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Naloxone Reduces Fluid Intake in Rats with Open Gastric Fistulas

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ROCKWOOD, G. A., S. M. SIVIY AND L. D. REID. *Naloxone reduces fluid intake in rats with open gastric fistulas.* PHARMAC. BIOCHEM. BEHAV. 15(2) 319–321, 1981.—Water-deprived rats, fixed with chronically indwelling gastric fistulas, drank with the fistulas open (sham drinking) for 20 min. The subjects were given three doses of naloxone (0.0, 1.0, and 10 mg/kg, SC) 15 min before the opportunity to sham drink. The mean water intakes on days of 0.0, 1.0 and 10 mg/kg of naloxone were 43.3, 36.3, and 23.1 g, respectively. Naloxone clearly reduced fluid intake in rats engaged in sham drinking. This finding, that naloxone reduces fluid intake when post-ingestional absorption is prevented, lends support to the idea that naloxone modifies central neural regulatory processes. The results of these experiments do not support the hypothesis that the endorphins are involved exclusively with drive-reduction.

Drinking Endorphins Naloxone Sham drinking

WHEN individually housed rats have food always available, but have one brief daily opportunity to drink, they readily learn to take sufficient water to maintain their health and to gain weight. When rats are under such a regimen, injections of naloxone (in doses as low as 0.1 mg/kg) reliably lead to reduced drinking [5, 6, 14].

The finding that naloxone (NX) will lead to reduced drinking, plus additional data showing that the NX-effect is stereospecific and occurs with morphine-antagonists other than NX [5], supports a conclusion that the endorphins are somehow related to the behavioral control of water balance. While the NX-effect has been observed many times [3, 4, 7, 8, 11, 12, 14], questions concerning the precise way NX leads to reduced drinking remain unanswered. This brief note reports our initial observations of the effects of NX on rats' drinking with open gastric fistulas (sham drinking). The results from these procedures have a number of implications concerning the way NX may achieve its effects on drinking.

METHOD

The subjects were six male Sprague-Dawley derived rats (Taconic Farms, Germantown, NY). The rats were individually housed in a colony room maintained at 24°C, with a reversed 12-hr dark/12-hr light cycle (the lights on beginning at 2200 hr). Five of the subjects were used in the initial test, and the sixth subject was fixed for later testing.

Each rat was fixed with a chronically indwelling, stainless steel, gastric fistula. The rats weighed from 300–400 g on the day of surgery. The gastric fistulas, and the procedure for fixing them are similar to those used by Kraly, Carty, Resnick and Smith [10] and Paré [13]. The open fistula allows fluid to drain directly from the greater curvature of the stomach. When rats sham drank, virtually all that they drank was collected. Consequently, with open gastric fistulas, the animals drank without restoration of fluid deficits.

Following a 7 to 10 day recovery period from the fistula surgery, the rats were placed on a 23.75 hr water deprivation schedule, with a 15-min daily opportunity to drink (the schedule most often used in our laboratory). This general procedure was repeated until stable baseline rates were attained (5 days), at which time all animals were given the opportunity to sham drink. All sham drinking sessions lasted for 20 min. Following several days of sham drinking, testing for NX-effects began.

Four hr before the onset of sham drinking sessions, food was removed from the rats' home cages. To prepare a rat to sham drink, the fistula was opened, and the stomach of the rat was gently flushed with 10 ml saline, to clear the stomach of any remaining food. The fistula was then closed, and the rat was returned to its home cage. Injections, of either saline or naloxone, were administered 15 min after the stomach-flush. Fifteen min after the injection, the fistula was reopened, and a drainage tube (a short, stainless steel tube with a 22.8 cm section of flexible tubing) was attached to the fistula to allow for the collection of the draining fluid. The rat was then placed in an individual drinking chamber for the 20-min session, and the fluid was collected in a pan placed directly below the grid floor of the chamber. At the end of the session, the rat was removed from the drinking chamber, and after the fistula was closed, was returned to its home cage. Since the sham drinking procedure did not allow the rats to retain fluid, water bottles were put up on the rats' cages for 10 min, 15 min after their sham drinking session.

The initial testing spanned 6 days. Five animals served as subjects. On Days 1, 3, 4, and 6, the animals received no

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injection, but were allowed to sham drink. On Day 2, one half the animals received an injection of naloxone HCl, 10 mg/kg, the other half saline. On Day 5, the injections were reversed, with the group which had received saline on Day 2 receiving NX, and the group which had previously received NX receiving saline. Following the first sham drinking test, 2 of the 5 subjects developed leaks about their fistula, and were not used in any subsequent tests. Consequently, an additional rat, fixed with a gastric fistula participated in the second sham drinking test, and in the closed fistula test along with the 3 subjects remaining from the first test. The procedure used in the first test was repeated 3 weeks later, but a smaller concentration of NX (1.0 mg/kg) was used. Four animals served as subjects, three of which were previously used in the initial sham drinking test. All injections were administered subcutaneously, in a 1.0 ml/kg volume, 15 min prior to the onset of the sham drinking session.

The drinking of rats with closed gastric fistulas while under NX, 10 mg/kg, was also examined. Four animals served as subjects, all of which were used in sham drinking tests. The animals were maintained on a 23.67 hr water deprivation schedule, with a 20-min daily opportunity to drink. Once baseline rates stabilized, one half the animals received an injection of saline, while the other half received an injection of NX, 10 mg/kg, 15 min before the opportunity to drink. On the following day, the injections were reversed, with those animals which had received NX on the previous day receiving saline, and those animals which had previously received saline, now receiving NX.

RESULTS AND DISCUSSION

While under the influence of saline injections, or no injections, and while sham drinking, each rat drank nearly steadily throughout the 20-min sessions, usually taking over 40 g of water. The mean water intake, on the days before injections (sham drinking, 20 min/day, no injection), across all rats = 44.7 g, SD = 5.62. There were no reliable differences among mean intakes on days without injections, or between intakes on days without injection compared to days with saline injections. We took, therefore, the score of the day before injection as one index of the rats' sham drinking performance without NX.

A summary of the results are depicted in Fig. 1. As can be seen from the figure, NX, at doses of 1.0 and 10 mg/kg, reliably reduced sham drinking compared to the previous day's drinking. A one-way ANOVA among scores of tests using 0.0, 1.0 and 10 mg/kg NX, yielded a $F(2,11)=9.75$, $p<0.01$. Results of comparisons, by way of a t-test for paired comparisons, of drinking on the day before injection, and on the day of injection, are illustrated in Fig. 1.

As can be seen from the right hand pair of bars (labeled NORMAL), NX produced its characteristic reduction in drinking in the rats when the fistulas were closed, and the patterns of drinking in these animals fixed with gastric fistulas, are similar to those observed in rats without fistulas [14].

The drinking of the rats under saline and with open gastric fistulas spanned the entire 20-min session, whereas the drinking under NX was less consistent during the 20 min. Because rats with open gastric fistulas will drink for periods much longer than 20 min (for up to 2 hr [2]), the percent reduction under NX, compared to that under saline, could probably be modified by manipulating the length of the test-session. Consequently, the particular mean percent reductions found in these experiments are probably of little theoretical interest.

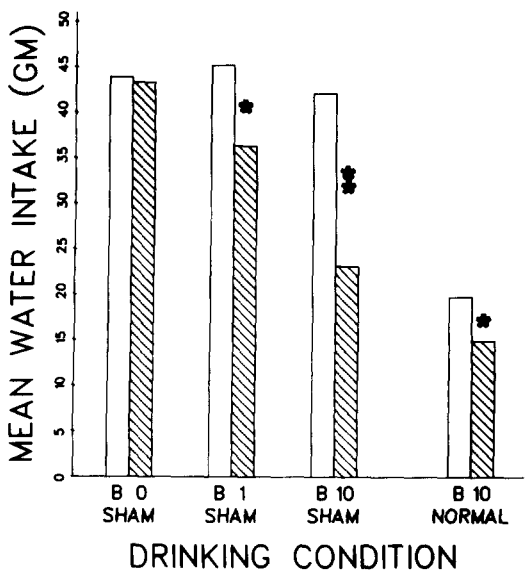

FIG. 1. Mean water intakes on the day before injections, and on the day of injections. B = baseline. 0, 1, and 10 = 0.0, 1.0, and 10.0 mg/kg doses of NX, respectively, 15 min before the opportunity to drink. The bars, labeled NORMAL, represent drinking with closed gastric fistulas. The remaining three sets of bars, labeled SHAM, represent drinking with open gastric fistulas. An asterisk between two bars indicates that a t-test for paired comparisons between scores of baseline and of injection day yielded a p value <0.05. Two asterisks indicate a p value <0.005.

While doing pilot work for this experiment, we found that if we flushed the stomach while rats were under the influence of NX (NX injection followed 15 min later by flushing the stomach and then the opportunity to drink), the resulting drinking was less than when we first prepared the subject and then gave NX (stomach flush, then 15 min later NX injection, followed by the opportunity to drink). Here, we used the procedure that produced the least reduction in drinking by NX and, of course, still achieved the NX-effect. We mention this observation to indicate that some of the procedural variables, other than test-duration, may be important in determining the amount of reduction that will be produced by NX.

There are a number of implications that follow from this simple demonstration that NX reduces intake of water in rats with open gastric fistulas. The finding indirectly confirms the hypothesis that NX modifies central neural regulatory processes, a notion supported by other findings [3,5]. It was reasonable to suppose, at an early stage of investigation, that NX, in the otherwise normal rat, could have its effects by affecting the dynamics of water moving from the gut and these events may, in turn, account for the modification of drinking. For example, NX may reduce the net holding capacity of the gut and thereby lead the rat to stop drinking sooner. Or conversely NX may facilitate water leaving the gut and reaching the brain quickly and, in turn, provide a "premature" satiety signal. Although there are opioid receptors within gut structures, and NX may indeed modify the way water is distributed once it is drunk, it is difficult to understand how these putative, NX-related activities would

lead to modification of drinking in rats with open gastric fistulas.

The baseline drinking of schedule-induced polydipsia is high, and NX does not reliably modify this type of drinking [6]. Deprived rats with open gastric fistulas also have very high baselines of drinking, yet NX does reliably modify this type of drinking. It follows, therefore, that baseline level itself is not the salient difference accounting for differential effects of NX on the drinking induced by various procedures.

Belluzzi and Stein [1] have suggested that the endorphins are the neurotransmitters of reinforcement associated with drive-reduction. The facts that the endorphin agonists can lead to ingestion [9], and antagonists to attenuation of intake [4], are contrary to their hypothesis. The fact that NX works in a circumstance where drive-reduction is not a factor [15], combined with our data that NX reduces fluid intake in rats with open gastric fistulas, renders it difficult to conclude that the endorphins are involved exclusively with drive-reduction.

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