Pharmacology Biochemistry & Behavior, Vol. 11, pp. 387–390. Printed in the U.S.A.

Suppressant Effects of Naltrexone on Water Intake in Rats

HANAN FRENK AND JEFFREY B. ROSEN

Psychobiology Research Unit, Department of Psychology, Tel-Aviv University
Ramat Aviv, Israel

Received 4 May 1979

FRENK, H. AND J. B. ROSEN. *Suppressant effects of naltrexone on water intake in rats.* PHARMAC. BIOCHEM. BEHAV. 11(4) 387–390, 1979.—Parenteral administration of naltrexone to water-deprived female rats suppressed water intake when injected 4, but not 12 hours prior to the drinking session. Intraperitoneal injection 5 mg/kg naltrexone twice daily or oral self-administration of naltrexone in doses sufficient to block morphine-induced analgesia failed to reduce water intake or to alter body weight in non-deprived animals. These findings suggest that the suppressant effects of naltrexone on appetitive behavior are mediated by a different population of opiate receptors than those mediating morphine-induced analgesia.

Analgesia Body weight Morphine Naltrexone Opiate Receptor Water intake

THE discovery of endogenous opioids in the normal mammalian brain [18] has stimulated various hypotheses concerning the role of these peptides in normal behavior. The analgesic and euphoric properties of opiates have been known for some time [21]. In view of the pharmacological similarities between the endogenous peptides and the exogenous alkaloids it is not surprising that much experimental evidence concerning the normal function of endogenous opioids pertains precisely to those two properties.

The analgesic action of both enkephalins and β-endorphin when applied directly to the brain has been demonstrated (e.g. [3, 8, 20, 31]). Analgesia induced by certain behavioral [1,6] or physiological [2] manipulations in rats may be partially reversed by the specific opiate antagonist, naloxone. In addition it has been reported that naloxone lowers the pain-threshold in normal animals [11,19] and humans [7] when administered in the absence of opiates.

Evidence pertaining to the euphoric properties of endogenous opioids has been presented in several experimental paradigms. It has been demonstrated that animals will self-administer both leucine- and methionine-enkephalin [4]. Electrical self-stimulation from the enkephalin-containing periaqueductal central grey was reduced by the administration of naloxone [4]. Finally, normal animal behavior assumed to produce drive-reduction reward such as eating and drinking [4], is suppressed by the prior administration of both naloxone and naltrexone [14, 15, 16, 24].

Whereas all this evidence supports the hypothesis, that endogenous enkephalin may mediate drive-reduction reward [4], other interpretations are possible. The aversive properties inherent to opiate antagonists as demonstrated in the conditioned taste aversion (CTA) paradigm [14, 22, 32] may induce sickness in rats, and because of this sickness animals will neither drink nor feed. Moreover, both morphine as well as opiate antagonists will suppress water intake in rats [16]. Whereas simultaneous administration of agonist and antagonist does not yield additive suppressant effects, no complete mutual antagonism has been found [14]. Therefore an alternative explanation that naloxone antagonizes the suppressant effects of morphine on food and water intake, but exerts similar suppressant effects not mediated by the same opiate receptors, cannot be excluded.

The present experiments were designed to accumulate further evidence pertaining to the question whether opiate antagonists suppress water intake by blocking specific opiate receptors.

EXPERIMENT 1: LONG-TERM NALTREXONE ADMINISTRATION TO NON-DEPRIVED RATS

The opiate antagonist naltrexone has been demonstrated to prevent opiate agonist action for a period of 12 hr and more following a single injection or oral administration in man [26,27]. If the suppressant effects naltrexone has on feeding and drinking in deprived animals are indeed mediated by specific opiate receptors, we would expect similar long-lasting effects of a single injection of naltrexone at comparable doses on these behaviors. Furthermore, injections of naltrexone at regular intervals should significantly suppress drinking and feeding, hence also weight gain, in non-deprived rats when compared to saline-injected controls. The present experiment was designed to test these hypotheses.

METHOD

Locally bred female Wistar rats, 120 days old at the beginning of the experiment, were individually housed in a room with a reversed light/dark cycle (light on from 8:00 p.m. to 8:00 a.m.). Food ad lib was always present. All animals were adapted to a 23 hr water-deprivation schedule (deprivation started at noon and continued until 11:00 a.m. the next day). During the 8-day period of adaptation to this

schedule, animals were weighed and injected intraperitoneally (IP) with physiological saline (1 ml/kg) 15 min before each hour-long drinking session. At 11:00 a.m. a calibrated Richter drinking tube was introduced into the home cage. The amount of water drunk was recorded at noon and the drinking tube was withdrawn. After the drinking session of the 8th day animals were assigned to three groups (n=5). These animals were injected IP with 5 mg/ml/kg naltrexone, either 12 hr, 4 hr, or 15 min prior to the 9th drinking session. An additional ten female and ten male Wistar rats of the same age were housed under conditions described above. However, these animals were not deprived and had both food and water ad lib. During the 8-day adaptation period the animals were weighed and their water intake was measured at 8:00 a.m., and injected IP with physiological saline (1 ml/kg) at 8:00 a.m. and 8:00 p.m. On the 9th day these animals were assigned to two groups. One group, consisting of five males and five females, now received injections of 5 mg/ml/kg of naltrexone IP following the schedule of the saline injections they received during the adaptation period. The second group, consisting of the remainder of the animals continued to be injected with 1 ml/kg of saline following identical procedures as during the adaptation period. The two groups were maintained on this schedule for eight consecutive days.

RESULTS AND DISCUSSION

Body weight and water intake remained stable for all animals during the adaptation period. The animals receiving injections of 5 mg/kg naltrexone every 12 hr did not reduce their body weight or water intake over the first day or any of the remaining days of the treatment when compared to the saline-injected controls, or when compared to their own baseline.

Deprived animals injected with 5 mg/kg naltrexone reduced water intake on the following drinking session when the injection was administered 15 min ($p<0.01$) or 4 hr ($p<0.05$), but not 12 hr before (see Fig. 1).

These results may be explained in several ways:

(1) Naltrexone is metabolized more rapidly in rats than in humans. If in humans naltrexone is still effective in blocking opiate effects after more than 12 hr [26,27], in rats naltrexone may be eliminated from the organism in less than 12 hr. This conclusion seems partially supported by Blumberg and Dayton [5] who found that naltrexone (2 mg/kg) loses in efficacy in antagonizing opiate effects after 4 hr. However, the dose of the drug was much lower than in the present study, and its route of administration was orally.

(2) Naltrexone is still actively present in the organism, but for several reasons the drug does not suppress drinking or feeding anymore. The following experiment attempted to answer these questions.

EXPERIMENT 2: ORAL SELF-ADMINISTRATION OF NALTREXONE

METHOD

Ten female Wistar rats, 120 days old at the onset of the experiment, were maintained and adapted under conditions identical to those described for the non-deprived animals of Experiment 1. After 8 days of adaptation animals were assigned to the experimental groups. Five animals now received instead of their normal drinking water a naltrexone-solution (1 mg/ml). The remaining animals were presented with a quinine-solution (0.25 mg/ml) to control for the bitter taste of naltrexone. Apart from the change in drinking regimens, which was maintained for 7 days, animals received the same treatment as during the adaptation period.

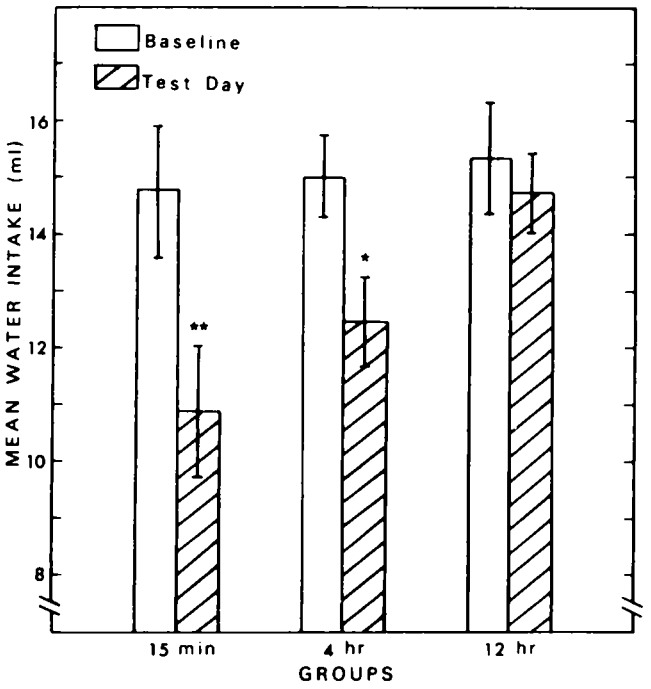

FIG. 1. Effects of naltrexone (5 mg/kg) on water intake in water-deprived rats when injected 15 min, 4 hr, or 12 hr prior to the drinking session. *$p<0.05$; **$p<0.01$.

Immediately after the animals were weighed and their water intake measured on the 7th day, the effectiveness of the consumed naltrexone in antagonizing morphine-induced analgesia was ascertained. A modified version of the tail-flick method [10,13] was used. The animals were placed in a homemade Plexiglas animal restrainer from which their tail extended. the tail was placed over a small opening in the top of a box containing a radiant heat source. When the heat source was switched on, a time counter was simultaneously activated. When the animal withdrew its tail, the experimenter deactivated both the heat source and the counter, the latter now indicating the latency until tail withdrawal. If the animal did not withdraw its tail within 10 sec, the heat source was switched off automatically to prevent tissue damage to the tail. Animals were now subjected to five trials on the tail-flick apparatus, separated by 3 min intervals. The last 4 of these 5 trials were averaged for each animal and were taken as a baseline estimate of its pain threshold. Immediately following baseline trials the animals were injected with 10 mg/ml/kg of morphine hydrochloride. Starting 3 min after the injection all animals were submitted to an additional 7 trials on the tail-flick apparatus following the same procedures as during baseline trials.

RESULTS

All animals used in this experiment showed stable water intake and body weight over the 8-day adaptation period.

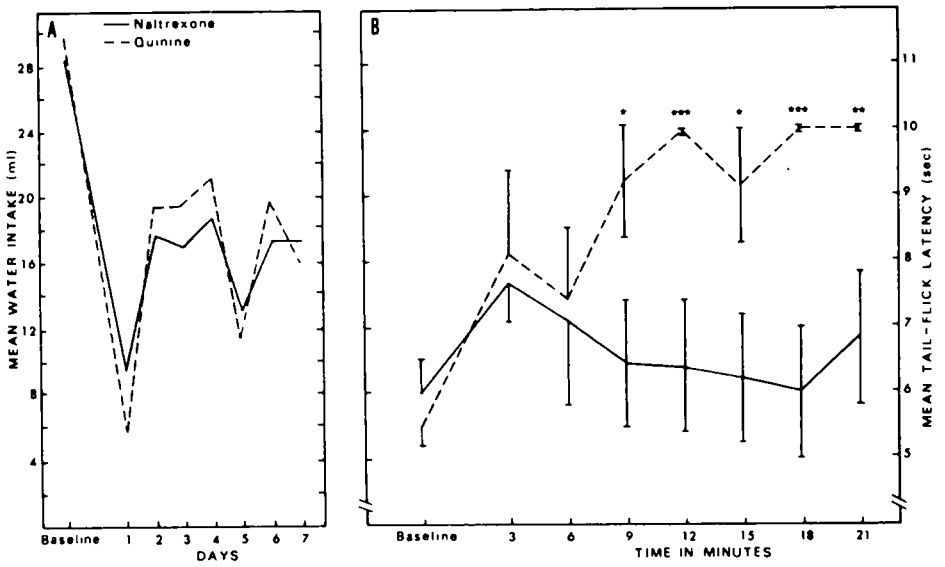

FIG. 2. A. Both naltrexone and quinine suppress water intake when administered in the drinking water. Naltrexone is not more effective than quinine though B. naltrexone is effective in antagonizing analgesia induced by morphine (10 mg/kg). *$p<0.05$; **$p<0.01$; ***$p<0.005$.

The introduction of the naltrexone and quinine solutions reduced water intake significantly (see Fig. 2A) in both groups ($p<0.005$). Whereas water intake was significantly higher on the second day of the new drinking regimen when compared to the first day for both groups ($p<0.01$), intake on this and the following days remained significantly lower when compared to baseline ($p<0.01$). There was no difference between naltrexone and quinine drinkers in respect to their water intake on any of the seven days. Over the seven days of reduced drinking no weight loss occurred in either group and no difference between the groups in respect to body weight was noticed.

The baseline threshold to pain induced by heat appeared to be no different for the naltrexone drinkers than for the quinine drinkers (see Fig. 2B). However, whereas the animals drinking quinine showed a good analgesic response following morphine (10 mg/kg), the naltrexone drinkers did not change their tail-flick latencies when compared to baseline. The quinine drinkers had significantly longer tail-flick latencies than the naltrexone drinkers from the 9th minute post-injection onwards.

GENERAL DISCUSSION

The parenteral administration of naltrexone (5 mg/kg) at 12 hr intervals did not interfere with normal water intake or cause any weight loss in non-deprived animals. It was found, that this same dose of naltrexone did reduce water intake in water-deprived rats when administered 4 hr, but not 12 hr before the drinking session. This finding suggests that this dose of naltrexone is eliminated from the organism in less than 12 hr, and could therefore explain the lack of effect of naltrexone in non-deprived animals. Consequently, animals were maintained with drinking water containing either naltrexone (1 mg/ml) or quinine. Whereas a reduction in water intake occurred in the naltrexone drinkers, this effect could not be attributed to any specific pharmacological action of naltrexone, since the quinine drinking animals showed a similarly reduced water intake. It seems therefore that the taste of the two drinking solutions, matched for aversiveness, was responsible for this reduction. This is supported by the finding that the naltrexone drinking animals did not lose weight, in spite of the fact that the average naltrexone intake of the animals over the last six days was 16.8 mg/animal/day, or about 93.5 mg/kg/day. This same dose of naltrexone blocked opiate receptors in those animals, as a dose of 10 mg/kg morphine induced analgesia in the quinine, but not in the naltrexone drinking rats. Two different explanations could account for the lack of effect on water intake and body weight in non-deprived animals by chronic naltrexone administration.

First, different opiate receptors may mediate the analgesic and euphoric effects of opioids. The existence of different types of opiate receptors has been suggested by Martin and coworkers [25]. Recently, additional evidence has been provided demonstrating that enkephalins and morphine act on different opiate receptors in selective ways [23]. Finally, support has been provided for the hypothesis that opiate receptors mediating analgesic and EEG responses to morphine and endogenous opioids are not only pharmacologically different, but concentrated in different brain areas [12]. If endogenous opioids do play a role in drinking and feeding, naltrexone could be expected to be less effective in blocking receptors mediating these behaviors than blocking the analgesic response to morphine, mediated by different receptors. The observation that relatively large quantities of naloxone are needed to antagonize the suppressant effects of morphine on drinking [14] or conditioned taste aversion (CTA) induced by morphine [22,32] supports this view. On the other hand, drinking in deprived rats is significantly reduced by doses of naltrexone as low as 0.1 mg/kg [16].

It is, however, possible to explain these results in a more simple fashion. Opiate antagonists, like opiate agonists, may cause nausea in humans [26,27] and may serve as UCS in the CTA paradigm [9, 14, 22, 32]. Presumably then, opiate antagonists have an aversive property which may be the cause of the suppressant effects on food and water intake. The

present findings indicate, that non-deprived animals habituate rather quickly to these aversive properties and normalize their water intake within 24 hr. This implies both that the suppressant effects of naltrexone are not mediated by opiate receptors, and therefore that endogenous opioids do not mediate normal water intake. This conclusion is supported by data recently reported by Holtzman [17]. One week following morphine withdrawal of dependent rats tolerance to the analgesic effects of morphine were still observed. However, at the same time the suppressant effects of naloxone on water intake in these animals were not different from those in morphine-naive animals. As previous experimentation [28, 29, 30] had shown that the morphine-antagonist activity of naloxone was enhanced in animals that had been pretreated with morphine when analgesic tests were used. Holtzman's data [17] indicate that the suppressant effects of naloxone on appetitive behavior are mediated by a different receptor system than its antagonism of morphine analgesia.

In conclusion, the major observation in the present experiments is that naltrexone suppresses appetitive behavior in deprived, but not in non-deprived rats, though effectively antagonizing morphine-induced analgesia in these animals. The suppressant effects of naltrexone do not seem to be mediated by the same opiate receptors which mediate morphine-induced analgesia.

ACKNOWLEDGEMENTS

These studies have been supported by a grant of the B. De Rothschild Foundation for the Advancement of Science in Israel, Inc. The naltrexone used in these studies was generously supplied by Endo Laboratories, Garden City, NY.

REFERENCES

1. Akil, H., J. Madden, R. L. Patrick and J. D. Barchas. Stress-induced increase in endogenous opiate peptides. In: *Opiates and Endogenous Opiate Peptides*, edited by H. W. Kosterlitz. Amsterdam: North-Holland Biomedical Press, 1976, pp. 63–70.
2. Akil, H., D. J. Mayer and J. C. Liebeskind. Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science* **191:** 961–962, 1976.
3. 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 the rat. *Nature* **260:** 625–626, 1976.
4. Belluzzi, J. D. and L. Stein. Enkephalin: possible mediator of euphoria and drive-reduction reward. *Nature* **266:** 556–558, 1977.
5. Blumberg, H. and H. B. Dayton. Naloxone, naltrexone and related noroxymorphones. *Adv. Biochem. Psychopharmac.* **8:** 33–43, 1974.
6. 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.
7. Buchsbaum, M. S., G. C. Davis and W. E. Bunney, Jr. Naloxone alters pain perception and somatosensory evoked potentials in normal subjects. *Nature* **270:** 620–622, 1977.
8. Büscher, H. H., R. C. Hill, D. Römer, F. Cardinaux, A. Closse, D. Hauser and J. Pless. Evidence for analgesic activity of enkephalin in the mouse. *Nature* **261:** 423–425, 1976.
9. Cappell, H. and A. E. LeBlanc. Aversive conditioning by psychoactive drugs: effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacologia* **29:** 239–246, 1973.
10. D'Amour, F. E. and D. L. Smith. A method for determining loss of pain sensation. *J. Pharmac. exp. Ther.* **72:** 74–79, 1941.
11. 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.
12. Frenk, H., B. C. McCarty and J. C. Liebeskind. Different brain areas mediate the analgesic and epileptic properties of enkephalin. *Science* **200:** 335–337, 1978.
13. Frenk, H., G. Urca and J. C. Liebeskind. Epileptic properties of leucine- and methionine-enkephalin: comparison with morphine and reversibility by naloxone. *Brain Res.* **147:** 327–337, 1978.
14. Frenk, H. and G. H. Rogers. The suppressant effects of naloxone on food and water intake in the rat. *Behav. Neural. Biol.* **26:** 23–40, 1979.
15. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *J. Pharmac. exp. Ther.* **189:** 51–60, 1974.
16. Holtzman, S. G. Effects of narcotic antagonists on fluid intake in the rat. *Life Sci.* **16:** 1465–1470, 1975.
17. 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.
18. 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.
19. Jacob, J. J., E. C. Tremblay and M. C. Colombel. Facilitation de réactions nociceptives par la naloxone chez la souris et chez le rat. *Psychopharmacologia* **37:** 217–223, 1974.
20. Jacquet, Y. F. and N. Marks. The C-fragment of β-Lipotropin: an endogenous neuroleptic or antipsychotogen? *Science* **194:** 630–632, 1976.
21. Jaffe, J. H. Drug addiction and drug abuse. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman and A. Gilman. London: MacMillan Co., 1970, pp. 276–313.
22. LeBlanc, A. E. and H. Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmac. Biochem. Behav.* **3:** 185–188, 1975.
23. Lord, J. A. H., A. A. Waterfield, J. Hughes and H. W. Kosterlitz. Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267:** 495–499, 1977.
24. Maickel, R. P., M. C. Braude and J. E. Zabik. The effects of various narcotic agonists and antagonists on deprivation-induced fluid consumption. *Neuropharmacology* **16:** 863–866, 1977.
25. Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler and P. E. Gilbert. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.* **197:** 517–532, 1976.
26. Martin, W. R., D. R. Jasinski and P. A. Mansky. Naltrexone, an antagonist for the treatment of heroin dependence. *Archs gen. Psychiat.* Chicago **28:** 784–791, 1973.
27. Resnick, R. B., J. Volavka, A. M. Freedman and M. Thomas. Studies of EN-1639A (naltrexone): a new narcotic antagonist. *Am. J. Psychiat.* **131:** 646–650, 1974.
28. Takemori, A. E., T. Oka and N. Nishiyama. Alteration of analgesic receptor-antagonist interaction induced by morphine. *J. Pharmac. exp. Ther.* **186:** 261–265, 1973.
29. Tulunay, F. C. and A. E. Takemori. The increased efficacy of narcotic antagonists induced by various narcotic analgesics. *J. Pharmac. exp. Ther.* **190:** 395–400, 1974.
30. Tulunay, F. C. and A. E. Takemori. Further studies on the alteration of analgesic receptor-antagonist interaction induced by morphine. *J. Pharmac. exp. Ther.* **190:** 401–407, 1974.
31. Urca, G., H. Frenk, J. C. Liebeskind and A. N. Taylor. Morphine and enkephalin: analgesic and epileptic properties. *Science* **197:** 83–86, 1977.
32. Van Der Kooy, D. and A. G. Phillips. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. *Pharmac. Biochem. Behav.* **6:** 637–641, 1977.