*Pharmacology Biochemistry & Behavior*, Vol. 11, pp. 227–229. Printed in the U.S.A.

# Naloxone Antagonism of Electrical Stimulation Induced Tail Erection in Mice[1,2]

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

*Department of Pharmacology, National Defense Medical Center*
*P. O. Box 8244, Taipei, Taiwan, Republic of China*

Received 21 May 1979

LEE, H. K., C. Y. CHAI, M. J. WAYNER AND L. C. KAO. *Naloxone antagonism of electrical stimulation induced tail erection in mice.* PHARMAC. BIOCHEM. BEHAV. 11(2) 227–229, 1979.—Tail erection was induced by focal electrical stimulation of the mesencephalic central gray through chronically implanted electrodes in mice. The response was current intensity dependent. Pretreatment with naloxone (5 mg/kg IP), a specific narcotic antagonist, abolished tail erection produced by low electrical current. In contrast, the tail response elicited by higher current was only partially blocked by naloxone. The results suggest that electrical stimulation induces tail erection by releasing an endogenous opioid peptide from the mesencephalic central gray.

Naloxone Tail erection Focal electrical stimulation Mesencephalic central gray
Endogenous opioid peptide

FOCAL electrical stimulation of the mesencephalic central gray produces tail erection in mice [12]. Electric stimulation induced tail erection exhibits some similarities to tail erection induced by narcotic agents. Both appear to exert their effects in periaqueductal gray of the mesencephalon [11,12]. In addition, drugs which affect dopaminergic transmission in the central nervous system, e.g., haloperidol, attenuate both morphine induced tail erection (MITE) and stimulation induced tail erection [10,12]. In the present study, another similarity between stimulation induced tail erection and MITE [1] was observed. Tail erection induced by electrical stimulation can be blocked by naloxone, a specific narcotic antagonist.

## METHOD

Male albino mice, weighing 20–25 g, were used. They were placed in individual cages for observation. The experimental room was kept at 25°C and relatively quiet.

The methods for producing focal electrical stimulation were previously described [12]. In brief, one bipolar electrode constructed of twisted stainless steel wire (0.2 mm dia.) teflon insulated except for the cut cross section at the tip was implanted in the midbrain in each animal under sodium pentobarbital anesthesia (36 mg/kg IP). The electrode was secured to the skull with a stainless steel screw and acrylic resin.

Animals were tested for tail erection one week after the electrode implantation. Brain stimulation was provided by a Grass S88 stimulator with an isolation unit and a constant current unit. Stimulation consisted of 100 msec trains of biphasic, rectangular wave pulse pairs. The frequency of pulse pairs was 50/sec. Each pulse pair consisted of two 50 μsec pulses of opposite polarity separated by 100 μsec. Three trains of pulses were delivered every sec. The duration of brain stimulation was 10 sec. Current intensities were 1.0, 2.0, and 3.0 mA. Tail response was evaluated according to the criteria described in previous articles [10–12]. Two latencies were measured: the first, from the time stimulation was turned on to when the tail was elevated to at least 45° to the horizontal; the second, from the time stimulation was turned on 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. Only mice which responded to brain stimulation of 3.0 mA by tail elevations of at least 90° to the horizontal were selected [12] and then subjected to the drug treatments.

Effects of naloxone on stimulation induced tail erection were studied as follows. Animals were given three testing sessions, each separated by 4 days. These are referred to as predrug, drug, and postdrug sessions. In the predrug session, animals were injected intraperitoneally with a matched volume of the naloxone vehicle (0.9% saline) and were tested 10 min later for tail response induced by brain stimulation. The drug session was identical except that animals received

[1]This study was supported by grants from the National Science Council of the Republic of China and the J. Aron Charitable Foundation, New York. We thank Endo Laboratories, Inc. for the naloxone.

[2]Send reprint requests to H. K. Lee.

[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.

TABLE 1

ANTAGONISTIC EFFECT OF NALOXONE (5 MG/KG IP) ON TAIL ERECTION INDUCED BY ELECTRICAL STIMULATION OF THE MESENCEPHALIC CENTRAL GRAY IN MICE*

| Current intensity (mA) | No. of mice responded (≥45°) / No. tested | Latency: median and range (sec) | No. of mice responded (≥90°) / No. tested | Latency: median and range (sec) | Duration: median and range (sec) |
|---|---|---|---|---|---|
| Predrug | | | | | |
| 1.0 | 4/9 | 3 (2–5) | 4/9 | 4 (3–6) | 6 (4–20) |
| 2.0 | 6/9 | 2 (1–7) | 6/9 | 3 (2–9) | 25 (7–77) |
| 3.0 | 9/9 | 1 (1–6) | 9/9 | 2 (2–8) | 35 (6–95) |
| Drug | | | | | |
| 1.0 | 0/9 | — | 0/9 | — | — |
| 2.0 | 0/9 | — | 0/9 | — | — |
| 3.0 | 2/9 | 1 & 2 | 2/9 | 2 & 3 | 7 & 10 |

*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 pulses of opposite polarity separated by 100 μsec. Trains of pulses were delivered at 3/sec. The duration of stimulation was 10 sec.

naloxone (5 mg/kg IP) instead of saline. The postdrug session was identical to the predrug session.

At the end of experiments, anatomical sites of electrical stimulation were confirmed histologically as described previously [13].

## RESULTS

In the present study, focal electrical stimulation of the midbrain produced a current dependent tail response, which confirmed a previous finding [12]. As shown in Table 1, electrical stimulation of 1.0 mA caused 4 out of 9 mice to elevate their tails to at least 45° with a median latency of 3 sec and to at least 90° with a median latency of 4 sec. Increasing the current intensity to 2.0 mA caused 6 out of 9 mice to elevate their tails to at least 90°. When 3.0 mA was used, all 9 animals displayed tail erection of at least 90°. Moreover, the duration of tail erection was also current dependent. The median duration of tail erection was 6, 25, and 35 sec for current intensity of 1.0, 2.0 and 3.0 mA, respectively.

Naloxone (5 mg/kg IP) antagonized the tail response produced by electrical stimulation. It abolished all tail erection produced by brain stimulation with current intensities of 1.0 and 2.0 mA (Table 1). With a higher stimulation intensity (3.0 mA), naloxone caused only a partial reversal, i.e., 7 out of 9 animals (78%). Increasing the dose of naloxone to 10 mg/kg IP failed to produce a greater inhibition of tail response in two animals which did not respond to a smaller dose of naloxone.

Tail response to brain stimulation fully recovered by the postdrug session. Histological examination revealed that sites of stimulation of these 9 animals were in the lateral part of the mesencephalic central gray, similar to those reported previously [12].

## DISCUSSION

Antagonism of electrical stimulation induced tail erection by naloxone supports an earlier contention [12] that similar mechanisms are involved in tail erection elicited by electrical stimulation and narcotic agents. Recently, several endogenous opioid peptides have been isolated from brain and pituitary [6, 8, 14]. Some of these substances are found in high concentrations in areas overlapping those where stereospecific binding of opiates occurs [16,17]. Periaqueductal gray of the mesencephalon is one of the areas showing such a close relationship between opioid peptides and opiate receptors [9,17]. In addition, endogenous opioid peptides might exert their physiological functions in the central nervous system as neurotransmitters [5]. Thus, tail erection induced by electrical stimulation of the mesencephalic central gray might result from the release of an opioid peptide and binding with opiate receptors.

The fact that tail erection at moderate stimulation intensities persists considerably beyond the duration of stimulation indicates the involvement of a chemical mediator. Naloxone would antagonize the action of this peptide as it does in many other central areas [2, 3, 7].

On the other hand, the present study indicates that tail erection produced by higher electrical stimulation currents cannot be completely prevented by naloxone. This cannot be due to an insufficient amount of the drug because 10 mg/kg of naloxone did not result in a greater inhibition than 5 mg/kg. It is possible that midbrain stimulation activates more than a single underlying mechanism. Strong electrical stimulation might induce release of other substrates of activation with which specific opiate antagonists do not interact. Dopamine is a strong possibility, since previous studies have indicated that this putative transmitter might play an important role in tail erection induced by electrical stimulation and narcotic agents [10,12].

Present evidence indicates that there is a neural pathway in the brain which utilizes an endogenous opioid peptide to activate tail movements. Activation of this particular locomotor system can be produced either pharmacologically by direct stimulation of opiate receptors or electrically by release of the opioid peptides. In both cases, tail responses are blocked by narcotic antagonists. Moreover, activation of

this locomotor system by opiates can be used as an indication of presumed euphoric activity in man [4]. At present, the exact mechanism of this opioid peptide modulated pathway in emotional behavior is not known. However, the periaqueductal gray in the mesencephalon is involved in self stimulation behavior [15].

## REFERENCES


1. Aceto, M. D., D. B. McKean and J. Pearl. Effects of opiates and opiate antagonists on the Straub tail reaction in mice. *Br. J. Pharmac.* **36**: 225–239, 1969.
2. Belluzzi, J. D., N. Grant, V. Garsky, D. Sarantakis, C. D. Wise and L. Stein. Analgesia induced in vivo by central administration of enkephalin in rat. *Nature* **260**: 625–626, 1976.
3. Chang, J. K., B. T. W. Fong, A. Pert and C. B. Pert. Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analogues. *Life Sci.* **18**: 1473–1482, 1976.
4. Collier, H. O. J. and C. Schneider. Profiles of activity in rodents of some narcotic and narcotic antagonist drugs. *Nature* **224**: 610–612, 1969.
5. Frederickson, R. C. A. Enkephalin pentapeptides—A review of current evidence for a physiological role in vertebrate neurotransmission. *Life Sci.* **21**: 23–42, 1977.
6. Goldstein, A. Opioid peptides (endorphins) in pituitary and brain. *Science* **193**: 1081–1086, 1976.
7. Haigler, H. J. and D. D. Spring. A comparison of the analgesic and behavioral effects of [D-Ala]-met-enkephalinamide and morphine in the mesencephalic reticular formation of rats. *Life Sci.* **23**: 1229–1240, 1978.
8. Hughes, J., T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**: 577–579, 1975.
9. Kuhar, M. J., C. B. Pert and S. H. Snyder. Regional distribution of opiate receptor binding in monkey and human brain. *Nature* **245**: 447–450, 1973.
10. Lee, H. K., C. Y. Chai, M. J. Wayner, P. M. Chung and C. H. Hsu. Effects of neuroleptics on morphine-induced tail erection in mice. *Pharmac. Biochem. Behav.* **7**: 153–157, 1977.
11. Lee, H. K., C. Y. Chai, M. J. Wayner, C. H. Hsu and P. M. Chung. Morphine-induced tail erection: site of action. *Pharmac. Biochem. Behav.* **8**: 69–73, 1978.
12. 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**: 221–226, 1978.
13. Lee, H. K. and S. C. Wang. Mechanism of morphine-induced miosis in the dog. *J. Pharmac. exp. Ther.* **192**: 415–431, 1975.
14. Li, C. H. and D. Chung. Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc. natn. Acad. Sci. U.S.A.* **73**: 1145–1148, 1976.
15. Liebman, J. M., D. J. Mayer and J. C. Liebeskind. Self-stimulation loci in the midbrain central gray matter of the rat. *Behav. Biol.* **9**: 299–306, 1973.
16. Pasternak, G. W., R. Simantov and S. H. Snyder. Characterization of an endogenous morphine-like factor (enkephalin) in mammalian brain. *Molec. Pharmac.* **12**: 504–513, 1976.
17. Simantov, R., M. J. Kuhar, G. R. Uhl and S. H. Snyder. Opioid peptide enkephalin: immunohistochemical mapping in rat central nervous system. *Proc. natn. Acad. Sci. U.S.A.* **74**: 2167–2171, 1977.