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Reinforcing Effects of Morphine Microinjection into the Ventral Tegmental Area

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PHILLIPS, A. G. AND F. G. LEPIANE. *Reinforcing effects of morphine microinjection into the ventral tegmental area.* PHARMAC. BIOCHEM. BEHAV. 12(6) 965–968, 1980.—A neural substrate for the reinforcing property of an opiate drug was identified in the ventral tegmental area (VTA) by establishing conditioned reinforcement to salient environmental stimuli paired with intracerebral microinjections of morphine. Bilateral microinjections of morphine into the VTA in doses of 0.2 μg and 1.0 μg produced a subsequent change in place preference to a distinctive compartment previously associated with the stimulant effects of morphine. Microinjection of 1.0 μg morphine at sites 2.5 mm dorsal to the VTA had no effect. Pretreatment with naloxone (2 mg/kg) antagonized the reinforcing effects of 1.0 μg morphine as this group showed no significant change in place preference. Nor did control groups receiving microinjections of sterile physiological saline. Taken together, these data suggest that opiate receptors, located in the ventral tegmental area, play an important role in mediating the reinforcing effects of morphine. The possible involvement of dopaminergic neurons in these effects is discussed.

Morphine Positive reinforcement Intracerebral microinjection Ventral tegmental area Naloxone Rats

THE reinforcing properties of opiates have been confirmed experimentally by a variety of procedures. Morphine and heroin have been self-administered intravenously by species ranging from rat to man [18]. Furthermore, when distinctive environmental stimuli were paired with opiate administration, they acquired conditioned reinforcing properties [9,16]. Endogenous ligands for opiate receptors have been identified in the brain [5], and rats will self-administer both Leu^5 and Met^5-enkephalin via intraventricular cannulae [1]. Direct microinjection of morphine (0.2–1.0 μg) and (D-Ala^2)-Met^5-enkephalinamide (1 μg) into the ventral tegmental area (VTA) produced a selective dose-related facilitation of intracranial self-stimulation from electrodes in the lateral hypothalamus [2]. In that study, the depression reported previously with systemic administration of opiates [10] was never observed. Microinjection of (D-Ala^2)-Met^5-enkephalinamide also elicited hypermotility which resembled the behavior produced by injection of dopamine (DA) into the mesolimbic DA pathway [3]. On the basis of these data we have conjectured that the reinforcing property of opiates may be mediated by neural pathways originating in the VTA [3]. Utilizing a procedure for establishing conditioned reinforcement with environmental stimuli [16] we now report reinforcing effects produced by microinjection of morphine into the VTA. These effects were antagonised by treatment with the opiate receptor blocker naloxone.

METHOD

Subjects

Fifty male Wistar rats (Woodlyn Labs, Guelph), weighing 300–320 g, were anaesthetized with Nembutal (50 mg/kg), and two stainless steel guide cannulae (23 ga.) were implanted stereotaxically into an area 0.5 mm dorsal to the VTA. Control placements were located 2.5 mm dorsal in the same anterior plane. Cannulae placements were confirmed histologically; the correct loci, quantified according to the coordinate system of König and Klippel [8] were A=2.1 ± 0.3 mm, L=0.9 ± 0.2 mm, and DV=−2.6 ± 0.5 mm. Each animal was housed individually in a stainless steel cage, located in a climatically controlled colony, with a 12 hr light/dark cycle.

Procedure

Behavioral testing began 10 days after surgery, and was conducted during the light phase of the diurnal cycle. Rats with VTA cannulae placements were assigned to one of 5 groups according to drug treatment: 1 μg morphine sulphate; 0.2 μg morphine sulphate; saline (0.9%); 1 μg morphine sulphate+naloxone (2 mg/kg, IP); saline+naloxone (2 mg/kg, IP). The control animals with dorsal cannulae placements received bilateral injections of 1 μg morphine sulphate. The behavioral tests were conducted in an aluminum chamber (80×25×36 cm) divided into 3 compartments. Two large compartments (34×25 cm) were separated by guillotine doors from a small area (11×25 cm) which served as a choice point in a later phase of the experiment. Each of the large compartments was distinctive in flooring and wall covering. One had a grid floor and black walls and the other had a mesh floor (1.2 cm square) and white walls. The testing boxes were housed in a quiet room illuminated by fluorescent overhead lighting.

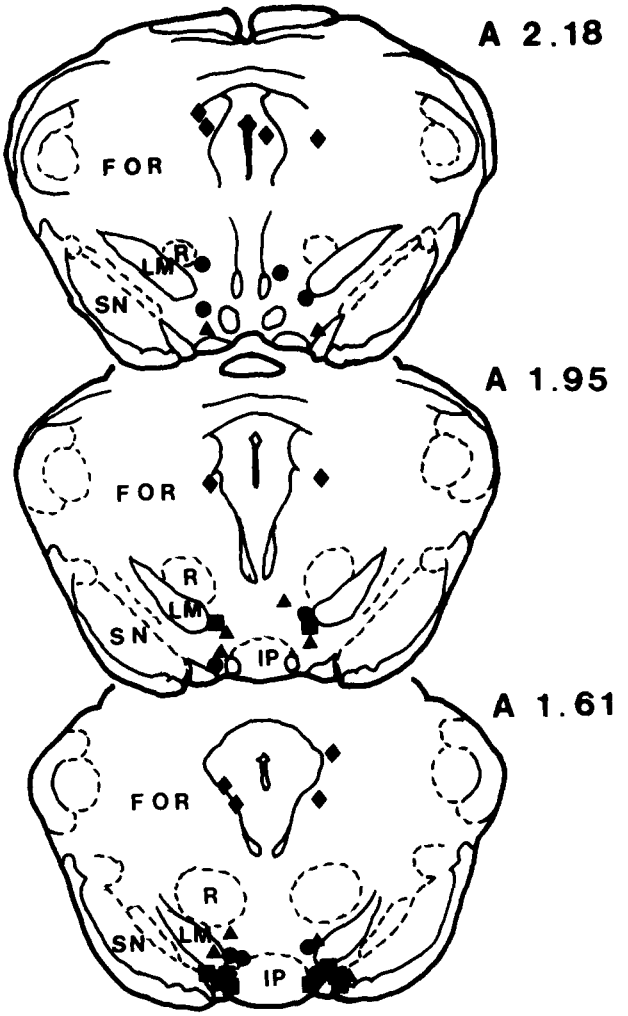

FIG. 1. Location of ventral tip of injection cannulae on coronal sections of rat mesencephalon. Abbreviations: FOR=reticular formation; IP=interpeduncular nucleus; LM=medial lemoniscus; SN=substantia nigra; R=red nucleus. Groups: (▲) 0.2 μg morphine, VTA; (■) 1.0 μg morphine, VTA; (△) saline (0.9%), VTA; (◆) 1.0 μg morphine, dorsal control. Behavioral data from these groups are shown in Fig. 2.

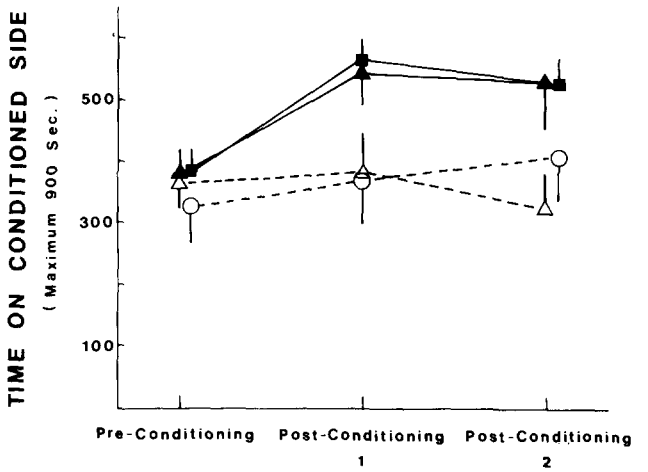

FIG. 2. Preference scores expressed as time spent on one side of the test chamber before and after intracerebral microinjection of morphine or saline. The pre-conditioning scores indicate that the "conditioned" side was the less preferred prior to drug treatment. The amount of time spent on the preferred side may be obtained by subtracting a given group score from the session length of 900 sec. Post-conditioning scores represent the amount of time spent on the same side after association between this environment and microinjection of morphine or saline. Groups: (▲) 0.2 μg morphine, VTA (N=5); (■) 1.0 μg morphine, VTA (N=5); (○) 1.0 μg morphine, dorsal placements; (△) 0.9% saline, VTA.

On the first 3 days of behavioral testing, each animal was allowed to explore all compartments of the chamber, for 15 min. Time spent by each animal in the two end compartments was measured to the nearest second and this provided a measure of preference between the 2 compartments. Throughout the study, cannulae were sealed and kept patent by insertion of a 30 ga. inner stainless-steel stylet which protruded 0.5 mm beyond the outer guide. Microinjections were made by replacing each inner stylet with a 30-ga. needle attached to a microsyringe by polyethylene tubing. A volume of 0.5 μl was injected into each cannula over a 30-sec period. In the second phase of the study, microinjections of morphine or saline, with and without naloxone treatment were made 30 min prior to confining the rat into the less preferred compartment (i.e., the "conditioned" side) for a 1 hr period. The 30 min latency between injection and behavioral testing and the 1 hr test period coincided with the temporal parameters that produced maximal facilitation of self-stimulation after similar injections of morphine into the VTA [2]. This procedure was repeated for 3 days. If the rewarding effects of morphine are mediated by neural pathways in the VTA, this procedure would permit the animal to associate morphine-induced reward with the salient features of the test compartment, through the process of classical conditioning. On the fourth day, each animal received a sham-injection in which an empty injection needle was inserted partway into the guide cannulae. Thirty min later each animal was placed into the middle compartment, both guillotine doors were raised, and the amount of time spent in each compartment was recorded over a 15 min period. The conditioning procedure and preference tests were repeated a second time over the next 4 days. A successful demonstration of the reinforcing property of morphine would be indicated by a significant shift in side preference to the compartment associated with morphine microinjection.

RESULTS

Figure 1 illustrates the location of the tips of the injection cannulae in the VTA and at dorsal control sites in and lateral to the central gray. Only data from subjects with symmetrical bilateral placements in one of the two target sites were included in the statistical analyses. Prior to conditioning, the amount of time spent in the less preferred compartment, (i.e., the "conditioned" side) by each group, ranged from a mean of 327 to 383 sec and did not differ significantly. After experiencing the stimulant effects of intracerebral morphine into the VTA, for 3 daily sessions on the 'conditioned' side of the chamber, two groups changed their preference to this side. When compared to saline controls, the change in preference to the 'conditioned' side was statistically significant for the subjects receiving 0.2 μg morphine, $F(2,24)=3.72$, $p<0.05$, and 1.0 μg morphine, $F(2,24)=3.80$, $p<0.05$ (see

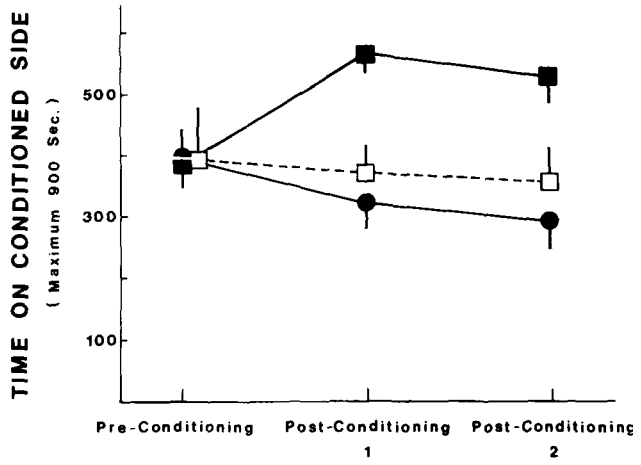

FIG. 3. Effects of naloxone (2 mg/kg) on changes in preference for side of test chamber associated with intracerebral injection of morphine (1.0 μg) or saline (0.9%). Groups: (■) 1.0 μg morphine VTA; (□) 1.0 μg morphine VTA+naloxone; (●) saline (0.9%) VTA+naloxone. For explanation of ordinate and abscissa, see Fig. 2.

Fig. 2). These changes in preference were confirmed on the second post-conditioning test. No significant change in side preference was observed in either the saline control or the dorsal morphine groups.

Pretreatment with naloxone antagonized the effects of 1.0 μg morphine into the VTA, as this group showed no change in place preference and the time measures were not significantly different from the control scores. The antagonism cannot be attributed to an aversive effect of naloxone which counteracted the reward effect of morphine, as the group treated with microinjections of saline and systemic naloxone did not develop a conditioned aversion to the compartment in which they were placed during drug treatment (see Fig. 3).

DISCUSSION

The significant change in place preference to a distinctive test environment paired previously with intracerebral microinjections of morphine into the VTA, is a clear demonstration of conditioned reinforcement; with morphine serving as the primary reward. This procedure has been used previously to confirm the reward properties of morphine administered peripherally [9,16] and (D-Ala^2)-Met^5-enkephalinamide injected by the intraventricular route [7]. The antagonism of the reward effects of morphine by naloxone suggests an important role for opiate receptors in mediating this effect.

It is significant that the present results are obtained with injection loci in the VTA but not at loci 2.5 mm dorsal. The A10 DA neurons originate in the VTA and they mediate certain aspects of brain-stimulation reward [11]. Microinjection of morphine into the VTA also facilitates brain-stimulation reward [2], suggesting a possible interaction between opiates and a DA reward pathway. In this regard, the possible role of the adjacent nigrostriatal DA reward pathway [10] cannot be precluded as the infusion could have reached the substantia nigra. There is mounting evidence for an interaction between DA neurons and those containing enkephalin, particularly at the level of the striatum [14]. Neurophysiological data [6,11] have shown that single unit firing rates of dopamine neurons are increased significantly following systemic injections of morphine. More specifically it has been postulated that presynaptic enkephalin interneurons facilitate synaptic transmission in the nigrostriatal DA pathway. In a recent study, Finnerty and Chan [4] have presented further neurophysiological evidence that morphine may disinhibit the DA-containing cells of the substantia nigra pars compacta (SNC) by depressing inhibitory neurons in the par reticulata that serve as part of a striatonigral feedback mechanism. In this regard it is significant that immunohistochemical mapping of enkephalin containing neurons has identified fluorescent fibers in the SNC and in the region between the substantia nigra and medial lemniscus [17,19].

In view of the neuroanatomical [17,19] and neurophysiological [4, 6, 11] data, it remains a distinct possibility that the rewarding effects of opiates are mediated by an interaction of enkephalinergic neurons with the A10 or A9 DA neurons. Lesions of the mesocorticolimbic DA pathway disrupt cocaine self-administration [15] raising further conjecture that the reward or euphoric properties of both psychomotor stimulants and opiates are mediated, at least in part, by this DA pathway.

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REFERENCES

1. Belluzzi, J. D., C. D. Wise and L. Stein. Enkephalin may mediate euphoria and drive-reduction reward. *Nature* **260**: 556–558, 1979.
2. Broekkamp, C. L., A. G. Phillips and A. R. Cools. Facilitation of self-stimulation behavior following intracerebral microinjection of opioids into the ventral tegmental area. *Pharmac. Biochem. Behav.* **11**: 289–295, 1979.
3. Broekkamp, C. L., A. G. Phillips and A. R. Cools. Stimulant effects of enkephalin micro-injection into the dopaminergic A10 area. *Nature* **278**: 560–562, 1979.
4. Finnerty, E. P. and S. H. H. Chan. Morphine suppression of substantia nigra zona reticulata neurons in the rat: Implicated role for a novel striato-nigral feedback mechanism. *Eur. J. Pharmac.* **59**: 307–310, 1979.
5. Hughes, J., T. Smith, B. Morgan and L. Fothergill. Purification and properties of enkephalin, the possible endogenous ligand for the morphine receptor. *Life Sci.* **16**: 1753–1758, 1975.
6. Iwatsubo, K. Effect of morphine and haloperidol on single cell activity of nigrostriatal neurons. *Jap. J. Pharmac.* **26**: Suppl. 17P, 1976.
7. Katz, R. J. and G. Gormezano. A rapid and inexpensive technique for assessing the reinforcing effects of opiate drugs. *Pharmac. Biochem. Behav.* **11**: 231–234, 1979.
8. König, J. F. R. and R. A. Klippel. *The Rat Brain*. Baltimore: William and Wilkins, 1963.
9. Kumar, R. Morphine dependence in rats: Secondary reinforcement from environmental stimuli. *Psychopharmacologia* **25**: 332–338, 1972.
10. Lorens, S. A. and C. L. Mitchell. Influence of morphine on lateral hypothalamic self-stimulation in the rat. *Psychopharmacologia* **32**: 271–277, 1973.

11. Nowycky, M. C., J. R. Walters and R. H. Roth. Dopaminergic neurons—Effects of acute and chronic morphine administration on single cell activity and transmitter metabolism. *J. Neural Trans.* **42:** 99–116, 1978.
12. Phillips, A. G., D. A. Carter and H. C. Fibiger. Dopaminergic substrates of intracranial self-stimulation in the caudate-putamen. *Brain Res.* **104:** 221–232, 1976.
13. Phillips, A. G. and H. C. Fibiger. The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum, nucleus accumbens and medial prefrontal cortex. *Can. J. Psychol.* **32:** 58–66, 1978.
14. Pollard, H., C. Llorens, J. C. Schwartz, C. Gros and F. Dray. Localization of opiate receptors and enkephalins in the rat striatum in relationship with the nigro-striatal system: Lesion studies. *Brain Res.* **151:** 392–398, 1978.
15. Roberts, D. C. S., M. E. Corcoran and H. C. Fibiger. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmac. Biochem. Behav.* **6:** 615–620, 1977.
16. Rossi, N. A. and L. D. Reid. Affective states associated with morphine injections. *Physiol. Psychol.* **4:** 269–274, 1976.
17. Sar, M., W. E. Stumpf, R. J. Miller, K. J. Chang and P. Cuatrecasas. Immunohistochemical localization of enkephalin in rat brain and spinal cord. *J. comp. Neurol.* **182:** 15–38, 1978.
18. Schuster, C. R. and T. Thompson. Self-administration of and behavioral dependence on drugs. *A. Rev. Pharmac.* **9:** 483–502, 1969.
19. Uhl, G. R., R. R. Goodman, M. J. Kuhar, S. R. Childers and S. H. Snyder. Immunohistochemical mapping of enkephalin containing cell bodies, fibers, and nerve terminals in the brain stem of the rat. *Brain Res.* **166:** 75–94, 1979.