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Naloxone Depresses Osmoregulatory Drinking in Rats[1,2]

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CZECH, D. A. AND E. A. STEIN. *Naloxone depresses osmoregulatory drinking in rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 987–989, 1980.—The effect of an opiate antagonist, naloxone, on hypertonic NaCl-induced drinking was studied in rats in a within-subject design. Naloxone reduced drinking at all dosage levels used (0.3–10.0 mg/kg) when compared to a control condition. These results extend previous findings of naloxone mediated reduction in fluid intake in water deprived and osmotically challenged animals. Naloxone's effect on fluid intake seems to be independent of procedure employed, and thus quite general. Possible mechanisms were considered.

Drinking Naloxone Water intake Narcotic antagonist Hypertonic NaCl

A NUMBER of recent studies have indicated that the narcotic antagonists, naloxone and naltrexone, can suppress appetitive behaviors in both rats and mice [1–9,11]. Drinking behavior has been shown to be particularly sensitive; as little as 0.3 mg/kg of naloxone was shown to reduce water consumption in 23.5 hr deprived rats to 76% of isotonic saline control intake [7] and 0.1 mg/kg reduced water intake in 12 hr deprived rats to approximately 77% of control intake [4]. Holtzman points out that naloxone-induced suppression of drinking appears to be a robust effect. It appears to be relatively independent of small variations in experimental protocol, such as number of times the animal is tested or whether or not the animal is first adapted to a deprivation schedule [7]. The current investigation attempted to extend these observations, using a procedure other than deprivation to induce drinking. Rather, drinking was induced by challenging the animal with a hypertonic solution of an osmotically effective substance, hypertonic NaCl. Although the stimulus conditions associated with water deprivation (both cellular dehydration and hypovolemia) and hypertonic saline challenge (cellular dehydration without reduced body fluid) differ, it was predicted that naloxone would similarly attenuate drinking. Two recent observations indicate that naloxone can depress hypertonic saline-induced drinking [2,11].

METHOD

Animals

Twelve male rats of the Sprague-Dawley strain (Holtzman, Madison, WI) weighing 240–290 g at the start of the experiment were used. Only eleven completed the procedure and are reported below. Animals were housed individually in suspended wire mesh cages in a temperature controlled room maintained on a 12 hr light-dark cycle (0800–2000). Standard laboratory chow pellets (Purina) and tap water were available ad lib except during drinking tests.

Procedure

Upon arrival at the laboratory, animals were placed in their home cages and allowed a 7 day period to acclimate to the environment. During this period they were handled daily. Rats were also given a single pretest acclimation trial in the test cage without injections. Tests were begun on Day 8.

On a given test day, an animal was first injected intraperitoneally (IP) with isotonic saline (0.15 M NaCl) or one of four doses of naloxone hydrochloride dissolved in saline, and returned to its home cage. Naloxone (Nx) doses were 0.3, 1.0, 3.0 and 10.0 mg/kg of body weight. Animals were weighed prior to test trials and solutions were prepared such that volume injected would be approximately 0.5 ml (actual range was 0.45–0.59 ml). Fifteen min later, the rat received an IP injection of either 12 ml/kg of a 1 M NaCl solution or an equivalent volume of isotonic saline and was immediately placed in a test cage similar to the home cage. Treatments thus were: 0.15 M NaCl/0.15 M NaCl, 0.15 M NaCl/1 M NaCl, 0.3 Nx/1 M NaCl, 1.0 Nx/1 M NaCl, 3.0 Nx/1 M NaCl, and 10.0 Nx/1 M NaCl. All injections were given between 0915–1030 hr. A graduated (0.1 ml) eudiometer tube, fitted with a stainless steel drinking spout, was attached to the center of the back wall of the cage for measuring water intake. The spout protruded 1 cm into the cage 7 cm above the grid floor. No food was available during drinking tests. Tests were separated by 3–4 days. All animals

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[2]Naloxone hydrochloride was generously provided by Endo Labs.

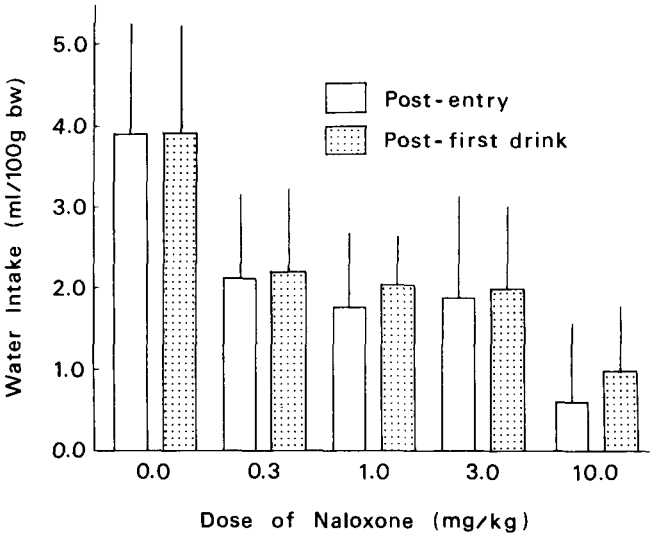

FIG. 1. Mean water intakes following 1 M NaCl challenge expressed as an increase over baseline control (double isotonic saline control) intake for doses of naloxone for the first hour after entry into test cage (open bars) and after first drinking response (strippled bars). The 0.0 condition corresponds to the 0.15 M NaCl/1 M NaCl condition. Vertical lines represent SEM.

were tested under all treatment conditions in an incomplete counterbalanced (Latin square) design. Two rats were randomly assigned to each of the six ordered sequences. Intake of room temperature tap water was recorded to the nearest 0.1 ml at 1.0, 2.0 and 3.0 hr after both entry into the test cage and first drinking response. The double isotonic saline treatment was used to measure baseline fluid intake over a 3 hr period.

All water intakes were converted to ml/100 g of body weight. In order to compensate for individual animal variability in drinking behavior or to injection procedures, differences between a rat's baseline water intake (double isotonic saline control) and its intake following hypertonic saline challenge in each of the five other treatments was determined. All response measures were evaluated with repeated measures analysis of variance (ANOVA) procedures and Duncan's multiple range tests.

RESULTS AND DISCUSSION

Differences between baseline and hypertonic saline induced water intakes over the 3 hr post-entry period were analyzed with an ANOVA with repeated measures on both dose and time. The ANOVA revealed significant main effects in water intake for both dosage level of drug, $F(4,40)=11.88$, $p<0.001$ and for time after entry into the test cage, $F(2,20)=82.29$, $p<0.001$, and a significant time×dose interaction, $F(8,80)=9.40$, $p<0.001$. Figure 1 shows mean differences in water intake during the first hour for all treatment conditions. Individual comparisons, using Duncan's procedure, revealed that water intake for all dosage levels of naloxone was significantly lower than for the placebo (0.15 M NaCl/1 M NaCl) condition (all $p<0.01$). In addition, 10 mg/kg of naloxone depressed drinking significantly more than did any of the other doses (all $p<0.01$). Means were 3.9, 2.2, 1.7, 1.9 and 0.6 ml/100 g for the 0.0–10.0 mg/kg conditions, respectively, for the first hour. With the exception of the 10 mg/kg naloxone condition, little additional water was consumed during the second and third test hours. Renal mechanisms and dilutional factors associated with drinking could account, at least in part, for low intakes during this period; however, this cannot be determined in the present study. Means for the entire 3-hour test period were 4.1, 2.4, 2.2, 2.2 and 1.3 ml/100 g.

It was also of interest to determine the amount of fluid consumed once an initial drinking response was made. If this issue is important, fluid consumption could have been partially confounded in the 10 mg/kg condition in the post-entry analysis, since 3 animals did not drink during the first hour after entry into the test cage. To test this issue, differences between baseline and hypertonic saline induced drinking over the 3 hr post-first drink period were also analyzed. The ANOVA revealed significant main effects for both dosage level of drug, $F(4,40)=11.12$, $p<0.001$ and time after first drink, $F(2,20)=125.53$, $p<0.001$. Again, the time×dose interaction was significant, $F(8,80)=9.36$, $p<0.001$. Figure 1 shows mean differences for the initial post-first drink hour for all treatment conditions. Post-hoc comparisons again showed that water intakes for all doses of naloxone were significantly lower than the placebo condition and that 10.0 mg/kg of naloxone depressed drinking more than did any of the other doses (all $p<0.01$). Apparently, magnitude of intake was not confounded by latency to drink for the test duration used.

The present results thus extend the findings of previous studies which showed that naloxone can exert a strong suppressant effect on drinking in rats. Since the current experiment employed an osmoregulatory challenge, it appears that the effect is quite general. The mechanism is presently unknown, although several possibilities have been advanced. One suggestion is that naloxone suppresses drinking (as well as feeding) by interfering with an endorphin system. Endorphin is suspected of mediating reward processes [12,13]. Thus, a decrease in water intake induced by naloxone in animals otherwise expected to drink may be interpreted as a diminution of the rewarding properties of water to these deprived animals. Naloxone may also have aversive properties, perhaps producing gastrointestinal discomfort to the animal. A high dose of the drug (10 mg/kg) has been reported to be both an effective [4, 14, 15] and an ineffective [10] stimulus in producing conditioned taste aversion. Further, Wu *et al.* [15] report that rats receiving 10 mg/kg exhibited considerable individual differences in magnitude of conditioned taste aversion. Attempts to use lower doses in the conditioned taste aversion paradigm have been unsuccessful [4]. Consequently, the present data are supportive of the concept of an endorphin-reward system.

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