Pharmacology Biochemistry & Behavior, Vol. 23, pp. 1065–1068, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

BRIEF COMMUNICATION

Naloxone and Fluid Consumption in Rats: Dose-Response Relationships for 15 Days

GAYLE A. OLSON, SARAH W. DELATTE, ABBA J. KASTIN,*
JACK H. McLEAN, DONNA F. PHILLPOTT AND RICHARD D. OLSON

Department of Psychology, University of New Orleans, New Orleans, LA 70148
*and *Veterans Administration Medical Center*
and Tulane University School of Medicine, New Orleans, LA 70146

OLSON, G. A., S. W. DELATTE, A. J. KASTIN, J. H. McLEAN, D. F. PHILLPOTT AND R. D. OLSON. *Naloxone and fluid-consumption in rats: Dose-response relationships for 15 days.* PHARMACOL BIOCHEM BEHAV **23**(6) 1065–1068, 1985.—Rats were given daily intraperitoneal injections of 10.0, 1.0, 0.1, 0.01, 0.001 or 0.0 mg/kg naloxone for 15 days. Each day after the injections, animals were allowed access to a 20% sucrose solution for two hours and to tap water for the subsequent 10 hours. Consumption of the sucrose solution by the group that received 1.0 mg/kg was reliably decreased on Day 1 and 2, reflecting the suppressive effect of naloxone at that dose. By Day 3 until the end of the experiment, however, the suppression was no longer significant, suggesting that tolerance had developed. A similar effect was seen with the group given the highest dose, 10.0 mg/kg; although drinking was significantly less than the control in each of the 15 sessions, this group showed a trend to increase intake over the days of the experiment, thus also indicating possible tolerance to the effect of naloxone. Drinking patterns of the other groups did not differ statistically from the control. Thus, the low doses had no ability to suppress consumption, and the lowest dose that did lower it soon lost that ability; the highest dose continued to suppress drinking throughout the study but with decreasing efficacy. High performance liquid chromatography (HPLC) demonstrated that the naloxone remained intact over the 15 days of the experiment, supporting the suggestion that tolerance to naloxone might have developed.

Naloxone Drinking Tolerance

THE relationship between the endogenous opiate system and consummatory behavior is an area of peptide reasearch subjected to extensive investigation. Interest began when Holtzman demonstrated that administration of the opiate antagonist, naloxone, led to reduced food intake by rats fasted for 48 hours [10]. The report by Margules *et al.* of elevated levels of β-endorphin in the pituitaries of genetically obese mice and rats [15] further suggested an association between the endogenous opiate system and consumption.

Subsequent reports have shown naloxone to reduce drinking induced by many conditions, including hypertonic saline [3, 8, 18], polyethylene glycol [18], and angiotensin II [18]. Naloxone has attenuated fluid consumption in deprived [6, 14, 17, 20] as well as non-deprived [5, 6, 20] rats, and its suppressant effect also has been demonstrated in other species [4, 7, 9].

In most studies, the effects of naloxone have been measured for a short time. Only a few experimenters have extended their focus over days [1, 11, 18, 19]. The purpose of the present research was to investigate the effects of injections of naloxone given daily for a two-week period. In addition, a wide range of doses was used to evaluate the effects of doses of naloxone lower than those most frequently tested.

METHOD

Animals

Male, albino, Sprague-Dawley-derived rats (N=60) were obtained from King Laboratories (Oregon, WI) and housed individually in a temperature-controlled colony (22–24°C) with a 12-hour light/dark cycle (light onset 8:00 a.m.) throughout the experiment. The experimentally-naive rats weighed about 225 g at the start of testing.

Drugs

Animals (n=10) received 0.0, 0.001, 0.01, 0.1, 1.0 or 10.0 mg/kg naloxone (Endo Laboratories) dissolved in a vehicle consisting of 0.9% NaCl made to 0.01 M with acetic acid. The injections were given in a volume proportional to weight of 1.0 ml/kg and were administered intraperitoneally (IP). To prevent experimenter bias, the vials containing the solutions were coded and the code was not broken until the data were collected.

Procedure

The animals were allowed free acess to food and water for 6 days after their arrival. During this acclimation period,

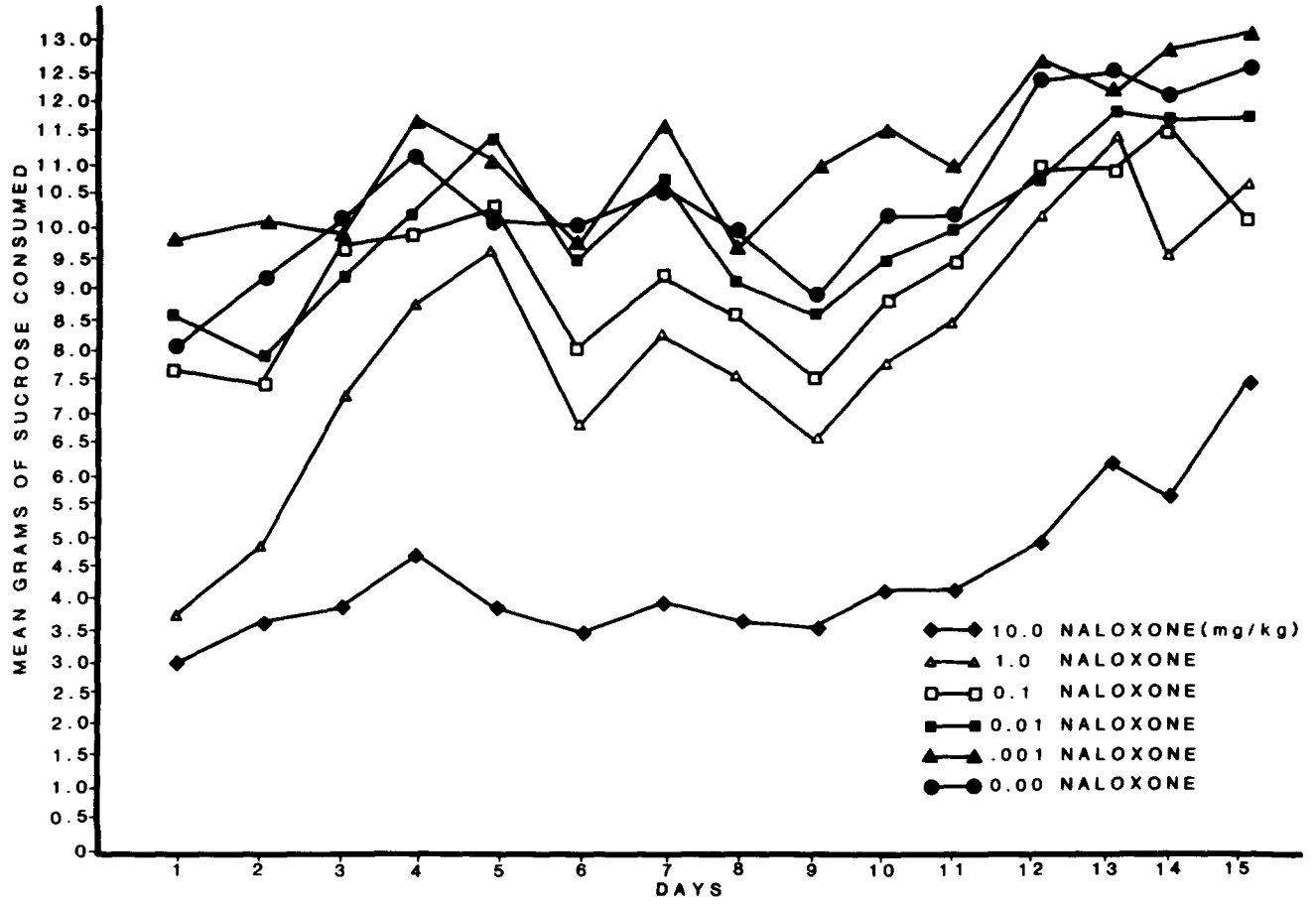

FIG. 1. Mean grams of sucrose consumed in the two-hour test period immediately after injection of various doses of naloxone administered each day for 15 days.

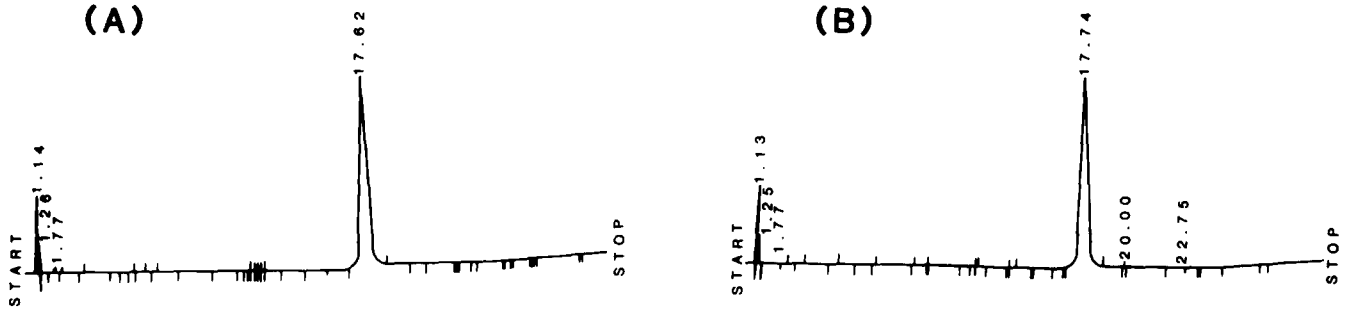

FIG. 2. Analysis by high performance liquid chromatography of (A) the naloxone after the 15 day study and of (B) a freshly prepared standard of naloxone, showing no significant difference between the two single peaks.

water consumption was monitored to provide baseline measurements, on the basis of which groups were balanced before testing began. At 8:00 p.m. on Day 6, water bottles were removed from all cages. Starting at 8:00 a.m. on Day 7, the rats received the appropriate injections, were returned to their home cages, and 10 min later were given access to bottles containing a 20% sucrose solution. Measurements of intake of the solution were taken after 30, 60, 90 and 120 min. After this 2-hour interval, animals were provided access to tap water for 10 hours. At 8:00 p.m. water consumption was determined, and 12-hour fluid deprivation initiated. This procedure remained the same for 15 consecutive days, each animal receiving the same dose of naloxone each day.

HPLC

After obtaining the behavioral data, the 1.0 mg/kg dose of naloxone in diluent solution was analyzed by high performance liquid chromatography (HPLC) on a Beckman model 344 system. This sample of naloxone was applied to a column of C-18 ODS in a solution of 0.1% trifluoroacetic acid (TFA) in 3% methanol. After two minutes under these initial

conditions, the concentration of methanol was raised to 16% over 11 min followed by a further increase to 40% over 10 min, and finally to 100% methanol over one min. The flow rate was 1.5 ml per min throughout the procedure.

RESULTS

Figure 1 shows consumption of the 20% sucrose solution over the 15 days. A mixed analysis of variance (doses by days by time intervals) was performed on the amounts of solution consumed. The main effect for doses was reliable, $F(5,54)=16.212$, $p<0.00001$, with overall fluid consumption indicating a dose-response curve: the group that received 10.0 mg/kg had the lowest mean fluid intake for the test sessions, with the next lowest being the group given 1.0 mg/kg, followed in order by the groups treated with 0.1, 0.01, 0.0, and 0.001 mg/kg. Scheffe's test showed the consumption by the group injected with 10.0 mg/kg to be significantly less than all other groups. Analysis of the comparisons on a daily basis revealed that the group given 1.0 mg/kg drank significantly less than the control on Days 1 and 2, whereas the difference between the groups injected with 10.0 and 0.0 mg/kg was significant on each of the 15 test days.

The main effect for days was reliable, $F(14,756)=30.183$, $p<0.00001$. The least consumption occurred on Day 1 and the most on Day 15, with a significant difference between the two revealed by Scheffe's test. The main effect for time intervals was significant, also, $F(3,162)=647.578$, $p<0.00001$, with the greatest intake having occurred during the first 30 min.

There were two two-way interactions that were reliable. The doses by time interaction was significant, $F(15,162)=27.001$, $p<0.00001$. As illustrated in Fig. 2, the same dose-response relationship appeared among groups for the first 30-min interval as was apparent for overall mean consumption, with the group that received 10.0 mg/kg having drunk the least, followed in order by the groups injected with 1.0, 0.1, 0.01, 0.0 and 0.001 mg/kg. All groups drank the most during the first 30 min; the second-highest interval was the final 30 min. The days by time interaction also was significant, $F(42,2268)=7.601$, $p<0.00001$, indicative of an increase in average consumption at times 30 min and 120 min as days progressed.

The drinking patterns produced over the daily two-hour test sessions were remarkably similar for the groups. One distinction is that the animals given 1.0 mg/kg drank more during the second hour than any other group, as reflected by the significant three-way interaction of doses by days by time, $F(210,1168)=1.233$, $p<0.05$. No other results were reliable.

A mixed analysis of variance (doses by days) was performed on the amounts of tap water consumed each day from 10:00 a.m. until 8:00 p.m. The main effect for doses was significant, $F(5,54)=11.473$, $p<0.00001$. Mean consumption was highest for the group injected with 10.0 mg/kg naloxone, followed in order by the groups injected with 1.0, 0.1, 0.0, 0.001 and 0.01 mg/kg. Scheffe's test showed the overall mean consumption of the groups injected with 10.0 mg/kg naloxone to be significantly higher than that of the groups given 0.1, 0.0, 0.001 and 0.01 mg/kg. These were the only significant comparisons obtained from Scheffe's test. The main effect for days was reliable also, $F(14,756)=12.337$, $p<0.00001$. The lowest daily average consumption occurred on Day 1 and the highest on Day 8. From Days 8 to 15, mean tap-water consumption progressively decreased. This trend coincided with a steady increase in the average daily intake of the sucrose solution. No other significant results were observed.

For the HPLC, the retention time of the naloxone used in the experiment was 17.62 min as compared to the retention time of 17.74 min for the freshly prepared standard (Fig. 2). Only one peak was observed, the area of which was 98.3% of the standard.

DISCUSSION

Observations for a period of two weeks revealed an unusual pattern of consummatory behavior for the groups injected with the two highest doses of naloxone. On Days 1 and 2, consumption by the group treated with 1.0 mg/kg was significantly suppressed, but on Day 3 their consumption increased, and never again did this group drink reliably less than the control. Since the naloxone at this dose lost its ability to lower intake, the possibility exists that tolerance to the antagonist developed by the third day of injections. Consumption by the group that received 10.0 mg/kg, although always reliably less than the control, showed an increasing trend, especially during the last three days of the study. On Day 15, the differences in consumption between the groups given 10.0 and 0.0 mg/kg barely reached significance by Scheffe's test. Thus, the lowered level of intake over days in this group also indicates the possibility that tolerance was developing. The higher the dose of naloxone administered, therefore, the longer this proposed tolerance appeared to take.

A similar drinking pattern was reported by Ostrowski *et al.* for a 10-day period [18]. As in the present study, the group given 10.0 mg/kg drank significantly less than the control on the final day, but there was a clear trend toward increased consumption over days. Comparable results have been reported for food consumption. One study reported feeding above the control level by Day 10 by rats [1], and another showed this increase as early as Day 4 in mice [19]. Another group, however, reported a sustained supressant effect of naloxone over a period of 8 days [11]. Since the dose used in their experiment was 2.5 mg/kg, intermediate between the two high doses used here, a longer period of time may have been necessary to observe indications of tolerance. No significant tolerance was demonstrated here for the highest dose until after the eleventh day.

The paradigm used in the present research measured consumption of 20% sucrose solutions for two hours and of tap water for the ten hours afterwards. Over the 12 hours observed, total fluid consumption was similar in all groups. As the groups treated with 1.0 and 10.0 mg/kg began to drink more sucrose solution over days, they drank less tap water. For example, on Days 5, 10 and 15, the mean 12-hour intakes for the group given 1.0 mg/kg were 27.62, 27.40 and 26.89 and for the group given 10.0 mg/kg were 25.62, 27.06 and 26.07. Other authors also have reported that treatment groups did not differ in total 24-hour consumption [1, 5, 6, 11]. These findings suggest that the animals are later able to compensate for the suppression induced by naloxone.

Another aspect also examined in this study was the effect of small doses of naloxone. In agreement with a previous investigation [15], overall mean consumption by the groups receiving less than 1.0 mg/kg of naloxone was dose-related, with smaller doses producing relatively more drinking. Although the differences were not statistically significant, consumption by the animals injected with 0.001 mg/kg naloxone

was higher than that by the control group, and on 11 of the 15 test days the group given that small dose drank more sucrose solution than did the control. The rats that received 0.01 mg/kg had a higher overall mean intake than those given 0.1 mg/kg, and on all but two of the test days drank more solution.

The effect of small doses of the opiate antagonist increasing consumption, significant in previous experiments [16,17], was not reliable in this study. Other experimenters have observed intake higher than controls resulting from low doses of naloxone [2, 4, 13, 21, 22], but few discussed the observation. It does not appear to be a robust phenomenon but seems to be affected by methodological variables like handling and habituation [12].

Thus, the ability of naloxone to suppress sucrose consumption in rats appeared to be highly dose-dependent and to develop tolerance with repeated administration. The lowest doses showed no suppression at all. The smallest dose that did decrease drinking did so for only two days before losing its effectiveness. The highest dose continued to suppress drinking throughout the 15-day study, but the suppression decreased significantly over time. The increased consumption could not be attributed to degradation of the naloxone, since HPLC showed that it remained intact throughout the study, further supporting the possibility that tolerance to naloxone might account for the results.

ACKNOWLEDGEMENTS

This work was sponsored in part by a University of New Orleans Faculty Summer Scholar Award to the first author, the VA and ONR. We thank Endo Labs for the naloxone and Dr. Alan Fischman for performing the HPLC.

REFERENCES

1. 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.
2. Brown, D. R. and S. G. Holtzman. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. *Pharmacol Biochem Behav* **11:** 567–573, 1979.
3. Brown, D. R. and S. G. Holtzman. Evidence that opiate receptors mediate suppression of hypertonic saline-induced drinking in the mouse by narcotic antagonists. *Life Sci* **26:** 1543–1550, 1980.
4. Brown, D. R. and S. G. Holtzman. Narcotic antagonists attenuate drinking induced by water deprivation in a primate. *Life Sci* **28:** 1287–1294, 1981.
5. Carey, M. P., J. A. Ross and M. P. Enns. Naloxone suppresses feeding and drinking but not wheel running in rats. *Pharmacol Biochem Behav* **14:** 569–571, 1981.
6. Cooper, S. J. Naloxone: Effects on food and water consumption in the non-deprived and deprived rat. *Psychopharmacology (Berlin)* **71:** 1–6, 1980.
7. Cooper, S. J. and S. Turkish. Food and water intake in the non-deprived pigeon after morphine or naloxone administration. *Neuropharmacology* **20:** 1053–1058, 1981.
8. Czech, D. A. and E. A. Stein. Naloxone depresses osmoregulatory drinking in rats. *Pharmacol Biochem Behav* **12:** 987–989, 1980.
9. Foster, J. A., M. Morrison, S. J. Dean, M. Hill and H. Frenk. Naloxone suppresses food/water consumption in the deprived cat. *Pharmacol Biochem Behav* **14:** 419–421, 1981.
10. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *J Pharmacol Exp Ther* **189:** 51–60, 1974.
11. Jalowiec, J. E., J. Panksepp, A. J. Zolovick, N. Najam and B. H. Herman. Opioid modulation of ingestive behavior. *Pharmacol Biochem Behav* **15:** 477–484, 1981.
12. Levine, A. S., J. E. Morley, J. Kneip, M. Grace and D. M. Brown. Environment modulates naloxone's suppressive effect on feeding in diabetic and non-diabetic rats. *Physiol Behav* **34:** 391–393, 1985.
13. Levine, A. S., S. S. Murray, J. Kneip, M. Grace and J. E. Morley. Flavor enhances the antidipsogenic effect of naloxone. *Physiol Behav* **28:** 23–25, 1982.
14. 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.
15. Margules, D. L., B. Moisset, M. J. Lewis, H. Shibuya and C. B. Pert. β-Endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* **202:** 988–991, 1978.
16. Olson, R. D., R. C. Fernandez, A. J. Kastin, G. A. Olson, S. W. Delatte, T. K. vonAlmen, D. G. Erickson, D. C. Hastings and D. H. Coy. Low doses of naloxone and MIF-1 peptides increase fluid consumption in rats. *Pharmacol Biochem Behav* **15:** 921–924, 1981.
17. Olson, R. D., A. J. Kastin, G. A. Olson, B. M. King, T. K. vonAlmen, M. C. Berzas, M. L. Ibanez and D. H. Coy. MIF-1 suppresses deprivation-induced fluid consumption in rats. *Peptides* **1:** 353–357, 1980.
18. Ostrowski, N. L., N. Rowland, T. L. Foley, J. L. Nelson and L. D. Reid. Morphine antagonists and consummatory behaviors. *Pharmacol Biochem Behav* **14:** 549–559, 1981.
19. Shimomura, Y., J. Oku, Z. Glick and G. A. Bray. Opiate receptors, food intake and obesity. *Physiol Behav* **28:** 441–445, 1982.
20. Stapleton, J. M., N. L. Ostrowski, V. J. Merriman, M. D. Lind and L. D. Reid. Naloxone reduces fluid consumption in water-deprived and nondeprived rats. *Bull Psychon Soc* **13:** 237–239, 1979.
21. Uemura, H., Y. Okawara, T. Tsukahara, N. Yanaihara and H. Kobayashi. Effects of Leu5-enkephalin on natural and angiotensin II-induced drinking in Japanese quail (*Coturnix coturnix japonica*). *Gen Comp Endocrinol* **56:** 240–245, 1984.
22. Wagner, G. C., D. B. Masters and A. Tomie. Effects of phencyclidine, haloperidol, and naloxone on fixed-interval performance in rats. *Pyschopharmacology (Berlin)* **84:** 32–38, 1984.