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Naloxone Persistently Modifies Water-Intake

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AKKOK, F., N. A. MANHA, S. A. CZIRR AND L. D. REID. *Naloxone persistently modifies water-intake.* PHARMACOL BIOCHEM BEHAV **29**(2) 331–334, 1988.—Rats, deprived of water for 19 hr, were given an opportunity to drink water for 30 min and for another 4.5 hr. Prior to drinking, rats were injected with saline or NX (2.5 mg/kg) to see if NX's effects persisted when given day after day. The findings confirmed that NX not only reduced intake but its effects showed no tolerance across consecutive administrations. Subsequently, when rats were deprived of water for 23.5 hr, NX produced a reliable decrease in intake. Intakes, however, were considerably greater than when subjects had an additional opportunity to drink.

Naloxone Water Opioids

IN what may be approaching hundreds of experiments, naloxone (NX), the prototypic antagonist at opioid receptors (opioceptors), has been shown to decrease intake of ingesta (see [8], for a review). The antagonist-effect of NX is pharmacologically specific, dose-related, and likely to be a central neural effect. The effect of NX is also behaviorally specific; it reduces the duration of a bout of ingestion rather than its initial features [2, 8, 10]. There remains, however, confusion concerning the topic of persistence of NX's effects when given day after day, or persistence of effects with chronic antagonism at opioceptors [8], as when long-lasting antagonists are given. Diminishing effects of repeated or chronic administration might be characterized as tolerance and the issue of tolerance to antagonists' effects is of considerable theoretical interest.

When adult rats are given only one brief (15 min to an hour) opportunity a day to drink, they soon take sufficient water (15 to 25 g depending on their size) to maintain their bodyweight and grow at rates similar to rats having water always available. They do take less water than rats having water always available, but evidently sufficient amounts to maintain a normal rate of growth. When NX, in doses from 1.5 to 10.0 mg/kg, is given before one of these limited periods, rats reduce their intake 20 to 60% [11]. The question addressed here is whether or not NX's effects persist when NX is given day after day before a restricted opportunity to drink.

METHOD

Subjects and Apparatus

The subjects were 20 male, Sprague-Dawley rats purchased from Taconic Farms (Germantown, NY) (mean weight at purchase = 165.8 g, S.D. = 8.2). They were individually housed in standard hanging cages in a room maintained at 24°C and with 12 hr of daily light, beginning at 0900 hr. Water was presented in bottles with ballpoint sipping tubes. To measure intakes, bottles were weighed before and after their presentation with scores corrected for spillage [3].

Procedure

Upon arrival, rats had a 5-day adaptation period with food and water always available. Following this, rats began a daily regimen of 19 hr of water-deprivation followed by a test-session with presentation of water for 30 min, beginning at 1430 hr. Subjects were then given water for another 4.5 hr.

After 5 days of the daily regimen, rats were randomly assigned to 2 groups of 10 each: one to receive saline and the other NX. NX hydrochloride was 2.5 mg/kg, the smallest dose for maximal effects without the possibility of side-effects [8,12]. Data [3] indicate that the dose is not sufficient to provide antagonism throughout the 4.5-hr period following testing, but does provide antagonism across the test-session. Placebo-injections were physiological saline, NX's vehicle. All injections were given subcutaneously, 1.0 ml/kg, 15 min before presentation of water.

During the first 3 days and the last 3 days of a 16-day period, while the daily regimen was continued, all subjects received saline. Across the middle of 10 days, half continued to receive saline while the others received NX. At the conclusion of the first phase, subjects began a new schedule of 23.5 hr of deprivation and 30 min of water. After 2 days to

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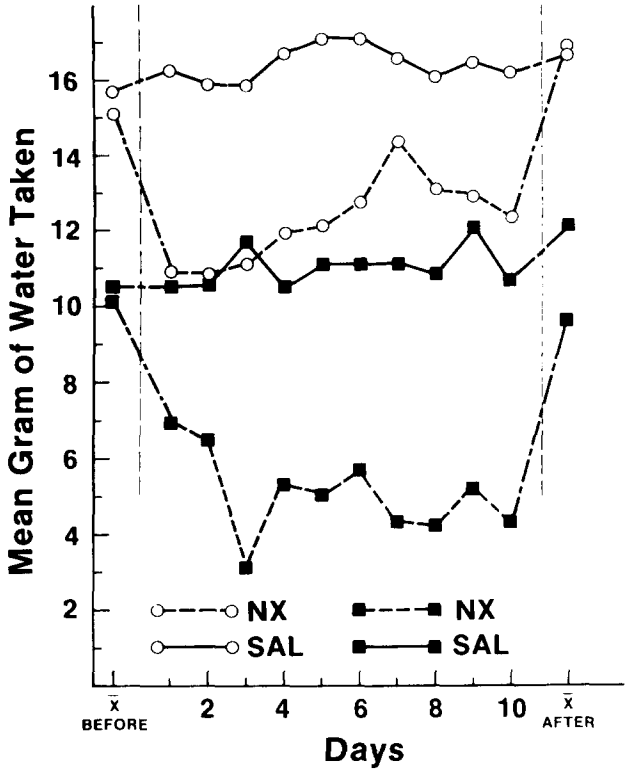

FIG. 1. Mean grams of water-intake, taken during 30 min, are depicted for each phase of the experiment. The closed squares summarize data of Phase I and the open circles, Phase II.

adjust, rats were given another series of injections spanning 16 days (3 days under saline, 10 days under NX or saline, 3 days under saline). In brief, rats were given an additional opportunity to drink following the test-session during the first phase but not during the second.

RESULTS

Across the initial 3 days with all rats getting saline, the two groups did not differ reliably in intake of water during either the 30-min test or the subsequent period, or in body-weights. A 2×3 ANOVA for repeated measures of intake-scores during the 30-min session, for example (means in Fig. 1), yields an F(1,18), for the group-effect=0.72, $p=0.41$, Since subjects were similar before the advent of NX, differences seen subsequently are apt to be due to NX.

Figure 1 depicts drinking under the two conditions during the 30-min sessions. An ANOVA of the data of Fig. 1 for the 1st 10-day phase yields an F(1,18), for saline versus NX=126.2, $p<0.001$, verifying that NX reduces water-intake. What is of interest, however, is whether or not the NX's effects diminish across consecutive administrations. The saline-subjects slightly increased their water-intake across days, while the NX-subjects tended to reduce their intakes during the 30-min test. On the 1st day, NX reduced intake by 32% compared to the mean of predrug placebos, while on the 10th day, intake was reduced by 59%. The differing trends are manifest in the reliable interaction term of the ANOVA, F(9,162)=2.88, $p<0.004$.

Across the 10 days of differential injections, during the 2nd part of a daily session, NX-subjects increased their intake (mean=15.8 g compared to 9.7 before injection), while the saline-subjects' intakes increased only slightly (mean=10.3 g compared to 9.5 before). The difference across the 10 days of dosing is reliable, F(1,18)=84.8, $p<0.001$. The interaction term is not reliable, F(9,162)=1.09, $p=0.37$.

Across the 10 days, rats gained weight as might be expected, since total fluid-intake across both parts of a daily session was maintained in both groups. Initially, the saline-group weighed 231.9 g compared to the NX-group's weight of 230.3 g. At the last measure of body-weight of the 10-day period, the 2 groups weighed 273.8 g and 271.4 g for the saline- and NX-groups, respectively, F(1,18)=0.02, $p=0.90$.

A prediction congruent with the idea of tolerance to NX would be that the NX-effect would wane during the 30-min session (i.e., 30-min intakes would increase across days), with comparable shifts during the subsequent period (i.e., subsequent intakes would decrease). This prediction was not met. The next question concerns how NX-effects change when there is no chance to drink except when NX is present.

During the last 3 placebo days of the 1st 16-day test, the NX-group tended to return to their performance levels prior to injections, but did drink less than the saline-group (Fig. 1), F(1,18), for the group-effect=10.77, $p=0.0041$. The means during the 2nd period (1st phase, last 3 placebo days) were 9.8 g for the saline-group and 11.4 g for the NX-group, F(1,18)=5.19, $p<0.04$. When the deprivation schedule was changed to 23.5 hr, the groups again consumed equal amounts of water (Fig. 1) when all subjects received placebos, mean of controls=15.7 g and the means of the experimental=15.1 g, F(1,18)=0.55, $p=0.47$.

In the 2nd 10 days of differential injections, NX produced a reliable decrease in intake, F(1,18)=60.24, $p<0.001$. Across days, the NX-subjects tended to slightly increase their water-intake while the controls remained the same. The interaction term, however, did not meet conventional standards for statistical significance, F(9,162)=1.82, $p=0.07$.

With the advent of NX-injections, subjects lost weight in comparison to their previous weights and compared to subjects getting saline. The NX-subjects, however, did not continue to lose weight and their weights remained relatively stable at about 277 g across the last 7 days of the 10 days. The controls tended to gain weight across the 10-day period. These effects are manifest in the results of the ANOVA which yielded an F(3,54)=24.74, $p<0.001$, for the interaction term. During the last 3 days of placebo for all subjects, the mean water-intakes were 17.0 g and 16.9 g for controls and experimentals, respectively, F(1,18)=0.04, $p=0.85$.

DISCUSSION

It is unlikely that giving NX day after day produces some changes at a molecular level that can be characterized as tolerance and manifest as drinking. One can, however, observe diminishing effects of chronic antagonism at the opioceptors on ingestion [1, 8, 9]. The usual circumstances for observing apparent tolerance is chronic antagonism at opioceptors among rodents having ready access to nutrients with measurements taken once a day across many days. On the 1st few days of such a regimen, the antagonists reduce intakes, but by the 3rd or 4th day intakes are at control-levels and rodents are maintaining or gaining weight as controls. Also, when the period to ingest is very limited, rats seem to overcome the antagonists' effects to maintain body weight [3]. A trend toward overcoming the effects of antagonism are seen with the highly restricted schedule of this experiment. Both of these circumstances (unlimited access or very lim-

ited access) produce signs of "tolerance," i.e., the antagonists' effects wane with repeated measures. The circumstances of the first phase of this experiment differed. The opportunity to ingest is limited, but rats had an opportunity to take nutrients after the antagonist was metabolized. Under these circumstances, NX's effects persisted without apparent tolerance. This persistence of NX's effects was also seen with rats having an opportunity to drink both water and a sweetened alcoholic beverage [3]. In that circumstance, total fluid-intake was decreased with NX and that effect persisted across many days when the rats had an opportunity to drink after NX was no longer effective. When rats had no additional opportunity to take water, they, as subjects of these procedures, took sufficient fluid to maintain body-weight but did not take the alcoholic beverage in the same amounts as before or after NX-injections.

If there was tolerance at a molecular level, one might presume that such an event would be manifest across various circumstances of presenting nutrients. It, however, does not. Therefore, we conclude that the changed patterns of intake seen in other studies is due to a compensatory intake to maintain body-weight rather than some specific tolerance. There is one study, however, that addresses the issue of tolerance with an experimental arrangement similar to ours, but comes to a very different conclusion.

Recently, Olson and colleagues [7] administered NX daily across 15 days while observing 12-hr deprived rats' drinking of a 20% solution of sucrose presented for 120 min. They measured drinking at intervals of 30, 60, 90 and 120 min. With the first dosing, only their two largest doses of NX, 1.0 and 10.0 mg/kg, produced a reliable suppression of intake while smaller doses (0.1 mg/kg or less) did not. This general finding confirms the general dose-response relationship observed often between subcutaneous or intraperitoneal administration of NX and suppression of intakes of ingesta (e.g., [12]).

The subjects of Olson *et al.*, in general, increased intakes across days. As subjects grow, they take more fluids. All subjects, including those getting 0.0 and 10 mg/kg, increased their intakes across days during the 120 min. As to be expected, the 10 mg/kg dose of NX significantly decreased intakes compared to those getting 0.0 mg/kg on each of the 15 days. Nevertheless, Olson *et al.* [12] stated that the gradually increasing intakes of those getting 10 mg/kg "indicates the possibility that tolerance was developing" (p. 1067). In the Abstract of their report, they stated "the highest dose continued to suppress drinking throughout the study but with decreasing efficacy" (p. 1065). The differences between means of those getting 0.0 and 10.0 mg/kg on the 1st day of administration and on the 15th day of administration, as measured from Fig. 1 of Olson *et al.*, are nearly identical. It is unusual, therefore, to conclude that the subjects of 10 mg/kg of NX show tolerance. In fact, there is no evidence in Olson *et al.*'s presented data germane to 10 mg/kg of NX to support a conclusion of diminished effectiveness ("decreasing efficacy") of NX across days of administration if one takes as the standard for comparison the performance of the control-group.

The subjects of Olson *et al.* getting 1.0 mg/kg did show more suppression on the 1st 2 days of administration than subsequently, in comparison to controls, when data were tabulated across 120 min. It is doubtful, however, whether the dose of 1.0 mg/kg is sufficiently large to sustain suppression of intakes across 120 min [3]. The dose may have been effective day after day in suppressing intakes at the 30-min interval. This is surely a reasonable possibility since the overall ANOVA of their data yields a "significant three-way interaction of doses by days by time, $F(210,1128)=1.233$, $p<0.05$" (p. 1067). Probably, the subjects getting 1.0 mg/kg had suppressed drinking during the 1st hour but as NX was metabolized they compensated by greater intakes during the 2nd hour when they were effectively free of NX. Olson *et al.* (p. 1067) said "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." So, theoretically, one should be able to see from their report if the dose of 1.0 mg/kg produced consistent levels of suppression of intakes day after day across the 1st 30 min. Figure 2, however, depicts the results of an analysis of high performance liquid chromatography and there is no other figure representing the results of the 30-min measurement. With some possibility for tolerance with the dose of 1.0 mg/kg, but with no statistical support for the possibility of tolerance with the dose of 10.0 mg/kg, Olson *et al.* (p. 1067) drew the unusual conclusion that "the higher dose of naloxone administered, therefore, the longer this proposed tolerance appeared to take."

There are reports that the effects of NX on ingestion wane with repeated or chronic dosing. Usually these reports are of measures of ingestion across 24 hr. In such a situation, NX could be reducing the size of a bout of ingestion, but there could be more bouts of ingestion.

The data confirm that NX's effects are modulatory and that full functioning of the endogenous opioid systems are not critical to maintaining adequate body-weight. Basic needs seem to override opioid antagonism. So, it is clearly possible to show apparent "tolerance" to NX's suppressent effects on intake of ingesta in certain tests. As need builds, NX is apparently no longer as effective, thereby giving the appearance of "tolerance." When, however, rats can meet their needs, opioid antagonist's effects persist [4–6]. Chronic antagonism may be, therefore, useful in treating a number of conditions without threatening the basic health of the individual. Indeed, NX's modulatory, rather than critical, effects are what leads to the conclusion that opioid antagonists may be useful adjuncts in treating a number of conditions best described as "gluttony," including the taking of alcoholic beverages [3].

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