Pharmacology Biochemistry & Behavior, Vol. 31, pp. 193–200. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Modulation by Morphine of Aversive-Like Behavior Induced by GABAergic Blockade in Periaqueductal Gray or Medial Hypothalamus

F. JENCK,[1] J. L. MOREAU[2] AND P. KARLI[3]

Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS
5, rue Blaise Pascal, 67084 Strasbourg, Cédex, France

Received 8 September 1987

JENCK, F., J. L. MOREAU AND P. KARLI. *Modulation by morphine of aversive-like behavior induced by GABAergic blockade in periaqueductal gray or medial hypothalamus.* PHARMACOL BIOCHEM BEHAV 31(1) 193–200, 1988.— Pretreatment with "analgesic" doses (15 nmoles) of morphine injected either into the periaqueductal gray (PAG) or into the medial hypothalamus (MH) were found to modulate flight behavior elicited by bicuculline injected into the same brain sites. When injected into the MH, morphine always suppressed bicuculline-induced flight, while PAG injections paradoxically either suppressed or facilitated the behavioral effects produced by bicuculline. Whenever a facilitation of the bicuculline-induced effects had been observed following pretreatment with 15 nmoles of morphine into the PAG, the infusion of lower doses (6 nmoles) did no longer induce facilitation but clear suppression. In those animals that had shown suppression of the aversive-like effects of bicuculline following the same 15 nmoles pretreatment, infusion of higher doses (24 nmoles) of morphine into the PAG still produced the same kind of suppression. And yet, when injected into the PAG, very high doses of morphine (50 nmoles) were found to induce, by themselves, flight behavior known as explosive motor behavior. In contrast, such high doses of morphine never induced comparable explosive motor behavior when injected into the MH. These data can be explained by the involvement of different types of receptors in the neural mechanisms subserving and controlling the generation of aversion in periventricular brain regions (PAG and MH).

Rat Periaqueductal gray Medial hypothalamus Bicuculline Morphine Escape Aversion

WHEN applied to periventricular brain regions such as the dorsal periaqueductal gray matter (PAG) or the medial hypothalamus (MH), electrical stimulation elicits behavioral reactions known as escape or flight behavior [29,30]. These reactions are generally considered to reflect pain and/or fear sensations induced by the neurostimulation, as such aversive effects prompt an animal to learn to switch-off the stimulation [5,21]. The behavioral flight or escape response is characterized by sudden and fast locomotor activity accompanied by jumps in an attempt to escape the experimental environment.

A similar behavioral pattern (escape-like behavior) can be observed when bicuculline (a GABA antagonist) is microinjected into the PAG or the MH [4, 7, 10]. When elicited from the MH, such escape-like behavior is characterized, as under electrical stimulation, by increased locomotion, a great number of rearings and some well-oriented jumps. When elicited from the PAG, less rearings and more jumps of an explosive type are observed under bicuculline as well as under electrical stimulation. The neuronal substrate involved in escape reactions within both the MH and PAG has been suggested to be under a tonic inhibitory influence mediated by GABA present in these brain regions [1, 8, 27, 28].

Escape behavior induced by PAG or MH electrical stimulation can be specifically suppressed by morphine microinjected into the PAG [16,17]. Such morphine microinjections are furthermore well known to exert an analgesic action as they also suppress the behavioral responses induced by pe-

[1]Present address: CNS Pharmacology Department, ORGANON International, P.O. Box 20, 5340 BH OSS, The Netherlands.
[2]Present address: Pharmacological Research Department, Hoffmann-La Roche & Co., Ltd., CH, 4002, Basle, Switzerland.
[3]Requests for reprints should be addressed to P. Karli.

ripheral nociceptive stimuli [9, 19, 24, 33]. The analgesic action of morphine may thus involve blockade of ascending nociceptive information at both a spinal and a supraspinal level.

Interestingly, some recent data have provided evidence for interactions to occur between opioids and GABA in the generation of analgesia. Thus, the antinociceptive effects of morphine injected systemically or into the midbrain can be reversed by microinjection of muscimol or THIP (two GABA receptor agonists) into the PAG [6, 26, 34]. Moreover, morphine is able to modify GABA receptor characteristics [31] and opioid peptides were shown to activate hippocampal pyramidal cells by inhibiting GABAergic interneurons [20].

In this context, the present study was designed to explore the possibility that interactions take place between opioids and GABA in the elaboration of escape behavior. As mentioned above, centrally applied morphine proved able to suppress escape induced by electrical brain stimulation [16,17]; we now report that a dose of 15 nmoles of morphine similarly suppresses escape-like behavior induced by bicuculline infused into the MH or the PAG. However, the PAG infusions brought into light some paradoxical effects of morphine: when injected into the PAG, morphine was found to exert a suppressant action in some of the animals whereas clear activating effects were observed in the remaining part. In an attempt to explain this discrepancy, additional experiments were performed using different doses of morphine. Finally, high doses (50 nmoles) of morphine known to induce explosive motor behavior when microinjected into the PAG [11], were tested as to their ability to produce comparable explosive motor behavior when injected into the MH.

METHOD

Animals

Male Wistar rats (400 g) were housed individually with free access to food and water and maintained on a 12 hr light-12 hr dark cycle. Each animal was implanted (under pentobarbital anaesthesia, 60 mg/kg IP) with guide cannulae (o.d.: 0.4 mm–i.d.: 0.3 mm) at a distance of 1 mm above target sites located in the dorsolateral periaqueductal gray and in the medial hypothalamus. The coordinates for the PAG guide cannula tip were 0.5 mm anterior to the lambda, 0.3 mm lateral and 4.6 mm ventral to the surface of the skull. The coordinates for the MH guide cannula tip were 4.5 mm anterior, 0.3 mm lateral and 7.6 mm ventral. The cannulae were angled at 10 degrees off vertical. Stainless steel blockers were kept in the guide cannulae between drug injections.

Testing

Global activity was recorded in an automated actometer (20×100×40 cm high) equipped with photocells. Four photocells spaced out 28 cm apart and placed on the length of the cage starting at 8 cm from each end measured horizontal locomotion. Two photocells were placed 10 cm apart on the cage width at a height of 15 cm in order to record rearings; two additional photocells were placed at a height of 30 cm in order to record jumps.

Pulses generated by the interruption of each infrared beam were recorded independently for locomotion, rearings and jumps for a one minute time period. Pulses recorded whenever jumps occurred were subtracted from the number of pulses recorded by the photocells measuring rearing activity.

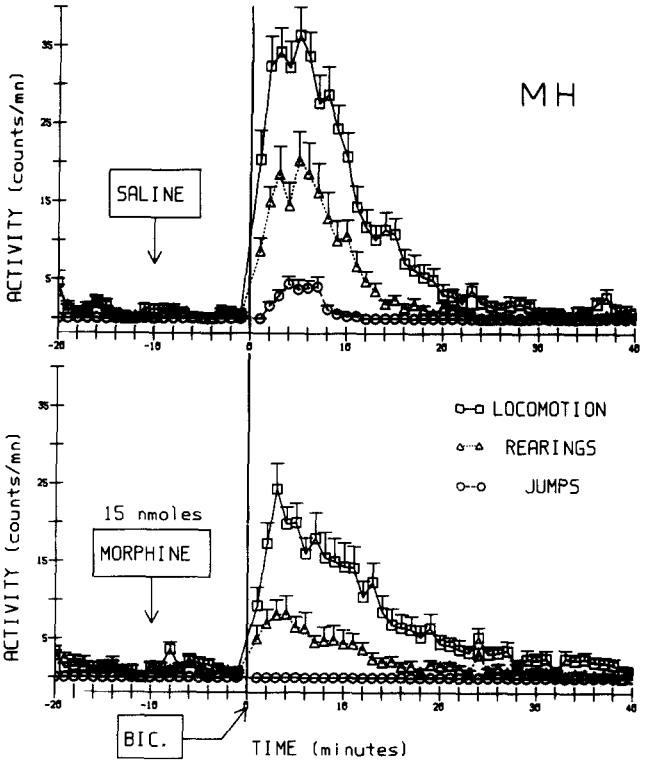

FIG. 1. Variations in activity (locomotion, rearings and jumps, n=13) after microinjection of bicuculline (BIC: 0.07 nmoles/0.25 μl) into the medial hypothalamus (MH). This injection was preceded either by a microinjection of saline 0.9% (top trace) or by a microinjection of morphine (15 nmoles/0.25 μl, bottom trace).

Drug Treatments

Injections were carried out by inserting in the guide cannula a stainless steel injection cannula (o.d.: 0.28 mm–i.d.: 0.18 mm) connected to a 1 μl Hamilton microsyringe by polyethylene tubing. The injection cannula penetrated 1 mm beyond the tip of the guide cannula and was left in place for 2 minutes.

Prior to any microinjection, the animals were placed in the actometer for 10 minutes in order to record their preinjection activity. Intracerebral (PAG or MH) infusions were then performed with either saline (0.25 μl) or morphine (15 nmoles in 0.25 μl of saline) administered in a counterbalanced order. These infusions were followed, 10 minutes later, by an infusion of bicuculline (0.07 nmoles in 0.25 μl of saline). Such a dose of bicuculline had been shown to reliably produce escape-like behavior when injected into these same brain regions [7]. The animal's activity was then recorded for 40 minutes following the bicuculline microinjection. Time intervals allowed between two consecutive treatments were ten to twelve days for any dose of morphine administered in this study. Activity scores recorded over time or at specific time bins were analysed with Wilcoxon's matched-pairs signed-ranks test [32].

Histology

After completion of the experiment, the animals were in-

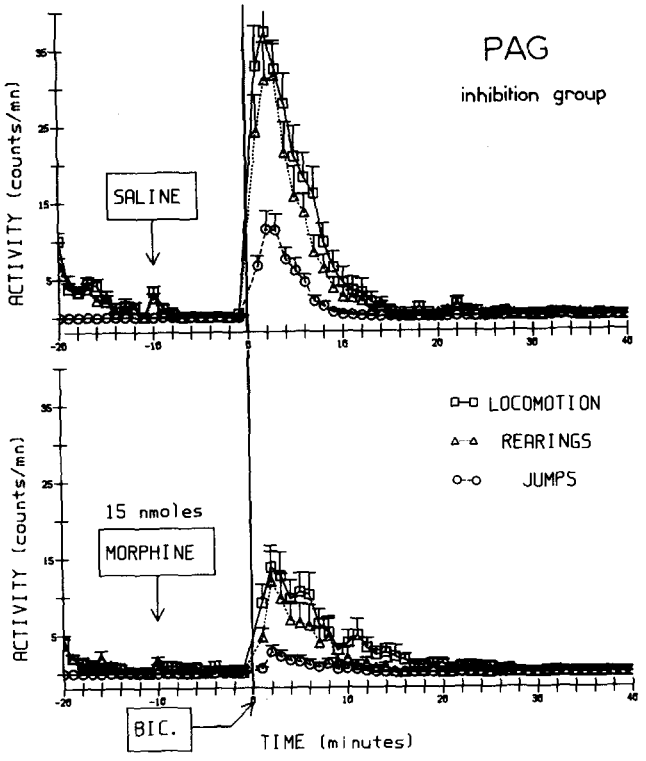

FIG. 2. Variations in activity (locomotion, rearings and jumps, n=14) after microinjection of bicuculline (BIC: 0.07 nmoles/0.25 μl) into the periaqueductal gray matter (PAG). This injection was preceded either by a microinjection of saline 0.9% (top trace) or by a microinjection of morphine (15 nmoles/0.25 μl, bottom trace). In this group of animals (inhibition group), morphine induced a clear suppression of the behavioral activation induced by bicuculline.

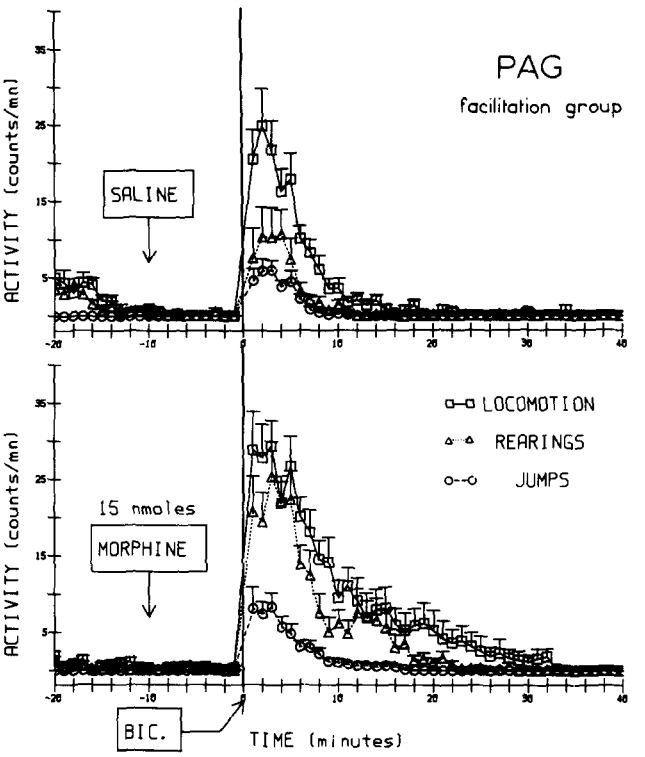

FIG. 3. Variations in activity (locomotion, rearings and jumps, n=11) after microinjection of bicuculline (BIC: 0.07 nmoles/0.25 μl) into the PAG. This injection was preceded either by a microinjection of saline 0.9% (top trace) or by a microinjection of morphine (15 nmoles/0.25 μl, bottom trace). In this group of animals (facilitation group), morphine induced a clear facilitation of the behavioral activation induced by bicuculline.

jected with an overdose of pentobarbital and intracardially perfused with NaCl 0.9% followed by 10% formalin. Serial brain sections were stained with cresyl violet and injection sites were localized on frontal planes of the König and Klippel atlas [18].

RESULTS

Pretreatment With 15 nmoles of Morphine Injected Into the MH or Into the PAG

Analgesic doses of morphine microinjected into either the MH or the PAG proved able to suppress the aversive-like effects of bicuculline infused 10 minutes later into the same brain regions.

Medial hypothalamus. When injected into the MH, bicuculline induced aversive-like effects that consisted, in the 13 animals tested, in an increased locomotion together with a number of rearings as well as jumps well oriented towards the top of the experimental enclosure. Pretreatment with saline did not affect any aspect of these bicuculline-induced effects (Fig. 1, top). On the other hand, pretreatment with 15 nmoles of morphine (Fig. 1, bottom) completely abolished the jumps observed when saline preceded bicuculline. The scores recorded over time for rearings as well as for locomotor activity were also decreased by 37% and 22%, respectively, under morphine pretreatment as compared to the same scores recorded under saline pretreatment (Wilcoxon test, T=401 and T=504.5, respectively; $p<0.05$). Nevertheless, the respective peak effects occurred with similar delays following the bicuculline injection (3–5 minutes for locomotion, 4–5 minutes for rearings) and scores at specific time bins obtained under morphine pretreatment did no longer differ from those recorded under saline pretreatment by the 12th minute (T=15, $p>0.05$) for rearings and by the 11th minute (T=13, $p>0.05$) for locomotion.

Periaqueductal gray. When injected into the PAG, bicuculline induced behavioral effects that likewise consisted, in the 25 animals tested, in increased locomotion, rearings against the walls of the cage and explosive jumps (Figs. 2 and 3, top). The overall effects of bicuculline injected into the PAG appeared of shorter duration than when injected into the MH. They further appeared less well oriented and of a more explosive kind. Local pretreatment with saline did not modify the effects induced by bicuculline injected into the same PAG site. On the other hand, pretreatment with 15 nmoles of morphine did affect the bicuculline-induced behavioral effects in a two-fold way. As a matter of fact, either suppression or facilitation was observed.

Suppression. In 14 out of 25 animals, morphine injected into the PAG provoked a clear suppression of the aversive-like effects of bicuculline (Fig. 2, bottom), at it did regularly when injected into the MH. Scores recorded over time for jumps, rearings and locomotion were decreased by 75%, 62%

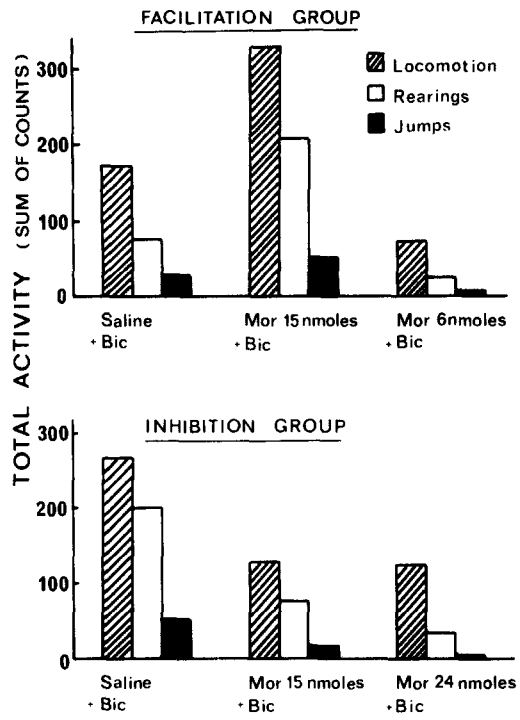

FIG. 4. Variations in total activity (cumulative over 40 minutes for locomotion, rearings and jumps) following microinjection of bicuculline (BIC: 0.07 nmoles/0.25 μl) into the PAG and local pretreatment with various doses of morphine. (Top) Facilitation group: following standard pretreatment with either saline or 15 nmoles of morphine (as described under Experiment 1), this group of animals was tested with a lower dose (6 nmoles) of morphine. (Bottom) Inhibition group: following the same standard pretreatment with either saline or morphine, this group of animals was tested with a higher dose (24 nmoles) of morphine.

and 51%, respectively, as compared to the same items recorded under saline pretreatment from the same animals (T=8 for jumps, T=50.5 for rearings, T=467.5 for locomotion; $p<0.05$).

However, such behaviors were never totally abolished: locomotion, rearings and even jumps were still recorded under morphine pretreatment. Whether saline or morphine had first been injected, the effects induced by the bicuculline microinjection reached their peak within 2–3 minutes and the scores at specific time bins obtained under morphine pretreatment no longer differed from those recorded under saline pretreatment by the 10th minute (T=22 for locomotion, T=6.5 for rearings, T=7 for jumps, $p>0.05$).

It should be noted that after the 15th minute, and following both types of pretreatment, the activity level of all the animals was particularly low, as they usually stayed quiet in a corner of the experimental chamber.

Facilitation. In the 11 remaining animals, a microinjection of 15 nmoles of morphine into the PAG surprisingly resulted in a facilitation of bicuculline-induced escape behavior (Fig. 3, bottom). Scores recorded over time for locomotion, rearings and jumps were increased by 90%, 168% and 69%, respectively, as compared to the saline pretreatment condition (T=220.5, T=229.5, T=0 respectively, $p<0.05$). Whether saline or morphine had first been injected, the effect of bicuculline reached a peak within 2–3 minutes with regard to locomotion and jumps, within 3–4 minutes with regard to rearings. Nevertheless, morphine appeared to increase the duration of the effects produced by bicuculline as the scores at specific time bins recorded under morphine pretreatment stopped differing from those recorded under saline pretreatment only by the 26th minute for locomotion (T=12.5, $p>0.05$) and by the 20th minute for rearings (T=16, $p>0.05$).

Pretreatment With 6 or 24 nmoles of Morphine Injected Into the PAG

The fact that morphine injected into the PAG was found to have both suppressant and facilitating effects led—in the light of previous reports of such paradoxical effects of morphine injected into the PAG—to carry out an additional experiment. Indeed, several authors reported [3,11] that high doses of morphine (30–40 nmoles and more) injected into the PAG would produce explosive motor behavior (EMB). EMB, which resembles the flight behavior elicited by locally applied bicuculline or electrical stimulation, was suggested to reflect the nonspecific activation of some ACTH receptors by high doses of morphine (see the Discussion section).

Thus, to further analyse the local action of morphine, we studied the effects of two additional doses of the drug. A pretreatment with a low dose (2 μg–6 nmoles) of morphine was applied to the rats in which a counterbalanced treatment with 15 nmoles of morphine and saline had previously produced a facilitation of the behavioral effect due to bicuculline. On the other hand, those rats in which the counterbalanced treatment with 15 nmoles of morphine and saline had previously produced an inhibition of the effect due to bicuculline were pretreated with a higher dose (8 μg–24 nmoles) of morphine.

Facilitation group. After pretreatment with a low dose (6 nmoles) of morphine (Fig. 4, top right), animals from the "facilitation" group showed a clear *inhibition* of the aversive-like effects of bicuculline.

A decrease by 59%, 66% and 77% was recorded for locomotion, rearings and jumps, respectively, as compared to saline pretreated animals (T=111 for locomotion, T=46 for rearings, T=0 for jumps, $p<0.05$). These values are quite similar to those previously obtained in the "inhibition" group (51%, 62% and 75%, respectively). Data obtained from animals pretreated with 6 nmoles of morphine did no longer differ from those recorded under saline pretreatment by the 10th minute following the injection of bicuculline (not displayed on the figure).

Inhibition group. Contrary to expectation, pretreatment with 24 nmoles of morphine in some animals from the "inhibition" group did not provoke any facilitation of bicuculline-induced escape-like responses, but still provoked *inhibition* (Fig. 4, bottom right). The effects of bicuculline were decreased by 53% for locomotion, by 83% for rearings and by 89% for jumps. This suppressant effect produced by 24 nmoles of morphine was more marked than that produced by 15 nmoles with regard to both rearings (T=180.5, $p<0.05$) and jumps (T=4.5, $p<0.05$) but not locomotion (T=703, $p>0.05$).

At this point, it should be emphasized that the sensitivity of PAG sites to the bicuculline treatment following a saline pretreatment clearly differed between animals from the "facilitation" group and animals from the "inhibition"

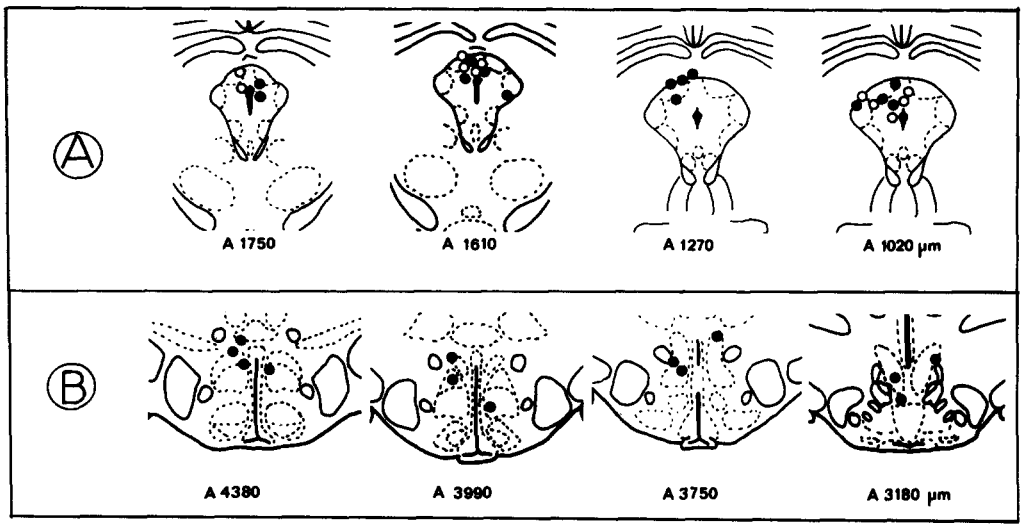

FIG. 5. Histological localization of microinjection sites on frontal planes of the König and Klippel atlas. (A) Microinjection sites at the level of the PAG. ●: Sites from which morphine induced a suppression of the behavioral activation induced by bicuculline; ○ Sites from which morphine induced a facilitation of the behavioral activation induced by bicuculline. (B) Microinjection sites at the level of the MH. ●: At this level, morphine induced only suppression of the behavioral activation induced by bicuculline.

group. Indeed, animals from the "inhibition" group proved more responsive to bicuculline: an injection of this GABA antagonist reliably induced more locomotion ($T=380$, $p<0.05$), more rearings ($T=212$, $p<0.05$) and more jumps ($T=9, p<0.05$) than did the same dose when microinjected to animals from the "facilitation" group.

However, the histological check of cannula placements on coronal sections of the König and Klippel atlas did not reveal significant differences between the two groups with regard to the location of the PAG injection sites. Nevertheless, sites from which facilitation was obtained seem to be preferentially located in the dorsomedial PAG. Cannulae tips were all located within the dorsal part of the PAG, above the aqueduct (Fig. 5A). MH injection sites were all found to be located within the dorsomedial part of the hypothalamus (Fig. 5B).

Injection of High Doses of Morphine (50 nmoles) Into the PAG or Into the MH

Since only suppression, but never facilitation had been obtained from MH sites with 15 nmoles of morphine, we further studied the effects produced by high doses of morphine (50 nmoles) injected into PAG and MH sites. Such high doses were expected to produce EMB when injected into PAG sites and the aim of the experiment was to check whether or not EMB would also be obtained from MH sites. The effects produced by a PAG or MH injection of 50 nmoles of morphine were recorded every 5 minutes for 265 minutes. Within each group of animals (PAG or MH) these effects were compared to those previously produced by 0.08 nmoles of bicuculline preceded by saline recorded every minute for 55 minutes (see Fig. 1, top and Fig. 3, top).

Periaqueductal gray. When injected into PAG sites of the animals of the facilitation group, such high doses of morphine elicited explosive motor behavior (EMB) characterized by sudden and fast locomotor activity for 40 minutes,

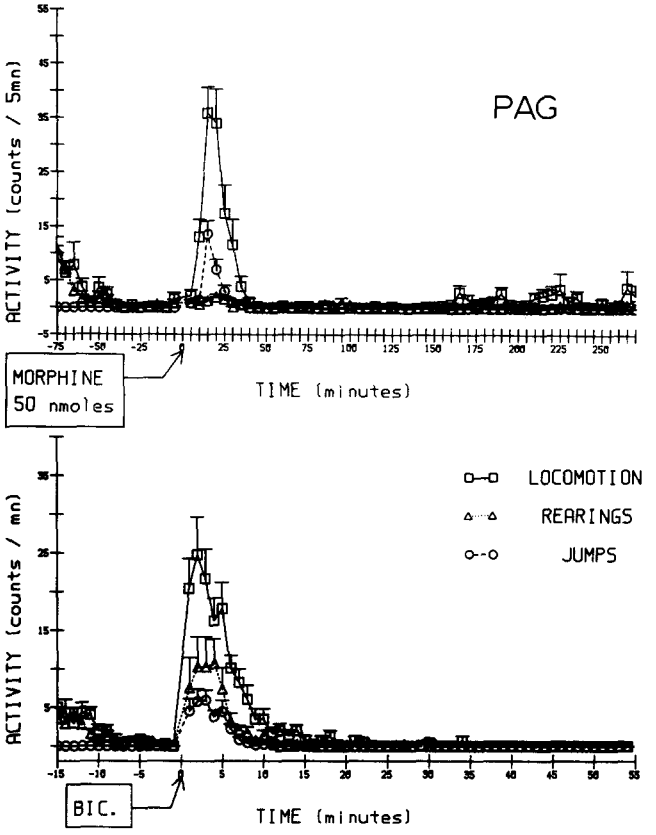

FIG. 6. Variations in activity (locomotion, rearings and jumps, $n=10$) after microinjection of a high dose of morphine (50 nmoles/0.25 µl, top trace, $n=10$) or of bicuculline (BIC: 0.7 nmoles/0.25 µl, bottom trace, $n=11$) into the PAG. Note that recordings took place every 5 minutes following a morphine injection and every minute following a bicuculline injection and that the time and activity scales are different for the two traces.

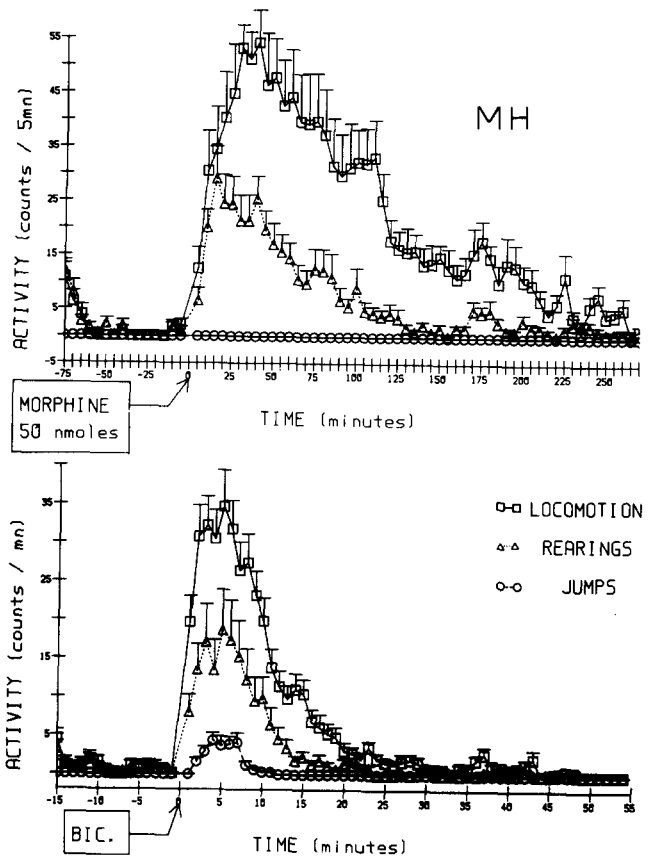

FIG. 7. Variations in activity (locomotion, rearings and jumps, n=10) after microinjection of a high dose of morphine (50 nmoles/0.25 μl, top trace, n=10) or of bicuculline (BIC: 0.07 nmoles/0.25 μl, bottom trace, n=13) into the MH. Note that recordings took place every 5 minutes following a morphine injection and every minute following a bicuculline injection and that the time and activity scales are different for the two traces.

explosive jumps for 25–30 minutes, and only a few rearings (Fig. 6, top).

A 2-hour period of almost complete inactivity followed that explosive behavior, as the animals appeared very quiet and froze in a corner of the experimental enclosure.

When bicuculline was previously microinjected into the same PAG sites, it induced a similar activity, but with more rearings and with a different time course (Fig. 6, bottom): increased locomotion was recorded for 15 minutes, rearings for 10 minutes and jumps for 8 minutes. The speed of activity induced by bicuculline was more than three times higher than that induced by morphine as can be seen when peak locomotor activity recorded under bicuculline (25 counts per minute) is compared to peak locomotor activity recorded under morphine (35 counts per five minutes or 7 counts per minute).

Medial hypothalamus. When injected into MH sites, high doses of morphine induced a marked increase in locomotion that reached its peak after 30 minutes and that lasted over four hours (Fig. 7, top). Numerous rearings were also recorded for two hours, a peak effect being reached after 15 minutes, but *no jump* was ever recorded. Both locomotion and rearings looked well oriented, with a lot of sniffing. The animals' behavior never appeared explosive as it did in the case of PAG injections.

In contrast, when bicuculline was previously microinjected into the same MH sites, it did induce jumps together with increased locomotion and rearings (Fig. 7, bottom). These jumps were recorded for 10 minutes only, while rearings were recorded for almost 20 minutes and increased locomotion for 30 minutes. The speed of activity induced by bicuculline was, in the case of MH injections, too, more than three times higher than that induced by morphine as can be seen when peak locomotor activity recorded under bicuculline (35 counts per minute) is compared to peak locomotor activity recorded under morphine (50 counts per five minutes or 10 counts per minute).

DISCUSSION

The results reported here show that "analgesic" doses (15 nmoles) of morphine injected into the dorsal part of the PAG or into the MH suppress escape-like behavior induced by bicuculline infused into the same brain regions. These results parallel those obtained with escape behavior electrically induced from the PAG which was similarly suppressed by identical doses of morphine [16,17].

In the present experiment, however, morphine did also, in some animals, enhance escape reactions induced by bicuculline injected into the PAG, while only the suppressant effect was obtained from MH sites.

These opposite and paradoxical effects of PAG morphine injections can be compared, as mentioned previously in the result section, with the paradoxical effects that high doses of morphine were shown to produce when injected into the PAG. Indeed, at a dose of 45 to 55 nmoles, morphine induces explosive motor behavior (EMB) [3,11]. This effect can be produced by morphine (−) as well as by morphine (+) and it is not reversed by naloxone [2,13]. EMB can also be observed following a local injection of ACTH, but never following a β-endorphin injection [12,15]. These authors have therefore proposed that morphine could act on two types of receptors within the PAG: One would be an opioid receptor, stereospecific, blocked by naloxone, involved in analgesia, with its endogenous ligand being β-endorphin. The other would be nonstereospecific, nonsensitive to naloxone, and involved in the elaboration of EMB; this nonopioid receptor would be nonspecifically activated by high doses of morphine and its endogenous ligand has been suggested to be ACTH. Another issue has recently been proposed by these authors who suggest that high doses of morphine may also block GABA-A receptors in the CNS [14].

Thus, a possible dual action of morphine within the PAG might be advanced to explain the heterogeneity of our results obtained following pretreatment with 15 nmoles of morphine into the PAG. According to the hypothesis, a dose of 15 nmoles of morphine should act on two types of receptors, though probably producing varying relative degrees of activation. The unexpected facilitating effect of morphine might then be due to morphine's action on some of the proposed ACTH-sensitive receptors and/or on the GABA receptors located at the injection site. This 15 nmoles dose of morphine would not be sufficient to produce EMB by itself, but summation or synergism with the effects produced by bicuculline would take place. Such a synergism could overcome the suppressant effect due to morphine and enhanced escape reactions could thus be obtained.

This hypothesis is supported by the data obtained in the

additional experiment in which lower doses of morphine (6 nmoles) were administered to animals that had previously shown a facilitation of the aversive-like effects induced by bicuculline. The previously observed facilitation did no longer occur, suggesting that 6 nmoles of morphine did not produce ACTH-like activation and/or GABA receptor blockade sufficient to potentiate the action of bicuculline. Under these conditions, the suppressant effects of morphine are likely to predominate and it is understandable that attenuated escape reactions were observed.

However, in those animals in which a clear suppressant effect had been obtained with 15 nmoles of morphine, injection of higher doses of morphine (24 nmoles) did not produce facilitation—but still suppression—of the aversive-like effects of bicuculline contrary to what one would have predicted from the hypothesis. This result would then suggest that morphine did never induce sufficient ACTH-like activation and/or sufficient GABA receptor blockade at any of the injection sites studied in the latter animals. The reason for that could lie in a low density of the putative ACTH-sensitive neurons and/or of GABAergic neurons at these injection sites. Unfortunately, the histological check of the cannula tip placements did not reveal that the location of the injection sites within the PAG did reliably differ depending on whether facilitation or suppression of the bicuculline-induced aversive-like effects was obtained under pretreatment with 15 nmoles of morphine. Moreover, animals of the PAG inhibition group showed a higher sensitivity to bicuculline than those of the PAG facilitation group (see Fig. 4) suggesting that a low density of GABAergic neurons at these injection sites is a very unlikely possibility. No clear regional differences have been shown to exist in the distribution of ACTH within the PAG. But the PAG happens to be one of the brainstem regions with the highest ACTH concentrations [22] and the highest number of ACTH immunostained-fibers [23]. Thus, even though morphine may well act within the PAG via an ACTH-mediated and/or a GABAergic mechanism and though the hypothesis involving these nonopioid receptors appears quite attractive, these explanations for the facilitatory effect of morphine need additional support. To our knowledge, no further evidence for a direct action of morphine on ACTH-neurones or receptors has yet been reported. Furthermore, it appears that GABA-A receptor blockade by high doses of morphine is not a general mechanism within the entire brain, since high doses of morphine injected into the MH did not at all—in our study—reproduce the effects of bicuculline which produces its aversive effects, in the MH too, via GABA-A receptors [28]. The fact remains nonetheless that high doses of morphine injected into the PAG probably induce effects via a nonopioid-specific mechanism.

When injected into the medial hypothalamus, 15 nmoles of morphine exclusively produced suppression of the aversive-like effects induced by bicuculline. One could again suggest here that only few or even none of the above-mentioned ACTH-sensitive and/or GABAergic receptors were activated by the infused morphine. This suggestion is well supported with regard to the ACTH-sensitive receptors by the fact that, in our study, high doses (50 nmoles) of morphine never induced EMB or any other aversive-like reaction when injected into the MH. This same suggestion is, however, not consistent with the clear behavioral effects produced by bicuculline, thus showing that high doses of morphine do not act by blocking GABA receptors in the MH.

The differential effects observed according as high doses of morphine were injected into the PAG or into the MH indicate that the generation of aversive effects is mediated within these two periventricular brain regions by—at least partly—independent neuronal mechanisms. Indeed, the comparable results obtained when either a high dose of morphine or bicuculline was injected into the PAG might have reflected an activation of one and the same neuronal substrate carrying both GABA- and ACTH-sensitive receptors. However, as high doses of morphine never elicited the behavioral responses induced by bicuculline when injected into the MH, it would rather appear that two distinct neuronal substrates subserve the induction of aversive-like effects by bicuculline and by high doses of morphine, respectively. Thus, the induction of aversive-like effects from the PAG would bring into play both GABAergic receptors and the putative ACTH-sensitive receptors located on distinct neuronal pathways. At the level of the MH, GABAergic receptors, but not ACTH-sensitive receptors, would be involved in the elaboration of flight behavior.

Moreover, it can be concluded from the present study that escape reactions, whether elicited from the PAG or from the MH, are under a clearly inhibitory opioid control, as morphine was found to suppress the aversive-like effects induced by bicuculline in both brain regions. This inhibitory opioid control is comparable to that exerted by morphine applied to the PAG on both centrally- (electrically) and peripherally-induced aversive effects. Whether this inhibitory control is of presynaptic or postsynaptic nature is not yet clear, even though morphine was already shown, in PAG or dorsal raphe slice perfusions, to induce increased GABA release [25]. Nevertheless, the occurrence of clear GABA/opioid interactions within these periventricular structures has been further evidenced here. The relative involvement of the various opioid receptor subtypes in these interactions remains to be elucidated.

ACKNOWLEDGEMENTS

This work was supported by grants from the DGRST (contract No. 81.E.0547), from the CNRS (ATP Neurobiologie sensorielle et comportementale) and from the Fondation pour la Recherche Medicale Française. The authors wish to thank Elisabeth Chau for excellent computer analysis, Gaby Rudolf for drawing the figures, Marie-Josée Angst and Any Boehrer for efficient technical assistance.

REFERENCES

1. Belin, M. F.; Aguera, M.; Tappaz, M.; McRae-Degueurce, A.; Bobillier, P.; Pujol, J. F. GABA-accumulating neurons in the nucleus raphe dorsalis and periaqueductal gray in the rat: a biochemical and radioautographic study. Brain Res. 170:279–297; 1979.

2. Blair, R.; Cytriniak, H.; Shizgal, P.; Amit, Z. Naloxone's antagonism of rigidity but not explosive motor behavior: possible evidence for two types of mechanisms underlying the actions of opiates and opioids. Behav. Biol. 24:24–31; 1978.

3. Blair, R.; Liran, J.; Cytriniak, H.; Shizgal, P.; Amit, Z. Explosive motor behaviour, rigidity and periaqueductal gray lesions. Neuropharmacology 17:205–209; 1978.
4. Brandao, M. L.; De Aguiar, J. C.; Graeff, F. G. GABA mediation of the anti-aversive action of minor tranquilizers. Pharmacol. Biochem. Behav. 16:397–402; 1982.
5. Delgado, J. M. R.; Roberts, W. W.; Miller, N. Learning motivated by electrical stimulation of the brain. Am. J. Physiol. 179:587–593; 1954.
6. Depaulis, A.; Morgan, M. M.; Liebeskind, J. C. GABAergic involvement in the antinociceptive effects of morphine at the level of the periaqueductal gray matter in the rat. Proc. West. Pharmacol. Soc. 28:143–145; 1985.
7. Di Scala, G.; Schmitt, P.; Karli, P. Flight induced by infusion of bicuculline methiodide into periventricular structures. Brain Res. 309:199–208; 1984.
8. Fahn, S. Regional distribution studies of GABA and other putative neurotransmitters and their enzymes. In: Roberts, E.; Chase, T. N.; Tower, D. B., eds. GABA in nervous system function. New York: Raven Press; 1976:169–186.
9. Herz, A.; Albus, K.; Metys, J.; Schubert, P.; Teschemacher, H. On the central sites for the antinociceptive action of morphine and fentanyl. Neuropharmacology 9:539–551; 1970.
10. Imperato, A.; Di Chiara, G. Behavioral effects of GABA-agonists and antagonists infused in the mesencephalic reticular formation-deep layers of superior colliculus. Brain Res. 224:185–194; 1981.
11. Jacquet, Y. F.; Lajtha, A. Paradoxical effects after microinjection of morphine in the periaqueductal gray matter in the rat. Science 185:1055–1057; 1974.
12. Jacquet, Y. F. Opiate effects of adrenocorticotropin or beta-endorphin injection in the periaqueductal gray matter of rats. Science 201:1032–1034; 1978.
13. Jacquet, Y. F.; Klee, W. A.; Rice, K. C.; Iijima, I.; Minamikama, J. Stereospecific and nonstereospecific effects of (+) and (−) morphine: evidence for a new class of receptors? Science 198:842–845; 1977.
14. Jacquet, Y. F.; Saederup, E.; Squires, R. F. Non-stereospecific excitatory actions of morphine may be due to GABA-A receptor blockade. Eur. J. Pharmacol. 138:285–288; 1987.
15. Jacquet, Y. F.; Wolf, G. Morphine and ACTH 1–24: correlative behavioral excitations following microinjections in rat periaqueductal gray. Brain Res. 219(1):214–218; 1981.
16. Jenck, F.; Schmitt, P.; Karli, P. Morphine applied to the mesencephalic central gray suppresses brain stimulation induced escape. Pharmacol. Biochem. Behav. 19:301–308; 1983.
17. Jenck, F.; Schmitt, P.; Karli, P. Morphine injected into the periaqueductal gray attenuates brain stimulation-induced aversive effects: an intensity discrimination study. Brain Res. 378:274–284; 1986.
18. König, J. F. R.; Klippel, R. A. The rat brain: a stereotaxic atlas of the forebrain and lower part of the brain stem. Huntington: Robert E. Krieger Publishing Company; 1970.
19. Mayer, D. J.; Price, D. D. Central nervous system mechanisms of analgesia. Pain 2:379–404; 1976.
20. Nicoll, R. A.; Alger, B. E.; Jahr, C. E. Enkephalin blocks inhibitory pathways in the vertebrate CNS. Nature 287:22–25; 1980.
21. Olds, M. E.; Olds, J. Approach-avoidance analysis of rat diencephalon. J. Comp. Neurol. 120:259–298; 1963.
22. Palkovits, M.; Eskay, R. L. Distribution and possible origin of beta-endorphin and ACTH in discrete brainstem nuclei of rats. Neuropeptides 9:123–137; 1987.
23. Pelletier, G.; Leclerc, R. Immunohistochemical localization of adrenocorticotropin in the rat brain. Endocrinology 104:1426–1433; 1979.
24. Pert, A.; Yaksh, T. L. Sites of morphine induced analgesia in the primate brain: relation to pain pathways. Brain Res. 80:135–140; 1974.
25. Pycock, C. J.; Burns, S.; Morris, R. In vitro release of 5-hydroxytryptamine and gamma aminobutyric acid from rat periaqueductal grey and raphe dorsalis region produced by morphine or an enkephalin analogue. Neurosci. Lett. 22(3):313–318; 1981.
26. Romandini, S.; Samanin, R. Muscimol injections in the nucleus raphe dorsalis block the antinociceptive effect of morphine in rat: apparent lack of 5-hydroxytryptamine involvement in muscimol's effects. Br. J. Pharmacol. 81:25–29, 1984.
27. Sandner, G.; Dessort, D.; Schmitt, P.; Karli, P. Distribution of GABA in the periaqueductal gray matter. Effects of medial hypothalamic lesions. Brain Res. 224:279–290; 1981.
28. Schmitt, P.; Carrive, P.; Di Scala, G.; Jenck, F.; Brandao, M.; Bagri, A.; Moreau, J.-L.; Sandner, G. A neuropharmacological study of the periventricular neural substrate involved in flight. Behav. Brain Res. 22:181–190; 1986.
29. Schmitt, P.; Abou-Hamed, H.; Karli, P. Evolution de la fuite et de l'approche en fonction du point de stimulation dans l'hypothalamus médian. Physiol. Behav. 22:275–281; 1979.
30. Schmitt, P.; Eclancher, F.; Karli, P. Etude des systèmes de renforcement négatif et de renforcement positif au niveau de la substance grise centrale chez le rat. Physiol. Behav. 12:271–279; 1974.
31. Sivam, S. P.; Nabeshima, T.; Ho, I. K. Alterations of regional gamma-aminobutyric acid receptors in morphine tolerant mice. Biochem. Pharmacol. 30:2187–2190; 1981.
32. Siegel, S. Nonparametric statistics for the behavioural sciences. New York: McGraw-Hill; 1956.
33. Yaksh, T. L.; Rudy, T. A. Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain 4:299–359; 1978.
34. Zambotti, F.; Zonta, N.; Parenti, M.; Tommasi, R.; Vincentini, L.; Conci, F.; Mantegazza, P. Periaqueductal gray matter involvement in the muscimol induced decrease of morphine antinociception. Naunyn Schmiedebergs Arch. Pharmacol. 318:368–369; 1982.