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Mesencephalic Central Gray: Locus of Morphine and Electrical Stimulation Induced Tail Erection[1,2]

H. K. LEE, C. Y. CHAI, M. J. WAYNER[3], L. C. KAO AND P. M. CHUNG

Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, Republic of China

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LEE, H. K., C. Y. CHAI, M. J. WAYNER, L. C. KAO AND P. M. CHUNG. *Mesencephalic central gray: Locus of morphine and electrical stimulation induced tail erection.* PHARMAC. BIOCHEM. BEHAV. 9(2) 221–226, 1978.—Previous ablation studies suggested that morphine most likely acts on the mesencephalic central gray producing tail erection. A microinjection technique, therefore, was used in the present investigation to further localize morphine induced tail erection (MITE) in the mesencephalon. Morphine, 1 to 10 μg, administered through chronically implanted injection cannula into the mesencephalic central gray elicited dose dependent tail erection in conscious mice equal to that produced by morphine, 20 to 100 mg/kg SC. In contrast, morphine 20 μg administered intracerebrally into areas outside the central gray in the mesencephalon did not produce any appreciable tail elevation. In addition, focal electrical stimulation was used to evaluate the physiological role of the mesencephalon in tail erection of the conscious mouse. Only stimulation of the mesencephalic central gray produced dose dependent tail erection. Stimulation of other brain areas never produced a tail response. Pretreatment with haloperidol abolished tail erection produced either by intracerebral morphine or by electrical stimulation. Tail elevation elicited by subcutaneous morphine was also antagonized by haloperidol administered into the mesencephalic central gray. Results indicate that the mesencephalic central gray is the locus of MITE in the central nervous system. By analogy with the site and mechanism of morphine action, it is proposed that focal stimulation activates a locomotor system concentrated in the periaqueductal region which elicits tail elevation by transmitting impulses through the spinal cord.

Tail erection Morphine Intracerebral microinjection Focal electrical stimulation Haloperidol Mesencaphalic central gray

ALTHOUGH morphine induced tail erection (MITE) is a well established phenomenon [2], only recently has it been studied in detail. First, MITE has been found to be a useful model for the evaluation of neuroleptic drugs [7]. Second, several dopamine receptor blocking agents clearly antagonize MITE which indicates that dopamine might be involved [7]. Third, the site of action of MITE has been investigated by ablation techniques and the results indicate that morphine acts on the mesencephalic central gray producing tail erection [8]. It has also been shown that decortication as well as high level decerebration did not interfere with tail erection induced by morphine. However, MITE was not elicited in spinal or low level inferior collicular decerebrate mice. In addition, lesions in various portions of the mesencephalon revealed that the degree of MITE was closely related to the size of the lesions of the central gray. In the present study, the role of the mesencephalic gray in tail erection was further investigated by intracerebral injection and electrical stimulation techniques. Apparently, tail erection can be elicited in mice either by microinjection of morphine into the mesencephalic gray or by focal electrical stimulation of the area. Under both conditions, tail erection was abolished by pretreatment with haloperidol, a dopamine receptor blocking agent [3,4].

METHOD

Male albino mice, weighing 20 to 25 g at the time of surgery, were used. The animals were housed in individual cages for observation and received ad lib food and water. The ambient temperature was kept at 25°C. To minimize effects of noise, the laboratory was kept relatively quiet.

Microinjection Experiments

A 9 mm stainless steel 23 ga guide cannula was stereotaxically implanted in the midbrain region of mice anesthetized

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[2]Send reprint requests to Dr. H. K. Lee, Department of Pharmacology, National Defense Medical Center, P.O. Box 8244, Taipei, Taiwan, Republic of China.

[3]Visiting Professor at the National Defense Medical Center, Taipei, sponsored by the Joint Research Program of the National Science Foundation, U.S.A. and the National Science Council, R.O.C.

with sodium pentobarbital (40 mg/kg IP). The coordinates in a David Kopf small animal stereotaxic instrument were: anterior 3.0 to 4.5 mm, lateral 0 to 1.5 mm and 1.5 to 2.0 mm in depth from the brain surface. The guide cannula was fastened to the skull with a stainless steel screw and acrylic resin (Repairsin, G-C Chemical Co., Japan). A 30 ga stainless steel stilette with 1 end attached to a short segment of PE-10 tube was inserted in the guide cannula to prevent blockage.

The microinjection experiments began 1 week after the surgical operation. For the injection, the stilette was removed and a 30 ga stainless steel inner injection cannula affixed to a 10 μl Hamilton syringe was inserted into the guide cannula to a predetermined depth in the mesencephalon, from 1.0 to 3.0 mm below the tip of the guide cannula. Saline or morphine HCl (Bureau of Narcotic Drugs, R.O.C.) was then administered. The volume of solutions injected into each site was 0.5 μl. The duration of injection was 10 sec. The injection cannula was quickly withdrawn after completion of delivery. Tail response was evaluated by several criteria as mentioned in previous articles [7,8]. Two latencies were measured; one, from when the test solution was administered to when the tail was elevated to at least 45° to the horizontal; second, from when the test solution was administered to when the tail was elevated to at least 90° to the horizontal. Duration of tail erection was defined as the total time the tail was elevated at least 45°. All times were measured by means of stop watches. The total test session was 60 min following the first latency or 120 min following the administration of the test solution in cases where there was no tail erection. If an animal was used more than once, the interval between each microinjection was at least 1 week.

Intraperitoneal haloperidol inhibits tail erection induced by morphine given subcutaneously in the mice [7]. In the present investigation, the relationship of haloperidol and MITE was further studied by 2 different approaches using microinjection techniques. In the first experiment, the morphine solution was injected intracerebrally to elicit tail erection. One week later the animals were pretreated with haloperidol (5 mg/kg IP, Janssen Pharmaceutica) 30 min before intracerebral morphine. In the second experiment, morphine 100 mg/kg SC was used to elicit tail erection. One week later the animals were pretreated with haloperidol (2 μg) injected into the mesencephalic central gray 15 min before subcutaneous morphine.

Electrical Stimulation Experiments

One bipolar electrode constructed of twisted stainless steel wire (0.2 mm diameter) teflon insulated except for the cut cross section at the tip was implanted in the midbrain in each animal. Both electrodes of the bipolar pair were the same length and their exposed tips were separated only by the layer of teflon insulation surrounding each wire. The electrode was secured to the skull as described previously for the microinjection cannula.

After a one week recovery period, animals were tested for tail erection. In all animals, brain stimulation was provided by a Grass S88 stimulator with an isolation unit and a constant current unit. The stimulation consisted of 100 msec trains of biphasic, rectangular wave pulse pairs. Pulse pairs were delivered at 50/sec. Each pulse pair consisted of two 50 or 500 μsec pulses of opposite polarity separated by 100 μsec. Three trains of pulses were delivered every sec. Cur-

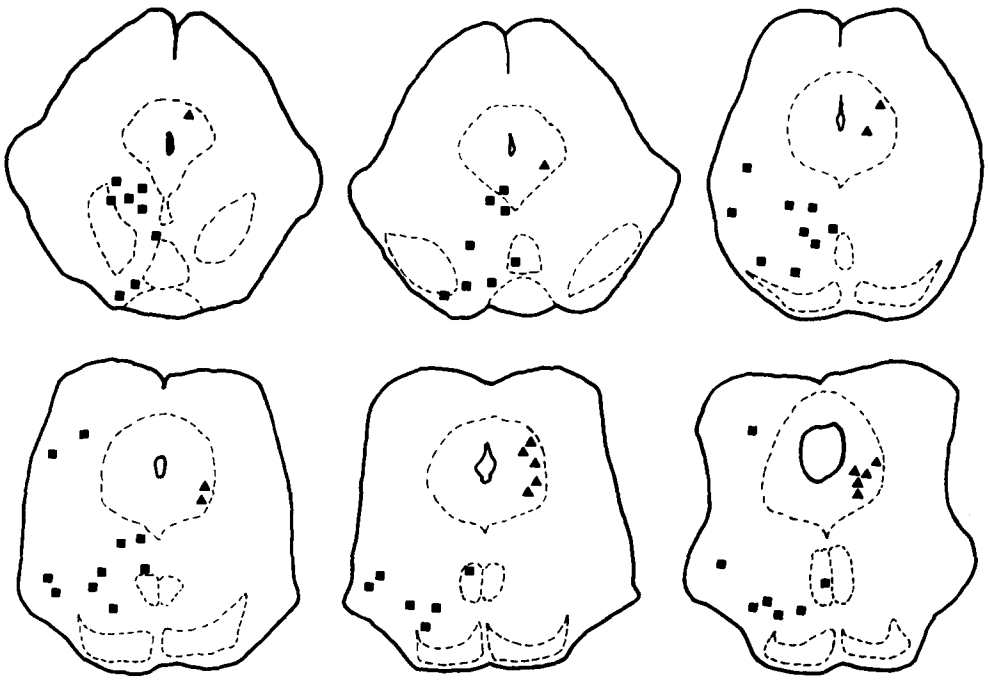

FIG. 1. Distribution of midbrain sites where microinjections of 20 μg of morphine were administered. Six serial coronal sections are presented, separated by 0.3 mm beginning 4.5 mm anterior to the ear bar of the David Kopf small animal stereotaxic instrument. ▲ on the right side of each panel represents the site where tail erection was produced by morphine microinjection. ■ on the left side of each panel represents where tail erection was not produced by morphine microinjection.

rent intensities ranged from 1.0 to 3.0 mA. The duration of brain stimulation was 10 sec. The criteria for evaluation of tail response were as described previously.

Effects of haloperidol on tail erection produced by electrical stimulation were also studied. One week after the tail response of the animals were determined, the animals were pretreated with haloperidol (5 mg/kg IP) 30 min before the stimulation test was performed again.

RESULTS

Tail Erection Produced by Microinjection of Morphine

At first, the effect of microinjection of a relatively large dose of morphine (20 μg) was studied in 64 mice. Each of the animals received only 1 injection of morphine into various midbrain sites. Sixteen out of 64 animals responded to morphine with a tail erection of greater than 90° to the horizontal with a median latency of 3.0 min (range 0.05 to 8.8 min) and a median duration of 60 min (range 25 to 60 min). Histological examination revealed the injection sites of these 16 positively responding mice were exclusively in the lateral portion of the mesencephalic central gray, indicated by solid triangles in the right side of each panel in Fig. 1. In contrast, the injection sites of all nonresponding animals were outside the central gray except 1 in the lower middle portion of the central gray, indicated by solid squares in the left side of each panel in Fig. 1. Therefore, it is apparent that the mesencephalic central gray is the most likely locus of action for MITE.

As shown in Table 1, a dose dependent response was found in these central gray reactive sites. A total of 7 mice which showed no tail response to intracerebral saline but responded to intracerebral microinjection of 10 μg morphine with elevation of tails at 90° to the horizontal and with a duration of erection of 60 min were used for the evaluation of a dose response relationship. Four of the 7 mice were then challenged with a dose regimen of decreasing amounts; i.e., 5, 2, and 1 μg, at intervals of 1 week. The remaining 3 mice were tested with a dose regimen of increasing amounts; i.e., 1, 2, and 5 μg. Table 1 summarizes the results. In a dose of 1 μg, morphine elicited tail erection of 45° or greater in 6 out of 7 mice with a median latency of 3.5 min. In addition, 3 mice reached a tail elevation of at least 90° with a median latency of 3.8 min. The median duration of tail erection was 44 min. A typical tail response produced by a microinjection of 1 μg of morphine in the brain is shown in Fig. 2. When the dose of morphine was increased to 2 or 5 μg, all animals displayed tail erection of at least 90°. With these doses, the duration of reaction was also appreciably prolonged. For animals treated with 10 μg of morphine, a drastic reduction of median latencies was noticed and the duration of the reaction for all animals reached a maximum. The injection sites of these 7 mice are shown in Fig. 3, all of them were in the lateral central gray.

Effects of Haloperidol on Tail Erection Induced by Morphine

Haloperidol abolished the tail erection induced by intracerebral injection of morphine. As shown in Table 2, 6 mice which responded to 20 μg of morphine given intracerebrally with tail elevations of at least 90° and with a duration of reaction of 60 min were used for further study. Haloperidol 5 mg/kg administered intraperitoneally 30 min before the same dose of intracerebral morphine, completely inhibited the tail response, see Table 2. However, 1 week after the haloperidol treatment, the tail response to intracerebral morphine had fully recovered in these animals. These data were not included in Table 2 in order to avoid unnecessary repetition.

In addition, haloperidol administered intracerebrally blocked tail erection induced by subcutaneous administration of morphine. As shown in Table 3, 5 mice which responded to morphine, 100 mg/kg SC, with tail elevation of 90° or greater and with a duration of reaction of 60 min were

FIG. 2. Tail erection produced by microinjection of 1 μg of morphine into the mesencephalic central gray in a 25 g mouse.

TABLE 1

DOSE DEPENDENT TAIL ERECTION PRODUCED BY MICROINJECTION OF MORPHINE INTO THE MESENCEPHALIC CENTRAL GRAY

Dose of morphine (μg)	No. of mice responded (≥45°) / No. tested	Latency: median and range (min)	No. of mice responded (≥90°) / No. tested	Latency: median and range (min)	Duration: median and range (min)
0 (saline)	0/7	—	0/7	—	—
1	6/7	3.5(0.03–10.8)	3/7	3.8(0.1–9.2)	44(5–60)
2	7/7	3.1(0.03– 6.0)	7/7	3.7(0.1–6.3)	60(15–60)
5	7/7	3.0(0.03– 4.3)	7/7	3.7(0.1–4.4)	60(52–60)
10	7/7	0.8(0.03– 3.8)	7/7	1.0(0.1–4.1)	60

TABLE 2

ANTAGONISTIC EFFECT OF HALOPERIDOL (5 MG/KG IP) ON TAIL ERECTION INDUCED BY INTRACEREBRAL INJECTION OF MORPHINE (20 μG)

Treatment	No. of mice responded (≥45°) / No. tested	Latency: median and range (min)	No. of mice responded (≥90°) / No. tested	Latency: median and range (min)	Duration: median and range (min)
Morphine controls	6/6	1.5(0.03–5.1)	6/6	3.0(0.05–8.0)	60
Haloperidol-pretreated	0/6	—	0/6	—	—

TABLE 3

ANTAGONISTIC EFFECT OF INTRACEREBRAL INJECTION OF HALOPERIDOL (2 μG) ON TAIL ERECTION INDUCED BY MORPHINE (100 MG/KG SC)

Treatment	No. of mice responded (≥45°) / No. tested	Latency: median and range (min)	No. of mice responded (≥90°) / No. tested	Latency: median and range (min)	Duration: median and range (min)
Morphine controls	5/5	6.8(3.0–8.5)	5/5	9.7(5.8–10.3)	60
Haloperidol-pretreated	0/5	—	0/5	—	—

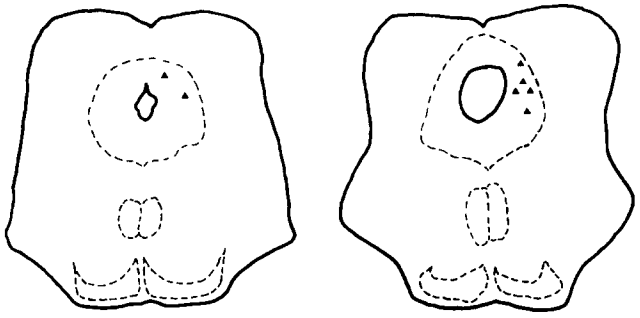

FIG. 3. Distribution of midbrain sites (▲) where microinjections of 1 to 10 μg of morphine produced marked and prolonged tail erection in 7 mice used to determine a dose response relationship. Two serial coronal sections are presented, separated by 0.3 mm beginning 3.3 mm anterior to the ear bar of the stereotaxic instrument.

used. Haloperidol, 2 μg, administered into the midbrain central gray 15 min before subcutaneous morphine, prevented the MITE completely. Similar to the previous experiment and 1 week after intracerebral haloperidol treatment, the tail response to subcutaneous morphine fully recovered in these animals. Histological examination revealed that the microinjection sites for haloperidol were in the lateral central gray.

Tail Erection Produced by Focal Electrical Stimulation of the Brain

Table 4 summarizes the results of tail erection produced by focal electrical stimulation of the midbrain. The effect was always dose dependent; i.e., stimulation with a high current intensity produced a greater response of tail elevation than stimulation with a lower current intensity. With pulse pairs, 50 μsec each of opposite polarity, electrical stimulation of 1.0 mA caused 5 out of 10 mice to elevate their tails to at least 45° with a median latency of 3.0 sec and to at least 90° with a median latency of 4.0 sec. A typical tail erection produced by 1.0 mA electrical stimulation is shown in Fig. 4. Increasing the current intensity to 2.0 mA caused 8 out of 10 mice to elevate their tails to at least 90°. When 3.0 mA was used, all 10 animals displayed tail erection of at least 90°. The median duration of tail erection with pulse pairs of 50 μsec pulses was 0.1, 0.3, and 0.4 min for current intensities of 1.0, 2.0, and 3.0 mA, respectively. The sites of stimulation in these 10 animals are indicated by solid circles in Fig. 5. All of them are in the mesencephalic central gray.

When the duration of pulse pair pulses was increased to 500 μsec, the tail response became more prominent (Table 4). Electrical stimulation of 1.0 mA caused 10 out of 12 animals to elevate their tails to at least 90° with a median latency of 4.5 sec and a median duration of 0.3 min. Increasing the current intensity to 2.0 mA caused all animals to display tail erection of greater than 90° with a median latency of 2.0 sec and a median duration of 1.2 min. On the other hand, when 3.0 mA was used, only 9 out of 12 animals displayed tail erection of at least 90° with a median latency of 2.0 sec and a median duration of 2.6 min. The reason for the decrease in percentage of response from 100% to 75% is not clear at the present. One possible explanation might be that the tail response is disrupted by the marked increase in locomotor activity produced by the 3.0 mA stimulation. The sites of

TABLE 4

CURRENT INTENSITY DEPENDENT TAIL ERECTION INDUCED BY ELECTRICAL STIMULATION OF THE MESENCEPHALIC CENTRAL GRAY*

Pulse duration (μsec)	Current intensity (mA)	No. of mice responded ($\geq$45°) / No. tested	Latency: median and range (sec)	No. of mice responded ($\geq$90°) / No. tested	Latency: median and range (sec)	Duration: median and range (min)
50	1.0	5/10	3.0(1–5)	5/10	4.0(2–6)	0.1(0.07–3.3)
	2.0	8/10	1.5(1–9)	8/10	2.5(2–10)	0.3(0.12–1.8)
	3.0	10/10	1.0(1–7)	10/10	2.0(2–8)	0.4(0.10–2.0)
500	1.0	10/12	3.5(1–10)	10/12	4.5(2–15)	0.3(0.06–45)
	2.0	12/12	1.0(1–9)	12/12	2.0(2–10)	1.2(0.25–29)
	3.0	9/12	1.0(1–8)	9/12	2.0(2–10)	2.6(0.25–60)

*Brain stimulation consisted of 100 msec trains of biphasic, rectangular wave pulse pairs. Pulse-pairs were delivered at 50/sec. Each pulse pair consisted of two 50 μsec or 500 μsec pulses of opposite polarity separated by 100 μsec. Trains of pulses were delivered at 3/sec. The duration of stimulation was 10 sec.

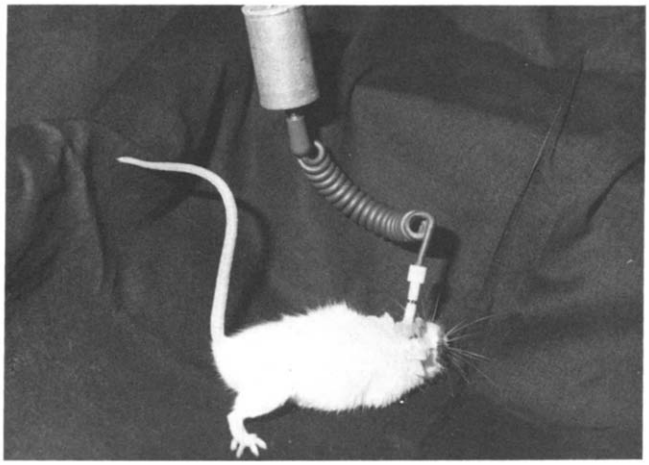

FIG. 4. Tail erection produced by focal electrical stimulation of the mesencepahlic central gray in a mouse. See text for stimulus parameters.

stimulation of these 12 animals are indicated by solid triangles in Fig. 5. They are intermingled with the sites of the previous group in the central gray.

In another 24 animals, stimulation electrodes were implanted outside the central gray and in the tegmentum of the midbrain. All of these animals displayed no tail erection in response to the electrical stimulation. These data were therefore excluded from the analysis.

Effects of Haloperidol on Tail Erection Produced by Electrical Stimulation of the Brain

Haloperidol also antagonized the tail erection produced by electrical stimulation. Seven animals which displayed typical responses to electrical stimulation with either pulse pairs of 50 or 500 μsec pulses were used. Haloperidol, 5 mg/kg IP, administered 30 min before electrical stimulation abolished all tail responses. One week after the haloperidol treatment, the tail response to electrical stimulation was completely restored. Histological study revealed that the sites of stimulation of these 7 animals were in the mesencepahlic central gray.

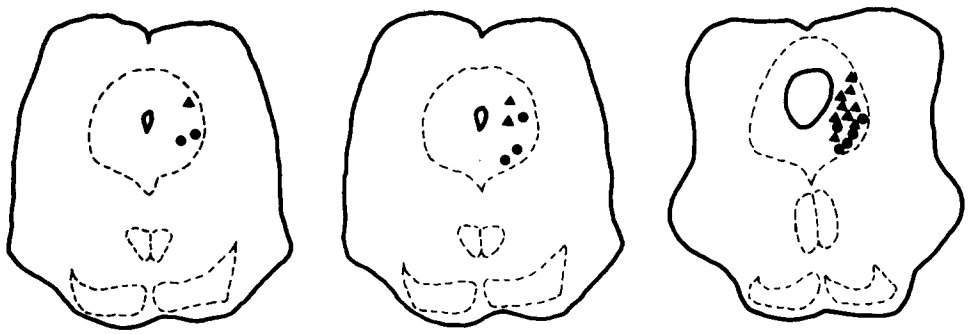

FIG. 5. Location of electrode tips where electrical stimulation produced tail erection. Three serial coronal sections are presented, separated by 0.3 mm beginning 3.6 mm anterior to the ear bar of the stereotaxic instrument. ●, stimulation site for pulse pairs of 50 μsec each; ▲, stimulation site for pulse pairs of 500 μsec each. See text for other stimulus parameters.

DISCUSSION

As demonstrated in the present study, morphine in a dose as small as 1 μg administered into the mesencephalic central gray can elicit a marked and prolonged tail elevation in mice. Compared with a dose of morphine 100 mg/kg SC required to elicit tail elevation in previous studies [2, 7, 8], the intracerebral dose is 2000 times less. These data implicate a central mechanism for MITE. In addition, microinjections of morphine into the mesencepahlic central gray produce a dose dependent tail erection in mice. Microinjections into other adjacent midbrain areas produce no MITE. These results confirm previous findings obtained by ablation techniques [8] and indicate that morphine acts specifically on the mesencepahlic central gray in producing tail erection.

Present results also demonstrate that focal electrical stimulation of the mesencephalic central gray, areas located identically to those reactive to morphine microinjections, produces a dose dependent tail elevation. Stimulation of other adjacent sites in the midbrain produced no tail erection. Thus, from these results a special role must be assigned to periaqueductal structures of the midbrain in the mediation of tail erection. Since tail erection elicited by morphine can be abolished by spinal transection at lower thoracic and lumbar segments [8], one would expect the responsible pathway to go downward from the midbrain to the spinal cord.

Results of the present investigation on tail erection produced by intracerebral injection of morphine and by electrical stimulation in mice are similar to those which have been found for the analgesic action of morphine. Microinjection of morphine into the mesencephalic central gray produced analgesic effects [6,12]. In addition, electrical stimulation of the mesencephalic central gray greatly reduced or totally abolished responsiveness to all noxious stimuli in rats [9] and results in pain reduction in man [11]. An important role of the mesencephalic central gray in the mediation of the pharmacological effects produced by morphine seems evident.

Some reports suggest that multiple brain sites are involved in morphine antinociception [13] as well as in electrical stimulation produced analgesia [10]; e.g., anterior and posterior hypothalamus, midline area of the caudal thalamus, pretectal area, etc. Although results of a previous ablation study indicate that structures rostral to the midbrain are probably not involved in MITE [8], it might still be worthwhile to utilize microinjection or electrical stimulation techniques in the study of brain tissues other than midbrain in the determination of MITE.

Haloperidol, a dopamine blocking agent [3,4], administered intraperitoneally antagonized tail erection induced by intracerebral microinjection of morphine. These results confirm previous findings that dopamine might be involved in MITE [7] and are consistent with the antagonizing effect haloperidol exhibits for continuous cycling behavior produced by microinjection of morphine into the substantia nigra of rats [5]. The fact that microinjection of haloperidol into the mesencepahlic central gray antagonizes tail erection induced by subcutaneous morphine strongly suggests a central action of haloperidol in that particular area.

It is interesting to observe that stimulation induced tail erection can be abolished by haloperidol pretreatment, which might not be surprising because the central site of action of stimulation induced tail erection is similar to that of MITE. There are other similarities between MITE and stimulation induced tail erection. For instance, MITE can be abolished by narcotic antagonists [1], and naloxone, a narcotic antagonist, is also effective in preventing tail erection produced by electrical stimulation (unpublished data). Since the sites and mechanisms of morphine and stimulation induced tail erection are similar, it is proposed that both forms of stimulation might activate a locomotor system concentrated in the periaqueductal region which transmits impulses to the spinal cord in the production of this phenomenon.

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