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BRIEF COMMUNICATION

Naloxone's Effects on Intake of Sequentially Presented Fluids

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TALANIAN, R. V., G. A. HUNTER, C. M. BEAMAN, G. L. REICHERT-HUNTER AND L. D. REID. *Naloxone's effects on intake of sequentially presented fluids.* PHARMACOL BIOCHEM BEHAV **25**(3) 697–700, 1986.—Fluid-deprived rats were presented with one of three types of palatable solutions for 15 min, followed by access to either the same solution or one of the other types for 15 min. The solutions were 5.3% sucrose, 0.9% salt solution, and tap water. Naloxone reduced intake of all solutions, as compared to placebo, regardless of type of fluid or order of presentation. Rats receiving water followed by sucrose solution reduced their intake of water under naloxone, but did subsequently take a considerable amount of sucrose solution showing that they were capable of drinking more. These findings indicate that naloxone's effects closely track ordinary satiation-effects, merely enhancing satiation-like functions at each instance of opportunity to take fluids.

Naloxone Eating Drinking Endogenous opioid peptides

THIS note reports the effects of naloxone (NX), the prototypic antagonist of morphine and related opioids, on the intake of flavored solutions in water-deprived rats. It is part of a program of study attempting to specify the behavioral effects of NX on intake of ingesta, thereby contributing to an understanding of the role of the endogenous opioid peptides (EOPs) in the mediation of ingestion.

Naloxone reduces intake of water and flavored water across a wide variety of experimental circumstances [1, 3, 5, 9, 13] and for recent reviews, see [8,11]. These effects are pharmacologically specific, i.e., dose-related and stereoselective. A reduction in intake is also observed with other antagonists of morphine and the EOPs [2,7].

Naloxone could be reducing the intake of fluids in a variety of ways. It could be interfering with the perception of thirst or the ability to drink, or it could be producing mild sickness or lethargy. It could also be engendering other motivational states (e.g., fear) incompatible with prolonged drinking, or it could be muting the ordinary reward value (palatability) of ingesta. Interestingly, the latter possibility seems to be supported by the extant evidence [8]. The inference may be drawn that one ordinary function of the EOPs is to act as a neurohumor related to the hedonic assessment of ingesta [8].

Mook and Brandsey [6], citing Lashley [4], pointed out that satiety is with respect to specific commodities. Body states, such as those associated with deprivation and repletion, apparently serve to modify responses to external stimulation. If NX is modifying perceived palatability, then it would be expected that a NX-induced suppression with respect to one commodity would not totally suppress intake of a 2nd. On the other hand if NX was reducing intake by rendering the rats incapable of further drinking, then presentation of a 2nd commodity would not lead to further intake. Following a smaller pilot study, we now report the effects of NX on the intake of two flavored solutions presented sequentially. Rats did reduce their intake of the initially presented solution under the influence of NX as expected, but also drank considerable amounts of subsequently presented solutions.

METHOD

Subjects

The subjects were male, Sprague-Dawley derived rats, weighing between 350 and 500 g at the start of these procedures. They were purchased from the supplier (Taconic Farms) as young adults and had been subjects of a study of benzodiazepines' and opioids' effects on drinking, i.e., they had been given limited time to drink water across 67 days and had been given injections prior to some of those opportunities to drink. Therefore, these rats had considerable experience with fluid-deprivation schedules, and they gained weight steadily throughout these procedures. The animals

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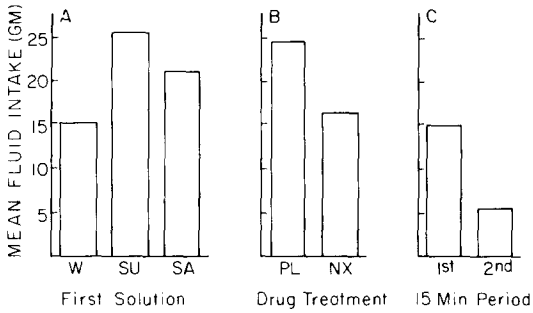

FIG. 1. Mean grams of intake of fluids with data grouped according to reliable main effects of the ANOVA. The data for panels A, B, and C yield: $F(2,45)=14.41$, $p<0.0001$; $F(1,45)=276.36$, $p<0.0001$; and $F(1,45)=437.21$, $p<0.0001$, respectively.

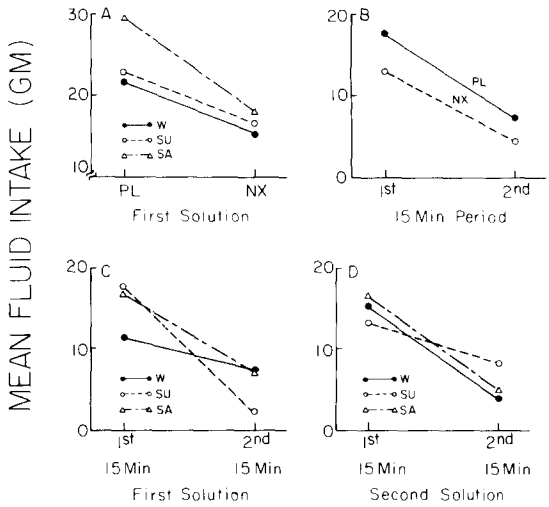

FIG. 2. Mean grams of intake of fluids with data grouped according to the reliable two-way interactions from the ANOVA which for panels A, B, C and D yields: $F(2,45)=12.63$, $p<0.0001$; $F(1,45)=30.51$, $p<0.0001$; $F(2,45)=55.20$, $p<0.0001$; and $F(2,45)=19.80$, $p<0.0001$, respectively.

were individually housed in a colony room, maintained at 24°C, with a 12-hr light/dark cycle (lights on at 1000 hr).

Procedure

Test solutions were presented by way of bottles which were equipped with stainless steel, ball-point sipping tubes and were weighed before and after presentation (to the nearest 0.1 g) to assess intake. Standard laboratory chow was always available.

For the 2 days before these procedures, rats had food and water always available. Then, they were deprived of all fluids for 3 days except for a 30-min period, when two bottles were presented sequentially for 15 min each, beginning at 1330 hr. Each of nine groups of subjects (6/group) received one of nine possible combinations of the three different test fluids (tap water, salt, and sucrose solutions). The salt solutions (SA) was NaCl in tap water, 0.9% (w/v or 0.154 M). The sucrose solution (SU) was made with commercially available sugar in tap water, 5.3% (w/v or 0.154 M).

On test days, rats were injected with either NX (naloxone hydrochloride, Endo) at 5 mg/ml or placebo (PL, physiological saline, the vehicle for NX). All injections were given subcutaneously, at a volume of 1 ml/kg, 30 min prior to the presentation of the 1st bottle. The regimen of injections for half of the rats of each group was PL, NX, NX, and PL, and for the other half NX, PL, PL, and NX. Two days separated the 2nd and 3rd day of injections (rats were maintained on the deprivation and fluid presentation schedule during these 2 days). Consequently, we have two measures of the effects of PL or NX with order of injections counterbalanced to control for effects associated with any particular day.

Data Reduction and Statistics

Since rats performed similarly regardless of the order of injections, we took the mean of their scores under PL and NX to represent their performance after these injections. With this data-reduction, the data (grams of fluid taken) conform to a matrix for an analysis of variance (ANOVA). The ANOVA is a 3 (1st kind of solution) by 3 (2nd kind of solution) by 2 (NX, placebo) by 2 (1st-15 min, 2nd-15 min) factorial design having repeated measures with respect to factors associated with injections and 1st- and 2nd-15 min. Selected comparisons of individual group means were done by appropriate *t*-tests.

RESULTS

Three main effects of the ANOVA were reliable sources of variance (ps<0.0001). NX reduces drinking across all solutions (Fig. 1, Panel B). The amount of drinking was related to kind of 1st solution (Fig. 1, Panel A), and there was considerably more drinking during 1st-15 min than 2nd-15 min of the session (Fig. 1, Panel C).

Figure 2 presents the relevant means of all of the reliable two-way interactions. Panel A represents total intake grouped according to kind of 1st solution and whether rats were under PL or NX. The high intake generated by presenting salty solutions 1st is evidently most sensitive to NX (Fig. 2, Panel A).

Figure 2, Panel B, depicts amount consumed during 1st-15 min and 2nd-15 min with respect to kind of injection. Under PL, the ratio of the number of grams taken in the 2nd period/1st period = .42; under NX, this ratio was .34. Panel C (Fig. 2) represents mean intake with respect to kind of 1st solution and with respect to 1st- and 2nd-15 min. When sucrose is presented 1st, the rats take most in the 1st-15 min and the least in the 2nd-15 min.

Panel D (Fig. 2) presents mean-intake with respect to kind of 2nd solution and period of presentation. When sucrose is the 2nd solution presented, rats drink the least from the 1st bottle as if they were anticipating the presentation of sucrose.

In addition to the interactions depicted in Fig. 2, the triple interactions involving the factor PL-NX were reliable sources of variance. As can be seen from Fig. 2, Panel C, when sucrose was presented 1st, little intake was obtained with the 2nd bottle and, therefore, there is little opportunity to see a drug-effect in comparison to the other two circumstances. The fact that these three-way interactions are reliable sources of variance allows detailed inspection of individual groups' performances.

Figure 3 depicts the results with selected groups of rats,

i.e., those getting water 1st. Panel A (Fig. 3) shows mean intake when water was followed by water under either PL or NX. Please notice that rats took a mean of 5 or fewer grams of water during the 2nd-15 min. NX reduced intake of water during 1st-15 min to about 10 g with all three groups getting water 1st (left values, Panels A, B, and C). Mean intake of SU and SA was greater under NX during the 2nd-15 min than intake of water during 2nd-15 min under PL. Consequently, it is difficult to maintain that rats' reduced intake during the 1st-15 min under NX is due to their inability to drink; since when presented a palatable solution, they do drink.

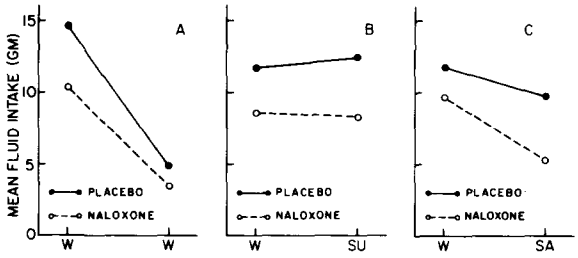

FIG. 3. Mean grams of intake of fluids when water is presented 1st (left values of all three panels are 1st-15 min intakes, right values 2nd-15 min intakes). Panel A depicts intakes when water is followed by water under PL and NX. Panel B and C depict intakes when sucrose or salt solution is presented during a 2nd-15 min opportunity to drink. A t-test comparing grams taken under NX during the 2nd presentation of water (right data point of Panel A) to g taken under NX when 2nd presentation was sucrose (right data point of Panel B) yielded, $t(10)=3.5$, $p<0.006$.

DISCUSSION

These data confirm that (a) satiation, in general, is with respect to commodities, (b) presentation of 0.154 M solutions of salt and sugar lead to enhanced intake in deprived rats compared to when only water is presented, (c) NX reduces intake of water, salt solution, and sucrose solution and (d) NX reduces, but does not eliminate, intake of fluids when there are inducements to drink (deprivation and palatable solutions presented). These data indicate that NX-effects are specific to each commodity, i.e., a NX-induced reduction in intake does not eliminate further ingestion.

At the beginning of the study of NX and the intake of nutrients, it seemed reasonable to suppose that NX was exerting its effects in a number of ways that now appear untenable, for example, by limiting the holding capacity of the gut. However, NX reduces intake in rats drinking with open gastric fistulas [9,10]. NX does not modify intake by interfering with the initiation of ingestion [2,12], it merely causes rats to stop drinking sooner. We now show that NX-treated rats are clearly capable of further intake when presented a 2nd fluid, a finding confirming the conclusion drawn from another procedure involving preloading rats before they drank [7].

Although the data of this study rule out a severe debility (such as extreme fatigue) in accounting for NX's reduction of intake, they do not completely rule out the possibility of a mild sickness or lethargy that is overcome by presentation of a 2nd palatable solution. The explanation of NX's reduction in intake, in terms of sickness, however, would have to take into account other findings (such as no reduction in latency to drink, a feature observed informally with these subjects) and also the remarkable specificity of findings presented here.

There seems to be an opioid system related to the process of modulation of incentive value. It is reasonable to conclude, given the extant evidence, that NX antagonizes the actions of EOPs acting at central neural sites. The EOPs somehow enhance the incentive value of ingesta and as a consequence, intake of commodities is enhanced with the expression of EOP-activity. Since NX usually merely reduces intake and does not eliminate it, it is presumed that the EOPs modulate other systems critical to ingestion. Even though we are reasonably confident that NX acts at central neural sites to produce its characteristic reduction in intake, it does not follow that the stimulus for release of EOPs, which act centrally, is of any particular origin. The stimulus for release of EOPs could be any of a number of factors postulated to be involved with the control of ingestion.

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