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Blockade of Endogenous Opiates Reduces Activity in the Rat

J. MICHAEL WALKER,[1] GARY G. BERNTSON,
THOMAS S. PAULUCCI AND TIMOTHY C. CHAMPNEY

The Ohio State University, 1314 Kinnear Road, Columbus, OH 43212

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WALKER, J. M., G. G. BERNTSON, T. S. PAULUCCI AND T. C. CHAMPNEY. *Blockade of endogenous opiates reduces activity in the rat.* PHARMAC. BIOCHEM. BEHAV. 14(1) 113–116, 1981.—Naloxone (2 mg/kg, SC) was found to result in a substantial and significant reduction in general activity levels in the rat (90–120 days old). This effect was seen both under baseline conditions and after stress manipulations which would be expected to result in elevated levels of endogenous opiate peptides. Thus, under baseline conditions general activity was reduced to less than half of the saline control value thirty min after injection. Similarly, a reduction was seen after stress induced by a 30 min swim. While naloxone may have some non-opiate effects, these results support the view that endogenous opiate systems may play an important activational role in behavioral regulation, under baseline conditions and conditions of stress.

Naloxone Endorphins Stress

OPIATE-like peptides and their analogs can induce marked analgesia and behavioral sedation when administered centrally [1, 3, 13, 15, 24, 26, 27]. Additional studies suggest that analgesia may also result from the natural action of endogenous peptides. This is indicated by the fact that blockade of opiate receptors by naloxone, in otherwise untreated animals, results in a heightened responsiveness to certain pain stimuli [2, 7, 11, 14, 18]. Furthermore, analgesia induced by stress manipulations appears to be mediated by a release of endogenous opiate-like compounds, since this analgesia shows cross tolerance with morphine and can be blocked by naloxone [5,21]. The natural action of endogenous opiates on behavioral activation, however, remains less clear. While high doses of opiate peptides produce clear sedational effects [3, 13, 26, 27], lower doses result in enhanced activity [9,16]. Further, blockade of opiate receptors by naloxone has been reported to decrease behavioral activity in novel situations [16,17].

In order to further examine this question, we tested the effects of naloxone on baseline activity levels. Since stress has been suggested to result in the central release or activation of endogenous opiate systems [5,21], we also examined the effects of naloxone on activity levels following stress. In both cases, naloxone was found to result in significant decreases in activity, supporting the view that endogenous opiate systems may have an activational influence on at least some classes of behavior.

METHODS

Six male and six female (90–120 day old) Holtzman albino rats were tested for activity levels after naloxone administration (2 mg/kg in 1 ml saline, SC), and after administration of the saline vehicle alone. Activity levels were measured by electrostatic field-type counters (Quartec). After an initial habituation session of 1 hr, each animal received four drug tests, two with naloxone and two with the saline vehicle. The habituation session and the drug tests were separated by 48 hr, and drug treatments were given in a balanced abba sequence with the order of drug treatments counterbalanced across animals. Each test session consisted of a five min preinjection baseline period, during which activity was monitored. After this baseline period, rats were given a drug treatment, order as between subjects variables, and replication (first or second drug test) and time interval (nine repeated measures over 45 min) as within subject variables. All tests were conducted between 8:00 a.m. and 2:00 p.m.

A separate group of 16 (90–120 day old) male albino rats was also used to examine the effects of naloxone on activity levels after stress manipulations. The present dose was used since it was found to readily block a variety of potent analogs of enkephalins and endorphins [26,27]. Activity was monitored with the activity counters described above. Two levels of stress manipulation were used; a three min warm water swim (23°C), and a three min cold water swim (2°C) in a 90×60 cm plastic cylinder. Each animal received four tests with all combinations of the treatments (cold water - naloxone, cold water - vehicle, warm water - naloxone, warm water - vehicle). Each test consisted of a three min swim, after which the animals were placed into the activity counters. Fifteen min of baseline activity measures were then taken (at five min intervals), and a drug treatment was given. Activity measures were subsequently taken in three blocks of three five min periods each, over the subsequent 45

[1]Send reprint requests to J. Michael Walker, Mental Health Research Institute, University of Michigan, 202 Washtenaw Pl., Ann Arbor, MI 48109.

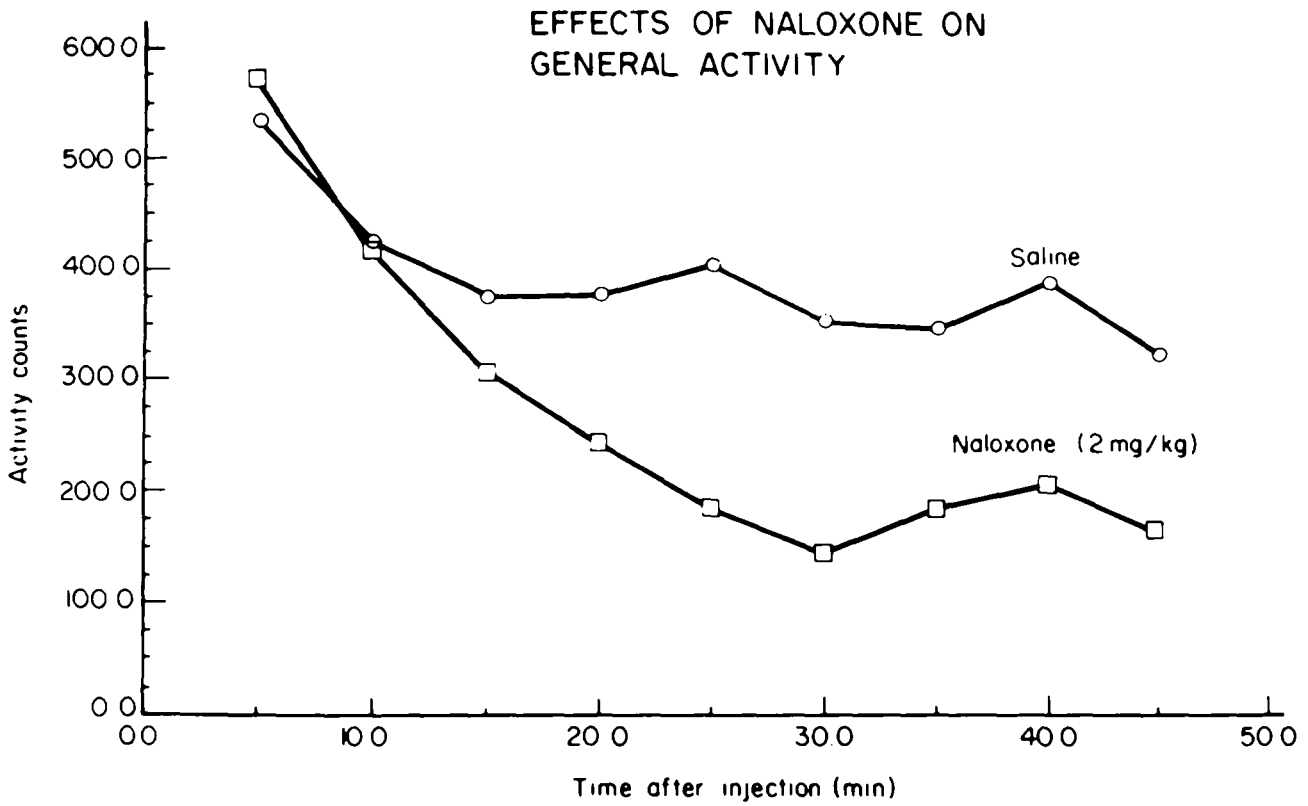

FIG. 1. Effects of naloxone or saline baseline activity levels over blocks of five minutes. Preinjection levels were not significantly different (Saline: mean=815 ± 144 S.E.M., naloxone: mean=826 ± 151 S.E.M.). An overall drug effect was revealed by analysis of variance ($p<0.005$).

min. The order of treatment was counterbalanced. Thus, half the animals received the two cold water tests, separated by 48 hr, and one month later were given the two warm water tests. The other half of the animals were given the warm and cold water tests in the opposite order. In all cases, order of drug treatments was also counterbalanced across animals. Results were analyzed by a repeated measures analysis of variance with drug treatment, level of stress, and time interval as within subjects variables.

RESULTS

As illustrated in Fig. 1, naloxone resulted in approximately a two-fold reduction in activity related to saline tests, $F(1,11)=8.58$, $p<0.15$. Activity declined over time under both conditions, with naloxone enhancing this decline in a time dependent fashion after injection, $F(8,88)=3.16$, $p=0.003$. The maximum drug effects was evident at about 30 min postinjection. Additional analysis revealed no significant effect of sex or order of drug treatment.

Figure 2 illustrates the effects of naloxone on activity after two levels of stress. As shown in Fig. 2 the cold water swim resulted in a greater reduction in activity than did the warm water swim, $F(1,15)=29.8$, $p<0.01$, and further, naloxone failed to reverse the stress-related depressions in activity. Indeed naloxone resulted in a significant time dependent depression of activity under both conditions of stress, $F(2,30)=7.83$, $p=0.002$, with no significant drug by water temperature interaction. As with baseline activity levels, this naloxone effect reached maximum at about 30 min after injection.

DISCUSSION

Results of the present study indicate that blockade of endogenous opiate receptors, by the opiate antagonist naloxone, leads to a significant reduction in the baseline activity levels in the rat. These results are generally consistent with the reports on the mouse that naloxone reduces exploration in novel situations [16,17]. However, since the animals in the present study received prior adaptation to the testing conditions, it does not appear that naloxone-induced reductions in activity are dependent upon novel testing situations. These findings support the suggestion that endogenous opiate systems can exert an activational influence on behavior [16,17], but also enhance grooming [9], and eating [10], behaviors. Conversely, blockade of opiate receptors by naloxone results in a reduction in eating and drinking and grooming [12,22].

While low doses of endorphins can have an activational effect on behavior [9,16], higher doses of these peptides produce clear sedational effects [2, 3, 13, 15]. Thus, it is possible that natural opioid peptides may exert a depressive

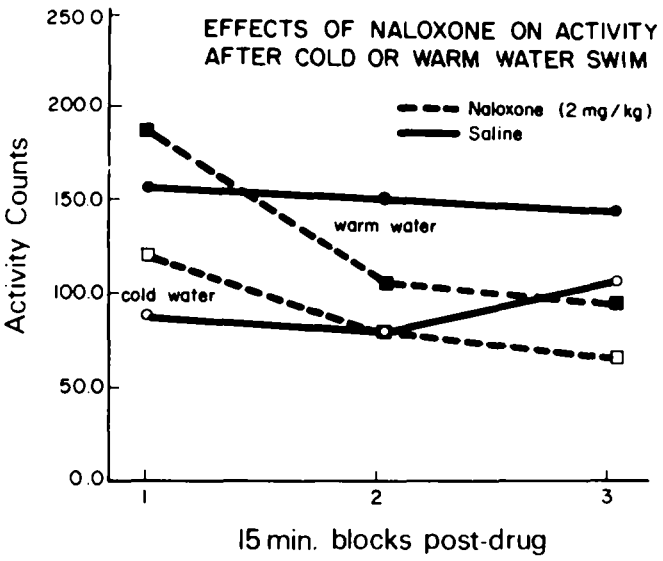

FIG. 2. Effect of naloxone or saline on activity after two levels of stress (cold water or warm water swim). Baseline measures were taken for fifteen min after stress manipulation, and drug treatment was then administered. For a given stress level preinjection baseline activities on saline or naloxone tests were not significantly different. Analysis of variance revealed a significant drug effect ($p<0.01$) and a significant depression of activity by cold water ($p<0.01$) but no interaction between water temperature and drug was observed. Baseline values were: warm water (saline: mean=254.1 ± 220 S.E.M., naloxone: mean=274.9 ± 22.6 S.E.M.), cold water (saline: mean=105 ± 23 S.E.M., naloxone: mean=99.9 ± 22.3 S.E.M.).

effect upon behavior at higher endogenous levels, such as those associated with stress [5,21]. Results of the present study, however, argue against this possibility. Blockade of opiate receptors by naloxone was found to significantly reduce behavioral activity levels even after stress manipulations.

The present data support the suggestion that endogenous opiate systems may play an important activational role in behavioral regulation. Indeed, there is a high correlation between the reported circadian rhythmicity in levels of endogenous opiates and circadian cycles of behavioral activity [7]. According to this general conception, a stress-related release of endorphins would be expected to result in behavioral activation, which could have substantial adaptive value to an organism in behavioral attempts to cope with the stressful condition.

These conclusions assume that the present effects of naloxone are mediated by opiate receptors. In that the opposite effect (activation) has been observed after administration with endogenous opiates, this assumption is probably not unwarranted. Nevertheless several effects of naloxone on other transmitter systems have been observed (Cf. [25]). For example at high concentrations naloxone appears to interact with GABA receptors [6]. Conversley, some effects of Met-Enkephalin are not reversed by naloxone. These include some effects on single neurons [8], on rotational behavior [4], and a local effect on the ear artery of the rabbit [19]. Naloxone has also been found to be much less potent when competing for κ- and δ-receptors [20,23]. Thus while naloxone remains one of the most specific pharmacological agents available, recent data open alternative explanations for effects observed after administration of naloxone.

REFERENCES

1. 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.
2. Berntson, G. G. and J. M. Walker. Effect of opiate receptor blockade on pain sensitivity in the rat. *Brain Res. Bull.* **2**: 157–159, 1977.
3. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **194**: 630–632, 1976.
4. Chang, J., T. W. Fong, A. Pert and C. Pert. Opiate receptor affinities and behavioral effects of enkephalin: Structure-activity relationship of ten synthetic peptide analogues. *Life Sci.* **18**: 1473, 1976.
5. Chesher, G. B. and B. Chan. Footshock induced analgesia in mice: its reversal by naloxone and cross tolerance with morphine. *Life Sci.* **21**: 1569–1574, 1978.
6. Dingledine, R., L. L. Iverson and E. Breuker. Naloxone as a GABA antagonist: Evidence from iontophoretic, receptor binding and convulsant studies. *Eur. J. Pharmac.* **47**: 19–29, 1978.
7. Frederickson, R. C., V. Burgis and J. D. Edwards. Hyperalgesia-induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. *Science* **198**: 756–758, 1977.
8. Gent, J. P. and J. H. Wolstencroft. Effects of methionine-enkephalin and leucine-enkephalin compared with those of morphine on brainstem neurones in cats. *Nature* **261**: 426, 1976.
9. Gispen, W. H., V. M. Wiegert, A. F. Bradbury, E. C. Hulme, D. G. Stein, C. R. Snell and D. Deinerd. Induction of excessive grooming in the rat by fragments of β-lipotropin. *Nature* **264**: 794–795, 1977.
10. Grandison, L. and A. Guidotti. Stimulation of food intake by muscuimol and β-endorphin. *Neuropharmacology* **16**: 533–536, 1977.
11. Grevert, P. and A. Goldstein. Some effects of naloxone on behavior in the mouse. *Psychopharmacology* **53**: 111–113, 1977.
12. Holtzman, S. G. Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. *Life Sci.* **24**: 219–226, 1979.
13. Izumi, K., T. Motamatsu, M. Chretien, R. F. Butterworth, M. Lis, N. Seidah and A. Barbeau. β-endorphin induced akinesia in rats: effects of apomorphine and α-methyl-P-tyrosine and related modifications of dopamine turnover in the basal ganglia. *Life Sci.* **20**: 1149–1156, 1977.
14. Jacob, J. J., E. C. Tremblay and C. Colmbel. Facilitation de reactions nociceptives par la naloxone chez la souris et chez le rat. *Psychopharmacologia* **37**: 217–223, 1974.
15. Jacquet, Y. and N. Marks. C-fragment of β-lipotropin: an endogenous neuroleptic or antipsychotogen? *Science* **194**: 632–635, 1976.
16. Katz, R. J., B. J. Carroll and G. Baldrighi. Behavioral activation by enkephalins in mice. *Pharmac. Biochem. Behav.* **8**: 493–496, 1978.
17. Katz, R. J. and J. Gelbart. Endogenous opiates and behavioral responses to environmental novelty. *Behav. Biol.* **24**: 338–348, 1978.
18. Kokka, N. and A. S. Fairhurst. Naloxone enhancement of acetic acid-induced writhing in rats. *Life Sci.* **265**: 358–360, 1978.
19. Knoll, J. Neuronal peptide (enkephalin) receptors on the ear artery of the rabbit. *Eur. J. Pharmac.* **39**: 403, 1976.
20. Lord, J. H., A. A. Waterfield, H. Hughes and H. A. Kosterlitz. Endogenous opioid peptides: Multiple agonists and receptors. *Nature* **267**: 495, 1977.
21. Madden, D. L., H. Akil, R. L. Patrick and J. D. Barchas. Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. *Nature* **265**: 358–360, 1977.

22. Margules, D. L., B. Moisset, M. J. Lewis, H. Shibuya and C. Pert. β-endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* **202:** 988–991, 1978.
23. Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler and P. E. Gilbert. Effect of morphine and nalorphine-like drugs in the nondependent and morphine dependent chronic spinal dog. *J. Pharmac. exp. Ther.* **197:** 517, 1976.
24. Pert, C. B., A. Pert, J. K. Chang and B. T. W. Fong (D-Ala2) Met-enkephalinamide: a potent, long-lasting synthetic pentapeptide analgesic. *Science* **194:** 330–332, 1976.
25. Sawynok, J., C. Pinsky and F. S. LaBelle. On the specificity of naloxone as an opiate antagonist. *Life Sci.* **25:** 1621–1632, 1979.
26. Walker, J. M., G. G. Berntson, C. A. Sandman, D. A. Coy, V. Shally and A. J. Kastin. An analog of enkephalin having prolonged opiate like effects *in vivo*. *Science* **196:** 85–87, 1977.
27. Walker, J. M., C. A. Sandman, G. G. Berntson, R. McGivern, D. H. Coy and A. J. Kastin. Endorphin analogs with potent and long lasting analgesic effects. *Pharmac. Biochem. Behav.* **7:** 543–548, 1977.