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Morphine Applied to the Mesencephalic Central Gray Suppresses Brain Stimulation Induced Escape[1]

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JENCK, F., P. SCHMITT AND P. KARLI. *Morphine applied to the mesencephalic central gray suppresses brain stimulation induced escape.* PHARMACOL BIOCHEM BEHAV **19**(2) 301–308, 1983.—In the rat, microinjections of morphine (1.5 to 15 nmoles) into the dorsal part of the mesencephalic centray gray (CG) were found to suppress both escape responding induced by electrical stimulations applied to either the medial hypothalamus (MH) or the CG and behavioral responsiveness to peripheral nociceptive stimulations. The time course of these two effects proved quite similar (Experiments 1 and 2). A systemic injection of naloxone reversed—in a dose dependent manner—the effects of morphine on the centrally induced escape responses (Experiment 3). The possibility that microinjections of morphine decrease both responsiveness to peripheral nociceptive stimulation and the reactivity of higher structures involved in the generation of aversive effects is discussed.

Central gray Medial hypothalamus Aversive effect Escape Electrical stimulation Morphine Microinjection Nociceptive stimuli

MICROINJECTIONS of morphine into various regions of the central nervous system are known to suppress the behavioral responses normally elicited by nociceptive stimuli [10, 21, 23, 35]. A number of results suggest this analgesic effect to be due to the activation of a descending inhibitory system which blocks the transmission of nociceptive input at the level of the first spinal relay [1, 4, 8, 15].

An electrical stimulation applied to various brain sites, in particular to the medial hypothalamus (MH) or to the dorsal part of the central gray (CG), elicits escape, pain and/or fear-like behavior and induces aversive effects which prompt the rat to switch-off the stimulation [22, 29, 30]. While affecting the reactions to peripheral nociceptive stimulations, microinjections of morphine into the ventral central gray failed to affect the threshold for escape induced by stimulation of the main trigeminal sensory nucleus [27]. Likewise, microinjections of D-Ala$_2$-metenkephaline or D-Ala$_2$-metenkephalinamide into the ventral part of the central gray [25] or of morphine into the nucleus giganto- or paragigantocellularis [26] failed to affect the threshold for CG induced aversion-like reactions.

These findings suggest the analgesic effect produced by central injection of opiates to result exclusively from an action at the spinal or trigeminal relay level, without any change affecting the reactivity of higher brain structures thought to be involved in the processing of nociceptive information. However, there are some data which actually lead to the suggestion that morphine may alter the processing of nociceptive input by acting at a supraspinal level. Thus, microinjections of morphine into the corticomedial amygdala were found to increase the jump threshold without affecting the reaction in response to tail immersion [24]. Furthermore, an intrathecal injection of noradrenergic and serotonergic antagonists was found to suppress the effect a CG morphine microinjection exerts in the tail flick situation whereas the effect in the hot plate test was affected for a very short time only [37]. The present study was undertaken to further verify the hypothesis as to whether morphine might not act on supraspinal structures thought to be involved in the processing of nociceptive inputs. Microinjections of morphine into—as well as electrical stimulation of—the dorsal part of the central gray were also found to produce analgesia [17, 20, 35]. However, the nature of the analgesic effect produced by such electrical stimulation seems to differ from that produced by electrical stimulation applied to the ventral central gray [6,7]. Also, dorsal central gray stimulation has always aversive effects, whereas ventral central gray stimulaton has clearly rewarding effects [18,30]. It was therefore of

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interest to verify whether and how microinjections of morphine into the dorsal part of the central gray would affect the behavioral responses to peripheral nociceptive stimulation as well as to aversive CG or MH stimulation.

EXPERIMENT 1: MICROINJECTION OF MORPHINE INTO CG. COMPARED EFFECTS ON SWITH-OFF RESPONDING INDUCED BY CG STIMULATION AND REACTIVITY TO NOCICEPTIVE STIMULI

METHOD

Subjects and Surgery

The experiment was performed on male Wistar rats (350–450 g) kept on a 12 hr light–12 hr dark cycle and housed in individual cages with an ad lib food and water supply.

Each animal was anesthetized with pentobarbital (40–50 mg/kg IP) and fixed into a stereotaxic apparatus so as to bring its skull to a horizontal position. A stainless steel guide-cannula (outer diameter: 0.4 mm; inner diameter: 0.3 mm) and an electrode made of 2 twisted stainless steel wires (0.12 mm in diameter) enameled except at the tip were then implanted into the central gray (CG) on either side of the midline. The following coordinates were used, the lambda serving as the reference in each plane: postero-anterior 0.5 mm; medio-lateral 1.2 mm (with a medio-lateral angle of 10°); dorso-ventral 5.6 mm. The tip of the guide-cannula was implanted 1 mm above the brain site aimed at, i.e., at a depth of 4.6 mm. The guide-cannula was then sealed with a steel wire. The whole was fixed on the skull by means of an autopolymerizing resin and 3 stainless steel screws.

Brain Stimulation

Following a postoperative delay of 1 week, each animal was put into a Plexiglas cage (25×25×35 cm high) equipped with a lever. By pressing the lever, the animal could interrupt a monopolar brain stimulation that consisted of rectangular cathodal pulses (one of the anchoring screws serving as the anode) of 0.1 msec duration delivered at a frequency of 50 pulses per second. A time counter (with a precision of 0.1 sec) allowed to measure the span of time that elapsed between the onset of the stimulation and the moment when the rat interrupted it by pressing the lever (escape latency: EL). The interruption lasted 15 sec during which a lever press was without any programmed effect. When the animal had learned to interrupt the brain stimulation, it underwent an additional training 3 hours a day for at least 3 days. Four stimulation intensities (I) were then chosen such as to induce escape latencies that would cover a range between 5 and 25 seconds. The effect of each of these intensities was assessed by determining the mean EL obtained from a series of 5 consecutive stimulations, a 15 sec delay elapsing between each lever press and the next stimulation onset. An additional group of five animals was submitted to the above-described procedure, except that a bipolar stimulation was applied to the dorsal part of the CG.

Peripheral Nociceptive Stimulation

Electric shocks of 1 sec duration were delivered to the rat's paws by means of a scrambler through the bars of the floor of the switch-off cage. Starting from 0.05 mA, the stimulation intensity was progressively increased. A threshold was considered to be reached when the rat reacted by raising its forelegs. This behavioral reaction was chosen in order to minimize possible interferences between peripheral and central stimulation. It should be noted that vocalisations were often associated with the paw lifting reaction.

Experimental Procedure

The aim was to determine whether and how a microinjection of morphine into the central gray would affect, in a given animal both the escape latencies (EL) induced by each of the 4 chosen brain stimulation intensities and the response threshold for the peripheral nociceptive stimulation.

The microinjection was carried out by inserting in the guide-cannula a stainless-steel injection cannula (outer diameter: 0.28 mm; inner diameter: 0.18 mm) connected to a 2 μl Hamilton syringe through a polyethylene tubing. The tip of the injection cannula jutted out from that of the guide-cannula by 1 mm. Ten nmoles (3 μg) of morphine sulfate dissolved in 0.2 μl of sterile saline (NaCl 9‰) were injected within 30 sec. This amount lies within the dose range known to raise the response threshold for nociceptive stimuli, when injected into the CG [35]. Prior to any daily experimental session, each rat underwent a warm-up period during which it interrupted 50 CG stimulations applied at a medium intensity. The 4 selected intensities were then applied in an increasing order, a 1 min delay elapsing between the applications of 2 successive intensities. Such series of CG stimulations were repeated every 30 min for 8 hours, and the microinjection took place between the 4th and the 5th series. The threshold intensity that would provoke a raising of the rat's forelegs was determined during the time span separating two successive series of CG stimulations.

Following a 1 week delay, a similar experiment was repeated on the same animals using a microinjection of 0.2 μl of saline.

The results were analysed by means of an analysis of variance or non parametric statistics [28].

After completion of the experiment, the animals were killed and intracardially perfused with NaCl 9‰ followed by 10% formalin. Serial brain sections were stained with cresyl violet in order to localize the stimulation and injection sites. The latter were then transferred to the corresponding frontal planes of the König and Klippel atlas [14].

RESULTS

The localization of both CG microinjection sites and CG or MH stimulation sites studied in the three experiments is shown in Fig. 1.

Figure 2A shows an example of how a microinjection of 10 nmoles of morphine into the CG affected the escape latencies (EL) induced by CG stimulation. Following the injection, the values of EL induced by the various stimulation intensities increased markedly and the duration of this effect was all the shorter as the stimulation intensity got higher. Figure 2B shows a series of EL=f (I) curves before (curve 1) and at various time intervals after the microinjection (curves 2, 3, 4 and 5). Right from the first post-injection session (curve 2) the curves markedly shifted away from the pre-injection curve, with a progressive return to pre-injection values (curves 3, 4 and 5). Such curves allow one to determine the stimulation intensity which is to be applied in order to obtain a constant behavioural output, for instance an EL of 8 seconds. The variation of that intensity—called I8—can be derived from the intersection points of the various exper-

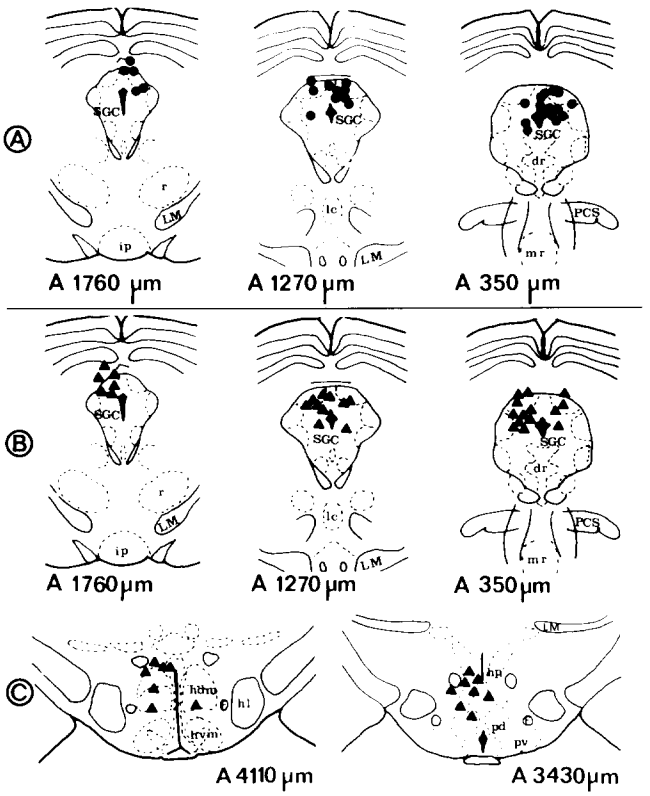

FIG. 1. Histological localization on frontal planes of the König and Klippel atlas [14] of the sites studied in the three experiments: A. microinjection sites (●) in the central gray; central gray B. and medial hypothalamic C. stimulation sites (▲). One MH stimulation site could not be localized. dr: nucleus dorsalis raphes; hdm: nucleus dorsomedialis (hypothalami); hl: nucleus lateralis (hypothalami); hp: nucleus posterior (hypothalami); hvm: nucleus ventromedialis (hypothalami); lc: nucleus linearis pars caudalis; LM: Lemniscus medialis; mr: nucleus medianus raphes; PCS: pedunculus cerebellaris superior; pd: nucleus prémamillaris dorsalis; pv: nucleus prémamillaris ventralis; r: nucleus ruber; SGC: substantia grisea centralis.

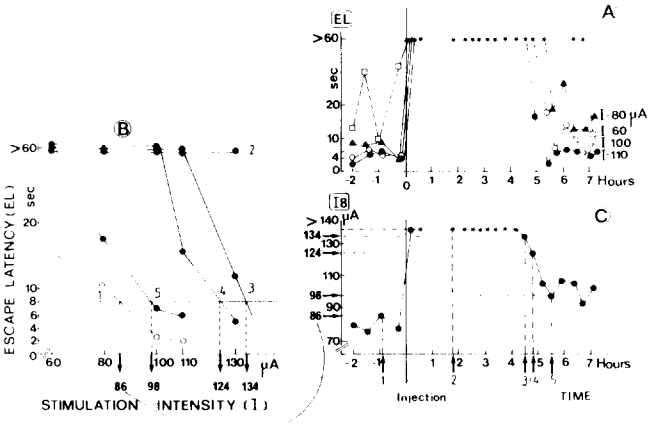

FIG. 2. Three different graphic expressions of the results obtained with rat B16: A. Variation over time of escape latencies (EL) induced by various stimulation intensities (I) applied to a CG site, before and after a microinjection of 10 nmoles of morphine into a contralateral CG site. B. Variation of escape latency as a function of stimulation intensity before (curve 1) and at different times after (curves 2, 3, 4, 5) the microinjection. This graphic expression allows to determine the I8 value which is the intensity required to induce an escape latency of 8 seconds (see text). C. Variation of I8 as a function of time. The stars indicate either undetermined escape latencies (>60 sec in panel A) or undetermined stimulation intensities (>140 µA, in panel C).

imental EL=f (I) curves with a straight line that runs parallel to the abscissa and intersects the ordinate at EL=8 sec. This I8 intensity changes over time, as is shown on Fig. 2C for the single rat previously considered and on Fig. 3A for a group of 14 animals submitted to monopolar stimulation. Right from the first session performed 30 min following the microinjection of morphine the stimulation intensity necessary to obtain a constant behavioural output, i.e., I8, reliably exceeds (Mann-Whitney $U=0$, $p<0.002$) that necessary following a microinjection of saline. This rise in I8 gets less over time and from the 5th hour following the injection of morphine the I8 values no longer differ from those obtained in the rats injected with saline (Mann-Whitney $U=43.5$, $p>0.05$). Figure 3A further shows that in the case of a bipolar stimulation, too, a microinjection of morphine is followed by a rise in I8. Although the resulting curve lies below that obtained in the case of a monopolar stimulation, these curves do not reliably differ from each other (Mann Whitney: $p>0.05$ at each of the time intervals considered after microinjection).

Figure 3B shows the results obtained with regard to the animals' reactivity to peripheral nociceptive stimuli. The use of foot-shocks revealed differential effects of morphine in two groups of animals. In 9 out of 14 animals, morphine induced a marked rise in the response threshold with a temporal course very similar to that of the effect on switch-off responding. In the remaining 5 rats, the microinjection of morphine into the CG did not affect the response threshold. It is noteworthy, however, that each of the 14 rats showed a marked reduction of its reactivity to a painful mechanical stimulus applied to the tail by means of a forceps.

EXPERIMENT 2: MICROINJECTION OF MORPHINE INTO CG. COMPARED EFFECTS ON CG-INDUCED AND MH-INDUCED ESCAPE

METHOD

The animals used in this experiment differed from those used in Experiment 1 only by the fact that an additional electrode was implanted into the medial hypothalamus (MH) at the following coordinates: postero-anterior 4.5 mm; medio-lateral 1.6 mm (with a medio-lateral angle of 10°); dorso-ventral 8.6 mm.

In a procedure similar to that used in Experiment 1, series of 4 stimulation intensities applied to the MH alternated with series of 4 stimulation intensities applied to the CG. Consequently, a delay of 2×30 min elapsed between two successive series of stimulations applied to the same brain site. Several doses of morphine (1.5, 7 and 15 nmoles) were injected in a constant volume of 0.2 µl, a 1 week delay elapsing between two successive injections.

One week after completion of these experimental sessions, the effects of microinjections of morphine on the rats' reactivity to painful thermal stimuli were studied. Each animal was put into a Plexiglas cage (21×11×21 cm high) kept at a height of 25 cm so as to allow the rat's tail to hang down between the bars of the cage floor. The tail was intro-

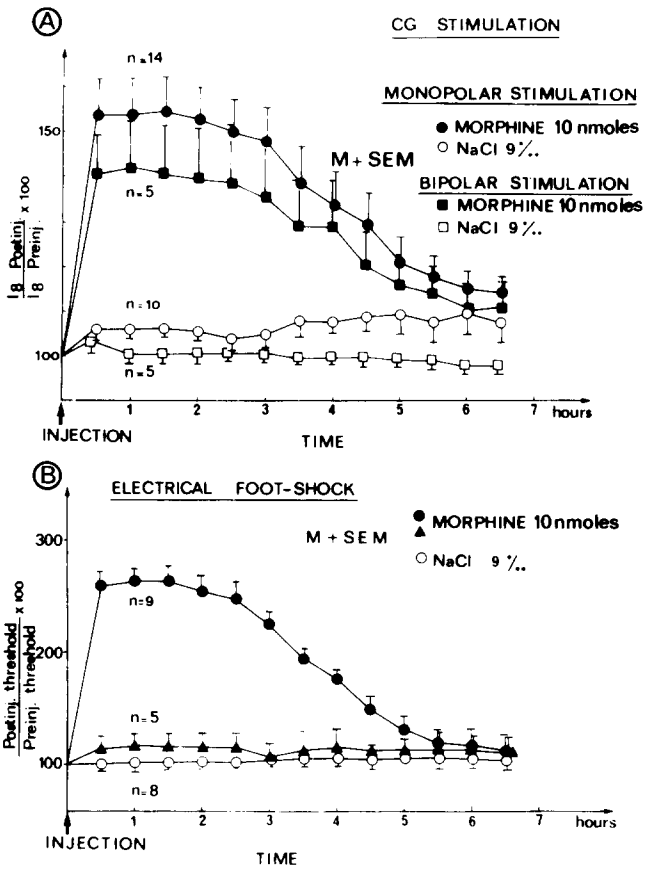

FIG. 3. Effects of morphine on centrally and peripherally induced escape. A. Variation of I8 (in percent of the mean preinjection value) after a microinjection of morphine (10 nmoles-filled symbols) or saline (open symbols) into the CG: results of a group of 14 rats submitted to monopolar CG stimulation (●) and of a group of 5 rats submitted to bipolar CG stimulation (■). B. Variation of the threshold (in percent of the mean preinjection value) of electrical footshock after a microinjection of morphine (10 nmoles) or saline into the CG: ● results of a group of 9 animals, in which the microinjection induced a marked increase in threshold; ▲ results of a group of 5 animals in which the microinjection did not modify the threshold.

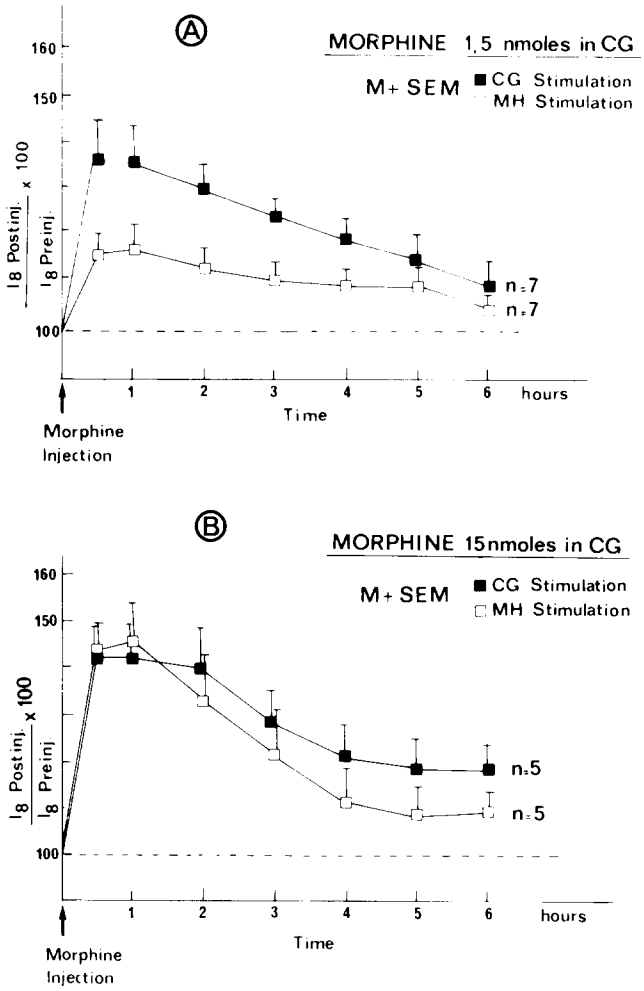

FIG. 4. Microinjection of morphine (A. 1.5 nmoles; B. 15 nmoles) into the central gray: resulting variation in the intensity required to induce an escape latency of 8 sec (I8) when stimulating either CG (■) or MH (□) sites.

duced into a receptacle containing hot water kept at 55°C and one recorded the time taken by the rat to remove its tail.

RESULTS

Figure 4 shows that microinjections of 1.5 nmoles (0.5 μg) or 15 nmoles (5 μg) of morphine provoked a rise in I8, irrespective of whether it was the MH or the CG that was stimulated. Both the maximal extent and the duration of the effect thus induced proved to depend on the dose injected (Fig. 5). Furthermore, following an injection of 1.5 nmoles, the maximal extent of the effect was greater and its duration longer in the case of a CG stimulation than in that of a MH stimulation (Wilcoxon, $T=1$, $p<0.05$). These differential effects appeared no longer when 15 nmoles had been injected.

In each animal, the latency with which it removed its tail from the water kept at 55°C was clearly increased (Fig. 6). The magnitude of this increase as well as its duration proved to depend on the dose injected (between 2 and 3 hours following an injection of 1.5 nmoles, between 4 and 5 hours following an injection of 7 nmoles).

EXPERIMENT 3: REVERSAL BY NALOXONE OF THE DEPRESSANT EFFECT OF MORPHINE

The aim of this experiment was to check whether the depressant effect of a microinjection of morphine into the CG on the escape responses induced by CG or MH stimulation could be reversed by a systemic administration of naloxone.

METHOD

The animals were prepared in the same way and submitted to the same procedure as in Experiment 2. For each CG or MH stimulation site, the values of I8 were determined both following the sole microinjection of morphine (7 nmoles) into the CG and following such a microinjection of

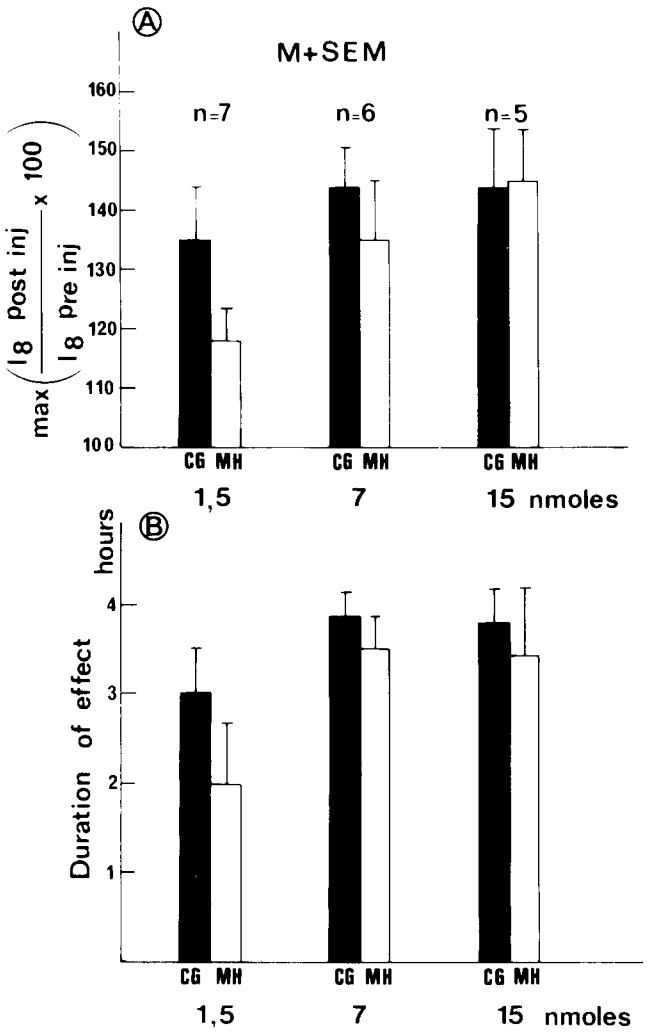

FIG. 5. Variation as a function of the dose of morphine injected (1.5-7-15 nmoles) of: A. the maximal effect (assessed by the maximal variation of the I8 value); B. the duration of effect (time for which the I8 value remains above the mean preinjection value). These two parameters were established for CG (black columns) and MH (white columns) stimulations.

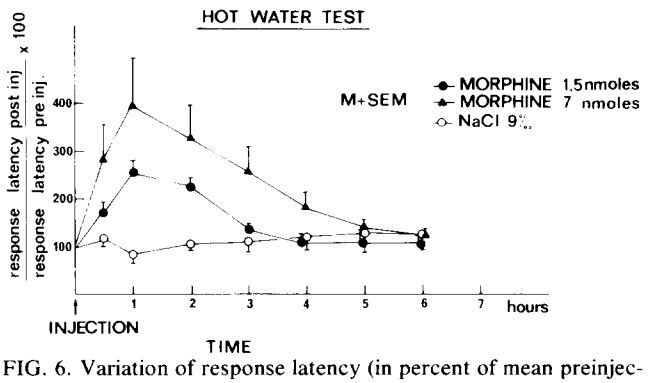

FIG. 6. Variation of response latency (in percent of mean preinjection latency) in the hot water test, after a microinjection of two different doses of morphine (1.5 and 7 nmoles) or saline into the CG.

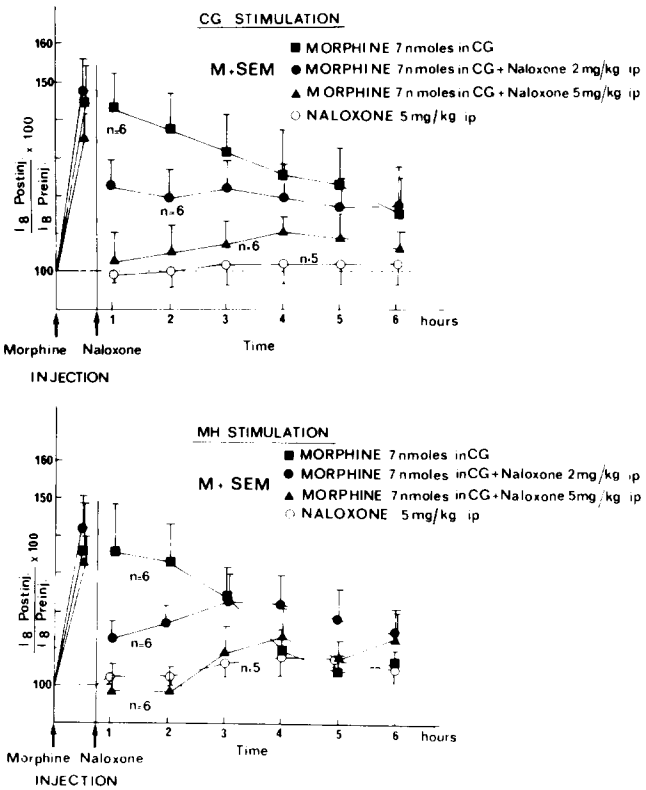

FIG. 7. Variation of I8 after a microinjection of morphine (7 nmoles) into the CG, and effect of two doses of IP naloxone injected 45 minutes later. The figure shows also the effects of naloxone administered alone. Top: CG stimulation; bottom: MH stimulation.

morphine followed—45 minutes later—by an intraperitoneal injection of 2 or 5 mg/kg of naloxone hydrochloride (Endo). The results were submitted to a 2 factors (treatment × post-naloxone delay) analysis of variance. The effects possibly induced by the sole intraperitoneal injection of 5 mg/kg of naloxone were studied in an additional group of rats.

RESULTS

Figure 7 shows that the rise in I8 provoked by the microinjection of morphine was clearly reversed by an intraperitoneal injection of naloxone, and all the more so as the dose of naloxone injected was higher. The analysis of variance showed I8 to vary over time, $F(6,105)=5.03$ and 4.14 for CG and MH stimulations, respectively; $p<0.001$ in either case, and this variation to depend on the treatment, $F(2,105)=15.10$ and 4.04 for CG and MH stimulations, respectively; $p<0.025$ in either case. The treatment × post-naloxone delay interaction did not reach the threshold of statistical significance, $F(12,105)=0.66$, $p>0.7$ and $F(12,105)=1.70$, $p>0.07$ for CG and MH stimulations, respectively. Figure 7 shows further that the action of naloxone is of shorter duration than that of morphine. As a matter of fact, the I8 values did not longer reliably differ 3 hours following the microinjection of morphine irrespective of whether or not 2 mg/kg of naloxone had further been injected (Mann-Whitney: $p>0.05$). And yet, a significant ef-

fect was found in previous experiments to still exist 3 hours following a microinjection of 7 nmoles of morphine. The sole intraperitoneal injection of naloxone did not affect the values of I8.

GENERAL DISCUSSION

It appears from the results reported that microinjections of morphine into the dorsal part of the central gray at doses varying from 1.5 to 15 nmoles (0.5 to 5 μg) not only reduce the rat's responsiveness to nociceptive stimulation [10, 21, 23, 35], but also suppress escape responding induced by CG or MH stimulation. This holds true whether the brain stimulation is monopolar or bipolar. The thus observed effects of morphine seem to be due to the activation of opioid receptors since the suppressant effect on escape responding as well as the analgesic effect [21,35] can be reversed by naloxone in a dose-dependent manner.

At least two hypotheses can be put forward when trying to explain the suppressant effect exerted on brain-stimulation induced escape responses. One can imagine either that a microinjection of morphine reduces the magnitude of the aversive effect induced by brain stimulation or that it impairs the animal's ability to interrupt an otherwise unchanged aversive effect. The following arguments suggest that the hypothesis of a mere motor impairment, although difficult to dismiss entirely, can hardly be retained to explain the suppressant effect on escape responding:

(1) Microinjections into the CG of etorphine which is 10 times more potent than morphine [11,13] are known indeed to result in catatonia [33,34], but morphine microinjected into the reticular formation was found to result in catalepsy only at doses greater than 5 μg [5].

(2) The doses of naloxone used in our experiments, though lying in the upper dose range known to reverse analgesia [9, 21, 23, 36], were smaller than those generally used to suppress the behavioral depression observed after centrally injected morphine [13,36].

(3) Some of our rats were tested in order to detect a possibly existing catatonia. They were submitted to the bar test [3] in which the animal's forepaws were gently placed on a 15 cm long bar (1 cm in diameter) placed 10 cm above the floor. Following a 15 nmoles microinjection of morphine, the tested rats stepped down from the bar after 1.4 ± 0.2 second much like they did (1.3 ± 0.2 second; $n=10$) when injected with saline (paired t test: $t=1.36$, $p>0.05$).

(4) When after a microinjection the rat did not press the lever even at the highest stimulation intensity, it remained quiet and did not show any unconditional escape reaction like trying to jump out of the cage. But when the intensity was then raised above this maximum, the animal actually showed a well oriented response toward the lever, similar to that observed before the microinjection at lower stimulation intensities. This indicates that the animal remembers the behavioral task it has to perform and that it is able to press the lever.

Even though these arguments do not rule out more subtle impairments of the animal's ability to respond, they certainly favour the hypothesis that morphine microinjections actually attenuate the aversive effects induced by CG or MH stimulation.

Morphine microinjections into the CG are known to produce analgesia, as confirmed by the present study. This analgesic effect was most often explained by the bringing into play of a descending inhibitory system which blocks the nociceptive input at the level of the first synaptic relay. One tends hardly to explain the suppressant effect on brain stimulation induced escape by a similar mechanism, except if one assumes that brain stimulation is not aversive per se [12]. In a few rats, the microinjection was effective on CG-induced responding, without affecting the responsiveness to electrical stimulation applied to the paws. However, in the same rats, the response to tail pinch or to tail heating was attenuated. Various data have demonstrated the existence of a somatotopic organization in the effects of intracerebrally injected morphine or D-Ala$_2$-metenkephalinamide on the reactions to peripheral nociceptive stimulation ([16, 25, 26, 36, see discussion in 25]). Such a somatotopic organization could well explain the differential results we obtained. It could also be that morphine injected at some sites differentially affected the reactions to nociceptive stimulation according as electrical stimulation versus heat or pinch was applied. The small number of rats showing such a differential responsiveness does not allow to clearly distinguish their injection sites from those found in the other group of rats.

In most animals, however, the effects of morphine on brain-stimulation induced escape and on the responsiveness to peripheral nociceptive stimulation showed a similar time course. This would suggest that morphine affects simultaneously both the transmission of nociceptive input at its first relay and the reactivity of higher brain structures thought to be involved in the elaboration of aversive effects. However, this suggestion is not in agreement with the findings of Rosenfeld [25, 26, 27]. As a matter of fact, he found a microinjection of either morphine (0.7 μg) or D-Ala$_2$metenkephalinamide into the ventral part of the CG to raise the response threshold for peripheral nociceptive stimulation without affecting the threshold for brain stimulation (applied at the level of the trigeminal sensory nucleus, in the case of morphine injection) induced escape. Since in our hands even a dose of morphine (0.5 μg) smaller than that used by Rosenfeld [27] was found to raise CG and MH escape thresholds, the discrepancy between our results and those of Rosenfeld might well be due to procedural differences. Indeed, there are many differences between the two studies with regard to the kind of stimulation (sinusoïdal vs. rectangular) applied, the stimulation parameters used, the behavioral reactions observed and the parameters recorded (threshold current producing aversion-like reactions vs. threshold current producing a given value of escape latency), the site of injection (ventral vs. dorsal central gray) and the site of stimulation (trigeminal nucleus vs. CG or MH). It should however be added that even in the case of D-Ala$_2$-metenkephalinamide injection into the ventral CG a 30% rise in CG induced aversion-like reactions threshold was noted, which was attributed however to a depression of motor activity [25].

Taken together, these data suggest that morphine and opiates act on both the responsiveness to peripheral nociceptive stimulation and escape responding induced by stimulation of at least certain brain sites. The discrepancy between our data and those obtained by Rosenfeld may be resolved by assuming that the magnitude of the effects exerted by morphine microinjections on the responses to aversive brain stimulation depends at the least on the site of injection as well as on the brain site where the stimulation producing aversive effects is being applied. In this context, it is worth noting that, in a given animal, a low dose morphine microinjection is more efficient on CG than on MH induced escape. The reason for such a differential effect remains to

be clarified. It should however be added that an electrical stimulation applied to the dorsal raphe was also found to exert a differential effect on MH and CG induced escape; but in this case, MH induced escape was more suppressed than CG induced escape [31].

The mechanism by which a CG microinjection of morphine affects CG or MH induced escape remains unclear. An explanation based merely on some local and direct effect of morphine cannot be retained, since MH induced escape was clearly affected by our CG microinjections, even though an injection of 1 μl was estimated to diffuse at a distance of no more than 1 mm [19]. The actual existence of such a direct effect can however not be excluded, since morphine proved to act more efficiently on CG than on MH induced escape. Microinjections of morphine into the CG affect not only behavioural responses induced by aversive or painful stimulations. As a matter of fact, lateral hypothalamic self-stimulation was found to be depressed [2] and, in the rabbit, slow wave sleep could be induced by an infusion of 15–30 nmoles of morphine [32]. Given the variety of effects observed after such a microinjection of morphine into the CG, it is possible that an activation of opiates receptors within the CG produces a more general suppressant effect—the nature of which remains to be specified—resulting in the various behavioral changes observed.

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