 ± 2.1[c]	16.1 ± 2.4[c]	20.0 ± 3.1	19.3 ± 2.1[c]
Methadone (13)	GHB	13.4 ± 2.0	5.1 ± 2.3[a]	2.8 ± 2.4[a]	1.4 ± 1.6[b]	2.1 ± 2.1	1.9 ± 1.2[b]	3.3 ± 1.3[b]
Methadone (6)	Placebo	12.3 ± 1.7	12.7 ± 1.4[c]	13.4 ± 1.2[c]	12.7 ± 2.5[c]	15.2 ± 1.1[c]	16.1 ± 1.8[c]	15.9 ± 1.7[c]

[a] $p < 0.01$
[b] $p < 0.001$ (Pratt's test for comparison of scores before and after treatment).
[c] $p < 0.01$ (Mann-Whitney test for comparison of placebo and gamma-hydroxybutyric acid (GHB) group).
Total withdrawal scores are mean ± SEM.

10), psychotic reactions ($n = 2$), convulsive reactions ($n = 2$), and diarrhea ($n = 24$). No cases of serious adverse reaction were observed.

Adverse reactions with varying degrees of severity occurred in all three groups of patients (alcoholics, opiate dependents, polydrug addicts) without any statistical difference. Nevertheless, symptoms completely disappeared without leaving any consequence.

5. Gamma-hydroxybutyric acid and abuse

Of the aforementioned 195 patients, 29 cases (14.9%) showed GHB abuse. It should be pointed out that, although several episodes of GHB abuse have been reported in the scientific literature (Galloway et al., 2000), the events described by us occurred within an integrated treatment for alcohol, heroin, or polydrug dependence.

We adopted a rather broad definition of abuse—namely, any use of GHB greater than the dose prescribed by the physician. According to this definition, three groups of GHB abusers were identified. Group 1 consisted of 7 patients who self-increased their daily dose of GHB (as much as two times the recommended dose) because they considered the dose prescribed by the physician insufficient; no sign of intoxication was ever observed in these patients. Group 2 included 12 patients who showed phenomena of acute intoxication from GHB, often after having increased the drug dose in a similar fashion to that by subjects in group 1. These episodes of intoxication varied widely in frequency (from 1 to 10 times per year) and severity. However, despite the episode of acute intoxication, the overall behavior of these patients greatly improved in comparison with that at the time of admittance to treatment. Patients seemed to behave in the same ways as drinkers who do not meet the diagnostic criteria for alcohol dependence but tend to get drunk a certain number of times per year. Group 3 was made up of 10 GHB-dependent patients, whose behavior resembled that observed in serious cases of alcoholism and drug addiction. Subjects appeared to be constantly engaged in searching for the euphoric, empathogenic, hypnoinducing, anxiolytic, and antidepressive effects of GHB. Life without GHB was described as being unacceptable.

The percentage of cases of GHB abuse identified in this 10-year survey (14.9%) is similar to that (10.1%) reported by Addolorato and colleagues in a study conducted to evaluate the anticraving effects in alcoholics (Addolorato et al., 2000). In the latter study, GHB abuse was defined as a drug intake six to seven times as high as the dose suggested by the physician.

6. Conclusions

Gamma-hydroxybutyric acid appears to meet at least four of the five criteria established by the American Psychiatric Association on the efficacy of pharmacotherapies to be employed in the treatment of alcohol and opiate dependence (American Psychiatric Association, 1995). Indeed GHB (1) is effective in the treatment of withdrawal (criterion 1), (2) possesses anticraving properties capable of diminishing the reinforcing effects of the drug (criterion 2), (3) can be used as a substituting drug (criterion 4), and (4) is effective in alleviating certain aspects of psychiatric disorders (namely, anxiety, depression, and insomnia), which show a high degree of comorbidity with alcohol and opiate dependence (criterion 5).

The abuse potential of the drug (limited, however, to 10%–15% of the subjects) should not endanger the profile of GHB as a safe and effective agent for the treatment of alcohol and heroin dependence. In our experience, a reduction in its abuse potential in outpatients can be obtained when the drug is entrusted to a referring familiar member of the patient.

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ALCOHOL

Abuse and therapeutic potential of gamma-hydroxybutyric acid

G.P. Galloway[a,b,*], S.L. Frederick-Osborne[a], Richard Seymour[c], Sarah E. Contini[c], David E. Smith[c,d]

[a]*Drug Detoxification, Rehabilitation, and Aftercare Program, Haight-Ashbury Free Clinics, 603 Clayton Street, San Francisco, CA 94117, USA*
[b]*Department of Clinical Pharmacy, University of California, San Francisco, CA, USA*
[c]*Office of the President, Haight-Asbury Free Clinics, 612 Clayton Street, San Francisco, CA 94117, USA*
[d]*Institute of Health and Aging, University of California, San Francisco, CA USA*

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Abstract

Gamma-hydroxbutyric acid is a compound found in mammalian brain that is structurally related to the neurotransmitters gamma-aminobutyric acid and glutamic acid. Gamma-hydroxybutyric acid effects dopaminergic systems in the brain and may be a neurotransmitter. Gamma-hydroxybutyric acid was first reported as a drug of abuse in 1990 and continues to be abused by bodybuilders, participants of "rave" dance parties, and polydrug abusers. Physical dependence can develop after prolonged, high-dose use, and overdoses have been widely reported. Its use in sexual assaults as a "date rape" drug and availability on the internet have recently emerged. Gamma-hydroxybutyric acid has established efficacy as an anesthetic agent, and preliminary evidence supports its utility in the treatment of alcohol dependence, opiate dependence, and narcolepsy.

Keywords: Gamma-hydroxbutyric acid; Abuse; Withdrawal syndrome; Internet; Date rape; Anesthesia; Alcohol dependence; Opiate dependence; Narcolepsy

1. Introduction

Gamma-hydroxbutyric acid (GHB) is a putative neurotransmitter, structurally related to gamma-aminobutyric acid (GABA) and glutamic acid, that has been the subject of investigation since 1960. Gamma-hydroxybutyric acid was first studied for its ability to induce short-term coma and surgical anesthesia. Subsequent work focused on its ability to create absence (petit mal) seizures and thus to facilitate evaluation of antiseizure medications. Despite potential adverse effects, including dependence and withdrawal, GHB has recently gained popularity on the illicit drug market in the United States. At the same time, it has also been studied as a potential therapeutic agent in such disparate conditions as narcolepsy, alcohol dependence, and opiate dependence. In this article, we will first describe the pharmacologic and pharmacokinetic activity of GHB and then review the findings regarding the potential abuse and therapeutic uses of this compound.

2. Neuropharmacology

Gamma-hydroxybutyric acid is a naturally occurring compound in mammalian brain, and data from animal studies indicate that it meets many of the criteria for being a neurotransmitter [(Benavides et al., 1982; Hechler et al., 1992; Roth & Giarman, 1970; Rumigny et al., 1981; Snead, 1987), but see also (Cash, 1994)]. Gamma-hydroxybutyric acid is, as mentioned earlier, structurally related to GABA and glutamic acid, is a metabolite of GABA, and binds noncompetitively to the $GABA_B$ but not to the $GABA_A$ receptor (Serra et al., 1991; Snead, 1996). However, the binding of GHB to the $GABA_B$ receptor has been demonstrated only at supraphysiologic (Serra et al., 1991; Xie & Smart, 1992a, 1992b) concentrations, and there are both high- and low-affinity GHB receptor sites whose distribution differs notably from that of GABA receptors (Snead et al., 1990). These receptors are highly specific for GHB (Benavides et al., 1982) and are most densely located in the hippocampus, in the cortex, and in dopaminergic areas such as the striatum, olfactory tracts, and substantia nigra (Hechler et al., 1992).

Gamma-hydroxybutyric acid has generally been reported to inhibit dopamine release (Ellinwood et al., 1983; Howard & Feigenbaum, 1997). This inhibition of dopamine impulse activity, accompanied by the activation of tyrosine hydroxylase (Gessa et al., 1966), results in the accumulation of striatal dopamine (Scrima et al., 1990). Some investigators have found increased dopamine release with GHB administration, but differences in study procedures, such as GHB dose and use of anesthesia, may explain these divergent results. An analysis by Howard and Feigenbaum (1997) sug-

* Corresponding author. Tel.: 415-487-3678; fax: 415-487-1081.
E-mail address: gantt@itsa.ucsf.edu (G.P. Galloway).

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gests that study results showing increased dopamine release (regardless of GHB dose) are confounded by the use of anesthetics that have been shown to create this very effect. Another finding that GHB eliminates burst activity in nigral dopamine neurons (Nissbrandt et al., 1994) may be similarly confounded by the use of a dialysate mixture that can produce the same result (Howard & Feigenbaum, 1997).

Doses used to assess the effect of GHB on dopamine activity have ranged from 50 mg/kg to 2000 mg/kg, resulting in brain levels notably higher than those that occur naturally. Diana and colleagues suggested that lower doses of GHB (50–400 mg/kg) increase rather than decrease the firing of dopaminergic neurons, specifically in the pars compacta of the substantia nigra (Diana et al., 1991). Although the present consensus is that GHB administration reduces the release and induces the accumulation of dopamine, lower doses may have the opposite effect.

Some of GHB's effects may be mediated by opiate pathways, though GHB is not a direct opiate receptor agonist (Feigenbaum & Simantov, 1996). Several GHB effects are blocked by naloxone hydrochloride, including electroencephalographic and behavioral effects (Snead & Bearden, 1980) and dopamine-modulation effects (Hechler et al., 1991). The GHB-induced reduction of local cerebral glucose utilization is blocked by naloxone only in some brain areas (Crosby et al., 1983). However, emergency-room reports have noted no effect of naloxone on GHB-related loss of consciousness in human beings (Anonymous, 1997; Ross, 1995). Gamma-hydroxybutyric acid has also been shown to cause the release of growth hormone and prolactin in human beings (Takahara et al., 1977). This effect is not blocked by naloxone but is blocked by the GABA antagonist flumazenil and the serotonin antagonist metergoline (Gerra et al., 1994, 1995).

3. Pharmacokinetics

Gamma-hydroxybutyric acid is rapidly absorbed from the human gastrointestinal tract, with peak plasma concentrations 20–60 min after oral administration. At a dose of 12.5 mg/kg, clearance is 14.0 mL $\cdot$ $min^{-1}kg^{-1}$, and half-life is 20 min. Gamma-hydroxybutyric acid is almost completely oxidized to carbon dioxide in the rat (Roth & Giarman, 1966). Only from 2% to 5% is eliminated in the urine in human beings. Pharmacokinetics appears to be similar in alcoholics (Ferrara et al., 1992). Gamma-butyrolactone, the prodrug of GHB, results in an initial blood level in rats that is 50% lower than that after GHB, followed by a more gradual fall in blood level (Roth & Giarman, 1966). Gamma-butyrolactone is more lipid soluble than GHB and is rapidly converted into GHB.

4. Potential therapeutic indications

4.1. Anesthesia

Because of its ability to induce both sleep and reversible coma, GHB was investigated for its potential as a hypnotic adjunct to surgical anesthetics, though its use is limited by the need for additional adjuncts to produce analgesia, brain stem reflex response diminution, and muscle relaxation to overcome GHB-induced clonic jerking movements (Vickers, 1969). However, GHB has been particularly useful in underdeveloped countries to induce a safe and effective surgical anesthesia in children for whom additional medications are apparently not needed (Lane, 1991). Although in limited use in other countries as a surgical anesthetic, GHB is not approved in the United States.

4.2. Narcolepsy

Gamma-hydroxybutyric acid alters sleep cycles, increasing slow-wave sleep (SWS) at the expense of sleep stages 1 and 2 with no effect on rapid-eye-movement sleep (Sériès et al., 1992). This observation has led to several small trials exploring the efficacy of GHB for the treatment of narcolepsy (Lammers et al., 1993; Scrima, 1992; Scrima et al., 1990). Each of these trials had positive outcomes on some of the outcome variables asessed.

4.3. Sleep apnea

Sleep apnea is a condition in which sleep is interrupted by episodes of breathing cessation. These episodes occur largely in sleep stages 1 and 2 and in rapid-eye-movement sleep, the stages during which physiologic fluctuations in respiration are most frequent. Consequent to frequent awakening, time spent in slow-wave sleep (during which apneas have been reported to occur infrequently) is diminished. Sériès and colleagues explored the use of GHB for sleep apnea on the basis of its ability to increase the proportion of SWS (Sériès et al., 1992). Although GHB did increase the amount of time spent in SWS, there was no reduction in the number of apneic episodes, and these episodes were found to occur in SWS as frequently as in other stages of sleep.

4.4. Schizophrenia

Gamma-hydroxybutyric acid blocks impulse flow and neurotransmitter release in dopaminergic tracts and may have a differential effect on limbic versus striatal pathways (Fuxe et al., 1977). The possibility that GHB might block dopamine activity, particularly in the mesolimbic dopamine tract, suggested that it might have antipsychotic effects (Fuxe et al., 1977). However, the results of small studies in which GHB was used for the treatment of schizophrenia were negative (Levy et al., 1983; Schulz et al., 1981).

4.5. Anabolic effects

Gamma-hydroxybutyric acid has been marketed as a health food product in the United States for its hypnotic and anabolic effects, a phenomenon that has persisted in the United States despite its removal from the over-the-counter market. Luby and colleagues reported that several gymnasiums surveyed in South Carolina were selling GHB for this purpose (Luby et al., 1992), and anecdotal reports indicate that it is still widely avail-

able from illicit sources (Chin et al., 1992). Although GHB has been demonstrated to increase the release of growth hormone (Gerra et al., 1994, 1995; Takahara et al., 1977), an action that may promote muscular development, the actual efficacy of GHB in this regard has not been documented.

4.6. Alcohol withdrawal and dependence

Gamma-hydroxybutyric acid and gamma-butyrolactone suppress alcohol withdrawal tremors and seizures in alcohol-dependent rats and reduce voluntary alcohol consumption in alcohol-preferring rats (Gessa et al., 2000). In addition, GHB receptor affinity in vitro is higher in alcohol-preferring rats than in alcohol-non-preferring rats (Frau et al., 1995). Gallimberti and colleagues have followed up on these observations with a series of clinical trials; their findings suggest that GHB may have utility in the treatment of alcohol dependence (Gallimberti et al., 2000). In a placebo-controlled trial of 23 subjects in alcohol withdrawal, GHB (50 mg/kg) markedly suppressed withdrawal for the 7-h observation period and was well tolerated. The long-term efficacy of GHB in alcohol dependence was tested in a 3-month randomized trial (Gallimberti et al., 2000). Subjects were administered placebo or GHB (50 mg/kg/day) in three divided doses. Subjects were told at study intake that they should abstain from drinking, but further psychosocial intervention was not noted. Of the 82 subjects who entered the study, results were reported for the 71 who completed it. Subjects in the GHB group had three times as many abstinent days and half as many drinks per day during the study period (both results were significant at or below a probability of 0.01). No data were presented on outcomes after GHB was discontinued, and biological indicators of alcohol consumption were not included.

Despite these promising findings, there are a number of caveats to be taken into consideration. The work conducted by Gallimberti and colleagues (Gallimberti et al., 2000) has not been replicated in a controlled trial by an independent investigator, although an open-label trial has been reported (Addolorato et al., 2000) and a large multicenter controlled clinical trial is underway in Italy (Beghè & Carpanini, 2000). Gamma-hydroxybutyric acid may have these effects on alcohol consumption and withdrawal symptoms by mimicking the action of alcohol (Gessa et al., 2000).

Other drugs that are well known to suppress alcohol withdrawal symptoms are GABAergic drugs such as barbiturates and benzodiazepines (Hechler et al., 1993), which have effects similar to those of ethanol. Ethanol and GHB have demonstrated cross tolerance, though the pattern of the development of tolerance to these agents does differ (Colombo et al., 1995). Likewise, results of a recent drug discrimination study demonstrated that GHB, at the dose (300 mg/kg) that reduced alcohol consumption in alcohol-preferring rats, substituted for a low dose of alcohol (1 g/kg), and vice versa (Gessa et al., 2000). Interactions of GHB and alcohol include potentiation of the loss of the righting reflex of each agent and slower metabolism of alcohol (McCabe et al., 1971). Thus, GHB may decrease the desire or need for alcohol simply by providing a substitute form of intoxication. Indeed, a few anecdotal reports by self-medicating alcoholics indicate that, though it does suppress desire for alcohol, GHB also provides comparable effects without some of the perceived consequences of alcohol—for example, hangover, aggressiveness, and overconfidence (Anonymous, 1994; Galloway et al., 1997).

The pleasurable intoxicating effects of GHB are now well documented (Chin et al., 1992; Galloway et al., 1997), and instances of tolerance, dependence, and a withdrawal syndrome associated with GHB abuse have been reported by several researchers (Dyer, 1991; Friedman et al., 1996; Galloway et al., 1994, 1997; Stell & Ryan, 1996). The results of recent work have also demonstrated that rats, particularly alcohol-preferring rats, self-administer GHB after a period during which the sole fluid presented contained GHB (Colombo et al., 1998). Gallimberti and colleagues suggest that, if the efficacy of GHB is due to an alcohol-mimicking quality, GHB treatment may be useful in much the same way as methadone is for heroin (Gallimberti et al., 2000). However, not only does GHB have clear abuse liability (perhaps particularly for those prone to addictive disease), the very short duration of action of GHB makes supervised use of GHB in an outpatient setting very difficult. Nonetheless, a number of GHB analogues have been synthesized (Hechler et al., 1993), so the development of a long-acting GHB agonist may be within the realm of possibility.

4.7. Gamma-hydroxybutyric acid for opiate withdrawal

In the course of their use of GHB in alcoholics, Gallimberti and colleagues noted that, in those patients with dual alcohol and heroin addictions, GHB also seemed to suppress heroin withdrawal. Consequently, they tested this observation in a double-blind study of 22 heroin-dependent subjects and 19 methadone-dependent subjects (Gallimberti et al., 2000). Subjects were hospitalized for 8 days, and their withdrawal symptoms were rated on multiple occasions for 3 h before and after administration of GHB (25 mg/kg) or placebo. Withdrawal signs and symptoms were markedly suppressed for the 3-h period of observation, and GHB was well tolerated. Subjects in the GHB group were given additional open-label doses every 2–6 h for the following 8 days, which continued to suppress withdrawal. No withdrawal symptoms were noted in response to naloxone injection at the end of this period. Although Hajra and associates, in a double blind trial, failed to find any difference between GHB (0, 15, and 30 mg/kg) in suppressing naloxone-precipitated withdrawal in an unspecified number of levorphanol-dependent subjects (Hajra et al., 1995), it is important to note that antagonist-precipitated withdrawal can be extremely difficult to treat (Galloway, 1993). The data that GHB suppresses opiate withdrawal need to be confirmed by a second group of investigators.

5. Abuse

Gamma-hydroxybutyric acid has recently gained notoriety for its popularity as a drug of abuse, initially among

bodybuilders and subsequently among participants of "rave" dance parties and polydrug abusers. Subjective reports suggest that a significant proportion of users experience a euphoric "high" from the drug; the effects have been described anecdotally as comparable to both alcohol intoxication (with disinhibition, drowsiness, and loss of motor control) and MDMA/ecstasy (enhanced sensuality, empathogenesis). Not surprisingly, some users take more than the recommended dose (approximately 2.5 g, or 36 mg/kg, for a 70-kg person) to enhance or prolong these effects (Dyer, 1991; Luby et al., 1992). We reported a series of case histories in which users took as much as 25 g of GHB per day (Galloway et al., 1997). Gamma-hydroxybutyric acid has also been touted for its hypnotic effects, and onset of sleep can occur in 10–15 min. However, a GHB-assisted sleep lasts only 3–4 h, after which the user reportedly awakes feeling unusually refreshed (Galloway et al., 1997; Morgenthaler & Joy, 1995). Gamma-hydroxybutyric acid is easily manufactured and has been sold under a variety of names including "GHB," "liquid ecstasy," 4-Hydroxy Butyrate, Gama Hydrate, Gamma OH, Sodium Oxybate, Sodium Oxybutyrate, and Somatomax PM. Illicit use has been reported only by the oral route.

Gamma-hydroxybutyric acid has gained limited popularity in the United Kingdom, where it has been sold at raves as "liquid ecstasy" (Anonymous, 1994), and reports of use in the United States are increasing. The introduction of GHB into the U.S. over-the-counter market in the spring of 1990 was rapidly followed by reports of adverse effects at doses from 1 teaspoon (approximately 2.5 g) to 4 tablespoons. (approximately 30 g) (Chin et al., 1992; Dyer, 1991; Dyer et al., 1990). Widespread reports of poisonings led to a U.S. Food and Drug Administration ban on distribution for human use outside of approved clinical trials in November 1990. At the same time, pro-GHB monographs are published on the Internet and in the popular literature, and proponents of GHB use suggest that the safety and efficacy profile of GHB is such that the ban is unwarranted (El-Sohly et al., 1997).

Haight-Ashbury Free Clinics field research has identified patterns of GHB abuse in specific regions of California. For example, among young gay males in the Los Angeles area, GHB is used in association with MDMA and alcohol in group sex involving high-risk sex. In San Francisco, the medical examiner has described GHB as a "date rape drug" in the San Francisco sexual assault program. As with most clinical observations, laboratory verification has not always been adequate, but it is clear that patterns of GHB abuse are developing in certain at-risk groups. The mass media in the United States have reported the dangers of several "date rape" drugs, including GHB, often with limited accuracy. Newspaper articles have described the drugs, their use at bars and parties throughout the United States, and ways in which readers can protect themselves from these drugs. These news articles at times present characteristics of GHB as belonging to the benzodiazepine hypnotic flunitrazepam (Rohypnol), and vice versa. Gamma-hydroxybutyric acid has been described as "nearly tasteless" (it has a salty taste) or, lumped together with flunitrazepam and other "date rape" drugs, as "colorless, odorless, and tasteless" (Louwagie, 1997). On the other hand, an article claiming that flunitrazepam may have been used in a drugging described it as having a bitter taste, which it does not have (Scott, 1997). The same article also described flunitrazepam as a weight-loss aid that was taken off the market when people began to abuse it. One of the few sources of objective, well-documented data on the phenomenon of GHB as a "date rape" drug is a free program for testing the urine of women who suspect that they have been drugged and then raped. Of the first 337 samples tested, 30 (9%) were positive for GHB, despite GHB's extremely short half-life (El-Sohly et al., 1997).

Referring to GHB as "nature's quaalude," Sanguineti and associates describe a case involving a 46-year-old male that shows how misinformation and "mislabeling" have created problems with GHB (Sanguineti et al., 1997). The authors point out that GHB has been extensively marketed among athletes and bodybuilders. They present the case of a bodybuilder, referred for depressive symptoms, insomnia, and decreased weight (a loss of approximately 25 pounds). Although he reported a "brief cycle" of anabolic steroid use several weeks before admission, he did not mention his use of a "health product" until his third day of hospitalization. By then, he was exhibiting symptoms of paranoia with ideas of reference and marked disorientation. Thinking that he had been using ephedra compounds, physicians treated him with haloperidol. It was eventually discovered that the patient had been using GHB and had been "lulled into a false sense of security" by the way in which the information in a pamphlet ("The Underground Steroid Handbook") encouraged users to consider any GHB side effects to be minor. Although this particular case showed indications of cardiovascular stress, the encephalopathic state of GHB intoxication appeared to be self-limited and with no long-term sequelae, treatable with low doses of neuroleptics for the psychotic symptoms. Sanguineti and colleagues suggest that patients, particularly athletic persons, suffering a cluster of drowsiness, intoxication, confusion, urgency, rapid and mumbling speech, and amnesia may be suffering from GHB intoxication (Sanguineti et al., 1997).

5.1. Gamma-hydroxybutyric acid and the Internet

The preceding case highlights a particular feature of the GHB abuse phenomenon in the United States, the role of the Internet and anonymous pamphleteering in the dissemination of information on the manufacture and use of GHB. Utilizing key words to access Internet information, we have found articles about GHB along with the benzodiazepine flunitrazepam (Rohypnol) as a "date rape drug," as well as articles and newsgroup postings about the use of GHB to detoxify from other addictions and about experimentation with GHB. Although many GHB sellers advertise on the Web, at least one has discontinued selling it because of several matters, including GHB's use as a "date rape drug."

A 1-day search on Dejanews in April 1998 showed that access to buying kits to make GHB is easily gained on the Internet. Fifteen of 20 listings of GHB on newsgroups referred participants to three sites where they could purchase GHB kits. In that same search, with the exception of one informational Web page on which it was mentioned that GHB had a reputation for possible use as a "date rape drug," no mention was found of possible GHB use in date rape. The most detailed listing was to be found on http://www.geocities.com/HotSprings/Spa/1646/. This Web page included a somewhat glorified description of the development of GHB by a Dr. Henri-Marie Laborit, a treatise on what is referred to as "The Demonization of GHB," and a "hot link:" "*** If you are interested in linking to a company that supplies the highest grade chemical for GHB kits and other research kits please click at North American Lab Services." The page also appears to downplay the risks of GHB use by describing the U.S. Food and Drug Administration's approval process and pointing out that "No pharmaceutical company likes throwing away money (not to mention its credibility) on useless or dangerous substances that are likely to be rejected. At an average approval cost of $250 to $350 million per drug, pharmaceutical companies have collectively bet many millions on GHB's safety and efficacy."

On 27 March 1998, both the *Philadelphia Daily News* and the *Philadelphia Inquirer* reported that two teenage boys "were found unconscious at home after downing unknown doses of homemade gammahydroxybutyric acid, known as GHB." It was reported that the boys had synthesized the drug with a kit that they had bought for $50 at an Internet Web site. The ease with which GHB kits can be obtained through the Internet has been noted regularly in news articles. The well-known availability of kits combined with the increasing number of states making possession of GHB illegal encourages potential users to pursue information about this drug on the Internet, where negative consequences of its use are often downplayed.

6. Adverse effects

Reported adverse effects of GHB include dizziness, nausea, vomiting, weakness, tonic-clonic seizurelike activity, loss of peripheral vision, confusion, agitation, hallucinations, bradycardia, decreased respiratory effort, unconsciousness, and coma (Chin et al., 1992; Dyer, 1991; Luby et al., 1992). These effects can appear within 15 min of oral ingestion of the drug, and acute symptoms resolve within 7 h, though some cases have reported lingering dizziness for as long as 2 weeks (Chin et al., 1992; Dyer, 1991). Although GHB users may come to the attention of emergency-room personnel because of seizurelike activity or loss of consciousness, most of the reported serious adverse events (including seizures, respiratory arrest, and death) have occurred when GHB was combined with other substances such as alcohol, methamphetamine, or heroin (Einspruch & Clark, 1992; Ferrara et al., 1995; James, 1996). Despite one misleadingly titled report, there is no convincing evidence that GHB causes Wernicke-Korsakoff syndrome (Friedman et al., 1996).

Stating that, "Although no deaths or long-term problems have been associated with GHB abuse, symptoms of GHB intoxication can be severe," Tunnicliff (1997) cautions clinical toxicologists to be aware of its central nervous system depressant properties. In voicing his concern, Tunnicliff cites a number of acute toxicological episodes involving GHB, including two for which overdoses were documented in which patients became comatose after ingesting GHB, with or without alcohol (Ross, 1995). Louagie and colleagues describe a case of sudden awakening from a near coma after combined intake of GHB, alcohol, and marijuana (Louagie et al., 1997). They recommend that GHB overdose be considered in all cases of unexplained sudden coma where there is no evidence of head injury, intake of coma-inducing drugs, or increasing intracranial pressure. Our field research located an overdose death involving GHB and alcohol in southern California, but toxicological verification was inadequate.

The dose–response curve for GHB is steep, and slightly exceeding the recommended or intoxicating dose can result in severe adverse effects. Whereas a 40–50 mg/kg dose of GHB in human beings causes somnolence leading to arousable sleep, 60–70 mg/kg causes coma for 1–2 h (generally without depression of the reticular activating system). The LD_{50} has been estimated in animal experiments at 5 to 15 times that inducing coma, though the author suggests that the deaths of these animals were "probably due to sodium intoxication rather than to any effect of the active drug" [(Vickers, 1969), p. 75]. Gamma-hydroxybutyric acid and alcohol have synergistic hypnotic effects (McCabe et al., 1971).

7. Conclusion

Gamma-hydroxybutyric acid is a naturally occurring brain chemical whose function is as yet unclear. Exogenously supplied at supraphysiologic concentrations, GHB has a unique combination of actions including hypnotic, euphorigenic, epileptigenic, and possible anabolic effects. Gamma-hydroxybutyric acid decreases dopamine release in the striatum and mesolimbic cortex; however, despite the activity of benzamide neuroleptics at GHB sites, GHB has no demonstrated antipsychotic activity at doses tested to date. Gamma-hydroxybutyric acid reportedly suppresses symptoms of withdrawal from alcohol, possibly owing to an alcohol-mimicking action, and may also have a role in long-term treatment of alcohol dependence. Gamma-hydroxybutyric acid may act indirectly on opiate pathways, and it has been noted to suppress symptoms of opiate withdrawal. The short half-life and abuse liability of GHB may limit its utility in the treatment of dependencies. Gamma-hydroxybutyric acid may be useful for the treatment of narcolepsy, although the trials conducted to date have included small numbers of subjects. Gamma-hydroxybutyric acid is a relatively inexpensive and effective surgical

anesthetic agent that has been invaluable in settings or countries where more expensive or difficult to obtain anesthetic agents may be unavailable. At this time, surgical anesthesia is the only established indication for GHB, although large clinical trials are warranted for alcohol dependence, opiate dependence, and narcolepsy. Clinical observation indicates that there is a growing pattern of GHB abuse in the United States, particularly as part of a polydrug abuse pattern including alcohol, methamphetamine, and MDMA.

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ELSEVIER

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ALCOHOL

Mechanism of the antialcohol effect of gamma-hydroxybutyric acid

Gian Luigi Gessa[a,b,c], Roberta Agabio[a], Mauro A.M. Carai[a,c], Carla Lobina[a,c], Marialaura Pani[a], Roberta Reali[c], Giancarlo Colombo[b,*]

[a]*Bernard B. Brodie Department of Neuroscience, University of Cagliari, Cagliari, Italy*
[b]*C.N.R. Center for Neuropharmacology, Via Porcell 4, I-09124 Cagliari, Italy*
[c]*Neuroscienze S.c.a. r.l., Via Palabanda 9, I-09123 Cagliari, Italy*

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Abstract

Treatment with gamma-hydroxybutyric acid has been reported to effectively decrease alcohol craving and consumption as well as alcohol withdrawal symptoms in alcoholics. We describe the results of animal studies demonstrating the ability of gamma-hydroxybutyric acid to reduce (1) the severity of ethanol withdrawal signs in rats rendered physically dependent on ethanol and (2) voluntary ethanol intake in selectively bred Sardinian alcohol-preferring rats. Furthermore, we review experimental data suggesting that gamma-hydroxybutyric acid and ethanol have several pharmacological effects in common. Relevant similarities are: (1) stimulation of firing rate of dopaminergic neurons and dopamine release in specific rat brain areas; (2) development of cross-tolerance to the motor-impairing effects after repeated administration in rats; 3) abuse potential, as indicated by self-administration of pharmacologically relevant doses of gamma-hydroxybutyric acid in rats and mice; (4) induction of anxiolytic effects in rats; and (5) induction of similar discriminative stimulus effects, as evidenced by symmetrical generalization in a drug discrimination study in rats. These lines of evidence are discussed in relation to gamma-hydroxybutyric acid exerting its antialcohol effects by a substitution mechanism.

Keywords: Gamma-hydroxybutyric acid; Ethanol; Reduction of ethanol intake; Alleviation of ethanol withdrawal signs; Cross-tolerance to the motor-impairing effects; Anxiolysis; Symmetrical generalization to the discriminative stimulus effects; Substitution mechanism; Rat

1. Introduction

Gamma-hydroxybutyric acid (GHB) has been featured as an effective agent in the pharmacotherapy of alcohol dependence owing to its ability to reduce alcohol craving and consumption, promote abstinence, and alleviate the symptoms of alcohol withdrawal syndrome (Addolorato et al., 2000; Gallimberti et al., 2000; Moncini et al., 2000).

The present paper first describes the experimental evidence demonstrating the reducing effect of GHB on (1) intensity of ethanol withdrawal syndrome in ethanol-dependent rats and (2) voluntary ethanol intake in ethanol-preferring rats, which actually instigated the evaluation of GHB efficacy in clinical trials. Then the different lines of evidence suggesting that GHB exerts its antialcohol effects by a substitution mechanism are reviewed.

2. Gamma-hydroxybutyric acid effect on ethanol withdrawal syndrome in ethanol-dependent rats

Laboratory rodents do not self-administer intoxicating quantities of ethanol for extensive periods of time and, therefore, do not develop any physical dependence and withdrawal syndrome on cessation of ethanol ingestion (Majchrowicz, 1981). However, different induction procedures, employing chronic and forced administration of ethanol, have been developed: after ethanol treatment has been discontinued, rats and mice exhibit a wide spectrum of neurological signs that closely model those occurring in human alcoholics (Majchrowicz, 1981).

The ability of GHB to reduce the intensity of ethanol withdrawal signs was evaluated in this laboratory in rats rendered physically dependent on ethanol with the use of the procedure conceived and validated by Majchrowicz (1975). Accordingly, rats received four daily ethanol administrations by intragastric gavage for 6 consecutive days. After a priming dose of 4 g/kg ethanol, all subsequent ethanol doses were determined individually for each rat on the basis of the observed degree of intoxication, so as to maintain constant and intoxicating blood-ethanol

* Corresponding author. Tel.: +39-070-6758422; fax: +39-070-657237.
E-mail address: colomb@unica.it (G. Colombo).
[1]Present affiliation: Neuroscienze S.c.ar.l., Cagliari, Italy.

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levels throughout the treatment period. Six successive stages of intoxication were defined: neutrality; sedation; ataxia 1, 2, and 3; and loss of righting reflex. Ethanol doses, ranging from 0 to 5 g/kg, were inversely related to the degree of intoxication.

In an initial study, three different doses of GHB (0.25, 0.5, and 1 g/kg) were administered intraperitoneally to ethanol-dependent Sprague-Dawley rats 7 h after the last ethanol treatment (Fadda et al., 1989). Gamma-hydroxybutyric acid dose dependently suppressed both tremors (evaluated on a 12-point rating scale) and audiogenic tonic-clonic seizures associated with ethanol withdrawal. The highest GHB dose tested completely protected from tremors and seizures, producing solely a mild sedation.

A subsequent study undertaken in this laboratory used ethanol-dependent Wistar rats and evaluated a wider range of withdrawal signs than in the previous investigation (Fadda et al., 1989). In the latest experiment, 11 different items (namely, general activity, shakes, jerks, general tremors, head tremors, tail tremors, rigidity of muscle tone, tail rigidity, bracing posture, vocalization, and spontaneous convulsions) were evaluated on the 4-point rating scale conceived by Lal and colleagues (0 to 3 for each item, paralleling increased frequency of occurrence and degree of severity) (Lal et al., 1988). The sum of the 11 values was the total score assigned to each rat at each observation. Gamma-hydroxybutyric acid was administered intraperitoneally at a dose of 1 g/kg 15 h after the last ethanol administration; that is, when signs of ethanol withdrawal syndrome had already reached their maximal intensity. Control rats received an equal volume of saline. Observations and scoring were carried out 1, 2, 3, 4, and 5 h after GHB or saline administration. The results of this experiment are shown in Fig. 1 and complement those previously reported by Fadda and associates (Fadda et al., 1989). The acute administration of GHB produced a marked reversal of ethanol withdrawal signs, as indicated by significantly lower scores in withdrawal severity in GHB-treated rats than in saline-treated rats. The effect of gamma-hydroxybutyric acid was maximal at the 1- and 2-h observation times and lasted for as long as 3 h. As in the previous study by Fadda and colleagues (Fadda et al., 1989), the tested dose of GHB, which would produce anesthesia in undrugged rats, induced only a modest sedation in the ethanol-dependent rats used in the present study; the reported development of cross-tolerance between ethanol and GHB (Colombo et al., 1995b) is the likely explanation of this phenomenon.

In close agreement with these data, the results of an earlier study by Poldrugo and Snead (1984) demonstrated that 1,4-butanediol, a naturally occurring aliphatic alcohol that is converted in GHB (Maitre, 1997), had a protective effect on ethanol withdrawal syndrome in rats.

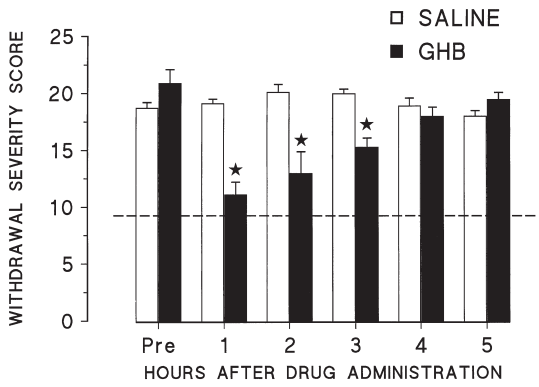

Fig. 1. Ability of acute gamma-hydroxybutyric acid to reverse ethanol withdrawal syndrome in ethanol-dependent rats. Rats were made physically dependent on ethanol by a 6-day treatment with intragastric administration of intoxicating doses of ethanol. Gamma-hydroxybutyric acid (1 g/kg) and saline were administered intraperitoneally 15 h after the last ethanol administration. Observations and scoring of severity of withdrawal signs were carried out 1, 2, 3, 4, and 5 h after gamma-hydroxybutyric acid or saline administration. The dashed line corresponds to a neutrality state (healthy, undrugged rat). Each bar is the mean ± SEM of seven rats; $*p < 0.05$ with respect to saline-treated rats (Newman-Keuls test).

3. Gamma-hydroxybutyric acid effect on voluntary ethanol intake in ethanol-preferring rats

Two studies carried out in this laboratory reported that the administration of the GHB precursors gamma-butyrolactone (Fadda et al., 1983) and 1,4-butanediol (Colombo et al., 1990) to ethanol-drinking rats markedly reduced voluntary ethanol intake and induced a compensatory increase in water consumption.

Subsequently, this laboratory investigated the effect of GHB on volitional ethanol intake by using rats from the Sardinian alcohol-preferring (sP) line, selectively bred in this laboratory for high ethanol preference and consumption (Colombo, 1997). These studies were of interest in view of the predictive validity of the sP rat line, as demonstrated by the evidence that agents reported to attenuate ethanol consumption in human alcoholics also reduced voluntary ethanol intake in these animals (this laboratory, unpublished results).

In the studies with GHB, sP rats were offered ethanol (10%, v/v) and tap water under the standard, two-bottle free-choice regimen with unlimited access for 24 h/day. Under this condition, sP rats habitually (1) consume approximately 6 g/kg ethanol per day; (2) avoid water almost completely (the daily ratio of ethanol solution to water preference is constantly higher than 90%); and (3) tend to divide daily ethanol intake in three or four separate binges during the nocturnal phase of the light/dark cycle, the first episode occurring in the first hour of the dark phase (Colombo, 1997).

Fig. 2 shows the results of a typical experiment conducted in this laboratory for evaluating the GHB effect on voluntary ethanol intake in sP rats. In this study, GHB was administered acutely by the intraperitoneal route from 15 to 20 min before lights off. Control rats received an equal volume of saline. Nonsedative doses of GHB (200 and 300 mg/kg) produced a significant dose-dependent reduction in voluntary ethanol intake, as much as 60% compared with the findings for saline-treated rats. However, the reducing ef-

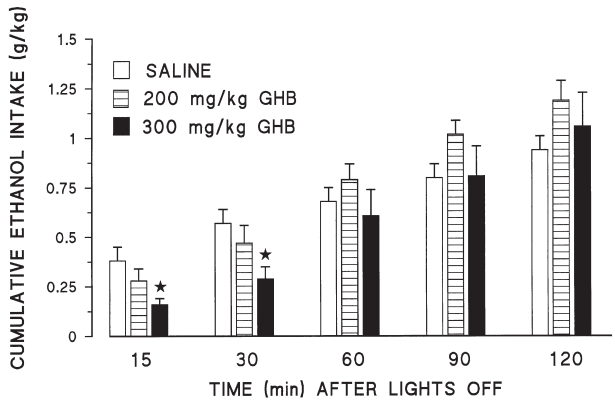

Fig. 2. Ability of acute gamma-hydroxybutyric acid to reduce voluntary ethanol intake in Sardinian ethanol-preferring rats. Sardinian ethanol-preferring rats had continuous access to ethanol (10% v/v, in tap water) and tap water under the two-bottle free-choice regimen for 3 consecutive months before the test with gamma-hydroxybutyric acid. On the test day, gamma-hydroxybutyric acid (200 and 300 mg/kg) and saline were injected intraperitoneally 15 to 20 min before lights off. After treatment, ethanol and water were withdrawn until lights off. Ethanol and water intakes were monitored 15, 30, 60, 90, and 120 min after lights off. Each bar is the mean ± SEM of nine rats; $^{*}p < 0.05$ with respect to saline-treated rats (Newman-Keuls test).

fect of GHB on ethanol intake occurred solely at 15- and 30-min observation times, being maximal at the former interval; indeed, ethanol intake in GHB-treated rats returned to control-group levels at the 60-min observation time, and no significant difference was subsequently recorded among GHB- and saline-treated rat groups. Finally, GHB administration did not affect water intake at the observation times when it reduced ethanol intake.

The reducing effect of GHB on voluntary ethanol intake in sP rats has been closely replicated in rats from the ethanol-preferring P line, selectively bred for high ethanol preference and consumption under the same criteria employed in the sP rat breeding (Li et al., 1987). In this study, the intraperitoneal administration of 300 mg/kg GHB reduced voluntary ethanol intake to approximately 70% of that of controls, exclusively during the initial 15 min of the daily 2-h drinking session (June et al., 1995).

The short duration of the GHB-reducing effect on ethanol intake in sP (Fig. 2) and P (June et al., 1995) rats is (1) a consequence of the short half-life of the drug [approximately 30 min in the rat plasma after parenteral administration (Lettieri & Fung, 1979)] and (2) consistent with the brief duration of GHB action observed in human alcoholics as well as the need, often encountered in clinical practice, to administer the drug six times a day to achieve the best treatment outcome (Addolorato et al., 2000).

4. Evidence in favor of a substitution mechanism

This laboratory has proposed that GHB may exert its effect on alcohol dependence by substituting for alcohol, like methadone in heroin addiction (Colombo et al., 1995c, 1998; Diana et al., 1991; Fadda et al., 1989). Consistently, ethanol and GHB have been reported to have a number of biochemical, electrophysiological, and pharmacological similarities (reviewed herein), suggesting that GHB may mimic ethanol actions in the central nervous system.

First, low doses of both GHB and ethanol have been reported to stimulate (1) the firing rate of dopaminergic neurons (Diana et al., 1991; Mereu et al., 1984) as well as the release of dopamine (Cheramy et al., 1977; Imperato & Di Chiara, 1986; Maitre et al., 1990; Signs et al., 1987) in specific brain areas and (2) spontaneous locomotor activity (Maitre, 1997; Pohorecky, 1977) in laboratory animals. These similarities are particularly relevant in view of the experimental evidence suggesting that (1) activation of the brain dopamine systems is part of a common link in the mediation of the reinforcing properties of various drugs of abuse, including ethanol (e.g., Bozarth, 1986; Di Chiara & Imperato, 1988), and (2) stimulation of locomotor activity reflects part of the positive reinforcing properties of ethanol and other addictive drugs (Wise & Bozarth, 1987). Consistently, several agents potentiating brain dopamine functioning have been reported to decrease ethanol consumption in ethanol-drinking laboratory animals (Dyr et al., 1993; George et al., 1995; McBride et al., 1990; Pfeffer & Samson, 1988; Weiss et al., 1990), likely through a direct activation of the "reward" pathway and subsequent replacement of the reinforcing properties of ethanol.

Second, cross-tolerance to the motor-impairing effects of GHB and ethanol has also been observed in a recent investigation by this laboratory. In this study, administration of an ataxic, though nonsedative, dose of GHB produced a significantly lower motor impairment, measured in a motor-coordination task with the use of a Rotarod, in ethanol-tolerant rats than in ethanol-naive rats. Conversely, administration of an equivalent (in regard to effects at the Rotarod) dose of ethanol induced a significantly lower impairment in GHB-tolerant rats than in GHB-naive rats (Colombo et al., 1995b). These results are suggestive of the presence of common adaptive changes in neural substrates to chronic ethanol and GHB.

Third, both GHB and ethanol have abuse potential, with GHB (1) inducing conditioned place preference in rats (Fattore et al., 2000), (2) being self-administered orally (Colombo et al., 1995a, 1998) and intravenously (Fattore et al., 2000) by rodents, and (3) abused by human beings for its alleged euphoric, anxiolytic, and relaxing effects, often described as resembling those of ethanol (Addolorato et al., 2000; Centers for Disease Control, 1991; U.S. Food and Drug Administration, 1991; Galloway et al., 2000). The results of a recent study in this laboratory demonstrated that preference for and high intake of ethanol and GHB were related in ethanol-preferring sP rats (Colombo et al., 1998). Indeed, the findings in this investigation showed that, when offered a free choice between GHB solution and water under the two-bottle procedure, ethanol-naive sP rats exhibited higher preference for and larger daily intakes of GHB than

did their Sardinian ethanol-nonpreferring counterpart. These results strengthen the hypothesis that GHB may possess reinforcing properties similar to those of ethanol.

Fourth, anxiolysis seems to be a common feature of the pharmacological profile of low to moderate doses of ethanol and GHB. The tension-reducing properties of ethanol have been experimentally demonstrated, for quite some time, with a number of different procedures (Becker & Flaherty, 1982; Blanchard et al., 1993; Blokland et al., 1992; Britton & Thatcher Britton, 1981; Colombo et al., 1995d; Conger, 1951; Costall et al., 1988; Durcan et al., 1988; Glowa & Barrett, 1976; Grant & Barrett, 1991; Hale et al., 1990; Lecci et al., 1990; Lister, 1987; McMillan & Leander, 1975;Vogel et al., 1980). In contrast, only a handful of studies have investigated the anxiolytic effects of GHB. The results of an initial study by Kršiak and colleagues demonstrated that nonsedative doses of GHB reduced anxiety in mice exposed to the social interaction test (Kršiak et al., 1974); more recently, the antianxiety effect of GHB has been shown in rats tested at the elevated plus maze (Schmidt-Mutter et al., 1998). This laboratory has recently investigated the anxiolytic effect of 300 mg/kg GHB in sP rats. As heretofore reported, this dose of GHB significantly reduced voluntary ethanol intake in sP rats (Fig. 2). Furthermore, this rat line has been featured as a valid animal model for investigating the association between ethanol drinking and anxiety (Colombo et al., 1998). Indeed, when tested at the elevated plus maze, sP rats behaved as being more anxious than ethanol-avoiding Sardinian ethanol-nonpreferring rats, and voluntary ethanol intake partly reversed their innate anxiety profile. In other words, anxiety is a genetic trait likely predisposing sP rats to ethanol drinking, whereas ethanol is voluntarily consumed for self-medicating anxiety. In this investigation, GHB was administered intraperitoneally 20 min before testing each rat at the elevated plus maze. For each rat, the time spent in and the number of entries into the open arms of the maze (i.e., the discomforting and anxiogenic spaces of the apparatus) were used as behavioral indexes of anxiety. As illustrated in Table 1, GHB-treated rats spent significantly more time in and made significantly more entries into the open arms of the maze than did saline-treated rats. In contrast, no significant difference was monitored between the two rat groups in the number of entries into the closed arms (i.e., a measure of general locomotor activity). Collectively, the results reported in Fig. 2 and Table 1 suggest that GHB, administered to sP rats at the anxiolytic dose of 300 mg/kg, substituted for the tension-reducing effects of ethanol usually sought by sP rats, rendering further ethanol intake redundant and, therefore, limiting ethanol consumption. Accordingly, the GHB-reducing effect on voluntary ethanol intake in sP rats has been observed to occur with a rapid onset (Fig. 2), suggestive of an immediate perception of effects similar to those sustaining ethanol drinking.

Fifth, a further confirmation of the substitution hypothesis is found in the results of a recent drug discrimination study demonstrating symmetrical generalization between the discriminative stimulus effects (i.e., the animal correlate of human subjective feelings elicited by a psychoactive drug) of ethanol and GHB in rats (Colombo et al., 1995c). In this study, 300 mg/kg GHB fully substituted for ethanol in rats trained to discriminate 1 g/kg ethanol from water, and 1 g/kg ethanol fully substituted for GHB in rats trained to discriminate 300 mg/kg GHB from water; no other dose of either drug substituted for the other training dose of the opposite drug. These results suggested that the effects exerted by GHB and ethanol, at least at doses of 300 mg/kg and 1 g/kg, respectively, are perceived as being similar by the rats. Interestingly, as noted heretofore, this dose of GHB produced anxiolysis and reduced voluntary ethanol intake in sP rats; 1 g/kg is the amount of ethanol usually consumed by “anxious” sP rats in each drinking episode (Agabio et al., 1996).

Table 1
Anxiolytic effect of gamma-hydroxybutyric acid in ethanol-preferring sP rats tested at the elevated plus maze

	% time in open arms	% entries into open arms	No. of entries into closed arms
Saline	7.0 ± 3.3	11.7 ± 4.8	7.0 ± 1.4
GHB	53.7 ± 12.9*	41.8 ± 2.1**	8.1 ± 2.1

sP rats had continuous access to ethanol (10% v/v, in tap water) and tap water under the two-bottle free choice regimen for 3 consecutive months before the test with gamma-hydroxybutyric acid. On the test day, ethanol was withdrawn 2 h before drug treatment. Gamma-hydroxybutyric acid (300 mg/kg) and saline were injected i.p. 20 min before the start of the test at the elevated plus maze. Spontaneous exploration at the elevated plus maze was monitored for 5 min for each rat; two measures of anxiety (percentage of time spent in and entries into the open arms) and one measure of locomotor activity (number of entries into the closed arms) were recorded. Each point is the mean ± SEM of 7 rats; *$p < 0.05$ (two-tailed) and **$p < 0.05$ (one-tailed) with respect to saline-treated rats (unpaired Mann-Whitney test).

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ALCOHOL

Gamma-hydroxybutyric acid as a signaling molecule in brain

Michel Maitre*, Christian Andriamampandry, Véronique Kemmel, Catherine Schmidt, Yann Hodé, Viviane Hechler, Serge Gobaille

Institute of Biochemistry and ER 2072 CNRS Faculty of Medicine, 11 Rue Humann, 67085 Strasbourg Cedex, France

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Abstract

Gamma-hydroxybutyric acid was synthesized 35 years ago to obtain a GABAergic substance that penetrates the brain freely. Since then, gamma-hydroxybutyric acid has been used in human beings for its sedative and anesthetic properties when administered at high doses, and most of the studies on gamma-hydroxybutyric acid have focused on its pharmacological effects. However, gamma-hydroxybutyric acid is also an endogenous substance, which is synthesized and released in the brain by specific neuronal pathways, implicated in the control of the GABAergic, dopaminergic, and opioid systems. This control is mediated by specific gamma-hydroxybutyric acid receptors with a unique distribution in brain and a specific ontogenesis and pharmacology. Stimulation of these receptors induces specific cellular responses. Taken together, these results suggest that gamma-hydroxybutyric acid possesses most of the properties required of a neurotransmitter/neuromodulator in the brain.

Keywords: Gamma-hydroxybutyric acid functions in brain; Gamma-hydroxybutyric acid receptors; Cloning of succinic semialdehyde reductase of rat brain

1. Introduction

Until recently, gamma-hydroxybutyric acid (GHB) has been considered a drug to be used in anesthesia and for the regulation of sleep patterns in patients with narcolepsy (Hoes et al., 1980; Mamelak et al., 1986). Even after the discovery of its natural occurrence in the brain and in several organs of human beings and various animals species (Roth & Giarman, 1970), its status has remained largely in the pharmacological domain. However, increasing evidence argues for the existence of a GHB system in the brain, implicated in specific signaling between neurons and perhaps between the brain and peripheral organs (Maitre, 1997). Gamma-hydroxybutyric acid administered peripherally penetrates freely into the brain and interacts locally with receptors whose distribution, ontogenesis, kinetics, and pharmacology are specific. These receptors are absent from peripheral organs and influence at least three major neurotransmitter systems in the brain: those of dopamine, opiates, and gamma-aminobutyric acid (GABA). Some brain compartments of this last substance are thought to be directly modulated by GHB acting as a precursor or as a presynaptic signal, leading to the modulation of anxiety, vigilance, and the electroencephalographic profile by means of $GABA_A$ and $GABA_B$ receptors (Schmidt-Mutter et al., 1998; Snead, 1992). These properties of GHB are used in some therapeutic indications in human beings. However, besides this GABAergic influence, a specific GHBergic entity exists through specific brain synthesis, release, transport, and receptors. At present, the functional specificity of this signal remains largely unknown, but recent study results shed some light on the molecular and cellular organization of the GHB system. This article will focus on new insights concerning GHB synthesis, GHB receptors, and functional links with the GABAergic system.

2. The reductive route of gamma-aminobutyric acid metabolism leads to GHB

Gamma-aminobutyric acid is first transaminated into an aldehydic product (succinic semialdehyde; SSA) by GABA-T in a manner similar to what has been described for the degradative pathways of catecholamines or serotonin. This transamination is thought to occur largely in the mitochondria. Then SSA either follows the oxidative route inside the mitochondria and is converted into succinic acid by succinic semialdehyde dehydrogenase and enters the Krebs cycle or it could leak out of the mitochondria into the cytosol, most probably by means of a regulated mechanism of transport,

* Corresponding author. Tel.: +33-3-88-243493; fax: +33-3-88-243501.
E-mail address: maitre@neurochem.u-strasbg.fr (M. Maitre).

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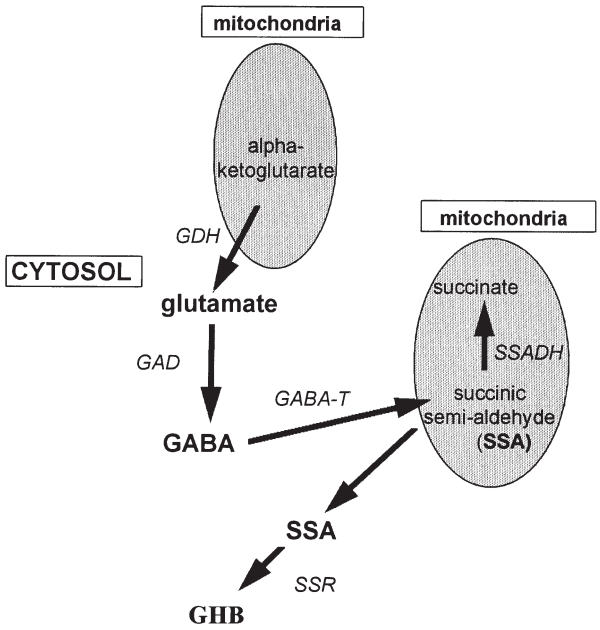

Fig. 1. Metabolic pathway for the synthesis of gamma-hydroxybutyric acid (GHB) in the neuron. Succinic semialdehyde (SSA) is formed in the mitochondria after transamination of gamma-aminobutyric acid (GABA) by GABA-transaminase (GABA-T). Succinic semialdehyde could either be oxidized to succinate by succinic semialdehyde dehydrogenase (SSADH) in the mitochondria or transported to the cytosol where it is reduced into gamma-hydroxybutyric acid by succinic semialdehyde reductase (SSR). The majority of SSR-immunoreactive neurons also contain glutamate decarboxylase (GAD), which synthesizes GABA from glutamate.

and is reduced by a specific reductase:succinic semialdehyde reductase (SSR; Fig. 1). This last pathway is about 1%–2% of the total metabolic flux through the GABA shunt and exists exclusively in neurons (Gold & Roth, 1977).

Succinic semialdehyde reductase has been purified from numerous sources, including the human brain. This enzyme is present in all rat brain regions that have been investigated, but its catalytic activity is heterogeneously distributed, suggesting the existence of a mechanism that regulates its activity (Rumigny et al., 1982). Subcellular fractionation has demonstrated that SSR-specific activities are highest in the soluble (cytosolic) and in the synaptosomal fractions (Rumigny et al., 1981). The nerve-ending fraction is also the brain compartment that contains the highest concentration of GHB (Snead, 1987).

Purified SSR from rat brain or from the brain of other species possesses a K_m for SSA of about 20 μM and a K_m for its cosubstrate NADPH of about 6 μM. Among a wide range of natural and synthetic aldehydes, SSR seems to be fairly specific for SSA (Rumigny et al., 1981). Phthalaldehydic acid, which is a structural analogue of SSA, also is reduced by SSR and behaves as a competitive inhibitor of SSA reduction with a K_i of about 500 μM. No other inhibitor of SSR activity has been described, and, in particular, none of the sedative or anticonvulsant compounds that are inhibitors of GHB degradation interfere with GHB synthesis (Maitre, 1997).

In contrast with the SSR from human brain, which appears to be a monomeric protein of about 80–90 KDa, the enzyme isolated from rat brain is a dimer of two similar (if not identical) polypeptide chains, each about 45 KDa (Cash et al., 1979; Rumigny et al., 1980). This last enzyme has been used as antigen to produce a polyclonal antibody after intramuscular administration to a rabbit. This antiserum labeled a single protein band of about 43 KDa on a Western blot made after SDS-PAGE of a crude rat brain homogenate. Thus, the antiserum was judged to be specific for SSR and used to carry out immunocytochemical work on rat brain slices or on neuronal cells in culture (Kemmel et al., 1998).

3. Succinic semialdehyde reductase is a neuronal enzyme present in axonal processes and synaptic structures

At the optical level, only neurons in the hypothalamus, cortex, and hippocampus seem to be labeled by SSR antibody. In these last two regions, pyramidal cells are heavily stained, particularly in the CA1 region of the hippocampus. The neuronal cytosol is strongly immunoreactive in general, with labeling of numerous processes and fibers. Glial cells appear not to be labeled.

Triple labeling in the rat substantia nigra and striatum was carried out at the confocal microscopic level with monoclonal antityrosine hydroxylase (TH) antibodies, anti-glutamate decarboxylase (GAD) antibodies from sheep, and anti-SSR from rabbit. Results of this study reveal that, in substantia nigra (pars compacta, SNpc), the majority of anti-tyrosine hydroxylase-immunoreactive neurons are also GAD and SSR positive, demonstrating the colocalization of TH, GAD, and SSR in the same neuron, in contradiction to the Dale's principle. Some neurons are only GAD and SSR positive. Because GHB is mainly synthesized form GABA, the coexistence of GAD and SSR seems logical in neurons that produce GHB. Some few neurons are only SSR reactive, suggesting the existence of an uptake of GABA by these cells and a conversion of GABA into SSA by GABA-T. Neurons in which GAD and SSR coexist could more appropriately be identified as GHBergic rather than GABAergic.

In the striatum, numerous TH immunoreactive terminals are visible, some of them surrounding SSR-positive neurons. In general, GAD and SSR seem to coexist, but a few cells are only GAD or only SSR immunoreactive. Punctuate images, processes, and fibers of SSR immunoreactive material are seen, sometimes in close contact with others cells and distinct from GAD-containing structures. These images suggest the existence of neuritic processes and synaptic contacts expressing SSR activity in the striatum of the rat. This type of organization could have a role in controlling, directly or indirectly, dopamine synthesis and release in this brain region.

Electron microscopic examinations confirmed the presence of immunoreactive products in various regions of the neuronal cytoplasm. Some punctuate deposits seem to be linked to the endoplasmic reticulum. Succinic semialdehyde

reductase immunoreactivity was also observed in selected postsynaptic and presynaptic elements. The immunoreactive synapses were all asymmetrical, indicating that the staining was selective. Several neuronal processes also were stained, often in a punctuate manner.

The existence of synaptic contacts expressing SSR was also confirmed by studies carried out on NCB-20 cells (Kemmel et al., 1998). This cell line, which expresses a neuronal phenotype, is a hybridoma of mouse neuroblastoma cells with brain embryonic cells from hamster. We have demonstrated that NCB-20 cells express both SSR activity and a population of GHB-binding sites whose kinetic characteristics were similar to those existing in the rat brain. A 3-day treatment with 1 mM dibutyryl cyclic AMP induced a morphological differentiation of NCB-20 together with a threefold increase in the cellular SSR activity. In parallel, a K^+-evoked, Ca^{2+}-dependent release of [^{3}H]GHB occurred, which was absent in undifferentiated cells. Thus, it could be argued that the cyclic AMP-induced differentiation of NCB-20 cells induces the development of synaptic contacts that parallel the development of SSR activity and of a depolarization-induced GHB release. This synaptogenesis and the colocalization of SSR with synaptic markers was demonstrated at the confocal microscopic level by using double labeling of SSR and synaptophysin. Results of this study reveal the existence of numerous punctuate structures, which were immunoreactive for both synaptophysin and SSR, whereas some others were reactive only for synaptophysin. These findings suggested the existence of synaptic terminals containing SSR in differentiated NCB-20 cells. Electron microscopic studies of these cultures showed large vesicular structures containing dense material that were labeled by the anti-SSR antibody together with images of synapse-like structures.

4. A succinic semialdehyde reductase, able to synthesize gamma-hydroxybutyric acid in the brain, was cloned from rat brain hippocampus

Cloning of brain SSR was undertaken to understand the mechanism of the regulation of GHB synthesis. The SSR was purified from total rat brain by using a series of chromatographic steps followed by a two-dimensional gel electrophoresis on SDS-PAGE. Then the SSR spot was digested in the gel with modified trypsin, and the peptide mixture was separated by high-performance liquid chromatography. Several homogeneous peptides were sequenced, and polymerase chain reaction oligonucleotide primers were designed on the basis of homology with some members of the aldo-keto-reductase family. With the use of these primers, a specific DNA fragment of 450 bp was amplified and used as a probe to screen a cDNA library from rat brain hippocampus, which is rich in SSR activity. One cDNA of 1341 bp was finally isolated, with an open reading frame of 447 amino acids and encoding a protein with a molecular weight of 47,967 that possessed SSR activity when expressed in *Escherichia coli* (Andriamampandry et al., 1998). The K_m values were 20 μM for succinic semialdehyde and 6 μM for NADPH. The enzyme activity was insensitive to valproic acid but was competitively inhibited by phthalaldehydic acid, similarly to what has been described for purified SSR from rat brain. Results of Northern blot experiments showed that mRNA for the cloned SSR was not present in peripheral tissues such as kidney or liver (Andriamampandry et al., 1998).

In situ hybridization on brain slices carried out with a complete cRNA sequence revealed the presence of SSR mRNA in some layers of the cortex, as well as in the hippocampus, thalamus, substantia nigra, pons-medulla, cerebellum, and olfactory tract. Curiously, the striatum and hypothalamus were not labeled, although striatum contains GHB, SSR, and GHB receptors. The cloned enzyme had 20% to 35% identity with four other proteins belonging to the aldo-keto-reductase family in highly conserved regions in which nicotinamide and substrates binding takes place. Some homologies were also found with the sequence of succinic semialdehyde dehydrogenase.

The SSR sequence analysis indicated the presence of several consensus sites for phosphorylations catalyzed by kinase C, tyrosine kinase, and casein kinase II. Some of these sites might be implicated in the controlled modulation of the enzyme activity. However, the control of GHB synthesis could be also due to the presence of several SSR isoenzymes, with different regional, cellular, or subcellular distribution. This hypothesis is supported by the fact that the structure of the cloned SSR did not exactly fit the partial sequence of purified SSR from rat brain. In addition, Northern blot of the whole brain RNA hybridized with the cRNA probe reveals a rather diffuse band, indicating that several mRNA species for SSR exist in a crude extract. The future cloning of a striatal or hippocampal cDNA library at lower stringency could reveal the existence of a specific succinic semialdehyde reductase family with particular regional, cellular, or subcellular distribution.

5. Some regions of the brain express specific receptors sites for gamma-hydroxybutyric acid that are absent from other organs

Radioactive GHB binds to a total membrane preparation of rat brain in a saturable, reversible manner with high affinity. Kinetic analysis supports the existence of two classes of binding sites, one of high affinity (K_d of 30 to 90 nM) and the other of lower affinity (K_d of about 16 μM). The corresponding binding capacities are $B_{max\text{-}1} = 0.5$ pmole/mg protein and $B_{max\text{-}2} = 46$ pmoles/mg protein, respectively (Benavides et al., 1982). However, if the membranes were washed with CHAPS or TRITON X-100, the percentage of specific binding increases from 40% to 70%–80%, and the two populations of binding sites are replaced by a single one with a K_d of about 1 μM (Maitre et al., 1983).

The binding of GHB is pH dependent, being absent at pH 5.0 or 8.0, maximum at pH 5.5, and very significant at phys-

iological pH (6.0–7.5), at which experiments are generally conducted. Gamma-hydroxybutyric acid-specific binding is not modified in the presence of several ions, including K^+, Ca^{2+}, Mn^{2+}, or Cl^-. Therefore, binding experiments are usually carried out in PIPES buffer or phosphate buffer, pH 6.0. Because GHB sites are very sensitive to hydrolysis by endogenous phospholipases and proteases, the brain homogenate and the membrane preparations must always be made in the presence of 5 mM EDTA and protease inhibitors (Hechler et al., 1990a).

The expression of GHB binding sites increases during development, the amount in an adult being approximately double that found in a 6-day-old animal (Snead, 1994). The regional distribution of GHB binding sites has been studied in human and rat brain. These studies relate essentially to high-affinity-binding sites because dissociation from low-affinity sites occurs easily, making binding more difficult to measure. Regional dissection of the rat brain showed a maximal density of sites in the hippocampus, followed by the cortex in general, the striatum, thalamus, and olfactory tract. The diencephalon binds radioactive GHB very slightly, and binding sites are virtually absent from the caudal regions of the brain (pons, cerebellum, and medulla). In the human brain, the distribution seems to be approximately the same, with the exception of the pons region, which seems to be richer in GHB receptors than the same region in the rat brain.

The distribution of high-affinity GHB sites has also been studied in consecutive slices of rat brain (20 μm thick) that were subjected to quantitative autoradiography followed by image analysis. The GHB sites are particularly dense in the superficial layers of the cortices (frontal, parietal, and temporal), in the hippocampus (dentate gyrus, regions CA_1, CA_2, and CA_3), and septum. The olfactory tract and the amygdala exhibit moderate concentrations of sites. The principal regions of the brain with a high dopaminergic innervation also strongly express GHB receptor sites: the entire striatum (including the nucleus accumbens), frontal cortex, and olfactory tubercles; but also dopaminergic nuclei: A_9, A_{10}, and A_{12}. Some nuclei of the thalamus also bind radioactive GHB (particularly the lateral posterior nucleus). The remainder of the brain does not show any labeling; in particular, this method does not lead to the detection of any receptor site in the hypothalamus, the pons-medulla, and the cerebellum (Hechler et al., 1992).

The possible existence of GHB receptor sites has been studied in peripheral organs. There are no high-affinity-binding sites in membrane preparations of hepatic, renal, or cardiac tissues. However, these tissues contain significant concentrations of GHB, which probably has a metabolic role whose origin is obscure (Nelson et al., 1981).

In cell cultures, the presence of high-affinity GHB-binding sites has been detected only in neurons or cell lines of neuronal origin (rat brain neurons in primary cultures or neuronal line NCB-20 or NH-25). The cells of glial origin (primary culture or C_6 line) do not possess membrane binding sites.

Undifferentiated NCB-20 cells bind [^{3}H]GHB in a saturable and reversible manner. In the range of concentration from 10 to 1100 nM, the Scatchard representation of specific binding indicates the presence of a single population of binding sites with a K_d of 250 ± 44 nM and a B_{max} of 180 ± 16 fmol/mg protein. If cultured in the presence of dibutyryl cyclic AMP, the specific [^{3}H]GHB binding was reduced (B_{max} = 74 ± 11 fmol/mg protein), and the K_d increased to 975 ± 236 nM after 2 days of treatment. This last value is very close to the K_d value measured on detergent-washed membranes from rat brain (about 1 μM). This desensitization could be due to phosphorylation of the agonist-occupied receptors because GHB release takes place in differentiated cells and could be accompanied by a downregulation due to agonist-induced receptor endocytosis after synaptogenesis. However, another class of GHB receptors could be expressed under the control of cyclic AMP-dependent transcription factors (Kemmel et al., 1998).

The subcellular distribution of GHB binding sites was studied after fractionation of a crude homogenate of rat brain. The various fractions obtained were not washed with detergent. Under these conditions, the highest densities of binding sites were present in the nerve endings fraction, which cosediments with choline acetyltransferase. The two classes of binding sites (high and low affinity) were found in this fraction in the same proportions as those in the unpurified membranes (Maitre et al., 1983).

6. Gamma-hydroxybutyric acid receptors possess a specific pharmacological profile

A number of substances have been tested for their ability to displace radioactive GHB from its binding sites. Of these substances, the principal ligands of GABA receptors (muscimol, isoguvacine, baclofen, bicuculline, picrotoxin) and GABA itself are without effect (Benavides et al., 1982). Substances structurally similar to GHB (ethanol) or capable of acting as precursors (butanediol, gamma-butyrolactone) also are inactive, as are the principal antiepileptics capable of interfering with GHBergic mechanisms in the production of petit mal seizures (valproate, ethosuximide, trimethadione).

On the other hand, a series of synthetic substances, structural analogues of GHB, possess varying degrees of affinity for the GHB site. These substances include trans-gamma-hydroxycrotonate, which is naturally present in the rat brain at levels 5 to 10 times lower than those of GHB and possesses an affinity about 10 times higher for the binding sites than GHB itself. It appears that trans-gamma-hydroxycrotonate recognizes only a fraction of the binding sites (about 1/10th), which suggests the existence of several classes of GHB sites, possibly with a regional specificity in the brain. Trans-gamma-hydroxycrotonate analogues are also potent ligands for GHB receptors (Hechler et al., 1990b).

Among the synthetic structural analogues of GHB, NCS-382 (sodium salt of 6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-4-ylidene acetic acid) is the first GHB receptor ligand

that possesses antagonistic properties. This compound displaces [^{3}H]GHB binding with two IC_{50} of low (130–300 nM) and high (5–8 μM) values and has no effect on [^{3}H]GABA binding ($GABA_A$ or $GABA_B$). In vitro, micromolar amounts of this substance inhibit the GHB-induced modification in cGMP hippocampal concentrations or in inositol phosphate accumulation. In vivo, NCS-382 protects against GHB-induced petit mal seizure and diminishes the sedative or cataleptic effects (or both) of GHB in a dose-dependent manner (Schmidt et al., 1991). Peripheral administration of NCS-382 to rats prevents the GHB-induced increase in dopamine release in striatum measured by in vivo microdialysis after local infusion of GHB (Maitre et al., 1990).

Several benzamide neuroleptics, including (−)sulpiride, sultopride, and amisulpride, also possess the ability to displace [^{3}H]GHB binding with two IC_{50}, in the nanomolar and micromolar range, respectively (Maitre et al., 1994). They exhibit specific therapeutic effects, especially on negative symptoms of schizophrenia. It may be postulated that these properties are due, at least in part, to their interactions with GHB receptors, which contribute to the regulation of dopaminergic activity not only in the striatum but also in the frontal cortex. Chronic (−)sulpiride treatment in rats induces hyperexpression of GHB receptors in the total brain, whereas chronic GHB treatment produces a downregulation of these same receptors. The same effects of (−)sulpiride and GHB have been obtained in NCB-20 cell cultures, in which GHB receptors were upregulated or downregulated by 24-h exposure to 100 μM (−)sulpiride or 1 mM GHB, respectively. This result confirms the interaction of (−)sulpiride with GHB receptors and could be considered a compensatory mechanism to functional blockade. Consequently, (−)sulpiride should be considered an antagonist at the GHB receptor, although some agonist-induced upregulation of receptors is thought to be produced by a stabilization of receptors by ligands (Ratomponirina et al., 1998).

7. Gamma-hydroxybutyric acid receptors are coupled to specific cellular responses

In vitro, the binding of radioactive GHB to a crude membrane fraction from the rat brain is sensitive to nonhydrolyzable analogues of GTP (GTPγS) and to pertussis toxin (Ratomponirina et al., 1995). In vivo, intraventricular pertussis toxin followed by autoradiographic study of the [^{3}H]GHB binding on brain slices of the treated rats showed a decrease in GHB-specific binding, which attained statistical significance only in the frontal cortex. These results suggest that GHB receptors belong to a family of receptors coupled to G proteins (either G_o or G_i).

The electrophysiological effect of GHB was studied in vivo (usually in the anesthesized animal) after local (microiontophoresis) or peripheral administration of GHB. Other studies have been conducted on viable brain tissue slices or on neuron cultures. In rats, microiontophoretic GHB depressed about half of the nigral and the majority of the cortical cells tested in rats, whereas GABA depressed the firing of all nigral and neocortical cells. The effect of GHB was resistant to bicuculline (Olpe & Koella, 1979). After application to guinea pig substantia nigra maintained in vitro, effective GHB concentrations of about 10 μM hyperpolarize cell membranes and facilitate calcium conductance (Harris et al., 1989). These effects were only partly reduced by bicuculline. Substantia nigra and cortex express not only a high density of GHB receptors, but also significant SSR activities and high concentrations of GHB.

In fact, results of several studies, including recent experiments in our laboratory, indicate a biphasic effect of GHB. Low doses of GHB generally induce effects opposite those of high doses. In the unanesthesized rat, doses of 100 to 200 mg/kg GHB increase the discharges in the dopaminergic neurons of the substantia nigra, whereas higher doses (1 g) hyperpolarize the neurons. In the anesthesized animal, either no effect or hyperpolarization is observed at a dose of 400 mg/kg (Diana et al., 1991, 1993). Findings from another study show that GHB administered at a low dose (5 to 10 mg/kg) increases the spontaneous discharge of neurons of the prefrontal cortex, this effect being antagonized by NCS-382. The highest doses elicit the hyperpolarizations commonly observed in the other studies, and these hyperpolarizations are not sensitive to NCS-382 (Godbout et al., 1995). Patch-clamp experiments carried out on NCB-20 cells differentiated by cyclic AMP have demonstrated the presence of calcium conductances, which were partly inhibited by low doses of GHB (25 μM). This GHB-induced effect was blocked by the GHB receptor antagonist NCS-382 but not by the $GABA_B$ antagonist CGP 55 845 (Kemmel et al., 1998).

The effect of GHB on second-messenger systems has not been explored in detail. However, the existing results in this field confirm the idea of a duality of GHB action, as a function not only of GHB concentrations but also of the brain region considered. In hippocampus, both in vivo and in vitro, GHB induced increases in cGMP and potentiated the turnover rate of inositol phosphates, which is suggestive of depolarizations and local influx of calcium ions (Vayer & Maitre, 1989, Vayer et al., 1987). On the other hand, GHB lowers cyclic GMP levels in the cerebellum where GHB receptors are absent, probably by exerting a hyperpolarizing role by means of other types of receptors, most probably $GABA_A$ or $GABA_B$.

8. Gamma-hydroxybutyric acid modulates GABAergic activity in some regions of the brain

No apparent direct interaction of GHB occurs with the $GABA_A$ receptor complex despite the fact that, in some studies, bicuculline partly reverses the inhibitory properties of GHB in electrophysiological tests (Kozhechkin, 1980). However, GHB might sometimes mimic $GABA_A$ receptor stimulation in tissue-slice experiments or in cell cultures or in pharmacological tests (Snead & Liu, 1993; Snead et al.,

1992). These results are thought to be due either to a GHB-induced modification of GABA release in some brain regions or to a GHB conversion into GABA. An increase in GABA release after microdialysis in awake rats has been recently described in the frontal cortex of the rat after administration of GHB (unpublished results). However, Banerjee and Snead (1995) demonstrated a GHB-induced reduction of GABA release in the thalamus of the rat. Both in vitro and in vivo, [^{3}H]GHB is generally converted into [^{3}H]GABA, which could be present in the extracellular space of some regions of the rat brain.

Several other reports argue for a role of GHB at the $GABA_B$ receptor(s) because sometimes GHB effects could be blocked by $GABA_B$ antagonists (generally CGP 35 348) (Engberg & Nissbrandt, 1993; Williams et al., 1995; Xie & Smart, 1992). Although the possible interaction of this last compound with GHB receptors has never been tested, these experiments seem to confirm an interaction between GHB and $GABA_B$ receptors. IC_{50} values of 150 (Bernasconi et al., 1992), 500 (Ito et al., 1995) and even 796 μM (Ishige et al., 1996) have been measured for the displacement of $GABA_B$ binding by GHB, and it is possible that this displacement was due to the GABA synthesized from GHB under the conditions used in vitro. The inhibition of GHB conversion into GABA by GHB dehydrogenase inhibitors abolishes the displacement of $GABA_B$ binding by high concentrations of GHB in vitro (Hechler et al., 1990b).

Thus it seems that the GABAergic effect of GHB is due to GHB metabolism or to GHB-induced release of GABA in vivo or in tissue slices or to both. A direct interaction of GHB on GABA sites is doubtful and could be hypothesized only for high concentrations of administered GHB.

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Gamma-hydroxybutyric acid and alcohol-related syndromes

M. Moncini, E. Masini, F. Gambassi, P.F. Mannaioni*

Department of Preclinical and Clinical Pharmacology, Toxicological Unit, Florence University, Via G. Pieraccini 6, 50139 Florence, Italy

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Abstract

We report on the effectiveness and safety of gamma-hydroxybutyric acid in the therapy of overt alcohol withdrawal syndromes, their prevention, and the prevention of relapses in formerly detoxified alcoholics. We studied 321 patients (236 men, 85 women), divided into two open-study groups for the treatment and prevention of alcohol withdrawal syndromes and one double-blind study group to evaluate the effects of gamma-hydroxybutyric acid versus placebo on alcoholic craving and relapses in detoxified patients. Gamma-hydroxybutyric acid treatment promptly reduced withdrawal symptoms in all patients and prevented alcohol withdrawal syndromes in 55% of cases. The attenuation of craving in detoxified patients was significantly greater in the gamma-hydroxybutyric acid-treated group in comparison with the placebo-treated group. The therapeutic use of gamma-hydroxybutyric acid was not accompanied by serious side effects. Gamma-hydroxybutyric acid diversion was poorly represented: gamma-hydroxybutyric acid-induced abuse was reported in 4 (1.1%) of 345 treated patients, and only 9 cases of gamma-hydroxybutyric acid acute poisoning were reported in the years 1992–1995. Our results suggest that gamma-hydroxybutyric acid, with a favorable risk/benefit ratio, is a clinically useful drug in the treatment of alcohol dependence.

Keywords: Gamma-hydroxybutyrate; Gamma-hydroxybutyric acid; Alcohol withdrawal syndrome; Craving; Gamma-hydroxybutyric acid diversion

1. Introduction

Gamma-hydroxybutyric acid (GHB) is a short-chain fatty acid derivative bearing an alcoholic function; it was reported by Bessman and Fishbein (1963) to be a natural constituent of the brain. The regional distribution is uneven, the greatest amount being in the substantia nigra, thalamus, and hypothalamus, and the lowest concentrations in certain areas of the cerebral cortex and the cerebellum. Gamma-hydroxybutyric acid is located mainly in the cytosol, where it is synthesized from succinic semialdehyde through the intervention of succinic semialdehyde reductase.

In experimental animals, GHB induces a sleeplike state after intravenous, oral, or intraperitoneal administration, at doses ranging from 0.1 to 1.5 g/kg (Laborit et al., 1962). Notwithstanding its neurodepressant effect, GHB produces abnormal electroencephalographic activity, similar to that observed in petit mal epilepsy, allowing the use of GHB as a tool to reproduce absent seizures experimentally (Bernasconi et al., 1992). These effects are thought to be mediated by interactions with gamma-aminobutyric acid B ($GABA_B$) receptors, with specific GHB receptors, and by the release of dopamine (Benavides et al., 1982; Howard & Feigenbaum, 1997; Mamelak et al., 1986).

The most accepted clinical use of GHB is in the treatment of narcolepsy, a neurological disorder characterized by frequent bouts of irresistible sleep, catalepsy, and sleep paralysis. A vast array of clinical investigations have reported that the symptoms of narcolepsy can be reduced substantially by GHB treatment [see Mamelak et al. (1986) for a review].

Experimental and clinical observations were reported on the effectiveness and safety of GHB in the treatment of alcoholism. In normal rats conditioned to choose between water and alcohol, the high ethanol intake is dramatically reduced by the administration of gamma-butyrolactone, a GHB prodrug (Fadda et al., 1983). More recently, these results have been confirmed by using GHB in an alcohol-preferring strain of rats (Gallimberti et al., 1992a; Gessa et al., 2000). The abstinence score of ethanol-dependent rats acutely withdrawn from alcohol is dose-dependently reduced by GHB in a fashion similar to that of ethanol (Fadda et al., 1989; Walter et al., in press). Similar results have been achieved with studies of human beings, showing that GHB can diminish the need for alcohol by alcoholics and can aid patients undergoing withdrawal from alcohol (Di Bello et al., 1995; Gallimberti et al., 1989, 1992b; Gessa et al.,

* Corresponding author. Tel.: +39-0-55-4271271; fax: +39-0-55-4271280.

E-mail address: mannaion@pharm.unifi.it (P.F. Mannaioni).

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1993). Results of an open multicentric study showed that GHB is capable of inducing short-term and medium-term abstinence from alcohol in about 60% to 70% of patients treated (Addolorato et al., 1996). In about 30% of nonresponder patients, a greater fractioning in the administration of GHB seems to benefit, owing to the short half-life of the drug (Addolorato et al., 1988). Gamma-hydroxybutyric acid has also been proposed in the treatment of opiate withdrawal syndrome (Gallimberti et al., 1993, 1994). However, the administration of GHB to heroin-dependent subjects receiving a diagnostic challenge with naloxone hydrochloride failed to modify any abstinence sign or symptom (personal observations). In a further clinical appraisal of the use of GHB in the treatment of alcoholic patients, here we report on the effectiveness and safety of GHB in the therapy of overt alcohol withdrawal syndromes, their prevention, and the prevention of relapses in formerly detoxified alcoholics.

2. Patients and methods

2.1. Patients

Patients included in this study were alcohol-dependent subjects admitted to the Toxicological Unit of the Department of Pharmacology, Florence University, School of Medicine, from June 1992 to June 1995. The group consisted of 321 patients (236 men, 73.6%, and 85 women, 26.4%) with a mean age of 40 years (range 18–65 years). All the patients entering the study had severe alcohol dependence, according to the *Diagnostic and Statistical Manual of Mental Disorders* DMS IV criteria, with an average ethanol intake of 150 ml pure ethanol per day.

Written informed consent was obtained from each subject, and the procedures followed were in accordance with the guidelines of the Ethical Committee of the University of Florence and approved by the responsible Institutional Committee for Human Experimentation.

2.2. Open study 1: therapy of overt alcohol withdrawal syndrome

Of 321 subjects, 22 (6.9%) were admitted with a diagnosis of overt alcohol withdrawal syndrome. They were treated with GHB at a daily oral dose of 50–150 mg/kg for 6 days. If signs and symptoms of abstinence were still present after the 6th day of treatment, the drug was administered until a complete remission of the syndrome was noted.

The rating of the abstinence syndrome was done according to the alcohol withdrawal scale. Eight main withdrawal symptoms and signs were evaluated (symptoms: clouding of consciousness; anxiety and panic attacks and paranoid delusions or ideation, visually unpleasant hallucinations; signs: restlessness, tremors, sweating, vomiting, epileptic seizures). Each symptom and sign was scored on a 4-point scale as follows: 0, not present; 1, mild; 2, moderate; and 3, severe. Seizures or hallucinations or both, when present, were scored 10 points each. The sum of these points gave the total score of signs and symptoms for each patient, the minimum being 0 and the maximum being 38 points. The total score was measured before and after 30, 60, and 120 min of medication.

Blood pressure and heart rate also were recorded daily. Routine laboratory tests were carried out on admission and were repeated if there were any abnormalities.

2.3. Open study 2: prevention of withdrawal syndrome

Two hundred eighty-two patients (87.8%) with a diagnosis of alcohol dependence (DMS IV criteria) were admitted for a clinical evaluation of their physical and psychological conditions and for detoxification. All patients were abruptly withdrawn from alcohol and treated with a daily oral dose of GHB (50–100 mg/kg). The incidence of abstinence was subsequently monitored. Patients were excluded from the study if they had seizures, had concurrent severe illnesses, abused other drugs, or were receiving antiepileptic treatment.

2.4. Double-blind study: prevention of relapses and relieve of craving

Seventeen patients (5.3%) were included in a double-blind study to evaluate the effect of GHB versus placebo on their craving for alcohol and relapse into drinking. The population entering the study included alcoholic patients previously detoxified with an inpatient protocol.

When discharged from the hospital, the patients were randomly divided into two groups, A and B. They received the first medical treatment in the hospital; thereafter, they were monitored as outpatients for 6 months. They were treated in a double-blind manner, with solutions A and B, and checked every 2 weeks. Patients were excluded if they had severe neurological or psychiatric illnesses.

The active medication consisted of the sodium salt of GHB dissolved in a brown cherry syrup, at a concentration of 175 mg/ml. Both the active medication and the placebo syrup were supplied by CT Laboratories [Sanremo (IM), Italy].

The intensity of alcohol craving was assessed with a questionnaire, the “Alcohol Craving Scale,” which had been devised and validated in Italy (Canton et al., 1991). The questionnaire contained 14 items, 11 of which required a positive or negative answer, corresponding to 1 or 0 points, respectively, whereas the last 3 items were in multiple-choice form, with 1 point assigned to an answer if present. The minimum craving score was 0 and the maximum score was 14 points.

3. Statistical analyses

We expressed the mean ± standard error of all our observations. Statistical analyses were performed by using Student’s t-test, the Chi-square test, and two-way analysis of variance: a p value of less than 0.05 was considered significant. For each table and figure, we report the test used for statistical analyses.

4. Results

4.1. Open study 1: therapy of overt alcohol withdrawal syndrome

Twenty-two patients (19 men, 3 women), with an average age of 43 years, were admitted to the Toxicological Unit with a diagnosis of alcohol withdrawal.

The individual and mean abstinence scores of this group of subjects on admission are shown in Table 1. The average score was 16.3 ± 2.6, with a maximum of 38 points in one case and a minimum of 10 points in nine cases. Gamma-hydroxybutyric acid was administered orally at the median dose of 125.6 mg/kg divided into three daily doses. After the first 3 days of treatment, the total daily dose of GHB was reduced by 30%. In a majority of patients, GHB was discontinued at day 6; however, 2 patients (admission abstinence score 38 and 32 points, respectively) were treated until the 10th day, because a complete remission of withdrawal symptoms was not observed at the 6th day of treatment.

Table 1
Individual and mean alcohol withdrawal syndrome scores of 22 patients admitted with the diagnosis of alcohol withdrawal/delirium tremens

Age	Signs and symptoms	Individual withdrawal score at admission
33	Tremors, anxiety, sweating	10
24	Tremors, anxiety, sweating, restlessness	13
29	Tremors, anxiety, sweating	10
38	Tremors, anxiety, sweating	10
48	Hallucinations, seizures, tremors, sweating, restlessness	29
53	Hallucinations, tremors, sweating, restlessness, clouding of consciousness	32
60	Hallucinations, seizures, tremors, anxiety, restlessness	31
24	Restlessness, tremors, anxiety, sweating	10
57	Hallucinations, seizures, tremors, anxiety, sweating, clouding of consciousness	38
24	Tremors, anxiety, restlessness	13
24	Hallucinations, tremors, restlessness, sweating	20
35	Tremors, anxiety, sweating	10
64	Hallucinations, tremors, sweating, clouding of consciousness	20
52	Tremors, sweating, restlessness	10
50	Sweating, tremors, restlessness, anxiety	10
56	Tremors, sweating, restlessness, anxiety	10
42	Hallucinations, tremors, clouding of consciousness	18
40	Tremors, restlessness, sweating, anxiety, clouding of consciousness	15
42	Tremors, sweating, anxiety, restlessness	10
61	Tremors, sweating, anxiety, restlessness	11
46	Tremors, sweating, anxiety, restlessness	12
67	Hallucinations, tremors, sweating, anxiety, restlessness	21
Mean 43.05 ± SE ± 4.7		16.3 ± 2.6

As shown in Table 2, there was a rapid decrease in the mean alcohol withdrawal score in all patients, with a significant effect within the first administration of the drug. Nearly all withdrawal signs and symptoms disappeared after 4 days (mean score, 0.9 ± 0.5), and total remission of the alcohol withdrawal syndrome was observed after 10 days.

In comparison with the standard therapy for alcohol withdrawal syndrome [i.e., substitution therapy with ethanol and benzodiazepines (Howard & Feigenbaum, 1997)], treatment with GHB reduced both the mortality rate and the duration of stay in intensive therapy. So far, no deaths from alcohol withdrawal syndromes and delirium tremens have occurred in our unit since the inception of the therapy with GHB, and the length of stay in intensive care has dropped from 87 ± 13 h for a homogeneous group of patients treated with the standard therapy to 47 ± 6 h for the group of patients treated with GHB.

Of 22 patients treated with GHB, 2 (9%) reported slight and transient diarrhea and gastric upset 30 min after the first administration. These signs and symptoms disappeared spontaneously within 1 day and did not recur on the subsequent days of treatment.

4.2. Open study 2: prevention of withdrawal syndrome

Two hundred eighty-two patients (202 men, 80 women) with a mean age of 39 years were admitted with a diagnosis of alcohol dependence, seeking medical evaluation for alcohol-related injuries and detoxification. On admission, none of the subjects showed any abstinence symptoms. However, the detoxification protocol entails abrupt discontinuation of ethanol intake, rendering possible the development of alcohol withdrawal syndromes. Therefore, all patients were treated with GHB from the first day of admission to prevent the onset of alcohol withdrawal syndromes. Gamma-hydroxybutyric acid was administered orally at a mean dose of 88 mg/kg divided into three daily doses. After the first 3 days of treatment, the total daily dose of the drug was reduced by 30% until the 6th day. On the 6th day, GHB was discontinued.

Of 282 patients, 161 (57%) did not present any withdrawal signs or symptoms during their stay in hospital (7–30 days). In the remaining 121 patients (42.9%), a mild abstinence syndrome was observed.

The mean abstinence score was 6.6 on days 1 and 2 of treatment, and a significant decrease in the score was observed on the 3rd day (mean abstinence score, 1.8 points). Nearly all the abstinence symptoms disappeared on the 4th day after admission (mean score, 0.2 points).

To sum up, GHB prevented the onset of alcohol withdrawal syndrome in 57% of 282 patients. The abstinence signs and symptoms observed in the remaining 42% were mild (mean score, 6.6 points) and promptly resolved in 72 h. Twenty (7%) of the 282 treated subjects showed some transient adverse effects (i.e., diarrhea, dizziness, and vomiting), which disappeared spontaneously within 1 day and did not necessitate the discontinuation of therapy.

Table 2
Time course of the effect of GHB (median dose 125.6 mg/kg/day[a] on the individual and mean withdrawal scores in 22 patients admitted with the diagnosis of alcohol withdrawal syndrome/delirium tremens: Individual withdrawal scores

		Days after starting therapy								
	At admission	1–2	3	4	5	6	7	8	9	10
	10	4	2	0	0	0	0	0	0	0
	13	1	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0
	10	1	1	1	0	0	0	0	0	0
	29	5	2	0	0	0	0	0	0	0
	32	16	2	2	2	2	0	0	0	0
	31	3	1	1	0	0	0	0	0	0
	10	1	1	0	0	0	0	0	0	0
	38	20	15	10	10	5	2	2	1	0
	13	8	3	0	0	0	0	0	0	0
	20	2	2	0	0	0	0	0	0	0
	10	7	0	0	0	0	0	0	0	0
	20	4	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0
	10	5	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0
	18	3	0	0	0	0	0	0	0	0
	15	8	6	0	0	0	0	0	0	0
	10	3	0	0	0	0	0	0	0	0
	10	3	0	0	0	0	0	0	0	0
	12	5	3	3	1	0	0	0	0	0
	21	5	0	0	0	0	0	0	0	0
Mean ± SE	16.3 ± 2.6	4.9 ± 1.7	2.0 ± 1.2	0.9 ± 0.5	0.6 ± 0.4	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.06 ± 0.04	0

[a] Oral administration. All patients were treated for 6 days. If signs and symptoms were still present, the treatment was continued until complete remission. See Methods for further details.

4.3. Double-blind study: prevention of relapses and relieve of craving

Seventeen alcoholic subjects (13 men, 4 women; mean age, 46.4 years) entered the double-blind study. After an inpatient stay at the Toxicological Unit for a 30-day detoxification, they were monitored as outpatients through medical and psychological examinations carried out every week for 6 months. They were randomly divided into two groups (group A and group B) and, when the code was opened, group A proved to have been treated with GHB (mean daily oral dose 50 mg/kg) and group B with placebo.

Figure 1 shows that the treatment with GHB was effective in reducing alcohol craving in formerly detoxified alcoholics. Both groups under study had a high comparable score on the Alcohol Craving Scale when the outpatient study began, although they had remained abstinent during their 30-day inpa-

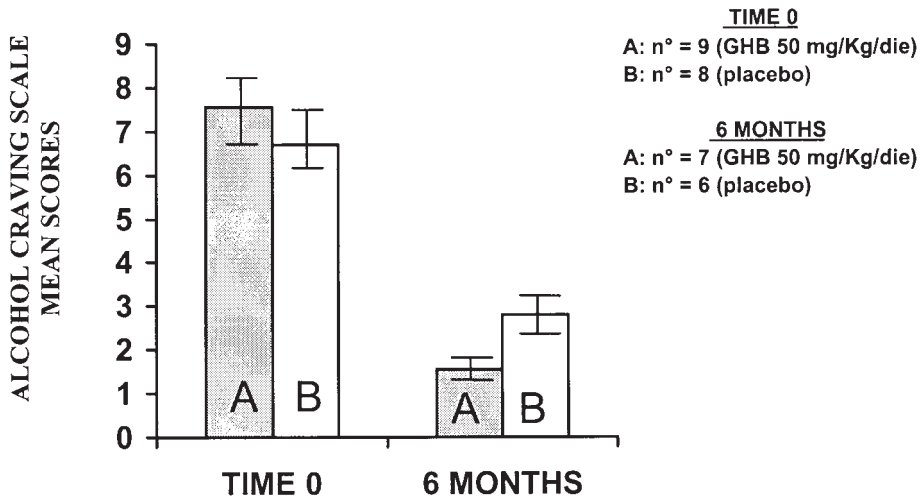

Fig. 1. Mean scores of Alcohol Craving Scale in GHB-treated and placebo-treated patients. Student's *t*-test: at time 0, A vs. B not significantly different (n.s.); A at time 0 vs. A at 6 months, $p < 0.05$; B at time 0 vs. B at 6 months, n.s.; at 6 months, A vs. B, $p < 0.05$.

Table 3
Drop-out, abstinence, and relapse in GHB- (50 mg/kg/day) and placebo-treated patients

	Number of patients	Drop-out[a]	%	Abstinent	%	Relapse[a]	%
Group A (GHB 50 mg/kg/day)	9	2	22	6	66.6	1	11.1
Group B (placebo)	8	2	25	4	50.0	2	25.0

[a] 6-month follow-up.
Chi-square test: n.s. group A vs. group B.

tient stay in the hospital. After a 6-month treatment, an overall decrease in the alcohol craving score was observed in both groups. However, the decrease in the alcohol craving score was significantly greater in the group treated with GHB than in the group treated with placebo (Fig. 1).

The effect of GHB on relapse in formerly detoxified alcoholics is shown in Table 3. Compliance with the therapy was satisfactory, as shown by the low drop-out rate, which is similar in the two groups. At the end of the study, the percentage of subjects remaining abstinent was higher in the group treated with GHB (66.6%) than in the placebo group (50%). The retention rate (Fig. 2) was significantly higher in the GHB-treated group, in comparison with the placebo-treated group.

Table 4 summarizes the differences between GHB-treated and placebo-treated patients. The data show that the cohort of patients receiving GHB has a better outcome in comparison with those treated with placebo. In fact, the percentage of patients who remained abstinent at the end of GHB treatment was higher and the percentage of relapses was lower. Moreover, the retention in treatment was higher in the GHB group (24 weeks vs. 16 weeks in the placebo group) and the retention rate at 6 months was accordingly higher.

Adverse effects were distributed as follows: two (18.2%) of the patients receiving placebo complained of nausea and vomiting after the first morning dose on the first 3 days of treatment; two (22.2%) of the patients receiving GHB complained of dizzines after the first morning dosage persisting for 3 to 4 h. These signs and symptoms disappeared after the 3rd day of treatment.

4.4. Evaluation of the GHB diversion

In recent years, GHB has been sold illicitly as a steroid alternative for body building (due to the induction of growth-hormone release), as a tryptophan replacement for weight control and sedation, and in the dance music scene as an alternative to ecstasy and speed. The administration of GHB has been shown to produce a significant stimulation of growth-hormone secretion in normal subjects (Takahara et al., 1977). In eight healthy young men, the stimulation of growth-hormone secretion was significantly correlated to a simultaneous increase in the amount of slow-wave sleep (Van Cauter et al., 1997). At fitness centers, the diverted use of GHB has spread as a recreational drug, owing to its euphoric effects. The diverted use of GHB raises the question of addiction liability and of acute poisoning in the recreational settings. A GHB-induced "high" was reported in subjects who chronically used GHB as a recreational drug. Many of them stated that the drug makes them feel good and produces a pleasurable experience (Mathivet et al., 1997). Possible physical dependence on GHB was reported in a woman attempting to withdraw from chronic GHB use and experiencing anxiety, tremor, and insomnia (Galloway et al., 1994). The same withdrawal syndrome was described

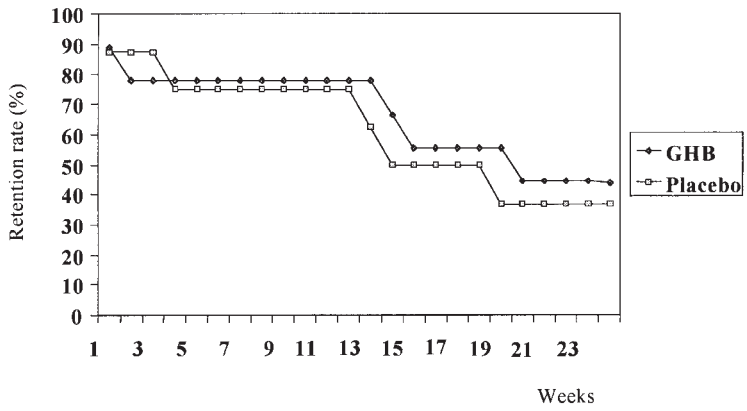

Fig. 2. Retention rate (%) in the program within GHB- and placebo-treated groups. Two-way ANOVA: GHB vs. placebo, $p < 0.05$.

Table 4
Patients treated with GHB and placebo

	GHB	Placebo
Average stay in the program (weeks)	24.9 ± 5.0[a]	16.6 ± 4.0
% abstinent patients at the end of the program	66.6[a]	50.0
% relapses	11.0[a]	25.0
Retention rate at 6 months (%)	44.0 ± 3.0[b]	37.0 ± 3.7

[a] Chi-square test: gamma-hydroxybutyric acid vs. placebo, not significantly different.
[b] Two-way ANOVA: gamma-hydroxybutyric acid vs. placebo, $p < 0.05$.

in a series of eight people chronically using GHB (Galloway et al., 1997). The withdrawal syndrome resolves without sequelae in 3 to 12 days (Galloway et al., 1997).

There is a growing number of reported cases of acute poisoning by GHB. Acute poisonings are characterized by seizures, coma, bradycardia, and dizziness. A simultaneous intake of alcohol worsens the symptoms, and a fatal case of combined use of GHB and heroin has been described (Ferrara et al., 1995; Louagie et al., 1997). Forty cases of neurotoxicity in recreational drug users have been described (Carter et al., 1997). An anecdotal observation of GHB-induced Wernicke-Korsakoff syndrome has been reported (Friedman et al., 1996). Facing these alarming trends in the spread of GHB as a drug of abuse, we have evaluated the numbers of acute poisoning by GHB and of GHB abuse among the population referred to our Toxicological Unit. There were 9 (0.26%) acute poisonings by GHB of 3389 drug-related poisonings in the years 1992–1995. Gamma-hydroxybutyric acid-induced abuse was reported in 4 (1.1%) of 354 treated patients in the years 1992–1995, rendering less alarming the toxicological profile of GHB in our setting.

5. Discussion

Our results show that GHB is effective in the suppression and prevention of abstinence syndrome in alcoholics. In fact, in all our patients treated with GHB for withdrawal syndrome, the decrease in symptoms was more rapid than that observed with the standard therapy and was not accompanied by serious side effects. Moreover, the administration of GHB to alcoholics abruptly withdrawn from drinking at hospital admission, prevented the onset of withdrawal syndrome in 55% of cases, and, in the remaining 45% of patients, the signs and symptoms were mild and promptly resolved.

Our results also show that GHB is effective in reducing ethanol craving. The results of the double-blind study after a 6-month follow-up demonstrate that GHB treatment was more successful than placebo. In fact, the percentage of patients who remained abstinent until the end of the trial was higher in the GHB group, even though the percentages of drop-out were similar. Moreover, the craving for alcohol was significantly lower in the GHB-treated group. However, our results have to take into account both the limited number of patients enrolled in the double-blind study and the short period (6 months) of evaluation and need more extended analyses.

The protective action of GHB against ethanol withdrawal syndrome and craving is not due to its sedative and hypnotic effects. Experimental evidence suggests that the effects of GHB are mediated by a GABAergic mechanism (interaction between GHB and the $GABA_B$ receptor) by which GHB can mimic the action of ethanol in the central nervous system. Indeed, ethanol and GHB have some pharmacological properties in common in that both suppress ethanol withdrawal syndrome, chronic ethanol consumption confers tolerance to GHB, GHB inhibits voluntary ethanol intake in alcohol-preferring rats, and a short-chain alcohol is present in GHB (Gessa et al., 2000). One possible reason for the similar mechanism of action of ethanol and GHB is that ethanol modulates chloride ion channels at $GABA_A$ receptors, whereas GHB modulates K^+ and Ca^{2+} channels at $GABA_B$ receptors (Tunnicliff, 1997). Both effects result in decreased neuronal excitability. Finally, it is relevant to point out that the protective action of GHB on ethanol craving might depend on its capacity to interfere with the release of the main modulators of the ethanol reward system, such as dopamine and serotonin.

Whatever its exact mechanism of action is, our results indicate that GHB is a clinically useful drug in the treatment of alcohol dependence.

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Gamma-hydroxybutyric acid and growth hormone secretion Studies in rats and dogs

Antonello E. Rigamonti*, Eugenio E. Müller

Department of Pharmacology, Chemotherapy, and Toxicology, University of Milan, via Vanvitelli 32, 20129 Milan, Italy

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Abstract

Gamma-hydroxybutyric acid, a gamma-aminobutyric acid metabolite, and baclofen, a gamma-aminobutyric acid B agonist, are endowed with a small growth hormone-releasing activity in human beings. In this study, we have investigated the reciprocal interactions of gamma-hydroxybutyric acid and the gamma-aminobutyric acid B system by evaluating the growth hormone-releasing activity of the two compounds and their respective antagonists in in vivo and in vitro experiments performed in rats and dogs. In in vivo experiments, neither gamma-hydroxybutyric acid (25, 100, 150, and 300 mg/kg, SC) nor baclofen (0.25, 1, 2, 4, and 8 mg/kg, SC) significantly modified growth hormone secretion in 9-day-old rat pups. Similarly, no growth hormone and prolactin release was observed in adult anesthetized rats after administration of gamma-hydroxybutyric acid (100 mg/kg, IP) or baclofen (10 mg/kg IP). Equally ineffective on the somatotropic response was the administration of gamma-hydroxybutyric acid (200 mg/kg, IP) alone or associated with its specific receptor antagonist NCS-382 (150 mg/kg, IP) given to adult anesthetized rats. In addition, a toxicological dose of gamma-hydroxybutyric acid (1500 mg/kg, IP) did not alter baseline growth hormone levels in adult conscious rats. gamma-Hydroxybutyric acid (50 mg/kg, IP) given for 10 days to adult conscious rats did not alter the growth hormone response to the same gamma-hydroxybutyric acid dose given acutely. In conscious dogs, gamma-hydroxybutyric acid (20 and 50 mg/kg, IV) and baclofen (0.15, 0.30 mg/kg, IV) also were ineffective in stimulating growth hormone secretion. In this species, growth hormone response to hexarelin (31.25 μg/kg, IV), a potent growth hormone-releasing peptide, was not modified by coadministration of gamma-hydroxybutyric acid (50 mg/kg, IV). In in vitro experiments, increasing doses of gamma-hydroxybutyric acid (10^{-7}, 10^{-5}, and 10^{-3} M) did not alter growth hormone concentrations in media of rat pituitary cell cultures. In contrast, growth hormone-releasing hormone (10^{-7} M) induced a significant growth hormone release into the media. In conclusion (1) gamma-hydroxybutyric acid is not an effective growth hormone secretagogue; (2) the reciprocal functional interactions between gamma-hydroxybutyric acid and the gamma-aminobutyric acid B system could not be investigated, due to the ineffectiveness of gamma-hydroxybutyric acid and baclofen to stimulate growth hormone release; and (3) short-term administration of gamma-hydroxybutyric acid does not induce adverse effects amenable to activation of the somatotropic function.

Keywords: Growth hormone; Prolactin; Gamma-hydroxybutyric acid; NCS-382; Hexarelin; Clonidine hydrochloride; Rats; Dogs; Pituitary cell cultures

1. Introduction

The secretion of growth hormone (GH) is regulated through a complex neuroendocrine control system and, especially, by the functional interplay of two hypothalamic hypophysiotropic hormones, GH-releasing hormone (GHRH) and somatostatin. These hormones exert, respectively, stimulatory and inhibitory influences on the somatotrope (Müller, 1987). In the past years, a new series of small peptides with strong GH-releasing properties have been identified, the GH-releasing peptides, which recognize specific receptors, whose endogenous ligands are yet to be known (Locatelli & Torsello, 1997). Growth hormone-releasing hormone and somatostatin are, in turn, subject to modulation by a host of neurotransmitters, especially those belonging to the catecholaminergic, colinergic, and GABAergic systems (Müller & Nisticò, 1989).

Gamma-hydroxybutyric acid (GHB), a breakdown product of gamma-aminobutyric acid (GABA), reportedly stimulates in human beings the secretion of GH and prolactin (PRL) (Gerra et al., 1994, 1995; Takahara et al., 1977; Vescovi & Di Gennaro, 1997; Volpi et al., 1997). A GABA-mediated mechanism for GHB is suggested by the finding that flumazenil, an antagonist of benzodiazepine receptors, counteracts the GH response to an oral dose of the compound (Gerra et al., 1994) and is consistent with the idea that GHB acts on a subpopulation of the GABA receptors

* Corresponding author. Tel.: +39-02-70146212; fax: +39-02-70106838.
E-mail address: antonello.rigamonti@unimi.it (A.E. Rigamonti).

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closely related to benzodiazepine receptors (Serra et al., 1991). The finding that bicuculline antagonizes the stimulatory effect of GHB on GH secretion (Vjayan & McCann, 1978) is consistent with a $GABA_A$ receptor-mediated mechanism. Some data, however, contradict this view and point to an involvement of the $GABA_B$ receptor in the GHB effects (Maitre, 1997). They include its inability to modify the chloride function coupled with the $GABA_A$ receptor (Serra et al., 1989) and the strong attenuation of some of its electrophysiological effects by $GABA_B$ receptor antagonists (Bernasconi et al., 1992; Engberg & Nissbrandt, 1993). Supporting this proposition, results of a series of studies have shown that, in healthy subjects, baclofen, a $GABA_B$ agonist, induced clear-cut GH increments in plasma with a peak after 60–90 min (Davis et al., 1996; Koulu et al., 1979).

To ascertain the existence of reciprocal functional interaction in the neuroendocrine effects of GHB and $GABA_B$, we evaluated the GH-releasing activity of GHB and baclofen given alone or together with their specific receptor antagonists NCS-382 (Maitre et al., 1990) and CGP35348 (Froestl et al., 1995), respectively. Either in vivo or in vitro acute experiments were performed, the former with the use of two different animal species (rat and dog). A second goal of this study was to investigate the safety and tolerability of GHB, after its short-term administration. Thus, we tested the GH pituitary responsiveness to an acute administration of GHB in rats treated with the compound for 10 days.

2. Materials and methods

2.1. In vivo experiments: rats

Infant (9-day-old, both sexes) and young adult (1 year-old, 250–250 g, both sexes) Sprague-Dawley rats (Charles River, Calco, Italy) were used in these experiments. Neonatal pups were left with the dams until 1 h before the experiment. Adult rats were housed under controlled conditions (23°C, 65% humidity, and artificial light from 07.00 to 19.00 h). They were fed with dry food (Mucedola srl, Settimo Milanese, Italy) and water ad libitum.

2.1.1. Acute experiments in infant rats

Rat pups, divided randomly in ten experimental groups of 11 pups each, were given GHB (Alcover, 25, 100, 150, and 300 mg/kg, SC; Laboratorio Farmaceutici CT srl, Sanremo, Italy), baclofen (0.25, 1, 2, 4, and 8 mg/kg, SC; Sigma Tau, Milan, Italy), or saline 0.9% (300 μl, SC). After 30 min postinjection, rats were killed by decapitation, and blood samples were collected from the trunks.

2.1.2. Acute experiments in anesthetized adult rats

Adult rats were anesthetized with ketamine (Inoketan, 0.05 ml/100 g, IP; Virbac, Milan, Italy) and xylazine (Rompun, 0.1 ml/100 gr, IP; Bayer, Milan, Italy), and then a jugular cannula was inserted into the right atrium to draw blood samples (300 μl) at different time intervals (−30, 0, 30, 60, and 120 min) after IP administration of:

1. Gamma-hydroxybutyric acid (100 mg/kg), baclofen (10 mg/kg), or saline (2 ml/kg) (eight rats per group: four males and four females)
2. Gamma-hydroxybutyric acid (200 mg/kg), NCS-382 (150 mg/kg; obtained through the courtesy of Dr. R. Clerici, Department of Organic Chemistry, School of Pharmacy, University of Milan, Milan, Italy), GHB + NCS-382, or saline (2 ml/kg) (four rats per group: two males and two females)

At the end of the experiments, animals were killed by decapitation, and blood samples were collected from the trunks.

2.1.3. Acute experiments in conscious adult rats

Three unanesthetized adult male rats were given GHB (1500 mg/kg, IP) or saline (2 ml/kg, IP). After 60 min, animals were decapitated, and blood samples were collected from the trunks.

2.1.4. Short-term experiments in conscious adult rats

Three adult male rats were administered a daily dose of GHB (50 mg/kg, IP) for 10 days or were injected with saline (2 ml/kg, IP). On the 11th day, 24 h after the last injection, all rats were given GHB (50 mg/kg, IP). After 30 min postinjection, animals were killed by decapitation, and blood samples were collected from the trunks.

In all these experiments, blood samples were collected in tubes containing EDTA, 0.15 M (Sigma Tau, Milan, Italy), and centrifuged, and plasma samples were frozen and kept at −20°C until assayed for rat GH (rGH) or rat PRL (rPRL) or both by radioimmunoassay (see Section 2.3.2).

2.2. In vivo experiments: dogs

Four young (age 3–4 years old; weight 8–12 kg; two females, two males) well-trained beagle dogs were used in this study. They were fed normal dry food (Diete Standard, Charles River, Italy) with water available ad libitum and were kept on a 12-h light:12-h dark regimen, with light on at 07.00 h. Body weights of the dogs were stable, and they had no observable diseases. All experiments were carried out in conscious animals after an overnight fast, starting at 09.00 h. Before the experiments, animals were kept at rest for at least 1 h. Blood samples were drawn at regular intervals from the cephalic vein through an indwelling nonthrombogenic catheter. An interval of at least 1 week was kept between individual experiments.

2.2.1. Acute experiments in conscious dogs: gamma-hydroxybutyric acid, baclofen, clonidine, or saline

Dogs were given, in a crossover randomized design, GHB (20 or 40 mg/kg, IV), baclofen (0.15 or 0.30 mg/kg, IV), clonidine (Catepresan, 4 μg/kg, IV; Boheringer Ingelheim, Milan, Italy), an alpha-2-adrenergic agonist, endowed with GH-releasing activity (Arce et al., 1990), or saline (0.1 mg/kg). Blood samples were drawn at −30 and 0 min and then at 15, 30, 45, 60, 90, 120, and 150 min postdrug administration.

2.2.2. Acute experiments in conscious dogs: gamma-hydroxybutyric acid, hexarelin, or gamma-hydroxybutyric acid plus hexarelin

Dogs were administered, in a crossover randomized design, GHB (50 mg/kg, IV), hexarelin (31.25 μg/kg, IV; Europeptides, Argenteuil, France), a potent analogue of GH-releasing peptides (Cella et al., 1995), or both compounds.

Blood samples were drawn at −30 and 0 min and then at 15, 30, 45, 60, 90, and 120 min after drug administration.

Blood samples (1.5 ml) were collected in tubes containing EDTA, 0.15 M (Sigma Tau, Milan, Italy). Plasma samples were frozen and kept at −20°C until assayed for canine GH by radioimmunoassay (see Section 2.3.2).

All protocols for rats and dogs had been previously authorized by the Committee on Animal Care and Use, University of Milan.

2.3. In vitro experiments

2.3.1. Effect of growth hormone releasing hormone and gamma-hydroxybutyric acid on growth hormone secretion in pituitary cell cultures

In these experiments, five adult female rats were decapitated, their pituitary glands were rapidly dissected from the sella turcica, and the posterior lobes were discarded. Briefly, the anterior pituitary glands were collected in sterile F-10 medium and, after they had been cut into small fragments, incubated twice at 37°C in F-10 medium containing 6% fetal calf serum and collagenase (2.5 mg/ml). Fragments were then washed in Dulbecco's phosphate buffered saline, Ca^{2+}- and Mg^{2+}-free medium, and mechanically dissociated. Single-cell suspension was plated onto 24-well (2×10^5 cells/well) culture plates. The cells were incubated in F-10 medium supplemented with 10% horse serum, 4% fetal calf serum, and gentamicin (25 μg/ml), in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. After 3 days, the medium was removed, and the cells were washed twice with serum-free F-10 and then incubated with 1 ml of F-10 containing 0.1% bovine serum albumin only or with added various concentrations of GHB (10^{-3}, 10^{-5}, and 10^{-7} M) and 10^{-7} M GHRH (Geref, Serono, Milan, Italy). After incubation for 4 h at 37°C, media were collected and stored frozen at −20°C until measurement of rGH concentrations. Five wells per group were used in each experiment.

2.3.2. Radioimmunoassays: plasma and medium rat growth hormone, rat prolactin, and canine growth hormone

Plasma and medium rGH and plasma rPRL and canine GH concentrations were determined by a double antibody radioimmunoassay (Cella et al., 1994, 1995). Highly purified amounts of these hormones as standard and for iodination and the respective antibodies were kindly provided by Dr. A. F. Parlow (Pituitary Hormones and Antisera Center, Torrance, CA, USA). The sensitivity of these assays was 0.39 ng/ml; intraassay variability was 5%. To prevent possible interassay variation, all samples of a given experiment were assayed in a single radioimmunoassay.

2.4. Statistical analysis

Growth hormone values were expressed either as mean area under the plasma concentration versus time curves [area under curve $(AUC)_{0–120}$; ng/ml × min)] ± SEM, calculated by the trapezoidal integration or as absolute mean peak values (ng × ml) ± SEM. Because no differences in hormone levels between male and female rats and in dogs were observed in the different experiments, data were pooled.

Statistical comparisons of the mean values were performed by the t-test for unpaired or paired (where necessary) data, preceded by analysis of variance. A p value of less than 0.05 was taken to be statistically significant.

3. Results

3.1. In vivo experiments: rats

3.1.1. Acute experiments in infant rats

In rat pups, administration of four scalar doses of GHB induced no significant GH release over that present in the control group (peak GH response: 19.1 ± 4.0 ng/ml, 22.7 ± 5.4 ng/ml, 14.1 ± 3.6 ng/ml, and 19.1 ± 4.4 ng/ml vs. 19.5 ± 4.9 ng/ml, respectively, p = NS; Fig. 1). Similar results were obtained when five scalar doses of baclofen were used. In fact, the GABAergic agonist did not significantly change GH secretion with respect to that present in rat pups treated with saline (peak GH response: 17.4 ± 5.0 ng/ml, 17.9 ± 5.6 ng/ml, 16.3 ± 3.4 ng/ml, 20.5 ± 6.5 ng/ml, and 17.8 ± 7.3 ng/ml vs. saline, respectively, p = NS; Fig. 1).

3.1.2. Acute experiments in anesthetized adult rats

Administration of GHB induced no increase in plasma GH and PRL concentrations over basal levels (0 min; p = NS; Fig. 2). Similar results were observed when rats were given baclofen (p = NS; Fig. 3). Similarly, the AUCs of GH and PRL responses to GHB or baclofen were not significantly different from those obtained after saline injection (AUC_{GH}: 4323.6 ± 596.8 ng/ml × min and 4580.9 ± 681.2 vs. 4394.9 ± 287.6 ng/ml × min, p = NS, respectively; AUC_{PRL}: 1989.9 ± 370.4 ng/ml × min and 2161.9 ± 373.1 vs. 2578.7 ± 240.6 ng/ml × min, p = NS, respectively; Fig. 4). Additionally, a higher dose of GHB (200 mg/kg, IP) did not increase GH secretion over basal levels (−30 min; p = NS; Fig. 5). Administration of the GHB receptor antagonist NCS-382 did not modify the basal GH levels (p = NS; Fig. 5), and similarly ineffective was the coadministration of GHB and NCS-382 (Fig. 5). The AUCs of GH responses to GHB or NCS-382 or both were not significantly different from those obtained after saline injection (AUC_{GH}: 4121.3 ± 413.4 ng/ml × min, 4455.6 ± 409.1 ng/ml × min, and 4872.5 ± 584.2 vs. 4864.8 ± 866.1 ng/ml × min, p = NS, respectively; Fig. 6).

3.1.3. Acute experiments in unanesthetized adult rats

Administration of a huge dose of GHB (1500 mg/kg, IP) was followed by a progressive time-dependent reduction in

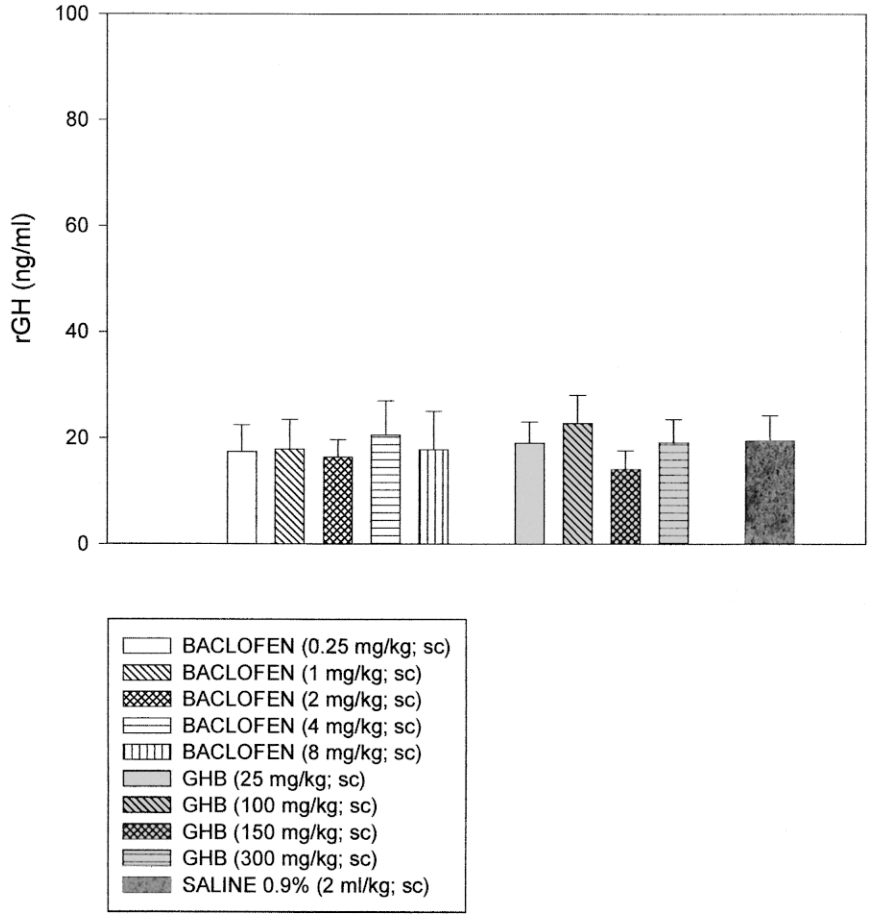

Fig. 1. Growth hormone plasma concentrations (ng/ml) ± SEM in 9-day-old rat pups given baclofen (0.25, 1, 2, 4, and 300 mg/kg, SC) or saline (0.9%; 2 ml/kg, SC).

wakefulness with loss of the righting reflexes, though complete anesthesia was not observed. Plasma GH concentration 60 min after GHB administration was not significantly different from that present after saline injection (43.8 ± 8.1 ng/ml vs. 46.1 ± 4.5, p = NS; Fig. 7).

3.1.4. Short-term experiments in adult rats

Short-term treatment with GHB (50 mg/kg, IP) did not modify the GH responsiveness to an acute administration of the same dose of GHB in comparison with the pattern present in rats treated with saline (30.9 ± 9.2 ng/ml vs. 37.7 ± 10.4, p = NS; Fig. 8).

3.2. In vivo experiments: dogs

3.2.1. Gamma-hydroxybutyric acid, baclofen, clonidine, or saline

In dogs, the administration of two doses of GHB induced no GH release (AUC_{GH}: 98.5 ± 18.7 ng/ml × min and 110.2 ± 6.4 ng/ml × min vs. 104.7 ± 6.0 ng/ml × min, p = NS, respectively; Figs. 9 and 10). Additionally, the GH responses to two doses of baclofen were not significantly different from those obtained in dogs treated with saline (AUC_{GH}: 106.8 ± 5.7 ng/ml × min, 113.3 ± 5.2 ng/ml × min vs. saline, p = NS, respectively; Figs. 9 and 10).

In contrast with GHB and baclofen, the administration of clonidine significantly stimulated GH secretion (AUC_{GH}: 373.0 ± 34.3 ng/ml × min vs. saline, $p < 0.01$; Figs. 9 and 10).

3.2.2. Gamma-hydroxybutyric acid, hexarelin, or gamma-hydroxybutyric acid plus hexarelin

The administration of hexarelin induced a rise in plasma GH concentrations in all dogs; GH peak was 7.9 ± 0.4 ng/ml (30 min; $p < 0.01$ vs. baseline; Fig. 11). In contrast, the administration of GHB did not modify basal GH concentrations (GH peak: 0.8 ± 0.2 ng/ml; AUC_{GH}: 77.8 ± 9.7 ng/ml × min; Figs. 11 and 12), and GHB failed to modify the GH response to hexarelin when administered together with the peptide (AUC_{GH}: 286.4 ± 22.0 ng/ml × min vs. 297.8 ± 27.0 ng/ml × min, p = NS, respectively; Figs. 11 and 12).

3.3. In vitro experiments: Effect of gamma-butyric acid and growth hormone-releasing hormone on growth hormone secretion in pituitary cell cultures

At no dose did gamma-hydroxybutyric acid induce an increase in GH concentrations in the pituitary cell culture medium (23.7 ± 2.0 ng/ml, 23.5 ± 4.0 ng/ml, and 29.1 ± 5.9 ng/ml vs. 27.0 ± 7.7 ng/ml, p = NS, respectively; Fig. 13). In contrast, GHRH significantly stimulated GH release from

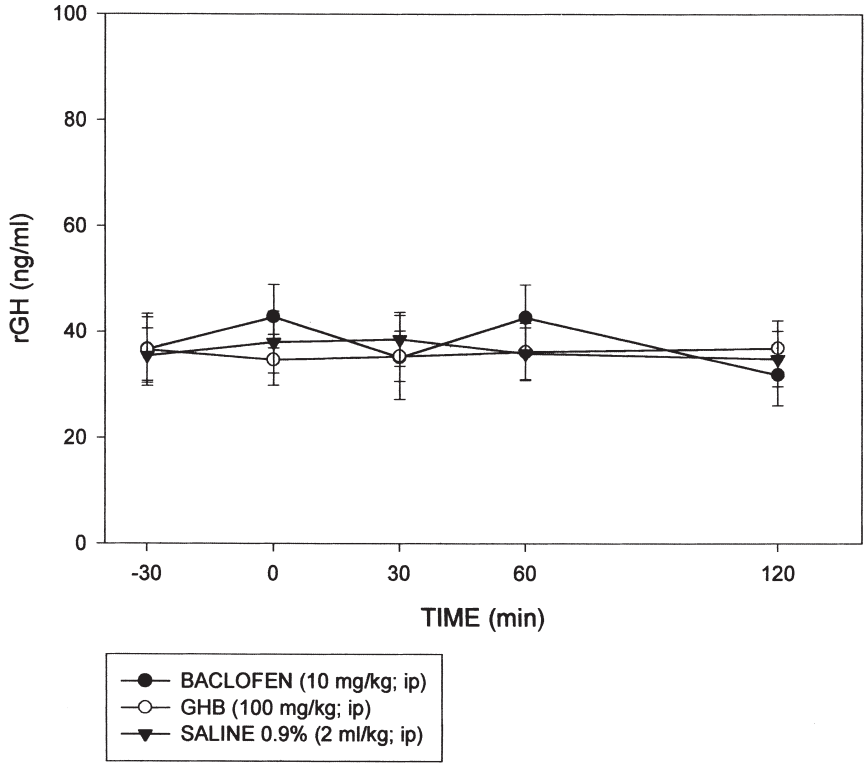

Fig. 2. Growth hormone and prolactin plasma concentrations and areas under the curve (ng/ml × min) ± SEM (AUC_{GH} and AUC_{PRL}) in anesthetized adult rats given baclofen (10 mg/kg, IP), gamma-hydroxybutyric acid (100 mg/kg, IP), or saline (2 ml/kg, IP).

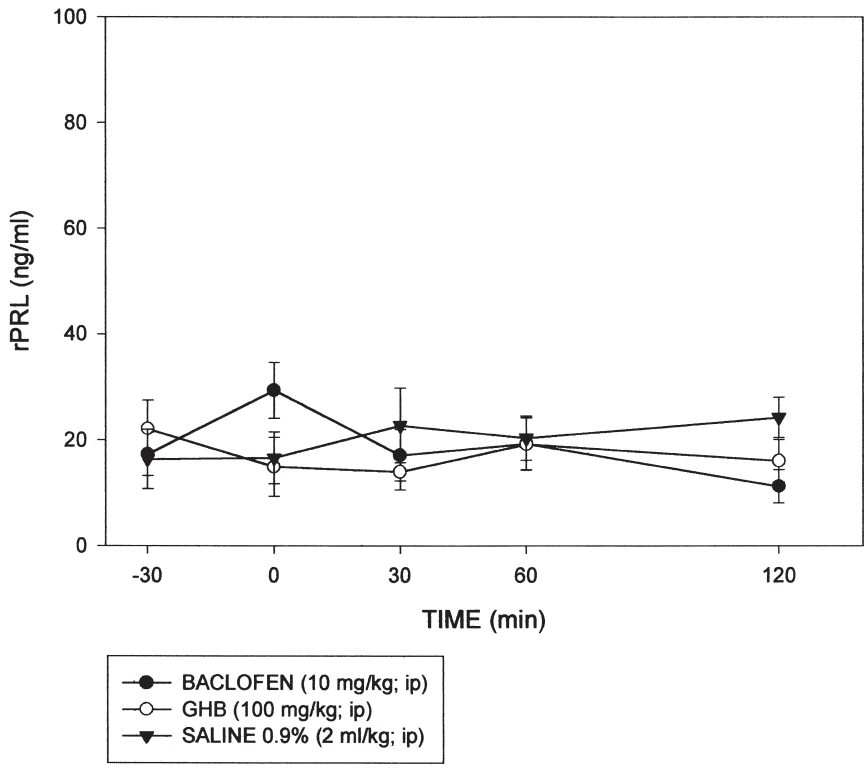

Fig. 3. Growth hormone and prolactin plasma concentrations and areas under the curve (ng/ml × min) ± SEM (AUC_{GH} and AUC_{PRL}) in anesthetized adult rats given baclofen (10 mg/kg, IP), gamma-hydroxybutyric acid (100 mg/kg, IP), or saline (2 ml/kg, IP).

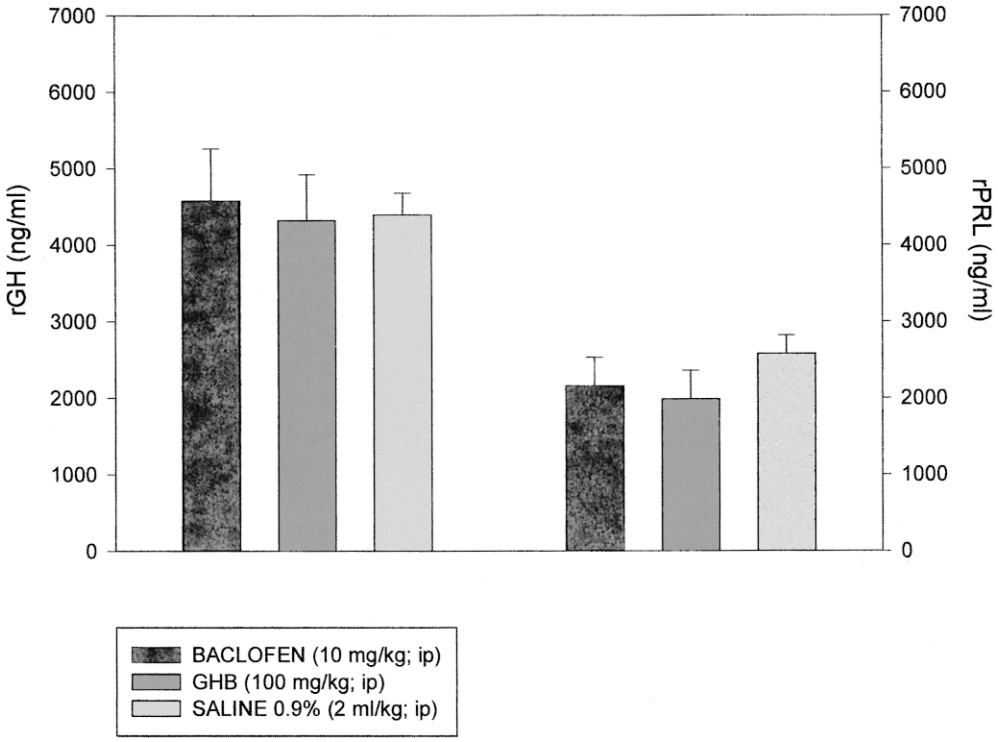

Fig. 4. Growth hormone and prolactin plasma concentrations and areas under the curve (ng/ml × min) ± SEM (AUC_{GH} and AUC_{PRL}) in anesthetized adult rats given baclofen (10 mg/kg, IP), gamma-hydroxybutyric acid (100 mg/kg, IP), or saline (2 ml/kg, IP).

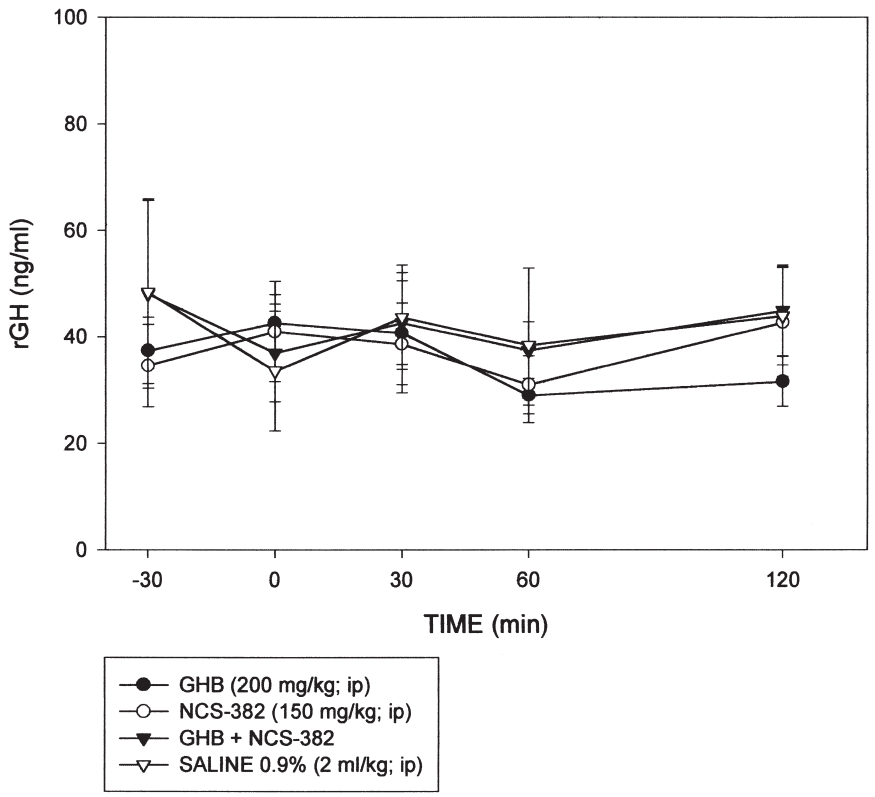

Fig. 5. Growth hormone plasma concentrations and AUC_{GH} in anesthetized adult rats given gamma-hydroxybutyric acid (200 mg/kg, IP), NCS-382 (150 mg/kg, IP), or gamma-hydroxybutyric acid plus NCS-382, or saline (2 ml/kg, IP).

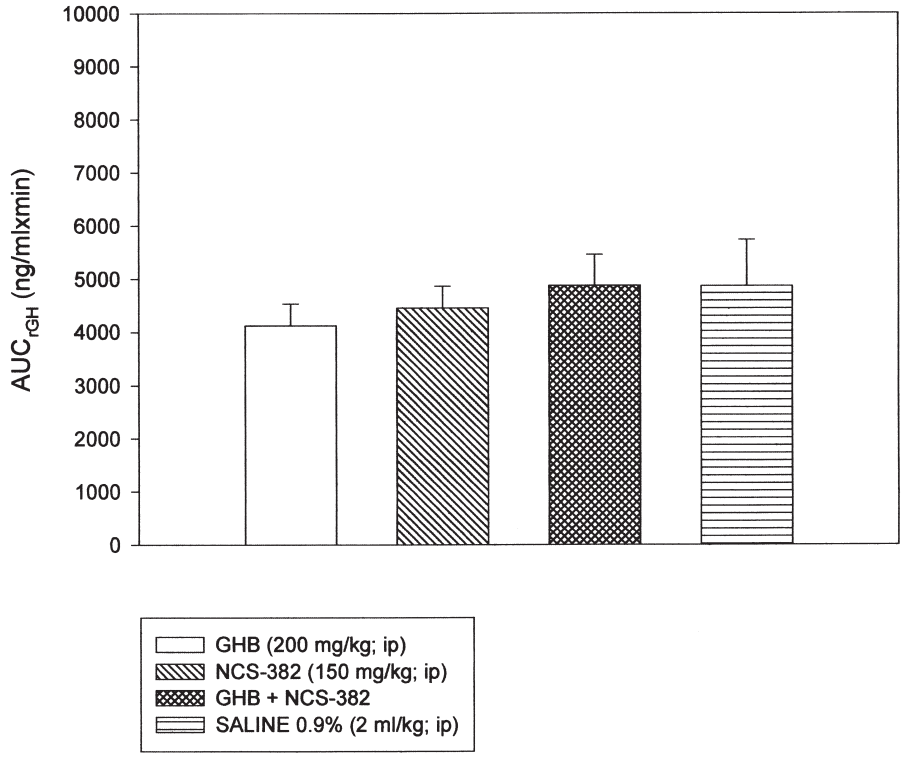

Fig. 6. Growth hormone plasma concentrations and AUC_{GH} in anesthetized adult rats given gamma-hydroxybutyric acid (200 mg/kg, IP), NCS-382 (150 mg/kg, IP), or gamma-hydroxybutyric acid plus NCS-382, or saline (2 ml/kg, IP).

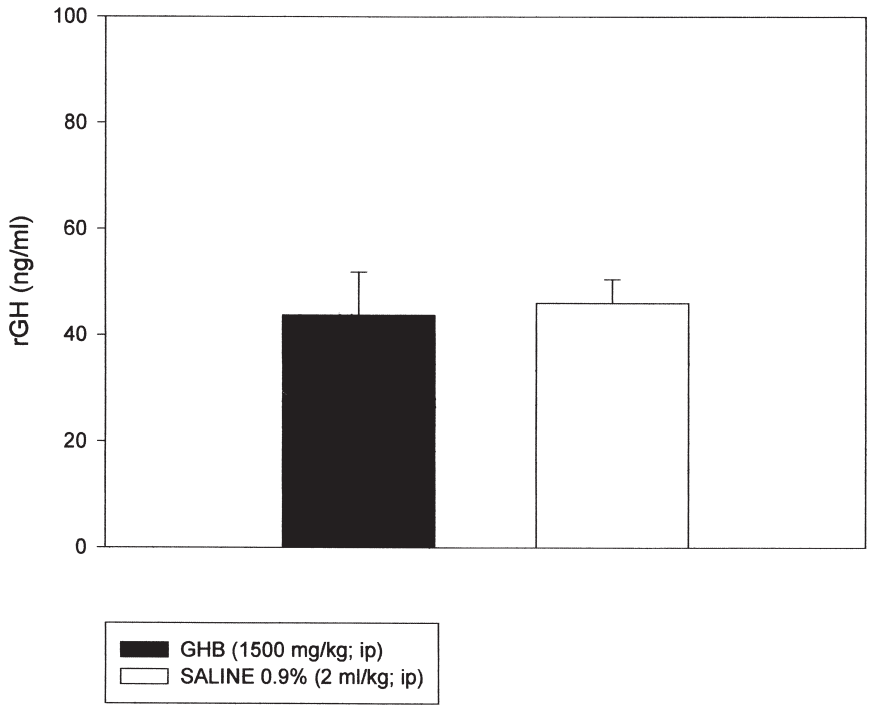

Fig. 7. Growth hormone plasma concentrations in unanesthetized adult rats given gamma-hydroxybutyric acid (1500 mg/kg, IP) or saline (2 ml/kg, IP).

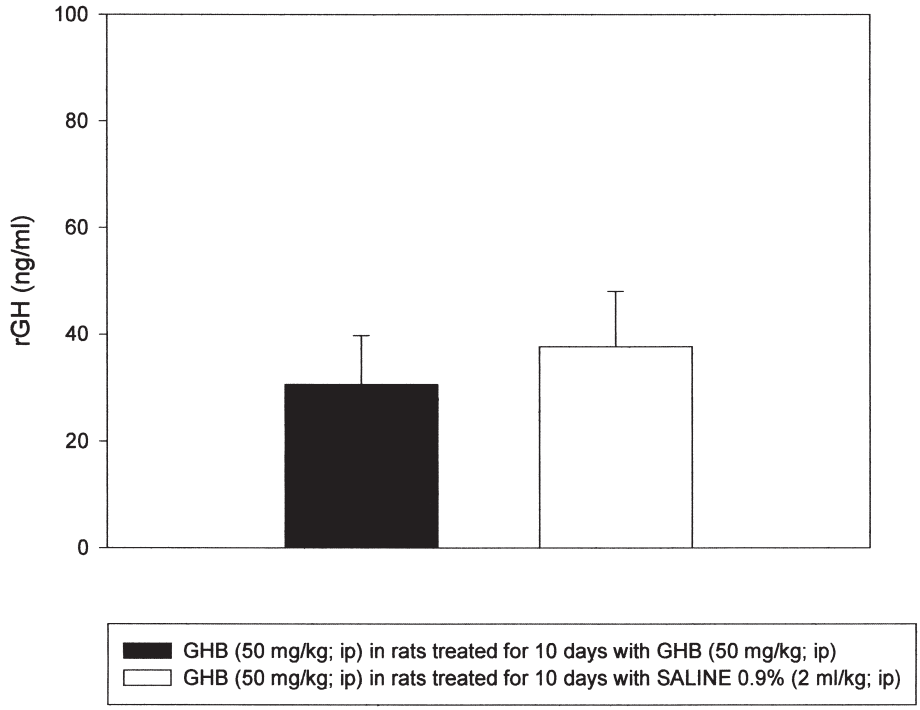

Fig. 8. Somatotropic responses to gamma-hydroxybutyric acid (50 mg/kg, IP) in unanesthetized adult rats, treated with gamma-hydroxybutyric acid (50 mg/kg, IP) or saline (2 ml/kg, IP) for 10 days.

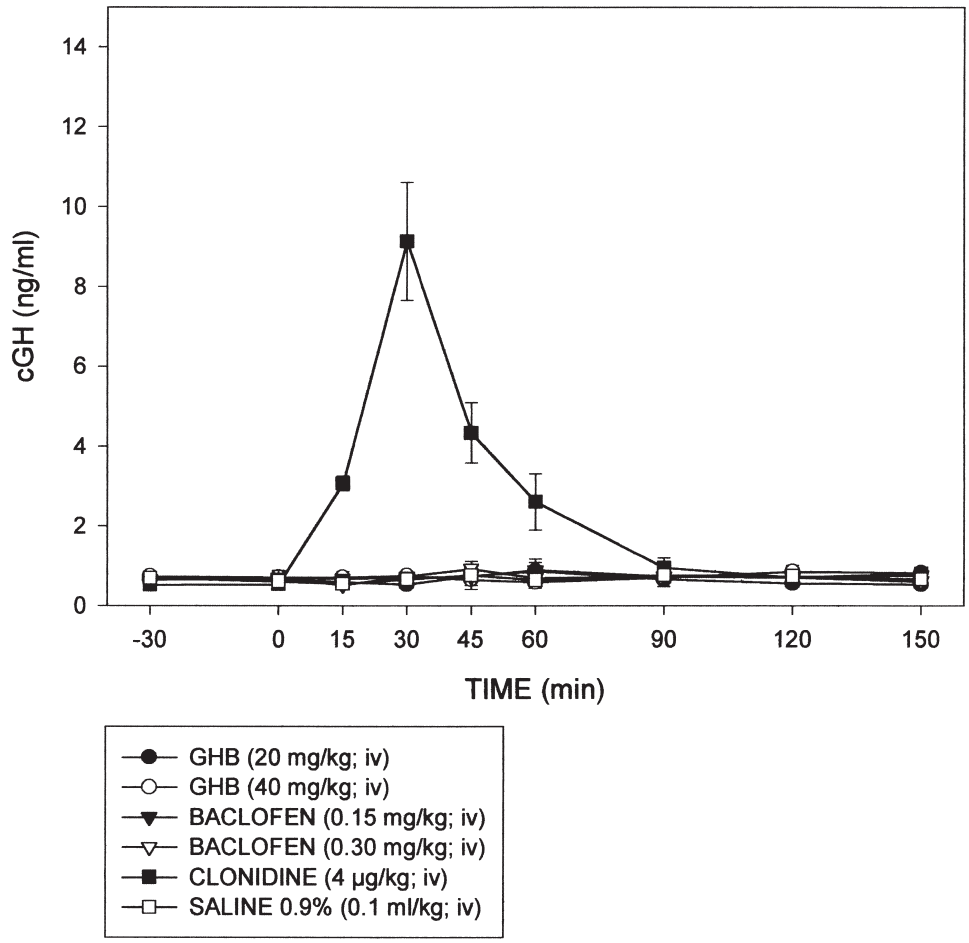

Fig. 9. Growth hormone plasma concentrations and AUC_{GH} in conscious dogs given baclofen (0.15 or 0.30 mg/kg, IV), gamma-hydroxybutyric acid (20 or 40 mg/kg, IV), clonidine (4 µg/kg, IV), or saline (2 ml/kg, IV).

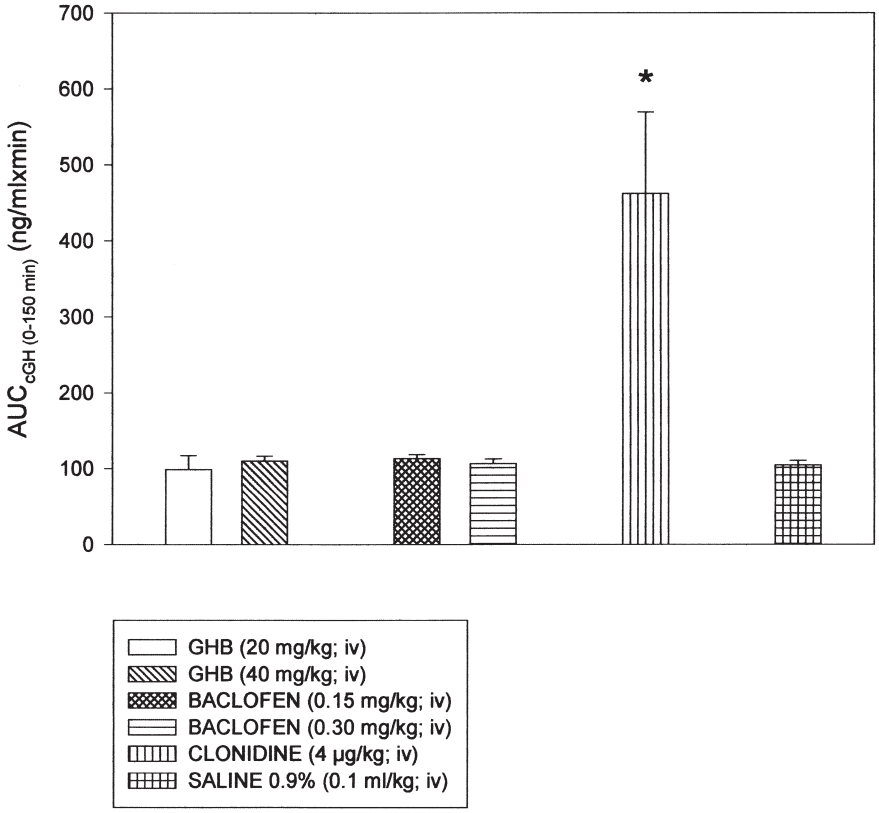

Fig. 10. Growth hormone plasma concentrations and AUC_{GH} in conscious dogs given baclofen (0.15 or 0.30 mg/kg, IV), gamma-hydroxybutyric acid (20 or 40 mg/kg, IV), clonidine (4 µg/kg, IV), or saline (2 ml/kg, IV).

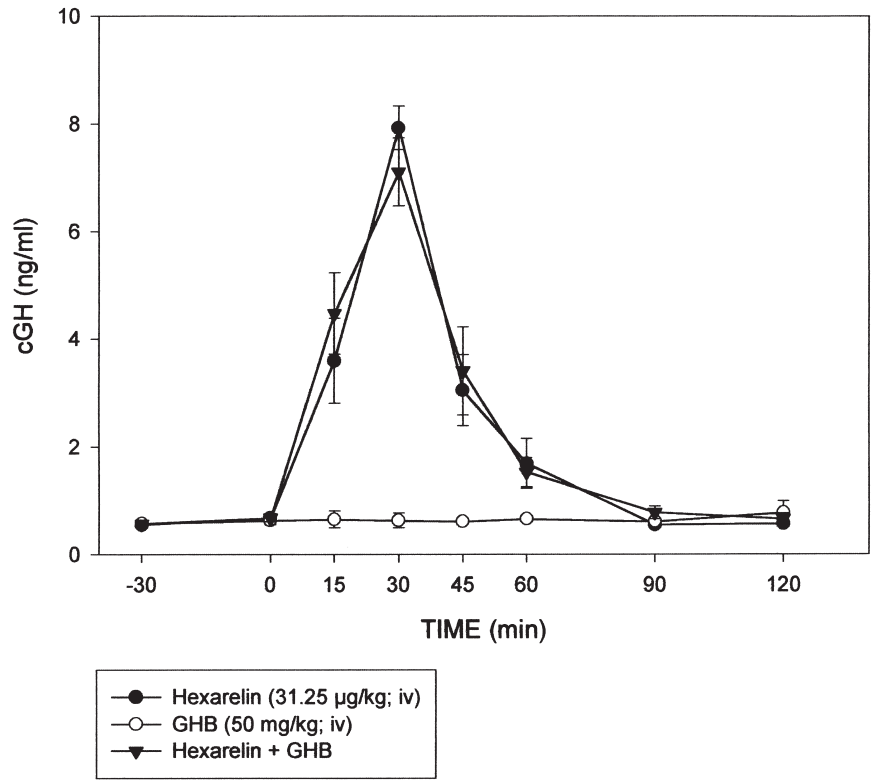

Fig. 11. Growth hormone plasma concentrations and AUC_{GH} in conscious dogs given hexarelin (31.25 µg/kg, IV), gamma-hydroxybutyric acid (50 mg/kg, IV), or hexarelin plus gamma-hydroxybutyric acid.

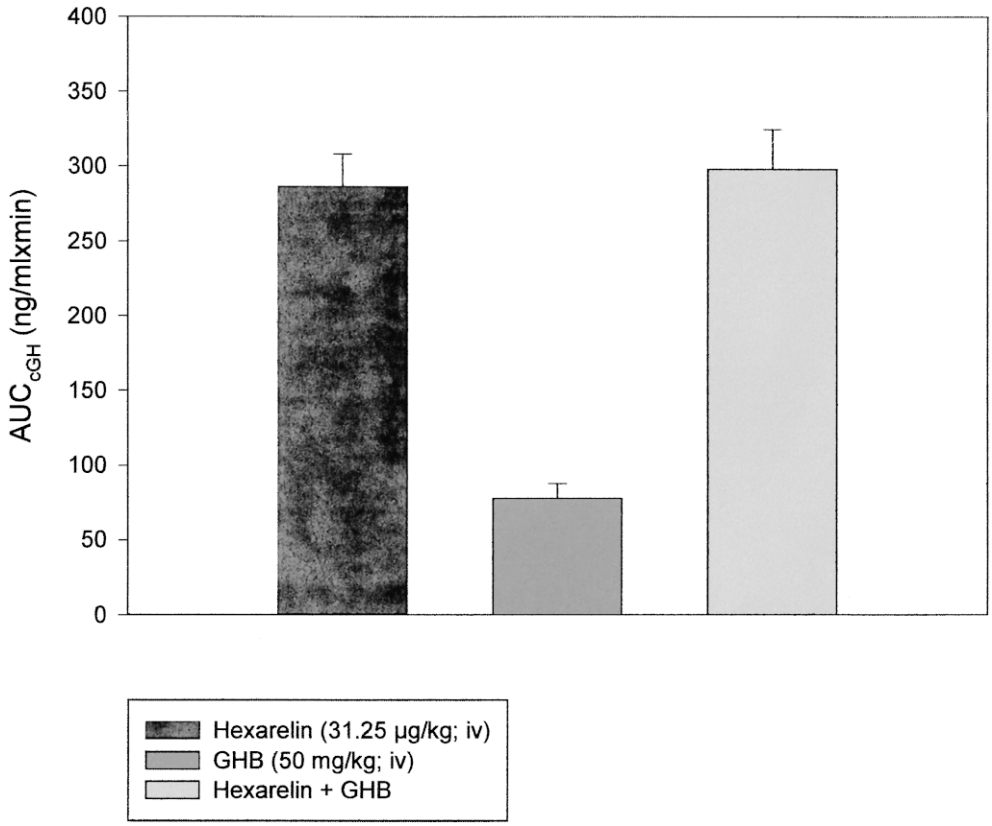

Fig. 12. Growth hormone plasma concentrations and AUC_{GH} in conscious dogs given hexarelin (31.25 μg/kg, IV), gamma-hydroxybutyric acid (50 mg/kg, IV), or hexarelin plus gamma-hydroxybutyric acid.

cell cultures in comparison with values present in control experiments (150.2 ± 4.1 ng/ml vs. control, $p < 0.01$).

4. Discussion

The results of the present study indicate that GHB lacks any GH-releasing activity in rats and dogs, even when a toxic dose as high as 1500 mg/kg, IP, was used in rats. Different in vivo and in vitro experimental models and conditions were used: infant rats, anesthetized and unanesthetized adult rats, conscious dogs, and rat pituitary cell cultures.

Rat pups represent an experimental model of physiological activation of the somatotropic axis, due to the relative immaturity of the somatostatinergic system (Cella et al., 1994). However, in this model, which has been previously exploited to unravel the action of GH secretagogues (Cella et al., 1987, 1988; Grilli et al., 1997; Locatelli et al., 1994), administration of GHB did not significantly affect basal GH secretion.

An ontogenetic development of the GHB-dependent neuronal circuits has been reported in rats (Snead, 1994). Therefore, to counter the objection that infant rats may not be an adequate experimental model, adult rats also were used. However, GHB also failed to stimulate GH secretion in adult anesthetized and unesthetized rats.

Reportedly, GABA modulates PRL secretion at different sites of the hypothalamo-pituitary axis (Ben-Jonathan et al., 1989). However, in contrast with GABA, no stimulation of PRL secretion was observed when GHB was administered to adult rats. The same negative findings obtained with GHB in rats were observed in dogs, a species that is similar to human beings in many aspects of the neuroendocrine regulation of the somatotropic axis (Müller, 1987). Also in this species, which as expected did respond to clonidine-induced alpha-2-adrenoceptor stimulation, GHB did not modify either basal or hexarelin-stimulated GH secretion (Cella et al., 1995).

Gamma-hydroxybutyric acid might have exerted a direct pituitary action, possibly masked in vivo by the inhibitory tone of somatostatin. However, in our study, unlike GHRH, GHB was unable to elicit GH release from pituitary cell cultures.

In view of the possibility that the $GABA_B$ receptors may be involved in the pharmacodynamics of GHB (Bernasconi et al., 1992; Engberg & Nissbrandt, 1993; Maitre, 1997), it was worthwhile testing a $GABA_B$ agonist. However, none of the scalar doses of baclofen that were used proved capable of releasing GH in rats or dogs.

The discrepancy between our results and the observations made in human beings, in whom baclofen stimulates, though poorly, GH release (Davis et al., 1996), could be due

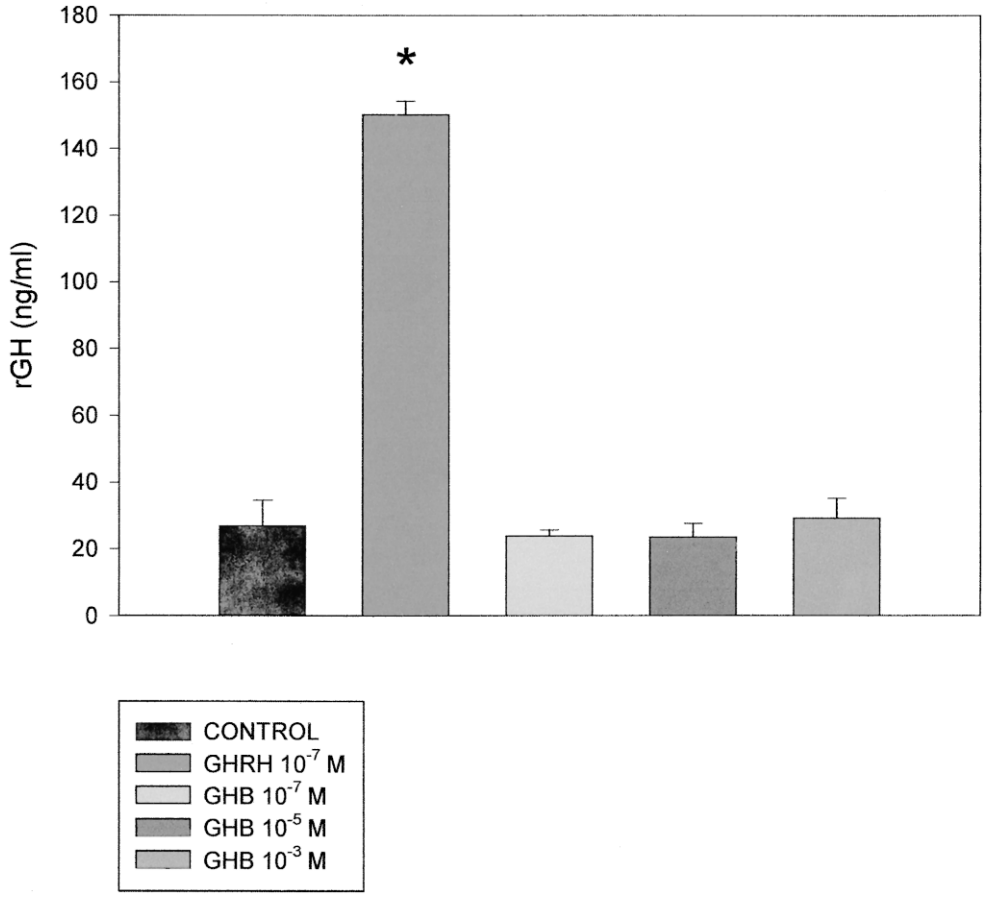

Fig. 13. Growth hormone concentrations in the medium of pituitary cell cultures after exposure to growth hormone-releasing hormone (10^{-7} M), gamma-hydroxybutyric acid (10^{-7}, 10^{-5}, and 10^{-3} M) or bovine serum albumin.

to the existence of a species difference. To our knowledge, there are no animal data on the GH-releasing activity of baclofen except those of our study. Because no GH response to GHB or baclofen was observed in our study, the postulated reciprocal interaction between GHB and the $GABA_B$ system could not be investigated with the two respective receptor antagonists NCS-382 and CGP35348.

Few investigators have studied the GH-releasing activity of GHB in human beings (Gerra et al., 1994, 1995; Takahara et al., 1977; Vescovi & Di Gennaro, 1997), and the doses of GHB used in these investigations were pharmacological in nature (2.5 g, IV) (Takahara et al., 1977). Because flumazenil, an antagonist of benzodiazepine receptors (Whitwam, 1988), abolished the GH secretion induced by a huge dose of GHB (1.5 g, PO) (Gerra et al., 1994), the GH-releasing activity of the latter would result not from a direct mechanism but from the endogenous conversion of GHB into GABA (Maitre, 1997). These observations, as well as the results of our study, would demonstrate that GHB itself is not a potent stimulus for GH secretion, and the latter event likely results in human beings because of the possibility that GHB may ultimately stimulate the $GABA_A$ receptor system (Maitre, 1997).

Allegedly, GHB has been foreseen as a potential doping drug in bodybuilders for its GH-releasing activity (Iven, 1998). Overall, previous data obtained from studies in human beings (Gerra et al., 1994, 1995; Takahara et al., 1977; Vescovi & Di Gennaro, 1997; Volpi et al., 1997) and our present results do not support this proposition. It seems more likely that the appeal of GHB as a doping drug may be due to its psychostimulant effect (Galloway et al., 1997; Tunnicliff, 1997). More interesting, in relation to the therapeutic use of the drug in alcoholic subjects (Gallimberti et al., 1989; Gessa, 1990), is the finding that short-term treatment of rats with a rather toxicological dose of the compound did not alter the original refractoriness of the GH response to its acute administration.

In conclusion, the (negative) results of the present animal studies coupled with the findings so far reported in research with human beings do not support the idea that acute or short-term administration of GHB may induce adverse effects through activation of the somatotropic axis.

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