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Naloxone Reduces Fluid Intake: Effects of Water and Food Deprivation[1]

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OSTROWSKI, N. L., T. L. FOLEY, M. D. LIND AND L. D. REID. *Naloxone reduces fluid intake: Effects of water and food deprivation.* PHARMAC. BIOCHEM. BEHAV. 12(3)431–435, 1980.—Food and fluid deprived and nondeprived male rats showed 36% and 46% decreases, respectively, in sucrose consumption 15-min after injection with 2 mg/kg of naloxone in one hr tests. The magnitude of this decrease was not correlated with an index of naloxone's ability to produce a sickness, as measured by the conditioned taste aversion test. Tests with animals scheduled to drink water in a 15-min daily session showed naloxone had similar effects in reducing water intake in 23-hr and 47-hr water deprived rats. Morphine, when self-administered, produced an increase in water intake during 6-hr sessions. The data support the idea that naloxone disrupts a component of normal regulation of ingestion.

Naloxone Opioids Morphine Ingestion Drinking

NALOXONE, the prototypic narcotic antagonist, reduces fluid intake in deprived and nondeprived, opioid naive rats [5, 6, 7, 9, 11]. Since the effect of naloxone, although highly reliable, is merely to reduce ingestion by a relatively small amount (about 30% reduction in water intake), we wondered whether severe deprivation might override this naloxone effect. If the naloxone-produced reduction is not greatly attenuated by a severe drive to consume, it may be that naloxone acts, under several conditions, in a robust and specific manner to decrease consumatory behavior. Two of the four experiments reported here assess the effects of deprivation on the naloxone-produced decreases in consumption.

EXPERIMENT 1

When sucrose solution is presented periodically to rats that have standard laboratory chow and water available continuously, they drink large quantities of solution. It has been shown that 10 mg/kg naloxone [11] as well as smaller doses [2] are effective in reducing fluid intake. The first experiment determines whether severe deprivation will alter the amount of sucrose solution consumed after rats receive 2 mg/kg of naloxone.

METHOD

Subjects and Apparatus

Forty, male, albino rats (Mean wt. = 344 g at the beginning of procedures) were used. Animals were Sprague-Dawley derived and were obtained from Taconic Farms. All animals were experimentally naive, as well as opioid naive, at the beginning of procedures. Subjects were individually housed in standard metal cages and had access to food and water as specified. The animals were housed in a room on a reverse light-dim light cycle (LD 12:12 with dim phase beginning at 1000 hr). All tests involving naloxone administration took place from 3 to 6 hr after the beginning of the dim phase, a period of greater activity for the subjects. Fluids were presented in bottles equipped with ball point sipping tubes.

Procedure

After random assignment, one-half of the rats (nondeprived group, n=20) were given free access to standard laboratory chow and water 23 hr/day for 5 days. At the same time each day, chow was removed from the cages and immediately thereafter water bottles replaced by bottles containing 10% (wt./vol) sucrose solution. Sucrose solution was available for an hr after which chow and water were again available until the next day. The other half of the rats (deprived group, n=20) were not given access to chow or water during the 23 hr between opportunities to take the sucrose solution, but they were given about 6 g of chow (1 medium block) after their opportunity to take sucrose.

After 5 days of the schedule, subjects within each group were ranked according to the amount of sucrose consumed and then assigned to either receive naloxone or saline injections in an ABBA fashion so that the amount consumed at the end of the 5th day was roughly equal for those that would eventually receive naloxone or saline. On the next day (6th day of procedure) each subject was injected with either naloxone hydrochloride, 2 mg/kg, subcutaneously (SC), or physiological saline, the carrier of naloxone. All injections were given 15 min before sucrose solution was presented and were 1 ml/kg.

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Across the next days, animals were maintained on their respective feeding schedules and all animals received the same drug injection again on the 5th day after their first injection. This day of injection was followed by two days of post-injection measures, making the procedures span a 13-day period. The amount of sucrose solution taken during each daily session was measured to the nearest 0.5 g by weighing the bottles before and after presentation.

On days when animals received no injections, Analyses of Variance (ANOVAs) indicated that the groups differed only with respect to the condition of deprivation. Therefore, only the sucrose consumption-data for days of injections are reported here. These scores (g consumed) conform to a 2×2×2 factorial experimental design having repeated measures with factors for deprived-nondeprived, naloxone-saline, and the two successive tests.

RESULTS AND DISCUSSION

The subjects of deprivation appeared healthy but steadily lost weight across the 13 days of the procedure. They lost a mean of 71 g. Consequently, it is assumed they were highly motivated to take nutrients. The nondeprived subjects weighed about the same as the other group at the beginning of the experiment but gained a mean of 32 g by the end of procedures. The nondeprived subjects were obviously not driven to take sucrose solution by virtue of deprivation.

Figure 1 summarizes the results. An ANOVA of the scores confirmed the expectation that deprived subjects would take more sucrose solution than nondeprived subjects: Mean for deprived subjects=22.5 g; mean for nondeprived subjects=8.9 g; $F(1,36)=155$, $p<0.001$. Also as expected, animals receiving naloxone drank less than animals receiving saline: Mean for saline-subjects=19.6 g; mean for naloxone-subjects=11.9 g, $F(1,36)=49.6$, $p<0.001$.

There was a reliable drug × deprivation interaction (Fig. 1), $F(1,36)=4.5$, $p<0.05$. Further analyses of the scores of the interaction indicate that naloxone reduced consumption compared to saline controls in both the deprived and nondeprived subjects: $t(18)=8.44$, $p<0.001$ for the deprived animals; $t(18)=5.44$, $p<0.001$ for the nondeprived animals. The issue of whether naloxone affects the deprived and the nondeprived animals differently is addressed and discussed in the report of Experiment 3.

There was a reliable trials-effect with subject taking more sucrose on the second test: Mean for the first treatment=13.6 g; mean for the second treatment=17.8 g; $F(1,36)=20.7$, $p<0.001$. In general across the 13 days of treatment, subjects increased consumption. This trials-effect did not reliably interact with any other factor and the triple interaction of the ANOVA was not a reliable source of variance.

When scores were transformed into g consumed/kg of body weight and analyzed, the same factors emerged as reliable sources of variance. When difference scores were used in an ANOVA (score after injection minus mean score for 2 days before injection) the same factors emerged except the trials effect. In summary, it can be confidently concluded that naloxone reliably reduces sucrose solution intake in both deprived and nondeprived subjects: a 36.5% decrease was seen in deprived animals, while a 46.2% decrease was observed in nondeprived subjects.

These data confirm the initial report [11] that naloxone reduces intake of sucrose solutions in nondeprived rats, although the extent of reduction was not quite as large as observed initially. The smaller reduction is probably due to the smaller dose of naloxone (10 versus 2 mg/kg). The effect of naloxone reducing fluid intake is dose-dependent and occurs with doses less than 1 mg/kg [9, unpublished data from this laboratory].

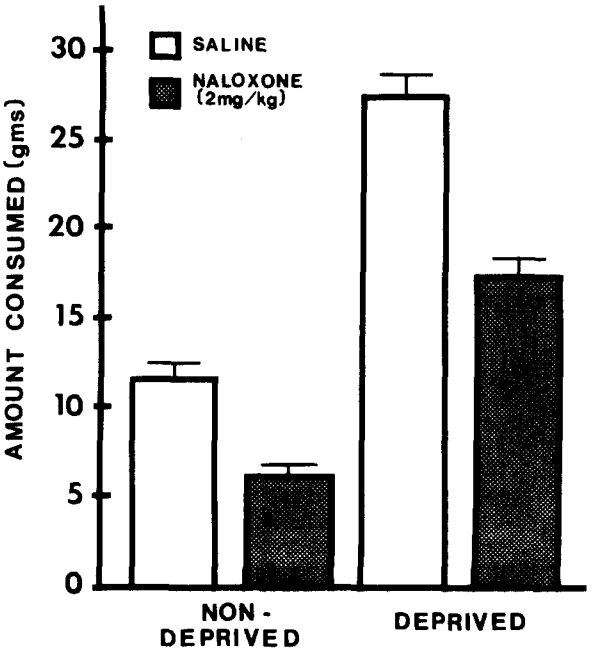

FIG. 1. Naloxone produces a decrease in sucrose solution intake in rats trained to consume the solution. Means and standard errors of the means are depicted for tests lasting one hr.

EXPERIMENT 2

It is clear, naloxone reduces intake of sucrose solution in 24-hr deprived rats. The question remains whether this effect is specific to naloxone's putative antagonism of endogenous opioid systems or to a non-specific effect. Since naloxone can be used as the unconditioned stimulus to establish a conditioned taste aversion (CTA) [8, 12, 13], it is possible that naloxone is merely inducing an illness or malaise that, in turn, results in reduced intake. To test this possibility, the same rats given naloxone in Experiment 1 were tested for the potential of naloxone, at the same dose, to establish a CTA. If the processes indexed by the CTA-test can account for any of the variance in naloxone's reduction of sucrose intake, there should be a correlation between the magnitude of reduction in sucrose solution intake and the magnitude of CTA.

METHOD

The same rats (n=20) which received naloxone in Experiment 1 began these procedures 2 days after the procedures of Experiment 1. During those 2 days, subjects had food and water available continuously. Animals were all given free access to food, but trained for 6 days to consume their daily ration of water during a 15-min period each day. Every 3rd day thereafter, animals were presented with a 0.1% saccharin solution, instead of water, and injected with 2 mg/kg of naloxone (SC) immediately after the drinking session. This saccharin presentation was repeated 4 times.

RESULTS AND DISCUSSION

The dose of 2 mg/kg of naloxone was not sufficient to sustain a reliable CTA, as would be indicated by reduced intake of saccharin solution of days following naloxone injections as the putative UCS. In fact, the subjects had a tendency to increase consumption of saccharin subsequent to naloxone injections, means for tests one to four were 14.5, 16.0, 16.6, and 14.7, respectively. The lack of a reliable taste aversion (using a test that is, in general, quite sensitive) argues against the notion that naloxone produces an illness and this illness in turn accounts for naloxone's ability to reduce sucrose consumption. There were, nevertheless, individual differences among subjects' CTA-scores (ranging from 5.6 to 22.4 g) and these differences might still be related to naloxone's ability to suppress intake of sucrose solution.

From previous work [4], it has been shown that the score of the last opportunity to take saccharin is highly correlated with other potential indices of the CTA and was most sensitive in predicting morphine consumption. Therefore, we used here the grams of saccharin solution/kg taken on the last opportunity to take saccharin as the CTA-score. The rank order correlation between the CTA-score and the sucrose reduction-score (mean intake of sucrose in g/kg of the second test of Experiment 1) across the subjects of deprivation is rho=0.05, $p>0.2$ and the rho for subjects of nondeprivation is -0.40, $p>0.2$. These observations do not lend support to the idea that processes indexed by the CTA-test can account for any significant proportion of the variance in naloxone's ability to suppress sucrose solution intake.

There have been two other tests of the relationship between naloxone's ability to produce a CTA and its ability to reduce fluid consumption. One test [16] found only a low, nonreliable correlation with water intake using similar procedures. The other test found that a substance which can produce a strong CTA (lithium chloride) did not result in decreased consumption when the agent was administered prior to a drinking session [2]. These observations show that even if naloxone can sustain a CTA such an event probably would not reduce fluid consumption. Give the extant constellation of results, it is difficult to conclude that naloxone reduces consumption merely because it might induce an illness.

EXPERIMENT 3

The purpose of this experiment is similar to that of Experiment 1, i.e., there is an interest in assessing whether severe deprivation will attenuate the naloxone-effect. From previous work [5, 6, 7, 9, 11], it is known that naloxone (10 mg/kg) reduces water intake about 30% in 24-hr deprived rats. Smaller doses are also effective in reducing fluid intake [2, 9, 16].

The reliable interaction obtained in Experiment 1 is difficult to interpret in terms of the question "Does naloxone produce more suppression in less deprived rats?" It is difficult because the extent of intake differs so radically between the nondeprived and deprived conditions. In order to ask whether degree of motivation modifies the naloxone-effect, what is needed is a circumstance where amount consumed is relatively constant but motivation varies.

Such a circumstance occurs when rats are compared under 23- and 47-hr water deprivation schedules. Subjects of these two deprivation schedules will consume about the same amount during brief opportunities to drink but latency to begin drinking is shorter in 47-hr deprived rats and bar presses for water are greater in 47-hr deprived subjects [1]. Consequently, it can be inferred that although consumption levels are similar, motivational levels are different with subjects of more severe deprivation being more highly driven.

METHOD

Subjects

Subjects were 12 experimentally naive, male, albino rats (Sprague-Dawley derived). Subjects weighed approximately 220 g at the beginning of procedures. Rats were housed under similar conditions to those described in Experiment 1. Standard laboratory chow was always available.

Procedure

All subjects were trained for 10 days to consume their daily ration of water during a 15-min session at the same time each day. Subjects were then assigned to conditions requiring either 23.75 or 47.75 hr of water deprivation prior to their drinking sessions and were injected 15 min prior to each session. The sequence of treatment for any particular test was placebo-placebo-drug treatment (naloxone/placebo)-placebo-placebo, under 23.75 or 47.75 hr of water deprivation. A within subjects design, with the order of tests counterbalanced (Latin Square arrangement) was used to provide for testing each subject under each deprivation condition (23 vs 47 hr deprived) and under each drug condition (naloxone vs saline). All injections were given, SC, 1 ml/kg and the dose of naloxone used was 2 mg/kg.

Since preliminary analyses indicated no order effects and treatments were counterbalanced, scores were collapsed across orders of treatments. There were no differences between groups on days when saline was given to all subjects, so only those scores for days when naloxone or saline were given are reported. These scores (g consumed) conform to a 2×2 design having repeated measures with a factor for deprivation schedule (23 vs 47 hr) and a factor for drug treatment (naloxone vs saline).

RESULTS AND DISCUSSION

The results are summarized in Fig. 2. Deprivation did not have an effect on the amount of water consumed during the drinking sessions, $F(1,11)=3.51$, $p>0.05$. There was a reliable drug-effect on the amount of water consumed, with animals receiving naloxone generally consuming an average of 4 g of water less than when they had received saline, $F(1,11)=38.2$, $p<0.001$. There was not a deprivation×drug interaction suggesting that the state of deprivation has little effect on the amount of water consumed after naloxone, $F(1,11)=0.2$, $p>0.5$ (see Fig. 2). The percent reduction in water intake after naloxone injection was 25.2% decrease in 23.75-hr deprived subjects and 24.2% decrease in 47.75-hr deprived rats.

The more severe deprivation surely did not attenuate the naloxone-effect and, in fact, the degree of deprivation hardly modified the naloxone-effect. Since the naloxone-effect does not interact dramatically with deprivation and since the naloxone-effect is so reliable, it is concluded that naloxone directly affects some component of the behavioral control of drinking that does not covary with motivational status.

EXPERIMENT 4

The idea that endogenous opioids are related to regulation

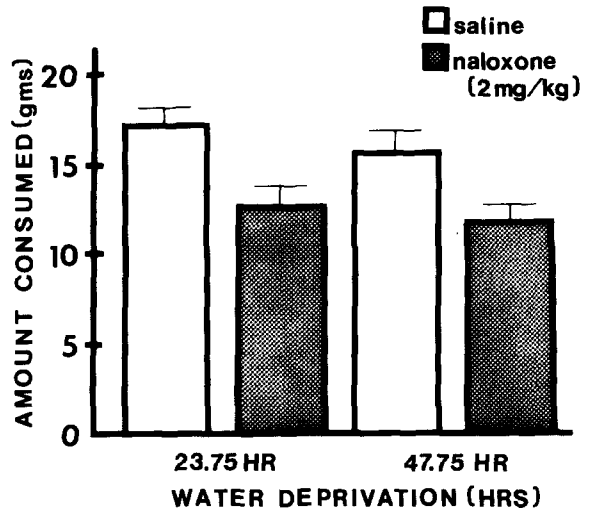

FIG. 2. Naloxone produces a decrease in water consumption in rats trained to consume their daily water ration. Means and standard errors of the means are depicted for tests lasting 15 min.

of water intake would be supported by results demonstrating that narcotic agonists increase water consumption. Maickel *et al.* [9] found that agonists increased water intake but Stapleton *et al.* [11] could not obtain the effect. The dose range for producing the effect may be narrow, and the effective dose may also differ between subjects. In this context, the following observations with rats self-administering morphine and the effects on water intake are important.

METHOD

Subjects

Four, male, experimentally naive, Sprague-Dawley rats weighing about 375 g at the start of the procedures were each fixed with a chronically indwelling jugular catheter. The catheter and procedures for fixing it were similar to those described in the literature [14,15]. The procedure and results to be described here are the first part of a larger experiment testing the effects of various manipulations on propensity to self-administer morphine. These subjects were given a test of propensity to self-deliver morphine across a four-day period (when these observations were made) as a preliminary test to subsequent manipulations which will not be described here. They were in the chamber 6 hr/day during the dim light phase.

Apparatus

The experimental chamber, a clear plastic box (34×20×24 cm), contained a lever (1×2.5×3 cm) extending through one wall, 7 cm above the floor. The subject's catheter system was attached to a swivel at the top of the test chamber, allowing for unhampered movement. The intravenous tubing was continuous with the infusion pump which delivered 0.25 ml of 0.9% physiological saline or 0.5 mg/kg morphine sulfate in saline across 10 sec upon activation. The water dispenser, attached to the outside of the chamber, contained a ballpoint sipping tube which extended slightly into the chamber. Food was available ad lib. The amount of food and water consumed was measured after every session. The chamber was well-illuminated and enclosed within a ventilated sound attenuated compartment. Lever presses were recorded hourly for a continuous reinforcement schedule of morphine administration.

RESULTS AND DISCUSSION

Three of the four subjects pressing rates were greater when morphine was the contingency than when saline was the contingency. Two of the four subjects greatly exceeded their pressing rates for saline when given the opportunity to press for morphine. Furthermore, the pattern of pressing for morphine differed from the pattern of pressing for saline. Pressing for morphine was characterized by regularly spaced presses while presses for saline generally occurred in one cluster. The mean number of presses across all trials with saline was 8.5/6 hr compared to the mean with morphine of 22.9/6 hr, or the equivalent of 11.4 mg of morphine/kg/6 hr session.

The rats increased their intake of water on days when morphine was available for self-administration compared to days when they received saline: Mean, 6 hr intake under morphine across 4 days of opportunity=15.3 g; mean intake with saline=8.6 g; $t(3)=3.72$, $p<0.05$. Although food intake was also increased, the degree of increase did not meet levels of statistical significance. With four subjects, there is, of course, a good change of making a Type II statistical error. Rats under saline usually took a meal or two during their time in the chamber. Rats under morphine seemed to nibble constantly at the food available during periods when they were not drinking or exploring the manipulandum. Informal observations then, on feeding behavior as well as the statistical results with drinking, support the idea that morphine seems to engage ingestive behaviors at doses which are self-administered by rats. Single large doses of morphine administered to rats reduces food and water intake presumably because these doses produce a number of additional events that are incompatible with ingestion.

GENERAL DISCUSSION

One might presume that the stress of privation might trigger the release of endorphins and that release, in turn, might affect ingestive behaviors. The finding, however, that naloxone effectively reduces sugar-water consumption in nondeprived rats and the finding that deprivation-level does not radically modify naloxone's actions lead to the suggestion that deprivation level is not a covariate of the naloxone effect. The fact that naloxone's effects are not related to the ability of naloxone to sustain a conditioned taste aversion provides evidence for a specific effect of naloxone on ingestive behaviors. Opioids modify ingestive behaviors and this effect is probably not due to some side-effect of stress or non-specific effect of the agents.

Although there may be opioid systems that are not antagonized by naloxone, there is general agreement that naloxone does antagonize the recently discovered but well-described endorphinergic system [3]. A naloxone effect in opioid naive subjects, therefore, is thought to reflect the disruption of the functioning of an endorphinergic system [3]. Naloxone reduces food and water intake, morphine in self-administered doses increases water intake, and, possibly food intake, and mice and rats that are fat seem to have more endogenous opioids than their lean counterparts [10].

Whether the putative regulatory process is a CNS-effect

or a peripheral (gut) effect, is related to perceptual events or response limitations, or is a specific component of the mechanism which maintains water balance or involves multiple, indirect effects, are issues that await further investigation. Nevertheless, it seems clear that naloxone reduces intake of fluids in a wide variety of circumstances including when there is severe deprivation. This observation may be important with respect to isolating an intrinsic property of opioids or as an intrusive variable confounding the search for other functions of the endorphinergic system.

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