*Pharmacology Biochemistry & Behavior*, Vol. 9, pp. 425–428. Printed in the U.S.A.

# Naloxone Reversal of Morphine Catatonia: Role of Caudate and Periaqueductal Gray

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(Received 28 November 1977)

WILCOX, R. E. AND R. A. LEVITT. *Naloxone reversal of morphine catatonia: Role of caudate nucleus and periaqueductal gray*. PHARMAC. BIOCHEM. BEHAV. **9**(4) 425–428, 1978.—The narcotic antagonist, naloxone, was microinjected into the head of the caudate nucleus (HC), periaqueductal gray matter (PG), and cerebellar white matter (CB) of rats to counteract the catatonia induced by systemic morphine. Naloxone produced a loss of the catatonic response when administered into HC or PG, but not when microinjected into CB. Isotonic saline in HC, PG, and CB did not counteract the catatonic effects of morphine. The reversal of catatonia was similar for naloxone injections in HC and PG. Both these areas have high concentrations of opiate receptors while CB has few opiate receptors. It is suggested that the HC and PG are involved in the reversal of the catatonic effects of morphine via the high concentrations of opiate receptors found there.

Catatonia Morphine Naloxone Caudate Periaqueductal gray

ONE FOCUS of recent research on the mechanisms of opiate action has been the search for brain areas which mediate the behavioral effects of these agents [3,13]. Depending on the nature and dose of opiate agonist used and the site of microinjection, various responses typically follow acute opiate administration—analgesia [4,5], euphoria [1,9], or catatonia [4, 14, 18, 19]. Opiate antagonists have also been microinjected into the brain to reverse a response produced by systemic administration of opiate agonists [16,17]. For example, the periaqueductal gray matter of the brainstem has been implicated in several opiate actions including catatonia [7], presumably due to the high concentration of opiate receptors found in this region [13,15]. However, high concentrations of opiate receptors are also found within limbic system and basal ganglia loci. The area of the rat brain containing the highest concentration of opiate receptors is the head of the caudate nucleus [13], a structure which has been much less thoroughly explored with regard to its role in the mediation of the actions of morphine. The present investigation was designed to evaluate the role of the head of the caudate nucleus in morphine-induced catatonia. Catatonia was induced by intraperitoneal injections of morphine and a comparison was made of the ability of the narcotic antagonist naloxone to produce a loss of the catatonic response when microinjected into the head of the caudate nucleus and the periaqueductal gray. The cerebellar white matter was selected as a control site for naloxone microinjection because it is a motor area of the brain containing few opiate receptors.

## METHOD

### *Animals*

Animals were 24 experimentally naive, male Long-Evans rats, weighing between 250 and 400 g at the time of surgery. Animals were individually housed and maintained on a 12 hr light-dark cycle (lights on 8 a.m. to 8 p.m.) throughout the experiment. Animals were food deprived for 24 hr prior to behavioral testing but otherwise given free access to food and water. A 23 ga guide shaft for a 30 ga microinjection needle was stereotaxically implanted in each animal aimed at the head of the caudate nucleus, periaqueductal gray, or cerebellar white matter.

### *Coordinates*

Coordinates for the 3 sites were as follows—head of the caudate nucleus (HC)= +2.0, ±3.0, 5.5 (skull); periaqueductal gray (PG)= -6.0, ±2.1 (12°), 9.0 (skull); cerebellar white matter (CB)= −9.0, ±3.0, 3.0 (skull) (all coordinates in mm relative to bregma and skull [12, 18, 19]. A minimum of 7 days was allowed for recovery from surgery before behavioral testing was begun.

### *Drugs*

Drugs used were morphine sulfate (80 mg/kg, dissolved in distilled water), naloxone hydrochloride (10 μg in 1 μl of sterile isotonic saline), and sterile isotonic saline (0.9% NaCl; 1 μl). The naloxone was generously supplied by Endo Laboratories. Other compounds were obtained from commercial sources.

### *Experimental Design and Controls*

Three experimental controls were used to minimize possible confounding from nonspecific effects of microinjections on the catatonia induced by morphine.

*Within site controls.* For each of the 3 groups (HC, PG

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and CB) an attempt was made to minimize the chance that the naloxone and saline microinjections sampled different receptor populations. This was accomplished by delivering both microinjections through the same 30 ga needle in each animal on 2 different occasions. In addition all solutions used for microinjection were coded so that the experimenter was unaware of their identity until the completion of the experiment.

*Between site controls.* Microinjections were made into PG, a brain locus containing a high concentration of opiate receptors (high binding control) and implicated in several responses of the opiates. A second control site, CB, was selected as a motor control area containing low concentrations of opiate receptors (low binding control). The effects of naloxone and saline microinjections into these structures were compared with the effects of similar microinjections into HC.

A completely randomized factorial design consisting of 1 between subjects factor (brain site of drug microinjection) [8] and one within subjects factor (drug used in microinjection) [18,19] was used. The use of this design made it possible to assess effects due to brain sites of drug microinjection, drug used in microinjection, and the effects for each experimental group (i.e., the interaction between brain sites and drugs administered) [20].

All animals received an intraperitoneal dose of morphine followed by microinjection of naloxone or saline on 2 test days 1 week apart. The ability of a single dose of morphine to induce tolerance is well documented [10]. In the present experiment a dose of the opiate was selected which reliably induced catatonia twice in a high percentage of pilot animals. In addition, the drug microinjections were completely counterbalanced to eliminate morphine tolerance as a source of confounding in the following way. Half of the animals implanted in each brain site received a naloxone microinjection following their first morphine injection. The other half of the animals implanted at each brain locus received a saline microinjection following their first morphine injection. Conditions for all animals were reversed on the second test day. Thus, all animals which had received naloxone following their first morphine injection received saline following their second morphine injection. All animals which had received saline following their first morphine injection received naloxone following their second morphine injection. For each of the 3 brain regions explored, half of the animals received naloxone following their first morphine injection while the other half received naloxone following their second morphine injection.

TABLE 1

EFFECT OF NALOXONE AND SALINE ON THE CATATONIA PRODUCED BY MORPHINE*

| Brain Site | Treatment | |
|---|---|---|
| | Naloxone | Saline |
| Head of the caudate nucleus | 22 | 30 |
| | 2† | 30 |
| | 30 | 30 |
| | 0† | 30 |
| | 0† | 30 |
| | 30 | 30 |
| | 0† | 30 |
| | 30 | 30 |
| Periaqueductal gray matter | 0† | 30 |
| | 0† | 30 |
| | 30 | 30 |
| | 5† | 30 |
| | 30 | 30 |
| | 0† | 30 |
| | 0† | 30 |
| | 30 | 30 |
| Cerebellar | 22 | 30 |
| | 15 | 30 |
| | 30 | 30 |
| | 30 | 30 |
| | 30 | 30 |
| | 30 | 30 |
| | 15 | 30 |
| | 30 | 30 |

*Rats were administered 80 mg/kg morphine sulfate intraperitoneally and tested for catatonia 30 min later using a bar test (see text). Animals spending 30 sec on the bar were microinjected with 10 μg naloxone hydrochloride (dissolved in sterile isotonic saline) or sterile isotonic saline in an injection volume of 1 μl. All animals received 2 morphine administrations and 2 brain microinjections (1 with naloxone and 1 with saline) 1 week apart [in counterbalanced order] (see text). Approximately 40 animals had surgeries and received morphine injections in order to have 3 groups of 8 animals which were catatonic twice and which had verified micorinjections in th intended brain sites.

†Animals showing loss of the catatonic response following microinjection.

*Procedure*

On the first test day animals were injected intraperitoneally with 80 mg/kg of morphine sulfate dissolved in distilled water. Thirty min later each animal was tested for catatonia by placing both forepaws over a horizontal bar 10 cm high and recording the number of sec this position was maintained. Characteristically, an adult rat given morphine maintains this position for increasingly longer periods as dose is increased, whereas a saline-injected animal dismounts from the bar immediately [2]. Animals keeping both front limbs on the bar for 30 sec immediately received a brain microinjection of naloxone hydrochloride (10 μg). Pilot work established an $ED_{50}$ for the ability of intraperitoneal naloxone to produce a loss of the catatonia (catatonia reversal) at 200 μg/kg. Microinjections were made using a Hamilton 30 ga needle (approximately 0.2 mm diameter) precut to the desired length for each rat and attached to a Hamilton microsyringe prefilled and set to deliver 1 μl of solution. The solution was allowed 15 sec to diffuse away from the needle before the animal was returned to its cage. Ten min after the brain microinjection the behavioral testing for catatonia was repeated. One week after the first behavioral test each rat was reinjected with 80 mg/kg of morphine and given a bar test for catatonia as before. Animals remaining on the bar for 30 sec were microinjected as before using the same 30 ga needle. Ten min after the microinjection the behavioral test for catatonia was repeated. Histological verification of all brain stimulation loci was obtained by microscopic inspection of thionin-stained 80 micron sections. At the time of verification, the brains were coded so that the experimenter was unaware of the results of the microinjections.

TABLE 2

BRAIN MICROINJECTION LOCI*

| Brain Site | Verified Micorinjection Coordinates Anterior-Posterior† | Lateral‡ | Dorsal-Ventral (dura)§ |
|---|---|---|---|
| Head of the caudate nucleus | +2.2 | 2.50 | 4.25 |
| | +2.2¶ | 3.25 | 3.50 |
| | +2.2 | 3.00 | 4.50 |
| | +2.0¶ | 3.25 | 3.50 |
| | +2.0¶ | 3.25 | 3.25 |
| | +2.0 | 3.00 | 4.50 |
| | +2.0¶ | 2.75 | 3.25 |
| | +1.8 | 2.50 | 5.25 |
| Periaqueductal gray matter | 5.8¶ | 0.75 | 4.25 |
| | −6.0¶ | 0.50 | 4.75 |
| | −6.0 | 1.00 | 6.00 |
| | 6.0¶ | 0.25 | 4.25 |
| | 6.0 | 1.00 | 5.00 |
| | 6.0¶ | 0.75 | 4.50 |
| | −6.0¶ | 0.75 | 4.75 |
| | −6.2 | 1.00 | 4.50 |
| Cerebellar white matter | −8.8 | 3.00 | 3.00 |
| | −9.0 | 3.50 | 3.00 |
| | 9.0 | 3.00 | 3.00 |
| | −9.0 | 3.00 | 3.00 |
| | 9.0 | 3.25 | 3.00 |
| | −9.0 | 2.75 | 3.75 |
| | −9.0 | 3.25 | 3.00 |
| | −9.2 | 2.75 | 3.50 |

*Following behavioral testing animals were sacrificed with an overdose of pentobarbital, perfused with 4% formalin solution, the brains rapidly removed and placed in formalin. Frozen sections were cut at 80$\mu$, stained with formal-thionin and verified microscopically using the Pellegrino and Cushman (1967) rat brain atlas. All verifications were carried out without knowledge of the results of microinjection.

†Anterior-posterior coordinates are given in mm relative to bregma. Coordinates for animals which spent less than 10 sec on the bar following naloxone, microinjection are indicated by ¶.

‡Lateral coordinates are given in mm relative to bregma. Guide shafts aimed for periaqueductal gray matter were implanted at 12° from vertical to avoid hitting the cerebral aqueduct. Verified coordinates in the table refer to the actual sites of microinjection.

§Dorsal-ventral coordinates are given in mm relative to dura.

## RESULTS

The catatonia bar test scores following naloxone and saline microinjection in each of the three brain sites are presented in Table 1. Coordinates of microinjection loci are given in Table 2. A second morphine administration was found to produce no differences in postnaloxone or postsaline bar test scores from those occurring following a single morphine administration ($\chi^2$ test). Therefore, the data in Table 1 have been pooled for the 2 test days. Previous work in our laboratory has suggested that animals spending 10 sec or less on the bar following naloxone are responding to this drug significantly more than animals spending more than 10 sec on the bar [18,19]. On this basis, animals with a postnaloxone bar test score of 0–10 sec were a priori defined as having shown a reversal of the morphine catatonia (i.e., naloxone produced a loss of the catatonic response). Animals spending 11–30 sec on the bar were classified as having failed to reverse the catatonia (i.e., naloxone did not result in loss of the catatonia). Statistical analyses of dichotomized data [11] were carried out using analysis of variance and a posteriori Scheffé tests for evaluating significant differences among means [8].

Analysis of variance revealed that the main effects of brain site of microinjection (Site) and that of drugs used in microinjection (Drug) were both significant (Site: $F(2,21)=4.74$, $p<0.02$; Drug: $F(1,21)=18.29$, $p<0.001$). The interaction of brain sites of microinjection with drugs used in microinjection was also significant, $F(2,21)=4.74$; $p<0.02$.

Naloxone administration in the HC was followed by a reversal of the morphine-induced catatonic response in fifty percent of the animals. In contrast, no animals showed a loss of the morphine catatonia following saline microinjections in this structure (Scheffé test for Naloxone-HC vs. Naloxone-CB, $p<0.01$). Naloxone microinjections into the PG pro-

duced similar effects (1). Naloxone in the PG resulted in a loss of the catatonic response in 67% of the animals while no animals showed a loss of the catatonic response following saline microinjections into the PG (Scheffé test for Naloxone-PG vs. Saline-PG, $p<0.01$). (2) Naloxone microinjections into the PG produced a significantly greater loss of the catatonia than did naloxone microinjections into the CB (Scheffé test for Naloxone-PG vs. Naloxone-CB, $p<0.01$). (3) Finally, the ability of naloxone to produce a loss of the catatonia when microinjected into the HC was indistinguishable from naloxone's ability to reverse the catatonia when microinjected into the PG (Scheffé test for Naloxone-HC vs. Naloxone-PG, $p>0.05$).

In summary, naloxone microinjection resulted in loss of the catatonia induced by morphine in 50% of the HC animals. Naloxone microinjection resulted in a loss of the morphine-induced catatonia in 67% of the PG animals. No animals showed a loss of the catatonic response to morphine following naloxone stimulation in the CB. None of the animals injected in the 3 brain sites showed a loss of the catatonic response following saline microinjections.

## DISCUSSION

Naloxone microinjections produce a loss of the catatonic response in 2 brain regions having high concentrations of opiate receptors. Since each animal was microinjected with naloxone and saline in the same locus it would be difficult to attribute the effects of naloxone in the HC and PG to nonspecific effects of microinjection. The differential response to naloxone microinjections in HC, PG, and CB could more easily be attributed to anatomical differences among these regions, specifically to their differential concentrations of opiate receptors.

These results confirm the importance of the PG in the catatonic response induced by morphine. They also suggest a role for the brain area containing the highest concentration of opiate receptors, the HC, in this action. These results in conjunction with the failure of naloxone to counteract catatonia when microinjected into the CB, are consistent with the hypothesis that the catatonia produced by morphine is mediated by the opiate receptor system. While the present experiment has not explored systematically the role of brain sites in morphine-induced catatonia, we have implicated the brain area containing the highest concentration of opiate receptors in this phenomenon and have compared the role of the HC with that of the PG.

## REFERENCES


1. Baltzer, J. H., R. A. Levitt and J. E. Furby. Etorphine and shuttle-box self-stimulation in the rat. *Pharmac. Biochem. Behav.* **7**: 413–416, 1977.
2. Costall, B. and R. J. Naylor. A role for the amygdala in the development of the cataleptic and stereotypic actions of the narcotic agonists and antagonists in the rat. *Psychopharmacologia* **35**: 203–213, 1974.
3. Criswell, H. E. and R. A. Levitt. The narcotic analgesics. In: *Psychopharmacology: A Biological Approach*, R. A. Levitt, Washington, D.C.: Hemisphere/Wiley, 1975.
4. Izumi, K., T., Motomatsu, M. Chretien, R. F. Butterworth, M. Lis, N. Ceidah and A. Barbeau. β-endorphin induced akinesia in rats: effect of apomorphine and α-methyl-p-tyrosine and related modifications of dopamine turnover in the basal ganglia. *Life Sci.* **20**: 1149–1156, 1977.
5. Jacquet, Y. F. and A. Lajtha. Morphine action at central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose. *Science* **182**: 490–492, 1973.
6. Jackquet, Y. F. and A. Lajtha. The periaqueductal gray: site of morphine analgesia and tolerance as shown by 2-way cross tolerance between systemic and intracerebral injections. *Brain Res.* **103**: 501–513, 1976.
7. Jacquet, Y. and N. Marks. The C-fragment of beta-lipotropin: an endogenous neuroleptic or antipsychotogen? *Science* **194**: 632–634, 1976.
8. Kirk, R. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, Calif: Brooks, 1968.
9. Levitt, R. A., J. H. Baltzer, T. M. Evers, D. J. Stilwell and J. E. Furby. Morphine and shuttle-box self-stimulation in the rat: a model for euphoria. *Psychopharmacologia* **54**: 307–311, 1977.
10. Misra, A., C. Mitchell and L. Woods. Persistence of morphine in central nervous system of rats after a single injection and its bearing on tolerance. *Nature* **232**: 49–50, 1971.
11. Nie, N. J., C. Hull, J. G. Jenkins, K. Steinbrenner and D. H. Debt. *Statistical Package for the Social Sciences*. New York: McGraw-Hill, 1975.
12. Pellegrino, L. and A. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967.
13. Pert, C. B., M. J. Kuhar and S. H. Snyder. Autoradiographic localization of the opiate receptor in rat brain. *Life Sci.* **16**: 1849–1854, 1975.
14. Segal, D. S., R. G. Brown, F. Bloom, N. Ling and R. Guillemin. β-endorphin: endogenous opiate or neuroleptic? *Science* **198**: 411–413, 1977.
15. Snyder, S. H. and S. Matthysse. Opiate receptor mechanisms. *Neurosci. Res. Prog. Bull.* **13**: 1–166, 1975.
16. Wei, E., H. H. Loh and E. L. Way. Neuroanatomical correlates of morphine dependence. *Science* **117**: 616–617, 1972.
17. Wei, E., H. H. Loh and E. L. Way. Brain sites of precipitated abstinence in morphine dependent rats. *J. Pharmac. exp. Ther.* **185**: 108–115, 1973.
18. Wilcox, R. E., M. McCoy, M. Bozarth and R. A. Levitt. Reversal of morphine catatonia by central naloxone. Paper presented at Society for Neuroscience, Toronto, 1976.
19. Wilcox, R. E., M. McCoy and R. A. Levitt. Reversal of morphine catatonia by central naloxone. Paper presented at Midwestern Psychological Association, Chicago, 1976.
20. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1971.