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Interacting Brain Stem Components of Opiate-Activated, Descending, Pain-Inhibitory Systems

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ROSENFELD, J. P. *Interacting brain stem components of opiate-activated, descending, pain-inhibitory systems*. NEUROSCI BIOBEHAV REV 18(3) 403-409, 1994.—This is a review of research aimed at elucidating how various opiate analgesia substrates in rat brain stem interact with one another to bring about opiate analgesia. The three substrates studied are the midbrain periaqueductal grey (PAG), the bulbar nucleus raphe magnus (RM), and the bulbar nucleus reticularis paragigantocellularis (PGC). The methods used in the reviewed studies are unique in that behavioral and neuronal responses are assessed in consequence of nanoinjecting opiates (met-enkephalin) into subset pairs of these structures. Responses to single and conjoint injections are compared. Effects on neuronal and behavioral responses in consequence of disruption of these structures with tetracaine block are also discussed. It is seen that PGC cannot serve as an opiate analgesia substrate if the functional integrity PAG is impaired. However PAG does not depend on PGC's functional integrity.

Pain Opiates Analgesia Brain stem Periaqueductal Gray Paragigantocellularis Raphe Magnus

UNTIL the 1970s, many neuroscientists studying pain and analgesia believed that opiate analgesics, such as morphine, had their effect by depressing pain perception systems located at higher levels of the neuraxis (e.g., the cortex). Then came the seminal discovery by Liebeskind, Mayer, and colleagues (16) that electrical stimulation in the ventral periaqueductal (PAG) gray matter of the midbrain produced a profound analgesia state, comparable to that achieved via standard peripheral (e.g., intravenous) injection of morphine. Later work extended this finding by showing that activation of PAG by direct application of opiates also produced analgesia (26, 27, 33, 41). These findings led to the descending inhibitory control hypothesis of opiate analgesia mechanisms. This model postulates that specific neuronal systems in the brain stem area have opiate receptors and are thus activated by opiates: When these systems are so activated, they project inhibition through their descending connections to primary-to-secondary synaptic areas, which otherwise (i.e., ordinarily) bring pain information into lower levels of the neuraxis such as the spinal cord or trigeminal nuclear complex. The PAG was known to have descending connections to these primary terminal regions via a relay in the bulbar nucleus raphe magnus (RM; 2, 3). However other routes to lower levels of pain input systems were also known. It was suggested that when driven as a result of PAG opiate activation, these documented anatomical con-

nections could presynaptically inhibit primary terminals on secondary ascending pain pathways (spinothalamic, spinoreticular, trigeminothalamic, trigeminoreticular) or directly inhibit secondary ascending neurons, thereby reducing the ascending pain message and producing analgesia.

The results of a series of studies from our laboratory in the 1970s were consistent with the descending inhibitory hypothesis. We found that a SC (i.e., peripheral) injection of morphine in a dose producing profound analgesia for foot shock pain in rats, had no effect on the pain-like experience presumably produced by electrical stimulation of ascending pain pathways. That is, the level of foot shock normally required to produce an escape response was tripled by 6 mg/kg of SC morphine, but there was no change in this escape reaction threshold to aversive stimulation of spino-reticular pain pathways in the midbrain (23). The midbrain neurons stimulated with electric shock are the pain projection neurons which connect the primary fibers activated by peripheral (e.g., foot shock) stimulation with the highest levels of the brain's pain systems. That is, these midbrain reticular fibers are higher level continuations of the incoming primary afferent pain pathways, whose most distal endings in peripheral skin receive direct painful stimulation. Thus our findings were explainable by the hypothesis that the hypothetical, opiate-activated, descending inhibitory fibers from PAG to primary afferent ter-

minal regions in the dorsal horn, exert their inhibitory effect sufficiently proximally within the CNS to block pain coming from the most distal loci (e.g., feet), but well below the mid-brain connections receiving our electrical stimulation. These stimulated fibers were thus presumed to be far enough "downstream" (though at high CNS levels) to be inaccessible to effects of descending inhibition.

There were other possible but less interesting explanations for our findings. Most troubling was the possibility that the brain stimulation was an artificial and unnatural method of activating ascending pain pathways, so much so, that opiates could not overcome its profoundly synchronizing effects. This possibility could have occurred in two ways: First, central stimulation of a pain pathway activates many more neurons at once than does stimulation of the nerve endings in a distal limb or set of limbs, which is accomplished by foot shock. In contrast, central pain pathways contain pain neurons coming from widespread regions of the somatic sensorium; shocking these pathways would be more like shocking all peripheral pain receptors at once. A second unnatural aspect of electrical stimulation is the intense, repetitive way it activates neurons. As was typical in other papers, we (23) utilized a 500 msec burst of square wave pulses at 200–300 Hz with immediate rise times in the leading edges. Natural synaptic transmission probably discharges neurons less synchronously and less often.

We did a second study (25) to control for these possibly trivial effects. In this study we used three stimulation frequencies; 5 Hz, 10 Hz, and 200 Hz. All were sinusoidal waves and all bursts were "soft-started" by a photoelectric switch which produced a 500 msec spindle-like envelope of sine waves, rising from zero to maximum in 100 msec, and falling to zero in the same time. The 3 different frequencies had expectedly different aversive reaction thresholds for midbrain spino-reticular pain path stimulation, with the lowest frequency requiring the highest current level to produce an escape reaction, and so on. However the lack of effect of morphine on all these central aversive reaction thresholds was total for each. If our earlier (23) result had been related to the unnatural synchronicity of stimulation, one might have expected some opiate analgesia with the lower frequency, soft-started stimulation in the more recent study (25).

In this first set of results in our 1976 paper (25), we tried to make the central stimulation more natural by reducing brain stimulation intensity and synchronicity. In the second part of this study, we tried to make "peripheral" stimulation more like brain stimulation, so as to let us see whether or not opiates would now fail to produce analgesia, as we had earlier found for actual central stimulation. The way we accomplished this was by utilizing the primary descending trigeminal tract (PDTT) as an electrical stimulation site. Physically, this tract carries primary afferent pain fibers whose distal endings innervate tooth pulp, head, face, mouth, and neck. It is "like a central pain pathway" in that it carries a heavy concentration of fibers from multiple peripheral sites (albeit all above the torso) within the skull. Yet according to the descending inhibitory hypothesis, it is outside or peripheral to the site where descending inhibitory fibers end on primary afferent terminals and/or secondary afferent neurons. The aversive reaction threshold for PDTT stimulation would be unchanged after morphine if the opiate could not overcome the synchronicity and unnaturalness of the stimulation. On the contrary, the reaction threshold for PDTT stimulation was in fact more than doubled by morphine, an analgesic effect which was no different than the effect of the opiate on the latency of tail or foot withdrawal from noxious heat (a clearly peripheral escape

response) in the same rats. Indeed many of the rats in this study had brain stimulation electrodes which missed the PDTT and located medially in the spinal trigeminal nucleus (TN)—where the secondary ascending pain neurons originate, having been synapsed upon with PDTT terminals. Stimulation in these more central loci was likewise profoundly affected by morphine. However still other rats had even more medial stimulation electrodes, locating in the subjacent bulbar reticular formation just medial to TN, i.e., just inside the presumed site of descending inhibition. For these sites, opiates had no effect on the aversive reaction threshold. This study strongly supported the descending inhibition hypothesis, and we have since done several others showing that no matter where in the ascending pain pathways one stimulates, (spino-reticular or spinothalamic paths, from diencephalon to medulla), various types of opiates (e.g., morphine, enkephalin analogs, etc.) given either systemically (21,22) or via direct microinjection in brain (26,29,30), will have no effects on the threshold for aversive reaction in doses producing clear analgesia for peripheral pain.

Many other approaches have provided more support for the descending inhibition hypothesis (2,3). For example, electrical or chemical stimulation of PAG or RM have been shown to alter neural activity in primary terminal regions, as predicted by the descending inhibitory hypothesis. It has also been shown that interference with descending connections from PAG and RM to lower levels of the neuraxis attenuates opiate analgesia.

In the later 1970s, other sites in brain were discovered to be potent opiate analgesia substrates. In particular, Satoh, Takagi and colleagues (33,35,36) discovered that the bulbar nucleus reticularis paragigantocellularis (PGC) was an even more sensitive opiate analgesia substrate than either PAG or RM, a finding that we replicated (26). It turned out that PGC, where microinjected opiates would produce analgesia at about half the dose required for the same level of analgesia from PAG microinjection, was also connected to RM and to lower levels of the neuraxis in the region of primary afferent terminals (1–3). This finding suggested the novel question of whether or not these emerging multiple opiate analgesia substrates of brain each comprised independent systems, each of which adds its inhibitory effects as each is, of necessity, reached by a systemic opiate injection. An alternative possibility is that the various substrates were redundant components of one system such that if one system were removed, another would be sufficient to mediate normal opiate analgesia. Lashley (14) established a similar principle of "equipotentiality" for other neural systems subserving other functions. If an interaction among opiate substrates were to be shown, a further question would arise. This question would ask where in the nervous system are the sites of convergence among opiate substrates. These have been the questions about opiate mechanisms with which our laboratory (and no others) has been mostly concerned in the past decade.

In our first examination of these issues we (27) did a straightforward experiment: On one day, we gave a single microinjection of morphine in rat PAG at a dose producing an average behavioral response latency (to noxious heat) across rats which was about 150% of the baseline value (i.e., 7.5 vs. 5 s). On another day we injected PGC with half the morphine dose used in PAG so as to produce approximately the same average analgesia level; (about 140% the baseline value was actually obtained). On yet a third day we gave a simultaneous conjoint injection of both sites (using the same doses as those used in the single injections). The order of these

treatments was counterbalanced across rats. The key question was whether or not the conjoint injection would have a greater analgesic effect than that of either single microinjection given singly. We found that there was indeed an apparently summing interaction between PAG and PGC, because the conjoint injection effect on baseline aversive heat reaction latency was at about 200% of baseline, which was significantly greater than either single injection effect. This finding strongly suggested that the maximum effect of a systemic (peripheral) opiate injection—still among our most powerful analgesic manipulations in most situations—depends on the participation of each opiate analgesia substrate in brain (including PAG, RM and PGC) which are, of course, all accessed by a systemic injection. Opiates have also been shown to produce analgesia when injected directly into primary afferent terminal regions (30,41). There may thus be a further interaction among opiate substrates involving these lower areas.

In any case, the summing behavioral effects resulting from conjoint PAG-PGC injection begged the question of where and how the conjoint effects were mediated. Figure 1 is

a diagram (based on existing studies) showing some of the relevant interconnections among the three opiate substrates which have received major study in recent years (PAG, RM, PGC) and the primary afferent terminal region ("PRIM AFF" in Fig. 1). This region would include the dorsal horns of spinal cord and medulla, the latter also known as the trigeminal nuclear complex. The primary afferent (bipolar) neuron at the lower right is shown to enter a nuclear area (either dorsal horn or trigeminal nucleus) diagrammed as a large rectangle into which various descending inhibitory neurons converge. Within this nuclear area, the primary afferent terminal region, the primary afferent terminals synapse on a representative secondary afferent neuron, shown as a long, thin, filled rectangle. Its output contributes to the pain pathways which ascend toward higher levels of the neuraxis. The figure shows that PAG sends some connections directly to primary afferent terminal areas (3,38), but sends others to RM (9,19), and still others to PGC (10). Because both RM and PGC connect also to primary afferent areas (3,-6,11-13,15,20,34), PAG can inhibit primary afferent pain input via RM, via PGC, or directly. Likewise PGC descending connections can go directly to primary afferent terminals or through RM; (4,9). Also, it may be that the same neurons in RM that connect PAG with primary afferent terminal regions similarly connect PGC. If so, these RM neurons could be the site where effects of PAG and PGC microinjection converge and summate. On the other hand it could be that PAG and PGC take independent routes through RM (or possibly not through RM) to primary terminal areas. In this case a site of convergent interaction could be in the primary terminal area. All these possible mechanisms (which are not mutually exclusive and could all co-exist) are represented in Fig. 1.

The first question we posed by way of dealing with the possible site(s) of interaction concerned RM: we first wanted to see whether or not the same RM neuron could be affected by separate, single injections of both PAG and PGC. Then we wanted to compare the effects of a conjoint PAG-PGC injection with the effects of each single injection. Obviously, to do such a study we needed to isolate an RM neuron and utilize its discharge pattern and/or rate as a dependent measure. We also needed to have at least some raphe-spinal neurons (those which descend without synapse from RM to primary afferent terminal areas) in our sample population. This latter requirement arose from the fact that not all RM neurons descend; some are ascending and probably not relevant to RM opiate analgesia mechanisms. By ascertaining that a studied neuron is raphe-spinal, one increases the likelihood of studying an appropriate member of the putative opiate-activated descending inhibitory mechanism. The method we ultimately used (28) to verify the spinal projection of an RM neuron was the classical antidromic stimulation/collision technique, which involves backfiring (antidromically activating) the descending axon fiber (in spinal cord) of the recorded RM cell. Our sites of spinal stimulation ranged from T1 to T12. Clearly, if one can backfire an RM neuron from such a cord location, this verifies raphe-spinality. However the failure to obtain an antidromic response does not necessarily mean that the unresponsive RM neuron is not part of the descending inhibitory mechanism: The RM neuron may be descending to a synapse above the site of antidromic stimulation upon a postsynaptic neuron, thus forming a continuation of the descending inhibitory route. Alternatively, the unresponsive RM neuron may be a legitimate part of the descending inhibitory mechanism which has terminated above the site of antidromic stimulation in locations such as the cervical cord or trigeminal nuclear complex.

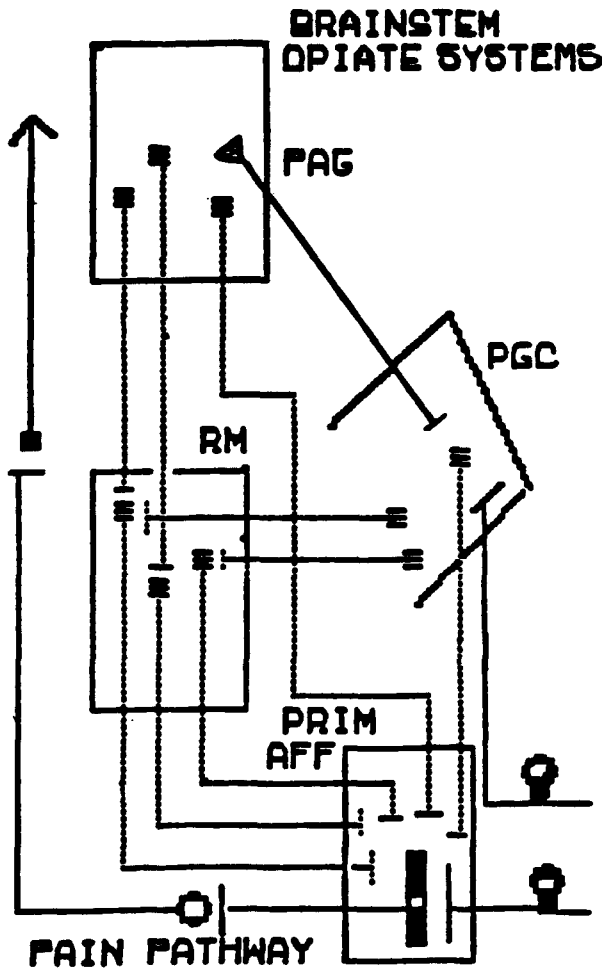


FIG. 1. A diagram of some brain stem opiate analgesia system member nuclei and their interconnections. Connections are documented in the text. PAG is periaqueductal gray; PGC is nucleus reticularis paragigantocellularis; RM is nucleus raphe magnus; PRIM AFF is a primary afferent synaptic area such as dorsal horn nucleus or trigeminal nucleus.

Thus in our second study (described below), we examined both RM neurons that were both clearly raphe-spinal, as well as those which did not respond to antidromic stimulation.

We did first a preliminary study (24) without the raphe-spinal classification, in which we demonstrated for the first time that RM neurons' firing rates were, in 11 of 14 cases, depressed in response to separate Met-enkephalin injections into PAG and PGC. This was the first demonstration that a given single RM neuron receives convergent input from both PAG and PGC. Moreover, in this study we were also able to hold 3 RM cells long enough to show that a simultaneous PAG + PGC injection produced (in all 3 cases) a greater depression than that obtained with injection of either single site. This was also one of the first demonstrations that RM cells were responsive to PGC opiate injection. This report was highly suggestive that PAG and PGC formed a convergent system with RM, but there were problems with it: There was no raphe-spinal classification as noted above, and there were not enough cells held to do a systematic study of conjoint effects. It was difficult to reliably hold a cell long enough to do 3 separate injection procedures.

An important decision regarding these studies concerned the specific dependent measure chosen for RM cells. Ordinarily one would simply look at spontaneous and noci-evoked activity (as we did in reference 24) without further classification of neurons. However in the early 1980s, H. Fields and colleagues made an important discovery regarding RM neurons (7,8,37). These workers identified 2 distinct subpopulations of nociresponse RM neurons including raphe-spinal cells. One cell type, which they call an "on-cell," shows an increase in spontaneous activity in tight temporal relationship to the behavioral tail flick response to noxious heat applied to the tail. This on-cell however is suppressed (i.e., its firing rate is reduced) by analgesic morphine injection. The other kind of RM neuron behaves oppositely; this "off-cell" shows a decrease in spontaneous activity in response to noxious stimulation, and opiates in analgesic doses enhance the off-cell's discharge rate. This discovery of these 2 cell types in RM accounted for much confusion in the earlier literature on RM responses to opiates. In these older studies, since there was no knowledge of 2 oppositely behaving RM cell types, investigators did not classify on-cells and off-cells, but probably grouped all RM data together, resulting in (now understandably) inconsistent results. It is noted that the studies of Fields and colleagues have involved rats in lightly anaesthetized states—light enough to allow the tail flick—and the generality of the results to the fully awake, pain-feeling organism has been challenged (18). Nevertheless, we decided in our next RM study to utilize the lightly barbiturated rat, and on-off cell classification, in addition to the raphe-spinal classification.

In this study (28), we utilized nonnoxious probe stimulation, and also noxious pinch, as well as noxious heat. All body surfaces were explored from face to tail. Also we used nano-injections (1–10 μ g in 100–200 nl of water) of the opiate Met-enkephalin. We chose this synthetic opiate analog because of its brief time course: since each neuron was to be tested with at least 2 injections (PAG or PGC and PAG + PGC together), it was important for effects of one injection to wear off prior to a second administration. Our plan was to inject half the rats with a single PAG or PGC nano-injection, followed later by the conjoint injection. Half the single injections

were PAG, half were PGC. We knew from our earlier (24) study that we could reliably hold an RM cell for at least 2 injection procedures. In some rats, however, we were able to give a third, single injection following the conjoint injection. This last was always the site other than the one receiving the first single injection.

We found that most RM neurons, including 8 raphe-spinal neurons, were unresponsive to nonnoxious touch. Approximately twice as many RM neurons (including 8 raphe-spinal neurons) responded to noxious heat with an increased (on-cell) discharge than with a decreased (off-cell) discharge to noxious heat. For noxious pinch, the same results obtained with all RM cells, however none of the raphe-spinal neurons responded as off-cells to noxious pinch. The major findings bearing on the detailed mechanisms of descending opiate inhibition were as follows: a) Extending the work of the Fields group which showed that systemic and PAG-injected opiates enhance off-cell activity and depress on-cell activity, we showed that PGC-injected opiates have exactly the same effects. b) In the clear majority (38 of 43) of cases, the effect of a single injection of one site (either PAG or PGC) was enhanced by the conjoint injection, whether the single effect was to increase the discharge of an off-cell, or to decrease the discharge of an on-cell. This was so for raphe-spinal as well as other RM cells. There were, however, 5 exceptions (in 43 cases) where the conjoint effect reversed the single effect. We verified, in a few cases, the implications of the conjoint effects with a final recording of a third, single injection. That is, in cases where the conjoint effect exceeded the single effect, implying that both single effects were in the same direction (increasing or decreasing), the final, single-injection experiment was confirmatory. Likewise, in the few cases where the conjoint effect was less than the single effect, the final experiment confirmed that PAG and PGC were acting in opposite ways, though still via a clear convergence on the same RM neuron.

We took these data to imply that PAG and PGC and RM form a convergent system within the opiate-activated, descending inhibitory/analgesia mechanism. It remained to inquire as to whether PAG and PGC formed an "AND-like gate" at RM, implying that both structures (PAG and PGC) were necessary for the action of the other to show, or whether either could function independently of the other. However, before we approached this problem (detailed below) we examined another possible site of convergence of PAG and PGC descending inhibitory pathways; the trigeminal nuclear complex where primary afferent terminals synapse upon secondary afferent ascending pain neurons of the trigeminohalamic and trigeminoreticular pathways.

In a series of studies (39,40) using methods very similar to those of the previously described RM study, (except, obviously, there were no backfiring manipulations), we recorded from secondary neurons in the spinal trigeminal nucleus, sub-nucleus caudalis (TNC) where pain information from the head and neck enter the CNS. Our findings may be summarized as follows: The vast majority (>90%) of TNC cells are "on-like" cells¹, whose discharge is increased by noxious stimulation. However in contrast to opiate effects in RM, in TNC, opiates do not effect systematic decreases in spontaneous activity of the on-like cells. We saw no systematic effects of PAG or PGC opiates on TNC spontaneous activity. There were about

¹ In trigeminal neurons "on-like" and "off-like" are adjectives used rather than "on" and "off" in respect of the distinction between pain modulating (RM) and pain transmission (TNC) neurons.

the same numbers of increased, decreased, and unchanged spontaneous discharge rates following opiate injections. We did see an expected depression of the on-like response to noxious facial stimulation following an injection of Met-enkephalin in either PAG or PGC, and there was a summation of the single effects produced by conjoint injection. This effect was seen behaviorally also. The off-like responses of the few off-like TNC cells found, however, were further depressed by Met-enkephalin. These data strongly suggested that the RM-independent routes from PAG and from PGC to TNC are probably not involved in an opiate descending inhibition mechanism which duplicates that of RM subsystems. The systematic effects of opiates on RM spontaneous activity imply that the analgesic state is set up in RM before a noxious stimulus is given. The same would be expected for TNC cells if opiate-activated PAG and PGC were setting up a directly inhibited state in TNC secondary neurons prior to noxious input. If secondary TNC cells were being directly inhibited, one would expect to see consistent effects of opiates on spontaneous activity. A model consistent with our findings is that the PAG and/or PGC activated RM-TNC fibers are ending presynaptically on primary afferent terminals. Thus one sees no spontaneous activity effects in secondary TNC neurons, which are nevertheless in the presynaptically inhibited state prior to noxious input, which when it does come in, is reduced. Thus the evoked noxious on-like response is reduced by opiates. The summing effects we saw on the evoked response are probably also mediated by summation at RM.

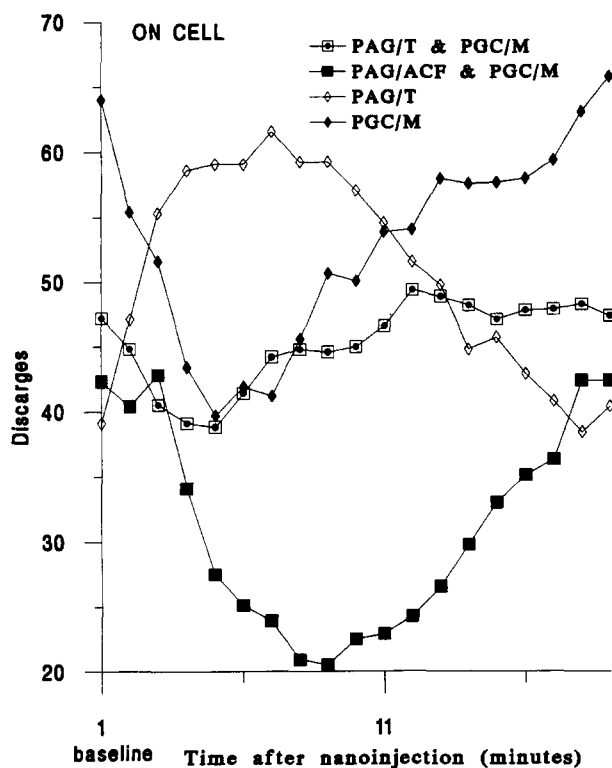


FIG. 2. Discharge frequency of an RM On-cell as a function of time after various nano-injections and injection combinations. PAG/T & PGC/M is tetracaine in PAG with met-enkephalin in PGC; PAG/ACF & PGC/M is artificial cerebrospinal fluid (control) in PAG with Met-enkephalin in PGC; PAG/T is tetracaine alone in PAG; PGC/M is Met-enkephalin alone in PGC.

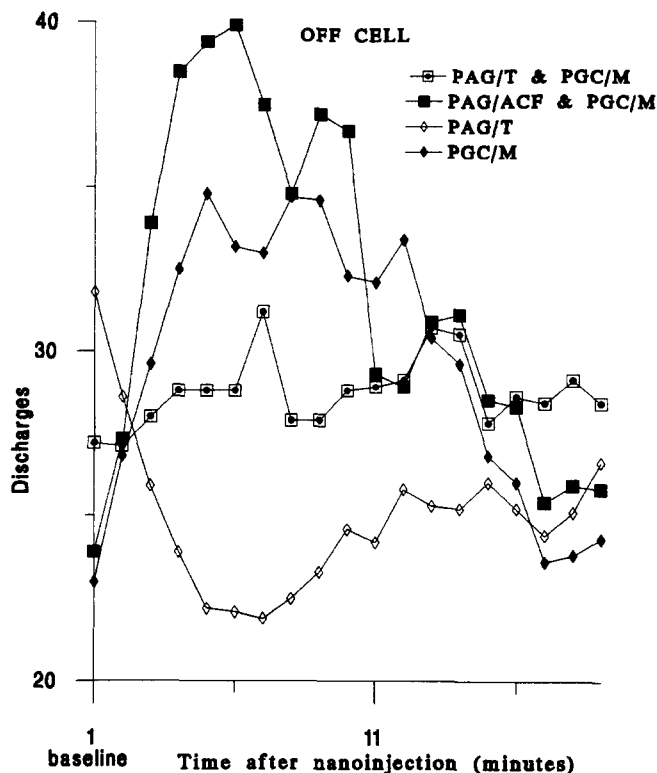


FIG. 3. Same plot of discharge frequency as a function of time, as in Fig. 2, but for an RM Off-cell.

The finding described above (28) that PAG and PGC seemed to work in concert at RM suggested the existence of an endogenous opiate analgesia system in which the 3 component parts are, in some sense, not mutually independent. A first hypothesis implied by this PAG-PGC system model is that the functional integrity of PAG is necessary for the analgesic action of opiates acting locally at PGC. We (32) tested this hypothesis by using barbiturate-sedated rats (again, light enough for us to reliably observe the noxious tail-flick response) subjected to various injections and injection combinations. Tetracaine was utilized to produce reversible lesions of PAG. In one experiment rats received (a) tetracaine injection alone in PAG; b) Met-enkephalin injection alone in PGC; (c) conjoint injections (a and b); (d) control solution injection (artificial cerebrospinal fluid) in PAG alone; and (e) in PGC alone. The results strongly supported the necessity of PAG for opiate effects in PGC, in that while injection (b) above (Met-enkephalin in PGC) produced the usual analgesic effect, the conjoint injection (c) above resulted in no change in tail-flick latencies. In other words, Met-enkephalin in PGC is without its usual profound analgesic effect if PAG is simultaneously blocked with tetracaine. The control injections had no effects. However, interestingly, we did observe (for the first time) that tetracaine alone in PAG [injection (a) above] produces a hyperalgesic decrease in tail-flick latencies (increased sensitivity). This unanticipated additional finding supported the idea that not only is there a descending inhibitory mechanism activatable by opiates, but that additionally, there is an endogenous, tonically active, descending pain inhibition system involving (perhaps originating from) PAG, the blocking of which releases the inhibition resulting in hyperalgesia.

The finding (described above) that the conjoint injection blocked PGC opiate effects could have been due to the confounded comparison of effects of a single injection with those of a conjoint injection. We did a second experiment which controlled for this possibility by comparing effects of 2 conjoint injections: (i) PAG tetracaine with PGC met-enkephalin [same as injection (c) above]; (ii) PAG control solution with PGC Met-enkephalin. The results were that injection (a) blocked the analgesia while injection (b) resulted in profound analgesia. These results could be unambiguously interpreted to mean that PAG is necessary for PGC to work as an opiate substrate. This begged the question of how PAG could exert this effect.

As noted above, PAG sends connections to PGC; it is thus possible that to properly function as an opiate substrate, PGC needs enabling via these PAG-PGC connections. Alternatively, it could be that connections ascending from PGC to PAG [e.g., in the spinoreticular path (17)], mediate analgesia from PGC opiate activation. Blocking PAG with tetracaine would interrupt this path. The final alternative explanation, which seemed to fit the earlier data described above in which PAG and PGC were seen to work in tandem at RM, is that for the ultimately effective descending inhibitory connections from RM to work, the RM neurons require AND-gate-like drive from both PAG and PGC. Taking away either PAG or PGC makes the AND effect impossible. An easy test of this last hypothesis would involve doing the Rosenfeld and Xia (32) experiment the other way around.

In our most recent study (31), the tetracaine block was applied to PGC while Met-enkephalin was injected into PAG. The complication in this study involved the fact that PGC is a bilaterally paired structure. Thus, one had to be certain that both left and right PGC nuclei were blocked. We actually did 2 experiments, examining both unilateral and bilateral effects—which turned out to be the same and which very clearly (to our surprise) disconfirmed the AND-gate hypothesis: Met-enkephalin in PAG with either bilateral PGC tetracaine or control solution injection produced clear, identical analgesia curves; PGC block was without effect. Incidentally, unilateral and bilateral PGC tetracaine alone produced no change in pain sensitivity (i.e., it did not produce the hyperalgesia seen with PAG tetracaine alone).

These data imply a more central, integrative role for PAG than for PGC in opiate descending inhibitory mechanisms. The two other hypotheses of PAG-PGC interaction presented earlier remain viable and are consistent with a more important role for PAG. In one hypothesis, PAG enables PGC, and in the other, PGC expresses its analgesic effects through PAG. It remains possible that both mechanisms may coexist.

Our work in progress provides clear electrophysiological correlates of the behavioral results (31,32) just given. In Fig. 2 it is seen that an RM on-cell's response is profoundly de-

pressed (as usual) by PGC Met-enkephalin injection, whether given as a single injection ("PGC/M" in Fig. 2) or with a conjoint control injection of artificial cerebrospinal fluid in PAG ("PAG/ACF & PGC/M" in Fig. 2). However when the conjoint injection involves tetracaine in PAG ("PAG/T & PGC/M" in Fig. 2), there is virtually no change in RM on-cell discharge rate. These results clearly implicate RM on-cells in mediating the PGC analgesic effects, which depend on PAG's functional integrity. An interesting additional finding is that tetracaine by itself in PAG ("PAG/T") produces an enhanced on-cell response—which perfectly correlates with the behavioral hyperalgesia we reported earlier (32) to follow tetracaine in PAG. This finding clearly implicates RM on-cells in the endogenous tonic descending inhibitory mechanism in PAG proposed above.

Remarkably, the effects on an off-cell in Fig. 3 show a near perfect inversion of the data of Fig. 2. Without tetracaine in PAG, the off-cell shows an enhanced response to met-enkephalin; this is blocked by tetracaine, which when given alone, produces a tonic reduction of off-cell activity.

It must be noted that these results are preliminary; we have studied only a few on-cells and off-cells, but the results have been quite consistent. The results of studies of effects of tetracaine in PGC combined with met-enkephalin in PAG are presently in a very preliminary state, but are so far quite consistent with the behavioral data of Rosenfeld & Xia (31): tetracaine in PGC does not alter effects of met-enkephalin in PAG on RM spontaneous discharge.

It is clear that we have only begun to work out the physiology of brain stem opiate analgesia substrates. In the past 25 years, we have learned about a descending inhibitory mechanism in brain stem forming a substrate of opiate analgesia. We have more recently learned that at least 3 interacting structures are involved in this mechanism, and that on-cells and off-cells in RM (and perhaps also in PAG and PGC) which appear to play a role in opiate analgesia, may also integrate interacting influences from PAG and PGC. However, there are many surprising results from this and other labs to be explained and integrated into modifications of existing theories. While many studies from other laboratories have provided and continue to provide crucial information on effects of actions of opiates applied at one site (sometimes in iontophoretic amounts that have electrophysiological effects but do not yield behavioral analgesia), we have remained committed to the idea that there is uniquely relevant information to be gained by manipulating more than one site at a time. We take this position because a systemic opiate injection—still the prototypically most effective clinical analgesia manipulation we have—must activate all opiate substrates at once, a fact which means that the normal (i.e., physiological) action of opiate analgesia involves simultaneously active, multiple opiate substrates.

REFERENCES

1. Abols I.; Basbaum, A. Afferent connections of the rostral medulla of the cat: A neural substrate for midbrain-medullary interactions in the modulation of pain. *J. Comp. Neurol.* 201:285-297; 1981.
2. Basbaum, A.; Fields, H. Endogenous pain control systems: brain stem spinal pathways and endorphin circuitry. *Annu. Rev. Neurosci.* 7:309-338; 1984.
3. Basbaum, A. I.; Fields, H. L. The origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat: Further studies on the anatomy of pain modulation. *J. Comp. Neurol.* 187:513-532; 1979.
4. Beitz, A. S.; Buggy, J. Brain functional activity during PAG stimulation-produced analgesia. *Brain Res. Bull.* 6:487-494; 1981.
5. Dostrovsky, J. P. et al. Presynaptic excitability changes produced in brain stem endings of tooth pulp afferents by raphe and other central and peripheral influences. *Brain Res.* 218 (1981) 141-160; 1981.
6. Fields, H. L.; Anderson, S. D. Evidence that raphe-spinal neurons mediate opiate and midbrain stimulation-produced analgesia. *Pain* 5:333-350; 1978.
7. Fields, H.; Bry, J.; Hentall, I.; Zorman, G. The activity of neu-

- rons in the rostral medulla of the rat during withdrawal from noxious heat. *J. Neurosci.* 3:545-552; 1983.
8. Fields, H.; Vanegas, H.; Hentall, I.; Zorman, G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature* 306:684-686; 1983.
 9. Gallager, D. W.; Pert, A. Afferents to brain stem nuclei (brain stem raphe, n. reticularis pontis caudalis, and n. gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. *Brain Res.* 144:257-275; 1978.
 10. Gebhart, G. F. et al. Inhibition in the spinal cord of nociceptive information by electrical stimulation and morphine microinjection at identical sites in the midbrain of the cat. *J. Neurophysiol.* 51:75-89; 1984.
 11. Giesler, G.; et al. Postsynaptic inhibition of primate spinothalamic neurons by stimulation in nucleus raphe magnus. *Brain Res.* 204:184-188; 1981.
 12. Hammond, D. Hypoalgesia induced by microinjection of a norepinephrine antagonist in the raphe magnus. *Brain Res.* 201:475; 1980.
 13. Hayes, D. L.; Rustinoni, A. Connections from nucleus paragigantocellularis to the trigeminal nucleus. *Exp. Brain Res.* 41:89-107; 1981.
 14. Lashley, K. S. *Brain mechanisms and intelligence.* N.Y., Dover Inc., 1963:25.
 15. Lovick, T. A.; West, D. C.; Wolstencroft, J. J. Bulbar raphe neurons with projections to the spinal trigeminal nucleus and the lumbar cord in the cat. *J. Physiol.* 277:61-62P; 1978.
 16. Mayer, D. J.; Liebeskind, J. C. Pain reduction by focal electrical stimulation of the brain. *Brain Res.* 68:73-93; 1974.
 17. Mehler, W. R. Some neurological species differences-a posteriori. *Ann. N.Y. Acad. Sci.* 167:424-467; 1969.
 18. Oliveras, J. L.; Montagne-Clavel, J.; Martin, G. Drastic changes of ventral medulla neuronal properties induced by barbiturate analgesia. *Brain Res.* 563:241-260; 1991.
 19. Pomeroy, S. L.; Behbehani, M. M. Physiologic evidence for a projection from periaqueductal gray to nucleus raphe magnus in the rat. *Brain Res.* 176:143-147; 1979.
 20. Rivot, J. P.; Chaouch, A.; Besson, J. M. Nucleus raphe magnus modulation of response of rat dorsal horn neurons to unmyelinated fiber inputs. *Neurophysiol.* 44:1039-1057; 1980.
 21. Rosenfeld, J. P.; Holtzman, B. S. Differential effect of morphine on stimulation of primary vs. higher order trigeminal terminals. *Brain Res.* 124:367-372; 1977.
 22. Rosenfeld, J. P.; Holtzman, B. S. Effects of morphine on medial thalamic and medial bulboreticular aversive stimulation thresholds. *Brain Res.* 150:436-440; 1978.
 23. Rosenfeld, J. P.; Kowatch, R. Differential effect of morphine on central vs. peripheral nociception. *Brain Res.* 88:181-185; 1975.
 24. Rosenfeld, J. P. The response of individual nucleus raphe magnus neurons to microinjections of met-enkephalin at midbrain and at bulbar loci. *Int. J. Neurosci.* 33:165-173; 1978.
 25. Rosenfeld, J. P.; Vickery, J. L. Differential effects of morphine on trigeminal nucleus vs. reticular aversive stimulation: Independence of negative effects from stimulation parameters. *Pain* 2: 405-416; 1976.
 26. Rosenfeld, J. P.; Stocco, S. Differential effects of systemic vs. intracranial injection of opiates on central, orofacial and lower body nociception: Somatotopy in bulbar analgesia systems. *Pain* 9:307-318; 1980.
 27. Rosenfeld, J. P.; Stocco, S. Effects of midbrain, bulbar, and combined morphine microinjections and systemic injections on orofacial nociception and rostral trigeminal stimulation: Independent midbrain and bulbar opiate analgesia systems? *Brain Res.* 215:342-348; 1981.
 28. Rosenfeld, J. P.; Huang, K. H.; Xia, L. Y. Effects of single and simultaneous combined nanoinjections of met-enkephalin into rat midbrain and medulla. *Brain Res.* 508:199-209; 1990.
 29. Rosenfeld, J. P.; Keresztes-Nagy, P. Differential effects of intracerebrally microinjected enkephalin analogs on centrally vs. peripherally induced pain, and evidence for a facial vs. lower body analgesic effect. *Pain* 9:171-182; 1980.
 30. Rosenfeld, J. P.; Pickrel, C.; Broton, J. G. Analgesia for orofacial nociception produced by morphine microinjection into the trigeminal complex. *Pain* 15:145-155; 1983.
 31. Rosenfeld, J. P.; Xia, L. Y. Lack of effects of tetracaine in rat nucleus paragigantocellularis (PGC) on analgesia from periaqueductal gray (PAG) met-enkephalin (ME) injections, and on baseline tail flick latency. *Int. J. Neurosci.* (in press).
 32. Rosenfeld, J. P.; Xia, L. Y. Reversible tetracaine block of rat periaqueductal gray. *Brain Res.* 605:57-66; 1993.
 33. Satoh, M.; Akaike, A.; Takagi, H. Excitation by morphine and enkephalin of single neurons of nucleus reticularis paragigantocellularis in the rat: a probable mechanism of analgesic action of opioids. *Brain Res.* 169:406-410; 1979.
 34. Sessle, B. J.; Hu, J. W. Raphe-induced suppression of the jaw-opening reflex and single neurons in trigeminal subnucleus oralis. *Pain* 10:19-36; 1981.
 35. Takagi, H.; Satoh, M.; Akaike, A.; Shibata, T.; Kuraishi, Y. The nucleus reticularis gigantocellularis of the medulla oblongata is a highly sensitive site in the production of morphine analgesia in the rat. *Eur. J. Pharmacol.* 45:91-92; 1977.
 36. Takagi, H.; Satoh, M.; Akaike, A.; Shibata, T.; Yajima, H.; Ogawa, H. Analgesia by enkephalins injected into the nucleus reticularis gigantocellularis of rat medulla oblongata. *Eur. J. Pharmacol.* 49:113-116; 1978.
 37. Vanegas, H.; Barbaro, N.; Fields, H. Tail-flick related activity in medullospinal neurons. *Brain Res.* 321:135-141; 1984.
 38. Watkins, L. R.; et al. Identification and somatotopic organization of nuclei projecting via the dorsolateral funiculus in rats. *Brain Res.* 223:237-255; 1981.
 39. Xia, L. Y.; Rosenfeld, J. P. Effects of single nanoinjections of met-enkephalin in the minimally anaesthetized rat brain stem on trigeminal nuclear neurons. *Brain Res.* 541:181-192; 1991.
 40. Xia, L. Y.; Huang, K. H.; Rosenfeld, J. P. Behavioral and trigeminal neuronal effects of rat brain stem-nanoinjected opiates. *Physiol. Behav.* 52:65-73; 1992.
 41. Yaksh, T. L.; Rudy, T. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. *J. Pharmacol. Exp. Ther.* 202:411-428; 1977.