Pharmacology Biochemistry & Behavior, Vol. 14, pp. 247–249. Printed in the U.S.A.

Effects of Chronic Naloxone Treatment on Brain-Stimulation Reward[1]

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Received 22 October 1980

PERRY, W., R. U. ESPOSITO AND C. KORNETSKY. *Effects of chronic naloxone treatment on brain-stimulation reward.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 247–249, 1981.—Rats were tested on a rate-free psychophysical procedure in order to determine the absolute reinforcement thresholds for self-stimulation behavior. The administration of naloxone (16 mg/kg) for five days failed to alter the reinforcement thresholds on this procedure. To the extent that naloxone is an effective antagonist of endogenous opioids, we conclude that central endorphin systems are not necessary to support self-stimulation behavior.

Brain-stimulation reward Reinforcement-thresholds Naloxone

BELLUZZI and Stein [3] initially reported that naloxone will produce dose-related decreases in the rate of rats' responding for intracranial self-stimulation (ICSS), and subsequently this finding was replicated by Stapelton *et al.* [19]. However, in contrast, several other investigators have failed to confirm this finding [7, 8, 12, 14, 15, 16, 22, 23, 24]. There are a number of reports indicating that acute and chronic naloxone treatments may have differential effects on certain pharmacological assays and behavioral tests. For example, studies concerned with receptor binding have reported supersensitivity to opioids following chronic naloxone administration [13,18]. In addition, it has been demonstrated that, in contrast to acute administration, chronic naloxone treatment will enhance the analgesic effects of morphine in rats [20]. Also, while acute naloxone administration failed to produce significant changes in rats' locomotor activity, chronic naloxone treatment resulted in marked reductions of this behavior [1]. The present study was undertaken, therefore, to investigate the effects of chronic naloxone treatment on reward thresholds for brain stimulation, and thus more thoroughly assess the effects of this agent on ICSS.

METHOD

Animals and Apparatus

Five male CDF strain rats (Charles River Breeding Laboratories), weighing approximately 300 g, were stereotaxically implanted bilaterally with bipolar stainless steel electrodes (0.01 cm in dia.). The animals were singly housed and provided ad lib access to food and water. The animal room was illuminated on a 12 hr light/dark cycle.

Prior to surgery all animals were anesthetized with Equi-Thesin® (0.3 ml/100 g body weight). The electrodes were aimed at either the medial forebrain bundle at the level of the lateral hypothalamus (MFB-LH) or the ventral tegmental area (VTA). The coordinates for the MFB-LH placements were: 4 mm posterior to bregma, 1.4 mm lateral from the midline suture, and 8.5 mm ventral from skull surface. The coordinates for the VTA were: +2 mm from lambda, 1.4 mm lateral from the midline suture, and 8.0 mm ventral from the skull surface. The electrodes were placed through small burr holes in the skull surface and attached to the skull with an acrylic platform. Bicillin® (60,000 units) was administered immediately following surgery. The animals were given 1 week for recovery before training began.

Animals were tested in a Plexiglas chamber (20×20 cm) with a cylindrical-wheel manipulandum (15 cm in length and 7.5 cm in dia.) mounted in an opening in one wall. Four equally spaced cams were positioned on one of the end plates such that they operated a microswitch when the wheel was rotated. Biphasic square wave stimuli were delivered by a constant current stimulator (Sunrise Systems, North Scituate, MA). Each stimulus consisted of a 500 msec train presented at 160 Hz, with a pulse width of 0.2 msec between the positive and negative pulses.

Procedure

Threshold determinations involved a discrete trial procedure. A trial began with a noncontingent 0.5 sec pulse train. A response within 7.5 sec of this stimulus resulted in a reward contingent stimulus, with parameters identical to the noncontingent stimulus. Failure to respond had no consequences and the trial terminated after 7.5 sec. Intertrial intervals varied around an average of 15 sec. Responses during the intertrial interval resulted in a 15 sec delay of the

[1]Work supported in part by a grant from NIDA, DA 02326.

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noncontingent stimulus. Stimulus intensities were varied according to the classical method of limits with slight modifcation. Stimuli were presented in alternating descending and ascending series, with a step size of 5 or 10 μA (depending on the individual animal's discriminative capabilities). The entire testing session lasted between 1.5–2 hours (see Esposito and Kornetsky [5] for further details). All experimental events and data were collected and stored by an online microcomputer. Subjects were run for four series pre-injection (2 ascending and 2 descending) and four series post-injection. Animals were tested on the above procedure until a stable performance was obtained, at which time saline injections were initiated. Threshold values were calculated for both sessions, with the difference between the two scores taken as the dependent measure. All the change (difference) scores were transformed to standard scores (Z scores) in order to make comparisons between drug-change scores and the distribution of change scores seen following saline injections. A Z score of 2.0 ($p<0.05$) was pre-selected as the level of significance. Animals received saline for at least 7 days in succession, following which they received daily naloxone injections (16 mg/kg) for 5 days. Naloxone hydrochloride was dissolved in 0.9% saline and injected intraperitoneally in a volume of 1.0 ml/kg of body weight.

After completion of behavioral testing the animals were sacrificed with an overdose of Equi-Thesin® and perfused intracardially with saline and then Formalin. The brains were removed from the skull, fixed, embedded and sectioned at 40 μ. Sections were stained with cresyl violet and Luxol Fast blue and examined under a light microscope to determine the site of electrode placement.

RESULTS

The mean standard scores (Z score) for 5 animals, for each of 5 days of naloxone administration, is displayed in Fig. 1. It is evident that repeated naloxone administration had no effect upon ICSS behavior. Histological analysis revealed that the electrode tips were either within the dorsal aspect of the medial forebrain bundle at the level of the zona incerta or dorsal to the ventral tegmentum nucleus, and medial to the substantia nigra.

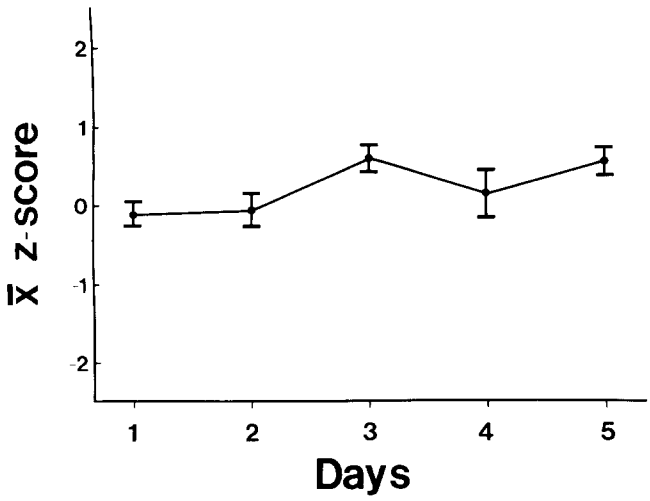

FIG. 1. The mean standard score (Z score) ± SEM for all subjects for five days of naloxone administration.

DISCUSSION

The present data demonstrate that repeated (5 days) naloxone administration fails to significantly affect self-stimulation behavior in the rat. These findings are in accordance with previous studies which have examined acute naloxone administration and brain stimulation reward to different brain sites, and have likewise failed to detect effects of naloxone (or naltrexone) [7, 8, 12, 14, 15, 16, 22, 23, 24]. Our failure to find significant naloxone effects cannot be attributed to the absence of enkephalin fibers or cell bodies at the site of stimulation since these areas demonstrate moderate to high enkephalin immunofluorescence [9,21]. Although two investigators [3,19], employing rate-dependent measures, have reported that naloxone will depress ICSS rates, the specificity of these findings can be questioned due to evidence demonstrating naloxone's modification of locomotor activity at the dose range reported to suppress ICSS [2,17]. Therefore, as previously suggested [6], tonic activation of endorphinergic systems does not seem to be necessary for maintaining ICSS in rats. Finally, since the brain-stimulation reward paradigm is generally regarded as a model for the effects of mood altering agents in man, it is interesting to note that recent studies have found naloxone to be without significant specific effects on mood in normal human volunteer subjects [4, 10, 11].

REFERENCES

1. Amir, S., M. Solomon and Z. Amit. The effect of acute and chronic naloxone administration on motor activation in the rat. *Neuropharmacology* **18:** 171–178, 1978.
2. Arnstein, A. T. and D. S. Segal. Naloxone alters locomotion and interaction with environmental stimuli. *Life Sci.* **25:** 1035–1042, 1979.
3. Belluzzi, J. D. and L. Stein. Enkephalin may mediate euphoria and drive reduction reward. *Nature* **266:** 556–558, 1977.
4. Davis, G. C., W. E. Bunney, Jr., M. S. Buchsbaum, E. G. Defraites, W. Duncan, J. C. Gillin, D. P. van Kammen, J. Kleinman, D. L. Murphy, R. M. Post, V. Reus and R. J. Wyatt. Use of narcotic antagonists to study the role of endorphins in normals and psychiatric patients. In: *Endorphins in Mental Health Research*, edited by E. Usdin, W. E. Bunney, Jr. and N. S. Kline. New York: Oxford University Press, 1979, pp. 393–406.

5. Esposito, R. U. and C. Kornetsky. Morphine lowering of self-stimulation thresholds: Lack of tolerance with long-term administration. *Science* **195:** 189–191, 1977.
6. Esposito, R. U., W. Perry and C. Kornetsky. Effects of d-amphetamine and naloxone on brain stimulation reward. *Psychopharmacology* **69:** 187–191, 1980.
7. Goldstein, J. M. and J. B. Malick. Effects of substance P on medial forebrain bundle self-stimulation in rats following intracerebral administration. *Pharmac. Biochem. Behav.* **7:** 475–478, 1977.
8. Holtzman, S. G. Comparison of the effects of morphine, pentazocine, cyclazocine and amphetamine on intracranial self-stimulation in the rat. *Psychopharmacologia* **46:** 223–227, 1976.
9. Jacobowitz, D. M., M. A. Silver and W. G. Soden. Mapping of leu-enkephalin-containing axons and cell bodies of the rat forebrain. In: *Endorphins in Mental Health Research*, edited by E. Usdin, W. E. Bunney, Jr. and N. S. Kline. New York: Oxford University Press, 1979, pp. 62–73.
10. Jones, R. T. and R. I. Herning. Naloxone-induced mood and physiologic changes in normal volunteers. In: *Endorphins in Mental Health Research*, edited by E. Usdin, W. E. Bunney, Jr. and N. S. Kline. New York: Oxford University Press, 1979, pp. 484–491.
11. Judd, L. L., D. S. Janowsky, D. S. Segal and L. Y. Huey. Naloxone-induced behavioral and physiological effects in normal and manic subjects. *Archs gen. Psychiat.* **37:** 583–586, 1980.
12. Kornetsky, C., R. U. Esposito, S. McLean and J. O. Jacobson. Intracranial self-stimulation thresholds: A model for the hedonic effects of drugs of abuse. *Archs gen. Psychiat.* **36:** 289–292, 1979.
13. Lahti, R. A. and J. R. Collins. Chronic naloxone results in prolonged increases in opiate binding sites in brain. *Eur. J. Pharmac.* **51:** 185–186, 1978.
14. Lorens, S. A. and S. M. Sainati. Naloxone blocks the excitatory effect of ethanol and chlordiazepoxide on lateral hypothalamic self-stimulation behavior. *Life Sci.* **23:** 1359–1364, 1978.
15. Nazarro, J. M., T. F. Seeger and E. L. Gardner. Differential opiate effects on intracranial self-stimulation thresholds in substantia nigra (A-9) and ventral tegmentum (A-10). *Soc. Neurosci. Abstr.* **5:** 658, 1979.
16. Pert, A. Effects of opiates on rewarding and aversive brain stimulation in the rat. In: *Problems of Drug Dependence*, National Research Council. Washington, D.C.: National Academy of Science, 1975, pp. 963–973.
17. Pert, A., L. A. DeWald, H. Liao and C. Sivit. Effects of opiates and opioid peptides on motor behaviors: Sites and mechanisms of action. In: *Endorphins in Mental Health Research*, edited by E. Usdin, W. E. Bunney, Jr. and N. S. Kline. New York: Oxford University Press, 1979, pp. 45–61.
18. Schulz, R., M. Wuster and A. Herz. Supersensitivity to opioids following the chronic blockade of endorphin action by naloxone. *Archs Pharmac.* **306:** 93–96, 1979.
19. Stapelton, J. M., V. J. Merriman, C. L. Coogle, S. D. Gelbard and L. D. Reid. Naloxone reduces pressing for intracranial stimulation of sites in the periaqueductal gray area, accumbens nucleus, substantia nigra and lateral hypothalamus. *Physiol. Psychol.* **7:** 427–436, 1979.
20. Tang, A. H. and R. J. Collins. Enhanced analgesic effects of morphine after chronic administration of naloxone in the rat. *Eur. J. Pharmac.* **47:** 473–474, 1978.
21. Uhl, G., M. J. Kuhar, R. R. Goodman and S. H. Snyder. Histochemical localization of the enkephalins. In: *Endorphins in Mental Health Research*, edited E. Usdin, W. E. Bunney, Jr. and N. S. Kline. New York: Oxford University Press, 1979, pp. 74–83.
22. Van derKooy, D., F. G. LePaine and A. G. Phillips. Apparent independence of opiate reinforcement and electrical self-stimulation systems in rat brain. *Life Sci.* **20:** 981–986, 1977.
23. Wauquier, A., C. J. E. Niemegeers and H. Lal. Differential antagonism by naloxone of inhibitory effects of haloperidol and morphine on brain self-stimulation. *Psychopharmacologia* **37:** 303–310, 1974.
24. Weibel, S. and H. Wolf. Opiate modification of intra-cranial self-stimulation in the rat. *Pharmac. Biochem. Behav.* **10:** 71–78, 1978.