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Morphine Applied to the Ventral Tegmentum Differentially Affects Centrally and Peripherally Induced Aversive Effects

JEAN-LUC MOREAU, PIERRE SCHMITT[1] AND PIERRE KARLI

Laboratoire de Neurophysiologie, Centre de Neurochimie du C.N.R.S., 5, rue Blaise Pascal 67084 Strasbourg Cédex, France

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MOREAU, J.-L., P. SCHMITT AND P. KARLI. *Morphine applied to the ventral tegmentum differentially affects centrally and peripherally induced aversive effects.* PHARMACOL BIOCHEM BEHAV **23**(6) 931–936, 1985.–In the rat, a microinjection of 15 nmoles of morphine into the ventral tegmentum (VT) was found to suppress escape responding induced by electrical stimulation applied to the dorsal part of the mesencephalic central gray. This suppressant effect (1) could be reversed by a systemic injection of naloxone and (2) was unlikely to be due to gross motor impairment since morphine injected into the VT provoked a behavioral activation. A similar microinjection of morphine into the VT did not induce analgesic effects since it did not affect the reaction thresholds to a nociceptive stimulus. The mechanisms underlying these differential effects of morphine applied to the VT are discussed.

Ventral tegmentum Periaqueductal gray Escape Aversion Electrical stimulation Microinjection Nociceptive stimuli Analgesia Morphine Opiates

WHEN applied to the dorsal part of the mesencephalic central gray (CG), an electrical stimulation is well known to elicit pain and/or fear like behavior such as escape, and to induce aversive effects which prompts the animal to put an end to the applied stimulation [15,18]. Electrical stimulation of the ventral mesencephalic tegmentum (VT) [14] as well as that of some other brain structures known to induce rewarding effects [10, 19, 20], proved to increase the rat's latency to stop such a CG stimulation (suppressant effect). Moreover, the magnitude of this suppressant effect was found to be correlated with the magnitude of the VT stimulation-induced rewarding effect, as assessed in a self-stimulation situation.

The ventral tegmentum has been described as a site involved in opiate reward. Thus, microinjection of morphine into the VT facilitates lateral hypothalamic self-stimulation [3] and microinjection of D-Ala2-met-enkephalinamide into the VT entails the development of a conditioned place preference [17]. Furthermore, rats readily learn to self-administer morphine into the VT [1].

Considering the correlation found to exist between suppressant and rewarding effects induced by electrical VT stimulation, we found it of interest to investigate, in a first experiment, if a microinjection of morphine into the VT would likewise increase the rat's latency to stop a CG stimulation (escape latency) and, if so, whether this increase could be reversed by administering naloxone.

In order to exclude the possibility of gross motor impairment being the cause of an occurring increase in the escape latency, the effects of a morphine microinjection into the VT on locomotor activity were tested in a second experiment.

An electrical VT stimulation was also found to decrease the rat's reactivity to a nociceptive stimulus [13]. Since morphine injected into another brain structure, namely the CG [5], proved to attenuate both centrally-induced escape and the reactivity to nociceptive stimuli, we investigated, in Experiment 3, the effects morphine injected into the VT would exert on the reactivity to a nociceptive stimulus.

EXPERIMENT 1: MICROINJECTION OF MORPHINE INTO VT: EFFECT ON ESCAPE RESPONDING INDUCED BY CG STIMULATION

The aim of this experiment was to determine whether a microinjection of morphine into the ventral tegmentum would affect escape responses induced by stimulating the mesencephalic central gray.

METHOD

Animals

The experiment was carried out on male Wistar rats (350–500 g) kept on a 12 hour-light/12 hour-dark cycle and

[1]Requests for reprints should be addressed to Pierre Schmitt.

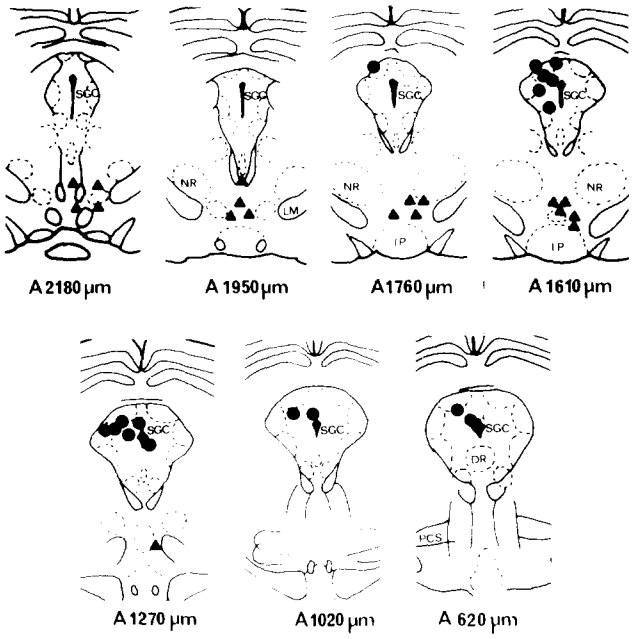

FIG. 1. Histological localization on frontal planes of the König and Klippel atlas [11] of the central gray stimulation sites (●) and the ventral tegmental microinjection sites (▲) studied in the three experiments. DR: nucleus dorsalis raphes; IP: nucleus interpeduncularis; LM: lemniscus medialis; NR: nucleus ruber; PCS: pedunculus cerebellaris superior; SGC: substantia grisea centralis.

housed in individual cages with an ad lib food and water supply.

Surgical and Histological Procedures

Each animal was anesthetized with sodium pentobarbital (40–50 mg/kg IP) and placed into a stereotaxic apparatus so as to have its skull in a horizontal position. A stainless steel guide-cannula (o.d.: 0.4 mm; i.d.: 0.3 mm) was implanted into the VT and an electrode made of 2 twisted stainless steel wires (0.12 mm in diameter), enameled except at the tip, was implanted into the CG. The following coordinates were used, the lambda serving as the reference for each plane:

	CG	VT
postero-anterior	0.3 mm	1.5–1.6 mm
medio-lateral	0.3 mm	1.6 mm
dorso-ventral	5.5 mm	7.6 mm

The guide cannula was inserted with a medio-lateral angle of 10° and its tip was located 1 mm above the aimed brain site. This guide cannula was then sealed with a stainless steel wire. The whole was secured to the skull by means of 3 stainless steel screws and an autopolymerising resin.

After completion of the experiments, all animals were overdosed with sodium pentobarbital and intracardially perfused with NaCl 9‰ followed by 10% formalin. The brains were embedded in paraffin and 20 µm serial sections were stained with cresyl violet. Stimulation and injection sites were localized and transferred onto the corresponding frontal planes of the König and Klippel atlas [11].

Escape Responding Measurement

Following a one-week postoperative delay, each animal was placed into a Plexiglas cage (25×25×35 cm high) equipped with a lever (switch-off lever). By making a lever press response, the animal could interrupt a bipolar brain stimulation for a 15 sec period during which any other lever press would remain ineffective. The stimulation consisted of 0.1 msec rectangular pulses delivered at 50 pulses/sec, the intensity depending on the site stimulated. A time counter (precision 0.01 sec) was used to measure the time that elapsed between the onset of the stimulation and the moment the rat interrupted it (escape latency). When the animal had learned to interrupt the brain stimulation, it underwent a 3 hours a day training for at least 4 days. Two stimulation intensities were then chosen such as to induce a swift escape response (escape latency of about 3 sec) and a slower escape response (escape latency of about 6 sec). The effect of each of these intensities was assessed by determining the mean escape latency obtained from a series of 5 consecutive stimulations, a 15 sec delay elapsing between each lever press and the onset of the next stimulation.

Experimental Program

Each rat underwent three experimental sessions, a delay of 5 days separating two consecutive sessions. In the first session, the effects of a 15 nmole morphine microinjection were assessed. In the second and third session, each rat was submitted either to a 0.25 µl saline microinjection or to a 15 nmole morphine microinjection followed 45 minutes later by a 5 mg/kg IP naloxone injection.

The microinjections 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 syringe through a polyethylene tubing. The tip of the injection cannula jutted out from that of the guide cannula by 1 mm. Fifteen nmoles (5 µg) of morphine sulfate dissolved in 0.25 µl of sterile saline were injected within 30 sec.

Prior to any daily experimental session, each rat underwent a warm-up period during which it had to interrupt 30 CG stimulations applied at an intensity chosen to lie between the two previous selected ones. The latter two intensities were then applied, each of them twice, a 1 min delay elapsing between the application of the two stimulation intensities. Such series of CG stimulations were repeated every 30 min for 5 hours, and the microinjection took place between the 3rd and the 4th series.

Following a logarithmic transformation, the results were submitted to an analysis of variance using the intensity as a grouping factor, the treatment and time as within factors [4,23]. This analysis was followed when applicable by the Neuman-Keuls test.

RESULTS

The location of both the VT microinjection sites and the CG stimulation sites used in the three experiments is shown in Fig. 1.

Nine animals completed this experiment. The low intensity used for CG stimulation was 102±9.8 µA (SEM) and the high intensity 137±12.2 µA. An analysis of variance performed on the escape latencies recorded before any treatment showed only an effect of the stimulation intensity, $F(1,18)=408.2$, $p<0.001$. Following the treatment, the escape latencies were found to depend on the treatment, $F(2,36)=22.83$, $p<0.001$, the time after treatment,

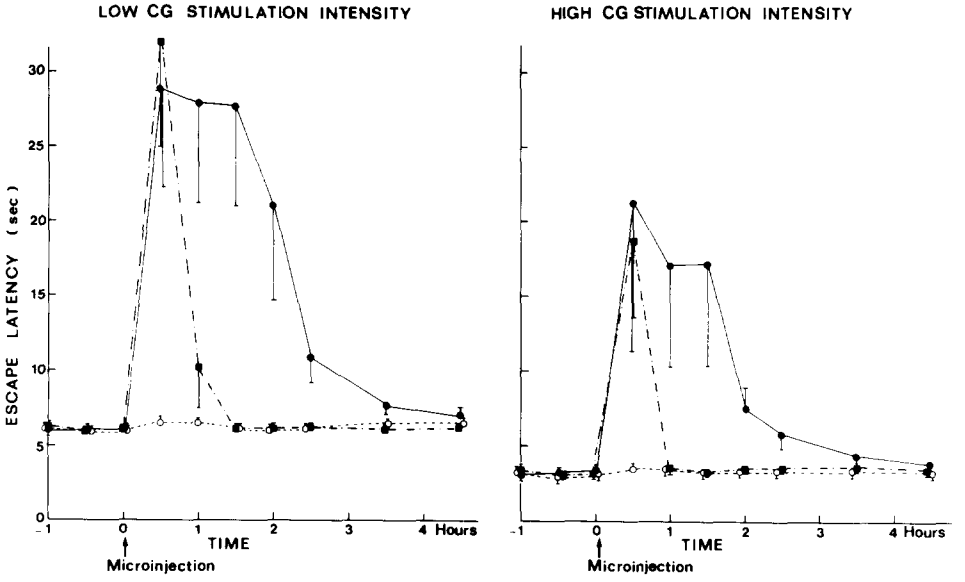

FIG. 2. Variations in the escape latencies induced by electrical stimulation of the central gray at low (left part) or high (right part) intensity following either a microinjection of 0.25 μl saline (○) or a microinjection of morphine (15 nmoles) (●) or a microinjection of morphine followed 45 min later by a 5 mg/kg IP naloxone injection (■).

$F(6,108)=27.56$, $p<0.001$, and the stimulation intensity applied, $F(1,18)=29.71$, $p<0.01$. The only significant interaction was the time × treatment interaction, $F(2,216)=26.56$, $p<0.001$.

The escape latencies recorded 30 minutes following a morphine microinjection were significantly longer (Neumann-Keuls, $p<0.05$) than those recorded following a saline microinjection, regardless of the stimulation intensity applied (Fig. 2). These escape latencies did no longer differ from those recorded following a saline injection $3^1/_2$ hours following the morphine microinjection.

When the morphine microinjection was followed 45 min later by a naloxone injection, the escape latencies significantly differed ($p<0.05$) from those recorded following a saline microinjection only in the session performed 30 minutes after the morphine microinjection (i.e., before the challenge by naloxone). Fifteen minutes following the naloxone injection (i.e., 60 min following the morphine microinjection), the escape latencies did no longer significantly differ from those recorded following a saline microinjection.

EXPERIMENT 2: MORPHINE INTO VT: EFFECT ON LOCOMOTOR ACTIVITY

In the course of Experiment 1, we often observed that, following a morphine microinjection into the VT, the rats displayed an increase in locomotor activity. The aim of the present experiment was to assess more clearly this morphine induced hyperactivity.

METHOD

This experiment was performed on the same 9 animals one week after completion of Experiment 1. Each rat was placed in an actometer (10×100×40 cm high). Four photocells were disposed along the length of the cage, with a spacing of 28 cm, 4 cm above the floor, so as to record the animals' locomotor activity. Each beam interruption elicited one pulse. The pulses were recorded on a graphic recorder.

Each rat was placed in the actometer and its basal activity rate was recorded during a half-hour period. Saline (0.25 μl) was then microinjected into the VT and the rat's activity was recorded during a 4 hour period. After a delay of at least 2 days, the same procedure was repeated on the same animals, using this time a microinjection of morphine (15 nmoles in 0.25 μl of saline).

The results were analyzed by means of nonparametric statistical tests [21].

RESULTS

Figure 3 shows the variations in locomotor activity as a function of time following a microinjection of morphine or saline into the VT. As compared to the saline treatment, the morphine microinjection provoked an increase in locomotor activity, the increase being significant as early as 20 min after the treatment (Wilcoxon, $p<0.05$). This effect lasted about 4 hours. As a rule, the rats exhibited forward locomotion together with sniffing and rearing. No tight turning nor excessive grooming were observed.

EXPERIMENT 3: MORPHINE INTO VT: COMPARED INFLUENCE ON CENTRALLY AND PERIPHERALLY INDUCED AVERSIVE EFFECTS

The aim of this experiment was to determine whether a morphine microinjection into VT would also affect the reactivity to nociceptive stimuli.

METHOD

To assess the reactivity to peripheral painful stimulation, electric shocks of 1 sec duration were delivered to the rat's paws through the bars of the floor of the switch-off cage by

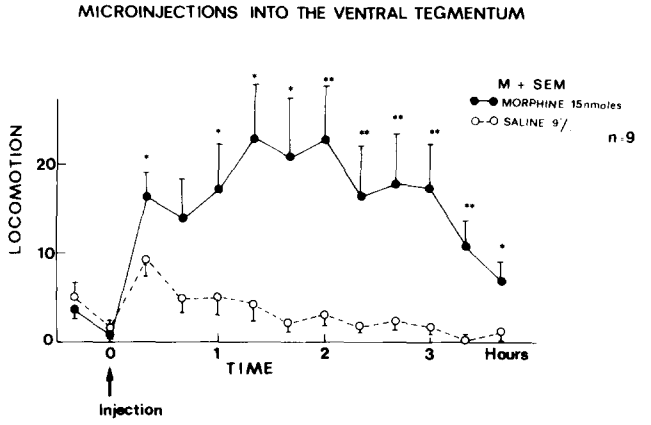

FIG. 3. Variations in the locomotor activity (total counts per 20 minutes) following a microinjection of 15 nmoles of morphine (●) or 0.25 μl of saline (○) into the ventral tegmentum (*$p<0.05$; **$p<0.01$).

means of a scrambler. Starting from 0.05 mA, the stimulation intensity was progressively increased. The threshold value was defined as the intensity at which the animal started to react by raising its forelegs.

Two groups of animals were used. In a first group (7 rats that had already participated in the previous experiments), threshold values were determined every 30 min for 4 and a half hours. The microinjection (either 15 nmoles of morphine or 0.25 μl of saline, with at least a 4 day interval) was performed between the 3rd and the 4th threshold measurement. In a second group (8 rats), the effects of a microinjection of morphine were studied in one and the same animal on both reactivity to nociceptive stimuli and CG induced escape responding. As described for Experiment 1, two different CG stimulation intensities were applied, each of them twice. Such series of CG stimulations were repeated every 30 min for 4 and a half hours, and the microinjection of morphine (or saline) took place between the 3rd and the 4th 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.

The data concerning the escape latencies and those concerning the response thresholds to footshock were separately submitted to analyses of variance similar to those used in Experiment 1.

RESULTS

In the first group of animals in which the sole reaction to nociceptive stimuli was tested, the response threshold to the nociceptive stimulation was not affected by the treatment, $F(1,6)=0.30$, $p>0.05$, and it did not vary over time, $F(5,30)=0.41$, $p>0.05$. The Treatment × Time interaction was not significant, $F(5,30)=0.46$, $p>0.05$.

Figure 4 shows the results obtained with the second group of animals which were submitted to both nociceptive stimuli and CG stimulation. The low stimulation intensity applied to the CG was 107±11.6 μA and the high intensity 151±14.2 μA. Concerning the escape latencies, the results were similar to those obtained in Experiment 1. Following the treatment, these escape latencies were found to depend on the stimulation intensity, $F(1,14)=117.3$, $p<0.001$, on the treatment, $F(1,14)=16.81$, $p=0.001$, and to vary over time,

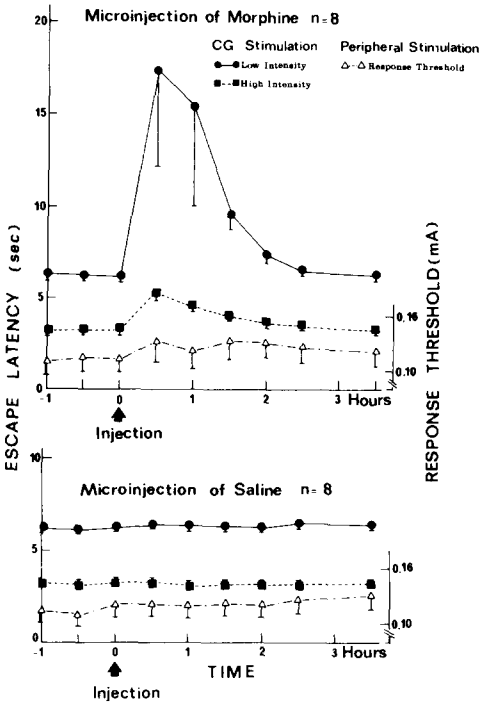

FIG. 4. Effects of a microinjection of 15 nmoles of morphine (upper part) or 0.25 μl of NaCl 9‰ (lower part) into the VT on: (1) the escape latencies induced by electrical stimulation of the central gray at a low (●) or a high (■) intensity (left ordinate); (2) the response thresholds to nociceptive stimuli (△) (right ordinate).

$F(5,70)=16.53$, $p<0.001$. Again, the only significant interaction was the Treatment × Time interaction, $F(5,70)=19.25$, $p<0.001$. As for the response threshold to nociceptive stimulation, the results were strictly similar to those reported for the first group of animals.

DISCUSSION

The present results demonstrate that morphine acts on the ventral tegmentum (VT) so as to increase the rat's latency to stop a central gray (CG) stimulation. This increase in escape latency can hardly be attributed to gross motor impairment since morphine microinjections were shown to provoke an increase in locomotor activity in agreement with other reports concerning microinjections of morphine [7] or enkephalins [2, 8, 9] into VT. Although the data obtained do not allow to completely rule out a response competition hypothesis, the two following facts suggest that the microinjections of morphine into the VT did not impair the rats' ability to press a lever. (1) Similar microinjections have been reported to increase the rate of lever-presses to self-stimulate the lateral hypothalamus [3]. (2) When following a morphine injection, the animal failed to press the lever to stop the CG stimulation, it did by no means exhibit any unconditioned fear-like behavior. Moreover, one had merely to raise the stimulation intensity above the highest value previously applied in order to induce the animal to display a well oriented response towards the lever and thus to stop the stimulation. This indicates, in addition, that the animal actually remembers the task it has to perform to interrupt the

stimulation. It is therefore most likely that the escape latency was found to be increased because the microinjection of morphine into the VT decreased the aversive effects normally induced by the CG stimulation.

It is of interest to note that a morphine microinjection entails an increase in escape latency just as does an electrical stimulation applied to the VT [14]. The two following observations suggest that at least some of the mechanisms brought into play in these two cases are similar: (1) Electrical VT stimulation was shown to have rewarding effects, and the magnitude of the escape suppressant effect was found to be correlated with the magnitude of this rewarding effect [14]. Morphine microinjections into the VT also induce rewarding effects as demonstrated in a spatial preference paradigm [16] as well as in a self-administration situation [1]; (2) Both the suppressant effect on centrally induced escape and the rewarding effect of morphine microinjections into the VT can be antagonized by naloxone, an opiate antagonist [1,16]. As the VT receives a particularly dense enkephalinergic innervation [6,22], it is quite possible that an electrical VT stimulation exerts its escape suppressant effect by acting on neural elements placed under an enkephalinergic control.

While producing a suppressant effect on centrally induced escape, a morphine microinjection into the VT does not affect the response threshold to a nociceptive stimulus. Such a differential effect can hardly be attributed to the fact that an operant response was used to assess the escape suppressant effect whereas a reflex like reaction was used to assess the analgesic effect. As a matter of fact, an electrical stimulation applied to the VT was shown to produce both an escape suppressant effect and an analgesic effect [13] even though a similar difference existed between the two experimental situations used. Therefore, it seems justified to assume that the analgesic effect induced by an electrical VT stimulation results from the activation of either cell bodies devoid of opiate receptors sensitive to morphine or fibers of passage or both. Previous data showing the analgesic effect produced by an electrical VT stimulation to be attenuated following a pCPA treatment [12] suggest that the latter effect could well be due to the activation of serotonergic fibers of passage.

When injected into the dorsal part of the central gray, the same dose of morphine was likewise found to increase the latency to stop aversive brain stimulation [5]. However, the following differences observed in the effects of morphine depending on whether it was injected into the CG or into the VT suggest that different mechanisms are involved in the two cases: (1) The suppressant effect on CG induced escape is of shorter duration in the case of VT injections. (2) There occurs a behavioral activation following VT injections whereas such is never the case following CG injections at least at the dose used. (3) In contrast to VT injections, CG injections produce not only an escape suppressant effect but also an analgesic effect. This proved to be the case both in animals tested in exactly the same conditions as those used in the present study and in animals submitted to a different kind of nociceptive stimuli, irrespective of whether the animals that had experienced aversive CG stimulations in a given environment were then submitted to the nociceptive stimuli in the same or in a different environment [5].

In conclusion, these findings suggest that opiate receptors sensitive to morphine play an important role in the expression of the escape suppressant effect induced by an electrical VT stimulation, whereas they are not involved in the development of its analgesic effect, at least when the nociceptive stimulus is an electrical footshock.

ACKNOWLEDGEMENTS

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