Pharmacology Biochemistry & Behavior, Vol. 17, pp. 389–392, 1982. Printed in the U.S.A.

Behavioral Alterations Produced by Chronic Naloxone Injections

DAVID H. MALIN,[1] MICHAEL P. LAYNG,
PAUL SWANK, MELANIE J. BAKER AND JOYCE L. HOOD

University of Houston at Clear Lake City, Houston, TX 77058

Received 8 May 1982

MALIN, D. H., M. P. LAYNG, P. SWANK, M. J. BAKER AND J. L. HOOD. *Behavioral alterations produced by chronic naloxone injections*. PHARMAC. BIOCHEM. BEHAV. 17(3) 389–392, 1982.—Repeated blockade of the endorphin receptors eventually induces symptoms resembling an opiate abstinence syndrome, despite the complete absence of opiate narcotics. Rats were injected with 0.6 mg/kg naloxone or with injection vehicle alone twice a day for six days. They were observed twice a day for four subsequent days. Body shakes, head shakes, scratches and total symptoms were significantly elevated in the naloxone treated group over controls. Symptoms were completely reversed by a small dose of morphine but not by naloxone. In a second experiment, rats were injected for ten days with the same dosage of naloxone. The abstinence-like syndrome began after six days of naloxone and continued for several days after cessation of injections. Total symptoms, body shakes, scratches and aggression were significantly elevated over controls and were reversed by morphine but not by naloxone.

Naloxone Endorphins Dependence Morphine Opiates Abstinence syndrome

NALOXONE effectively and specifically blocks endorphin receptors. Only at high doses are there marked effects directly on other neurohormonal systems [23].

Naloxone has been reported to antagonize endorphinergic behavioral responses to various specific stressors such as cold water [4], footshock [7], or maternal separation [13]. This indicates a role for the endorphin system as an emergency system for organisms coping with episodes of severe stress. However, the behavioral role of ongoing daily endorphin secretion in the absence of specific stressors is much less clear. An acute injection of naloxone in animals not subjected to stressors or painful stimuli fails to produce signs or symptoms of marked distress, although the acute injection has been reported to reduce food intake [14,15], to increase male sexual behavior [9], and to increase [11,16], decrease [24] or have no significant effect [6, 10, 12], on pain sensitivity depending on the specific dose of naloxone and the testing method employed. The comparatively mild effects of acute endorphinergic blockade have raised the possibility that "the putative endorphin system cannot be essential to life or to ongoing behavior" [10].

On the other hand, the lack of severe reaction to acute naloxone might be consistent with findings that endorphin release is not constant but cyclic [8,29] or episodic in response to stress [1,3]. It seems possible that some systems in the brain might require at least occasional endorphinergic stimulation for the long term maintenance of their normal functioning. In such a case, acute absence of endorphin receptor stimulation would not produce a marked change. However, a chronic lack of endorphin stimulation due to naloxone blockade might induce a gradual and prolonged alteration of function, perhaps similar to that which results from abstinence following chronic opiate exposure. Therefore, in the present experiments, rats chronically treated with naloxone were observed for those symptoms (wet dog shakes, head shakes, hind food scratches and aggression) typical of abstinence syndrome in morphine dependent rats.

EXPERIMENT 1

METHOD

Subjects were 39 male Sprague Dawley albino rats with an average weight of 130 grams. Throughout the experiment, rats were housed in groups of four, maintained on a twelve hour light and dark cycle (lights on from 9 a.m. to 9 p.m.), and on ad lib food and water. The animals were randomly divided into two groups. Group 1 (n=19) received a subcutaneous injection twice a day at 9 a.m. and 9 p.m. for six days with 0.6 mg/kg of naloxone in a 0.25 mg/ml distilled water solution. The 9 p.m. injection time was selected to shortly precede the major burst of endorphinergic activity detected by Frederickson [5] after lights out. Group 2 (n=20) received a similar volume of distilled water on the same injection schedule. Solutions and animals were coded so that the animals were observed under "blind" conditions. Both groups were observed after the six day injection series for opiate abstinence syndrome-like symptoms (body shakes, head shakes and scratches) in a 45 cm × 45 cm × 30 cm clear plastic chamber with an open top and a wire mesh floor. Rats were observed in groups of four by one trained observer assisted by a second investigator who recorded the above

[1]Send reprint requests to David Malin, Box 237, University of Houston at Clear Lake City, Houston, TX 77058.

behaviors on a check-list. This allowed the observer to devote continuous attention unhampered by recording duties. Inter-observer reliability has been established in separate experiments in which a high correlation (r=.903) was found between the data of two trained "blind" observers. All animals were observed for 15 min at 10 a.m. each day for four days. All animals were observed for the first time 18 hours after the last injection of naloxone or distilled water. (Timing and dosage parameters of this experiment were selected on the basis of small pilot experiments.)

On the fifth day of observations, a test was made of whether the symptoms were reversible by a small dose of morphine sulphate. Six of the naloxone pretreated animals were randomly selected to receive 4 mg/kg morphine sulphate (Merck) SC, while another six received an equivalent volume of distilled water alone. Similarly, six of the water pretreated animals received the same dose of morphine sulphate, while six received water alone. Animals were observed 30 min after injections for 15 min.

A further test was made of the ability of a renewed dose of naloxone to reverse the symptoms. The naloxone pretreated animals that served as the water controls in the previous reversal test were all injected with 0.6 mg/kg naloxone SC and observed 30 min later for 15 min.

RESULTS

Figure 1 summarizes the total abstinence-like symptoms (body shakes, head shakes and scratches) demonstrated by naloxone pretreated and placebo pretreated rats. A 2×4 analysis of variance with one factor having 4 repeated measures revealed a significant main effect of pretreatment: naloxone vs placebo, $F(1,37)=13.55$, $p<0.01$, as well as of days, $F(3,111)=3.21$, $p<0.05$, but no significant interaction effect: pretreatment × days, $F(3,111)=0.58$, NS. Figure 2 provides a breakdown of specific types of symptoms, accumulated through all four days. The naloxone pretreated group had significantly more wet dog shakes as compared with controls, $t(37)=3.12$, $p<0.005$, as well as head shakes, $t(37)=2.99$, $p<0.005$, and scratches, $t(37)=2.15$, $p<0.025$.

The symptom reversal tests of morphine and naloxone at the end of the experiment were analyzed in terms of difference scores, the difference between each animal's total symptoms before and after an acute injection. Figure 1 shows that the abstinence-like symptoms of the naloxone pretreated animals were virtually eliminated by morphine. The naloxone pretreated rats receiving morphine had significantly greater difference scores, $t(10)=2.0$, $p<0.01$, than those naloxone pretreated rats injected with water. Morphine produced a non-significant decline in the water pretreated animals, $t(10)=0.5$, NS.

In contrast, naloxone totally failed to reverse the symptoms in those naloxone pretreated animals not already treated with morphine. According to the within subjects-test the corresponding difference scores (pre vs post naloxone) were not significantly different from zero, $t(5)=0.0$, NS.

EXPERIMENT 2

METHOD

This experiment was performed to determine the effects of ten days of naloxone injection and to ascertain whether any resulting symptoms began during or after the naloxone treatment period. Procedures were the same as in Experi-

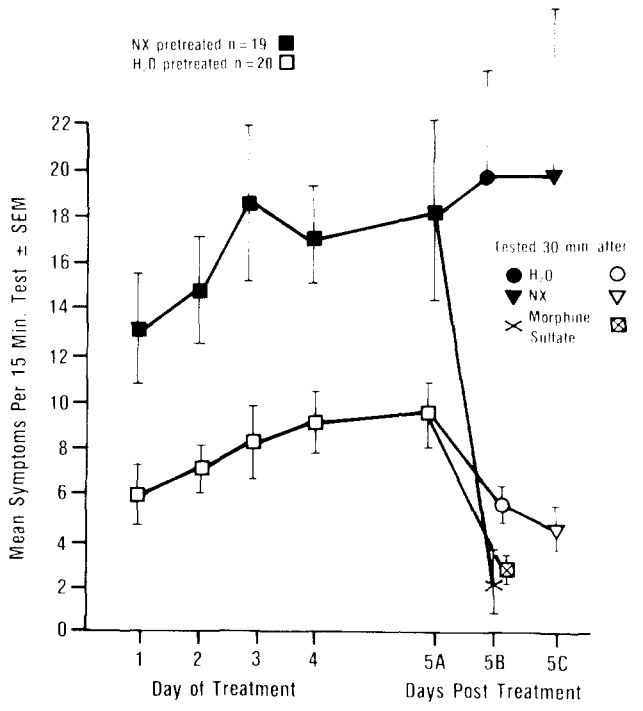

FIG. 1. Mean total abstinence-like symptoms per day in rats pretreated with distilled water or 0.6 mg/kg naloxone for 6 days. 5A is a 3 p.m. observation. 5B is a 5 p.m. MS reversal test. 5C is an 8 p.m. NX reversal test.

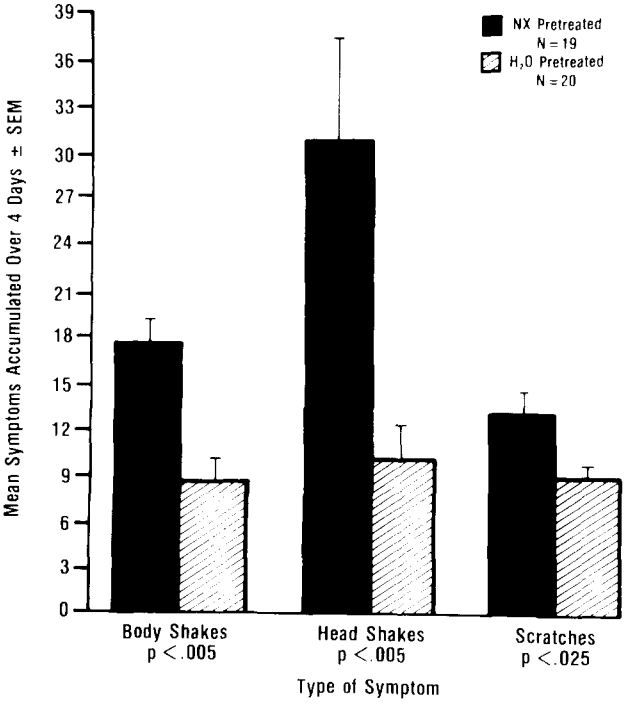

FIG. 2. Body shakes (wet dog shakes), head shakes and scratches cumulated over 4 days by rats pretreated for 6 days with naloxone (0.6 mg/kg) or H_2O.

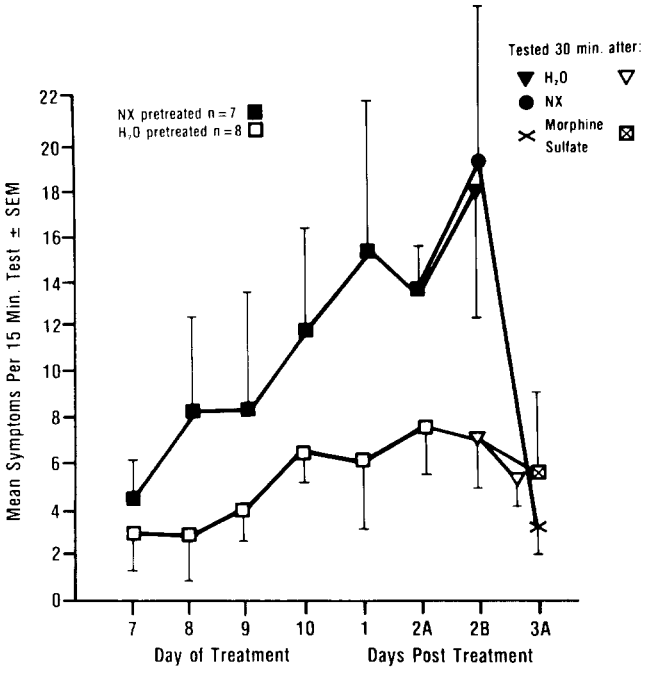

FIG. 3. Mean total abstinence-like symptoms in rats receiving distilled water or 0.6 mg/kg naloxone for 10 days. 2A is an 8 a.m. observation. 2B is a 9 p.m. NX reversal test. 3A is an 8 a.m. MS reversal test.

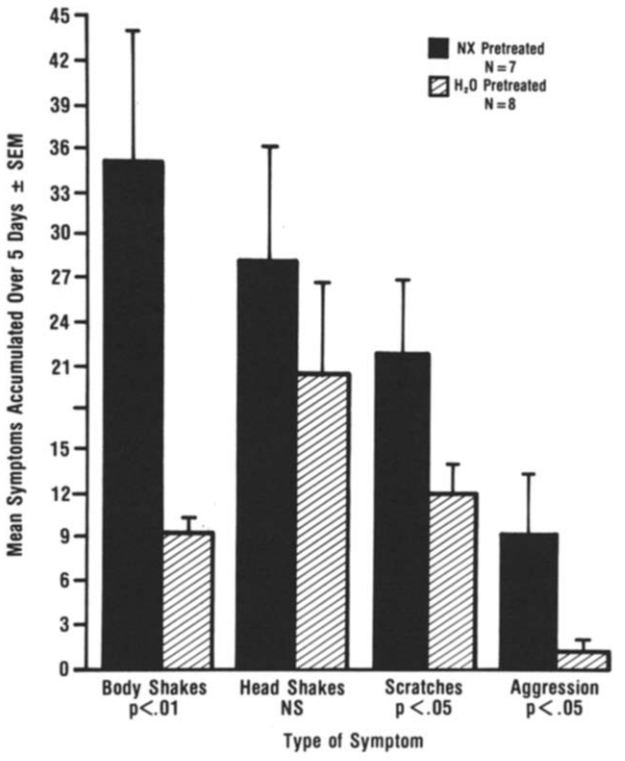

FIG. 4. Body shakes (wet dog shakes), head shakes, scratches, and acts of aggression cumulated over 5 days by rats pretreated for 10 days with naloxone (0.5 mg/kg) or H_2O.

ment 1 except as noted. Seven rats received the injections, while eight served as water injected controls. The injections continued for ten days and observations commenced on day seven. Observations were performed twice daily (8 a.m. and 8 p.m.), and each animal's total daily symptoms were tabulated. An additional symptom of "aggression" was recorded. This was defined as leaping on another rat and making biting motions with the head. This behavior is often seen in morphine abstinent rats in our laboratory.

On day two post treatment, four animals from each pretreatment group received a renewed dose of naloxone (0.6 mg/kg SC), while the remaining animals received water alone. All animals were observed 30 min post injection. Those receiving water during this test were injected the next day with 4 mg/kg morphine sulphate SC and observed 30 min afterward.

RESULTS

As Fig. 3 indicates, the naloxone-treated rats once more had consistently more symptoms than controls. (Since animals were tested twice daily, note that daily symptoms are twice the indicated value.) It is noteworthy that the upsurge in symptoms began during the naloxone treatment period and continued for several days after termination of treatment. A 2×5 analysis of variance with one factor having 5 repeated measures revealed a significant main effect of pre-treatment: naloxone vs placebo, $F(1,13)=5.8$, $p<0.05$, as well as days, $F(4,52)=4.39$, $p<0.05$, and a significant interaction: pre-treatment × days $F(4,52)=4.85$, $p<0.05$. Analysis of simple main effects [7] revealed significant differences (naloxone vs placebo) on day eight, $F(1,65)=4.4$, $p<0.05$, of treatment and day one post treatment, $F(1,65)=13.53$, $p<0.01$.

As Fig. 3 illustrates, the symptoms were once more virtually abolished by morphine sulphate while they were virtually unaffected by naloxone.

Figure 4 provides a breakdown of specific types of symptoms accumulated across days. The naloxone treated group had significantly more body shakes, $t(13)=3.01$, $p<0.01$, scratches, $t(13)=2.06$, $p<0.05$, and instances of aggression, $t(13)=1.87$, $p<0.05$. Head shakes were also elevated, but not significantly, $t(13)=0.62$, NS.

GENERAL DISCUSSION

The symptoms resulting from chronic naloxone pretreatment resembled an opiate abstinence syndrome in that they were completely reversed by a small dose of morphine. The symptoms cannot be interpreted as an indication of induced dependence on naloxone, since they began before naloxone treatment had ceased and were not reversed by naloxone. The present results suggest that a certain degree of ongoing natural stimulation of the endorphin system is necessary over the long run to prevent a pattern of abnormal behaviors or discomforts bearing some resemblance to those seen with opiate narcotic withdrawal. However, the degree of similarity of the physiological states underlying the two syndromes remains to be further investigated. To shed light on this question, studies are now in progress in our labora-

tory on the effects of chronic naloxone treatment on respiration, weight gain, and pain sensitivity.

One interesting aspect of the naloxone effect is its very gradual time course. It requires approximately six days of endorphin receptor blockade to produce the symptoms (four days injection series in other experiments resulted not in abstinence-like symptoms but in a condition of exaggerated startle and freezing responses). Furthermore, the abstinence-like symptoms persisted for several days after the cessation of naloxone treatment. This suggests that the endorphin system responds in a gradual manner to changes in receptor stimulation. It is interesting to recall the observation [19] that chronic naloxone infusion causes a gradual and persistent increase in the number of endorphin receptors.

The receptor blockade produced by two naloxone injections daily is far from continuous. If deprivation of endorphinergic stimulation produces abstinence-like symptoms, continuous naloxone blockade might well produce the symptoms more quickly and in greater number. Recent experiments in our laboratory with continuous infusion of naloxone via Alzet® osmotic minipump show this to be the case. After 27 hrs of naloxone infusion, rats exhibited a variety of abstinence-like phenomena such as body shakes, increased oxygen consumption and decreased weight gain [21].

It seems paradoxical that chronic administration of a pure opiate antagonist would have somewhat similar long term consequences to chronic administration of opiates themselves. This paradox would be resolved, however, if the body responded in negative feedback fashion to overstimulation of endorphin receptors by producing a naloxone-like blockade of the endorphin system. Indeed, it has been shown that administration of morphine renders animals more responsive to the effects of naloxone [26]. There is also some preliminary evidence [20, 27, 28] that brain extract from morphine dependent rats demonstrates some naloxone-like and morphine-dependence-promoting activity.

ACKNOWLEDGEMENTS

This study was supported by a University of Houston at Clear Lake City Organized Research Grant. Naloxone was donated by Endo Laboratories. We thank Suzanne H. Kurth for skillful editorial assistance.

REFERENCES

1. Baizman, E. R., B. M. Cox, O. H. Osman and A. Goldstein. Experimental alterations of endorphin levels in rat pituitary. *Neuroendocrinology* **28:** 402–424, 1979.
2. Berntson, G. G. and J. M. Walker. Effects of opiate receptor blockade on pain sensitivity in the rat. *Brain Res. Bull.* **2:** 157–159, 1977.
3. Blasig, J. *et al.* Involvement of endorphins in emotional hyperthermia of rats. *Life Sci.* **23:** 2525–2532, 1978.
4. Bodnar, R. J., D. D. Kelly, A. Spiaggia, C. Ehrenberg and M. Glusman. Dose-dependent reductions by naloxone of analgesia induced by cold-water stress. *Pharmac. Biochem. Behav.* **8:** 667–672, 1978.
5. Brands, B., J. A. Thornhill, M. Hirst and C. W. Gowdey. Suppression of food intake and body weight gain by naloxone in rats. *Life Sci.* **24:** 1773–1778, 1979.
6. El Sobky, A., J. O. Dostrovkky and P. D. Wall. Lack of effect of naloxone on pain perception in humans. *Nature, Lond.* **263:** 783–784, 1976.
7. Fanselow, M. S. and R. C. Bolles. Naloxone and shock-elicited freezing in the rat. *J. comp. physiol. Psychol.* **93:** 736–744, 1979.
8. Frederickson, R. C. A., V. Burgis and J. D. Edwards. Hyperalgesia induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. *Science* **198:** 756–758, 1977.
9. Gessa, G. L., E. Paglietti and B. Pellegrini Quarantotti. Induction of copulatory behavior in sexually inactive rats by naloxone. *Science* **204:** 203–205, 1978.
10. Goldstein, A., G. T. Pryor, L. S. Otis and F. Larsen. On the role of endogenous opiod peptides: Failure of naloxone to influence shock escape threshold in the rat. *Life Sci.* **18:** 599–604, 1976.
11. Grevert, P. and A. Goldstein. Some effects of naloxone on behavior in the mouse. *Psychopharmacology* **53:** 111–113, 1977.
12. Grevert, P. and A. Goldstein. Endorphins: Naloxone fails to alter experimental pain or mood in humans. *Science* **199:** 1093–1095, 1978.
13. Herman, B. H. and J. Panksepp. Effects of morphine and naloxone on separation distress and approach attachment: Evidence for opiate mediation of social affect. *Pharmac. Biochem. Behav.* **9:** 213–220, 1978.
14. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *J. Pharmac. exp. Ther.* **189:** 51–60, 1974.
15. Holtzman, S. G. Effects of narcotic antagonists on fluid intake in the rat. *Life Sci.* **16:** 1465–1470, 1975.
16. Jacob, J. J. and K. Ramabradran. Opioid antagonists, endogenous ligands and nociception. *Eur. J. Pharmac.* **46:** 393–394, 1977.
17. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences.* Belmont, CA: Wadsworth, 1968, pp. 263–266.
18. Kokka, N. and A. S. Fairhurst. Naloxone enhancement of acetic acid-induced writing in rats. *Life Sci.* **21:** 975–980, 1977.
19. Lahti, R. A. and R. J. Collins. Chronic naloxone results in prolonged increases in opiate binding sites in brain. *Eur. J. Physiol.* **51:** 185–186, 1978.
20. Malin, D. H. and G. J. Radcliffe. Brain extract from morphine dependent rats facilitates morphine dependence. *Soc. Neurosci. Abstr.* **1:** 296, 1975.
21. Malin, D. H., M. R. Reagan, J. Leavell and C. A. Westmoreland. Continuous naloxone infusion induces abstinence-like syndrome. *Proc. 11th A. Meeting. Soc. Neurosci.* **7:** 798, 1981.
22. Margules, D. L., B. Moisset, M. J. Lewis and C. B. Pert. Beta-Endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* **202:** 988–991, 1978.
23. Sawynok, J., G. Pinsky and F. S. LaBella. Minireview on the specificity of naloxone as an opiate antagonist. *Life Sci.* **25:** 1621–1632, 1979.
24. Sewell, R. D. E. and P. S. J. Spencer. Antinociceptive activity of narcotic antagonists and partial agonists analgesics and other agents in the tail immersion test in mice and rats. *Neuropharmacology* **15:** 683–688, 1976.
25. Sherman, J. E. and J. C. Liebeskind. An endorphinergic, centrifugal substrate of pain modulation: Recent findings, current concepts, and complexities. In: *Pain*, edited by J. J. Bonica. New York: Raven Press, 1980.
26. Takemori, A. E., T. Oka and N. Nishiyama. Alteration of analgesic receptor-antagonist interaction by morphine. *J. Pharmac. exp. Ther.* **186:** 261–265, 1973.
27. Ungar, G., A. Ungar and D. H. Malin. Brain peptides with opiate antagonist activity. In: *Opiates and Endogenous Opioid Peptides*, edited by H. Kosterlitz. Amsterdam: Elsevier, 1976.
28. Ungar, G., A. Ungar, D. H. Malin and G. Sarantakis. Brain peptides with opiate antagonist action: Possible role in tolerance and dependence. *Psychoneuroendocrinology* **2:** 1–10, 1977.
29. Wesche, D. L. and R. C. Frederickson. Diurnal differences in opioid peptide levels correlated with nociceptive specificity. *Life Sci.* **24:** 1861–1867, 1979.