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Morphine Antagonists and Consummatory Behaviors

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OSTROWSKI, N. L., N ROWLAND, T L FOLEY, J. L NELSON AND L D. REID *Morphine antagonists and consummatory behaviors*. PHARMAC BIOCHEM BEHAV **14**(4) 549–559, 1981 —Opiate antagonists were tested for their effects upon either drinking or eating in eight experiments. Naloxone, nalorphine, and the active isomer of WIN 44,441 all reduce drinking Neither an analog of nalorphine that does not cross the blood-brain barrier, nor the inactive isomer of WIN 44,441 is effective in reducing water intake. These data provide support for the conclusion that these antagonists have stereospecific effects within the central nervous system. Naloxone suppresses drinking following procedures inducing osmotic, volemic, or hormonal thirst. Naloxone suppresses eating following procedures inducing glucoprivation but does not alter eating elicited by tail-pressure Collectively, these data lead to the conclusion that endorphins play a role in the organization of ingestive behavior following challenges to homeostasis

Opioids Agonist Tolerance Opiates Opiate antagonists Naloxone Nalorphine
Quaternary salt WIN 44,441 Thirst Drinking Hyperosmotic Polyethylene glycol
Angiotensin Feeding Insulin 2-deoxyglucose Tail-pinch Motivated behavior

THE BEHAVIORAL functions of endogenous opioids are poorly understood. One approach to this problem is to observe behavioral changes when endogenous opioid effects are blocked pharmacologically. This method has problems similar to those of lesion studies, in that inferences must be drawn about the system in question from the behavior of animals functioning without that system. The method also has the problems of pharmacological studies in that drugs hardly ever have isolated effects [27]. Despite these problems, the procedure of giving antagonists and then observing behavior can provide clues to the function of endogenous opioids [10].

N-allyl-noroxymorphone (naloxone) antagonizes the effects of opioids in many procedures, and reliably reduces ingestive behavior in opiate-naive rats [12, 13, 14, 33]. For example, there is a reduction in water intake by rats deprived of water from 6 to 48 hr [9,24]. These reductions do not seem to be due to a potential nonspecific malaise induced by naloxone. While naloxone can produce varying degrees of conditioned taste aversions (CTA) in rats [9,37], the magnitude of the aversions does not correlate with the reductions in fluid consumption [24,36] Also, naloxone reduces fluid intake at small doses (<1 mg/kg) which generally do not produce marked signs of illness or malaise.

Naloxone also reduces food intake. Naloxone reduces nutrient intake after food deprivation as well as when nondeprived rats are given access to palatable substances (e.g., sucrose solutions [2, 9, 33]). Naloxone also decreases the feeding induced by diazepam [32]. Also of interest is the finding that intrahypothalamic application of β-endorphin increases food intake [11] and genetically obese rodents have higher levels of endorphins [21].

The experiments reported here examined the specificity of naloxone's effects in suppressing ingestive behavior. First, we tested whether the reduction in drinking was a stereospecific effect of an opioid antagonist. Next, we examined whether the effect was of central origin or peripherally mediated by using a partial agonist-antagonist which does not cross the blood-brain barrier. We then investigated the possibility that naloxone was simply rendering the animals incapable of drinking more than a fraction of their daily water using a procedure involving an orogastric preload. Finally, we tested naloxone's effects on drinking and feeding induced by various challenges. The results support the idea that the effectiveness of naloxone in reducing water and food intake is a specific effect related to naloxone's ability to stereoselectively occupy opiate-receptors within the central nervous system

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EXPERIMENT 1

9 β-(pent-3-one-5 cyclopentyl-ane)-metazocine (WIN 44,441, methanesulfonate salt) is a potent opioid antagonist with binding properties similar to naloxone, which is available in two enantiomeric forms [22,38]. The levorotatory form (levo-WIN) possesses full antagonistic activity in the guinea pig ileum and mouse vas deferens [22,38], while the dextrorotatory form (dextro-WIN) is inactive. If the reductions in water intake reflect specific drug effects at opioid binding sites, then only levo-WIN should be an effective antidipsogen. If, on the other hand, the reductions reflect a nonstereoselective or non-opioid action of the drug, then levo-WIN and dextro-WIN should have similar effects on drinking.

METHOD

Subjects and Apparatus

Twenty male Sprague-Dawley rats (from Taconic Farms, Germantown, NY), initially weighing about 185 g, were housed individually in standard metal cages. The colony room was maintained at 24°C, on a bright-light/dim-light cycle (dim period: 1000 to 2200 hr). Laboratory chow was available at all times except during the drinking sessions. Water bottles were equipped with sipping tubes with ball point tips and were weighed to the nearest 0.1 g before and after the opportunity to drink.

Procedure

Two days after their arrival at the laboratory, the rats started a 14-day training period during which they learned to consume a daily ration of water during a single 15-min presentation at 1200 hr. Based on the amounts consumed during the last two days of training, rats were divided into two matched groups (n=10). On the test day, 15 min before the drinking session, one group received a subcutaneous (SC) injection of 2 mg/kg of levo-WIN and the other received 2 mg/kg of dextro-WIN. The enantiomers were dissolved in 1 ml/kg of bacteriostatic water. Water intakes, in the absence of food, were measured for 15 min. The animals were maintained on this schedule, and 6 days later the test was repeated to replicate initial findings.

To ensure that these animals would respond as usual to naloxone, they were maintained on the drinking schedule for 6 more days. Then, one-half of the animals from each previous treatment group received 2 mg/kg of naloxone hydrochloride (SC) while the other half received injections of the carrier, 0.9% saline. The amount of water consumed during a 15-min test, 15 min after injection, was measured.

Data from one subject of each group were deleted from the initial test with the WIN compounds because their sipping tubes were blocked. Consequently, these same subjects' data were excluded from an overall analysis of variance (ANOVA) but were not excluded from comparisons involving the second (repeat with WIN compounds) and third (naloxone) tests of drug effects.

RESULTS AND DISCUSSION

With the initial tests, only the levo-WIN was effective in suppressing water intake, $F(1,16)=11.52$, $p<0.005$ (Fig. 1). The group receiving levo-WIN consumed 15% less water than the group receiving dextro-WIN on the first test day and 13% on the second test day. Reliable decreases were also found when intakes after injections with levo-WIN were compared (dependent *t*-tests) to that group's baseline intakes (mean intake across two days prior to drug injections). On the second test, for example, the intakes of the levo-WIN injected rats were decreased with respect to baseline by 10% (baseline mean=15.23, treatment mean=13.7, $p<0.05$). Intakes of rats after receiving dextro-WIN were not significantly different from their baseline intakes. At the dose used, therefore, levo-WIN produced a small, but reliable decrease in the water intake of deprived rats.

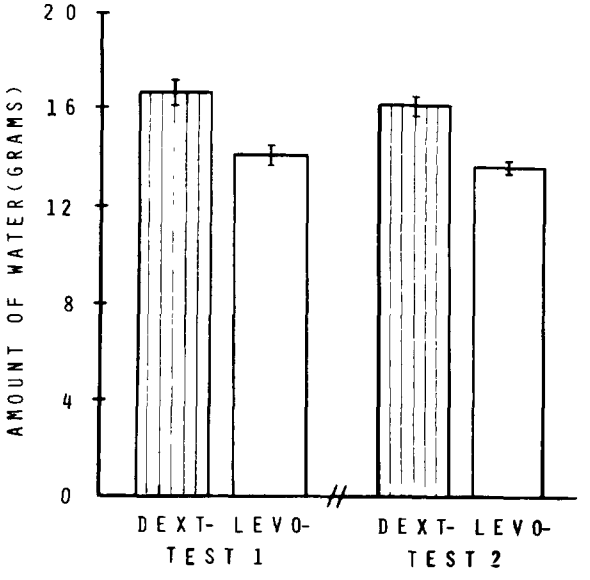

FIG. 1. Mean water intakes following injections of the two enantiomers of WIN 44,441. The lines extending from the bars are standard errors of the means for comparing independent groups. A 2 by 2 ANOVA having repeated measures with factors associated with the enantiomers and the repeated tests indicated that there was a reliable difference in the amount of water consumed with drug injections, $F(1,16)=11.52$, $p<0.005$. The factor of trials, $F(1,16)=1.16$, $p>0.20$, and the trials by group interaction, $F(1,16)=0.00$, $p=0.99$, were not reliable sources of variance.

With the test using naloxone, animals receiving naloxone drank 27.5% less water than those receiving saline (mean with saline=17.55, mean with naloxone=12.73, $p<0.002$). There were no reliable differences in the amount of water drunk between rats previously receiving the levo- and dextro-WIN enantiomers.

This dose of levo-WIN might not exert a full effect for some time, and we noticed that water intakes of levo-WIN-treated rats were still depressed ($p<0.05$) the next day. This is consistent with the finding that levo-WIN has a tenfold longer duration of action than naloxone in binding studies (Michne, personal communication). Since only the active enantiomer of WIN 44,441 and naloxone were effective in reducing water intake, these data support the suggestion that the reductions in water intake result from stereospecific activity of the antagonists. It remains to be seen whether larger, yet specific, reductions in drinking are obtained with larger doses of dextro- or levo-WIN and/or after longer delays between the injection and testing.

EXPERIMENT 2

N-allyl-normorphine (Nalorphine) is a partial agonist-antagonist which can generally block the effects of morphine, but by itself can produce analgesia [16]. It also reduces water intake [13] but it is not clear whether this effect is due to its antagonist properties, or due to a general behavioral depression characteristic of opioid agonists. In the present experiment, which is a logical precursor to Experiment 3, we have attempted to determine whether nalorphine's effects on fluid intake are due to its agonist or antagonist properties. To do this, we have made use of the fact that the sedative effects of agonists generally tolerate with repeated administrations, but antagonist (e.g. naloxone) effects on drinking typically show little or no tolerance (Merriman and Reid, in preparation). We therefore examined whether the effects on water intake of chronically administered nalorphine resembled more closely the effects produced by chronic regimens of a pure agonist (morphine) or antagonist (naloxone)

METHOD

Subject and Apparatus

Thirty-six male Sprague-Dawley derived rats (Zivic-Miller, Pittsburgh, PA) were individually housed in metal cages in a colony room maintained on a reversed light/dark schedule (L/D:12/12 hr) with the dark period beginning at 1200 hr). Water intakes were measured (±0.1 ml) by reading the graduations on a buret fitted with a metal sipping tube. Food was available except during the scheduled drinking sessions.

Procedure

For 15 consecutive days, the animals were maintained on a drinking schedule of 15 min water availability each day at 1630 hr. Rats were then assigned to one of six groups matched for baseline intakes, and the water restriction schedule was maintained. One group received injections of morphine sulfate (10 mg/kg in 2.0 ml/kg of saline) 15 min before the drinking session for each of the next 9 days. Its control group received vehicle (saline) injections for 9 days. On Day 10, both groups received morphine (10 mg/kg). The third and fourth groups received injections of 10 mg/kg of naloxone HCl, or saline for 9 days, and on Day 10 both groups received naloxone The fifth and sixth groups received daily injections of nalorphine (Nalline hydrochloride, 10 mg/kg) or saline for 9 days and on Day 10 both groups received nalorphine. All injections were given SC, 15 min before water presentation. Drug-injected animals which did not drink at least 8 ml of water on a given day were injected with saline (up to 8 ml/rat, SC) about 2 hr after the drinking session in order to prevent severe dehydration.

The data were analyzed using an ANOVA for a 2 by 3 by 9 factorial design having repeated measures with factors for (a) saline or drug injections (treatment), (b) type of injection (morphine, nalorphine, or naloxone), and (c) the nine daily measurements. There were ANOVAs for repeated measures comparing the effects of each drug administration to its respective control. The data for Day 10, when all subjects received drug injections, were analyzed separately using ANOVAs. Because 9 days of drug treatment resulted in lower body weights than in rats receiving saline injections, the water intakes were analyzed both as mls consumed and as ml/kg; both ANOVA's gave similar results, and only the latter are presented for brevity.

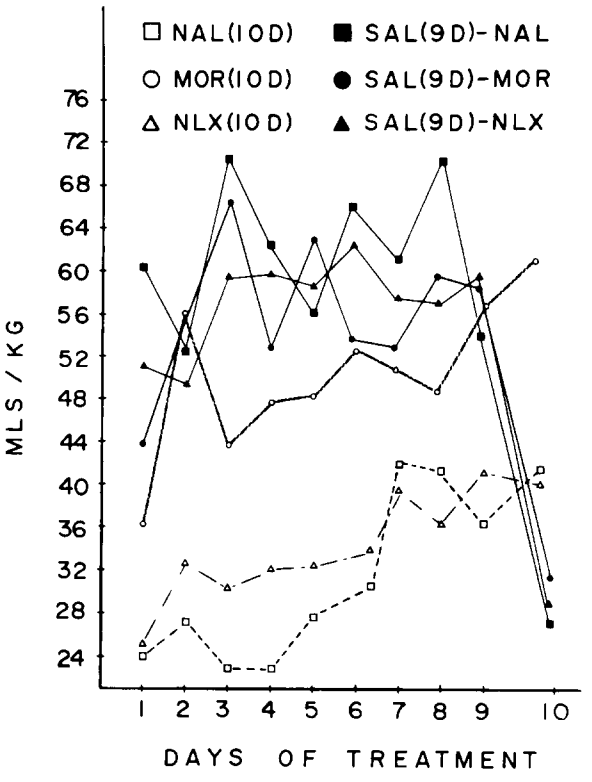

FIG. 2. Nalorphine and naloxone exert similar effects on drinking Depicted are group means (ml/kg of water consumed) for Experiment 2 Three groups received 9 days of saline injections followed by 10 mg/kg of morphine (MOR), nalorphine (NAL) or naloxone (NLX) on Day 10. The other three groups received 10 days of injections with 10 mg/kg morphine, nalorphine or naloxone Groups receiving 9 days of nalorphine or naloxone differed reliably from their respective control groups, and from animals receiving 9 days of morphine.

RESULTS AND DISCUSSION

As can be seen by inspecting Fig. 2, the subjects that received 9 days of drug-treatment consumed less water than subjects that received saline, $F(1,30)=35.5$, $p<0.001$. There was also a reliable effect associated with the factor of repeated tests, with animals consuming more water as testing continued, $F(8,240)=3.32$, $p<0.001$. More germane to the issue, however, are that the interactions associated with treatment by days of treatment ($p<0.004$) and treatment by particular drug ($p<0.02$) emerged as significant sources of variance.

The groups receiving saline for 9 days did not reliably differ from one another in amount consumed ($p<0.20$) but did consume increasing amounts of water as testing progressed ($p<0.02$) When each drug-group's scores were compared to its respective saline-control group's scores, it was found that both the nalorphine group and the naloxone group differed reliably from their respective control groups ($ps<0.001$) There was, however, no difference between the

group that received morphine for 9 days and its respective control group ($p>0.68$).

The data for Day 10, when all animals received drug, indicated that naloxone, nalorphine and morphine were equally effective in reducing water intake in animals that had not previously received drugs (Mean consumed: naloxone=29.1, nalorphine=27.6, morphine=29.8 mg/kg), $F(2,15)=0.04$, $p>0$ 96. On the other hand, scores for animals that had received the respective drugs for 9 previous days differed markedly, $F(2,15)=8.43$, $p<0.004$, their group means being naloxone=40.1, nalorphine=42.2, morphine=61.1 ml/kg. By Day 10, animals receiving morphine were comparable in their intake of water to animals receiving saline.

These observations confirm that the suppressive effects of morphine on water intake tolerate quickly. Naloxone and nalorphine retained their capacity to reduce intake to below control values and both produced similar effects This is supported by the finding that overall, the groups receiving 9 days of naloxone and nalorphine did not differ from each other ($p>0.68$) but did differ from the group receiving 9 days of morphine ($ps<0.05$). Nalorphine's effects on water intake thus resemble those of naloxone more closely than those of morphine. Based on these observations, it is inferred that it is the antagonist properties of nalorphine which are primarily responsible for its antidipsogenic effects. These results led us to the following experiment.

EXPERIMENT 3

Opioid antagonists reduce drinking in rats. No extant results, however, indicate the location of the critical receptors. In favor of a possible peripheral site of action is the fact that naloxone can increase peristalsis by direct action upon the intestine [20] as well as have effects on a wide variety of peripheral opioid receptors. In favor of a central nervous system site of action are the facts that (a) the general idea that a complex behavior, such as ingestion, involves central events [28], (b) opiate receptors are present in areas known to be involved with ingestion [15], and (c) naloxone is effective when applied centrally [33] even though the effective doses are quite high and thus do not provide unequivocal support for the central hypothesis.

Using our preceding result that nalorphine reduces water intake via its antagonist properties, we now report an experiment using N,N-diallyl-normorphinium bromide (FR 13-BR, Boehringer und Sohn, Ingelheim). This quaternary salt of nalorphine does not cross the blood brain barrier at low doses [17,20] Thus, if it has a suppressant effect on water intake this would constitute evidence in favor of a peripheral site of action of opiate antagonists in this paradigm. If, on the other hand, it has no such effect, then support is gained for the central hypothesis.

In a pilot study, using seven rats/group, we found no evidence that 2 or 10 mg/kg of quaternary nalorphine reduced intake of water, but as in Experiment 2, nalorphine and naloxone did reduce intake We report here the results of a more formal study.

METHOD

Forty male Sprague-Dawley derived rats (Taconic Farms) were maintained as described in Experiment 1 on a schedule of 15-min/day access to water. On days before and after the test day, rats received injections of placebo. On the test day, the groups (N=8) were randomly assigned to receive one of 5 injections, 2, 10 or 15 mg/kg of quaternary nalorphine, 10 mg/kg of nalorphine, or saline vehicle. All injections were given SC in volumes of 2 ml/kg, 15 min before access to water.

RESULTS AND DISCUSSION

An ANOVA of the difference scores (obtained by subtracting amount drunk on day of drug treatment from the mean amount drunk on placebo days before and after drug treatment yielded an $F(4,35)=4.48$, $p<0.01$. Nalorphine reduced drinking by a mean of 6.43 ml, or 59% (Dunnett test: $p<0.05$ vs saline [7]). The groups receiving each dose of quaternary nalorphine drank about 0.5 ml more than their baseline while under the influence of the drug (n.s.).

The failure of this peripheral antagonist to affect water intake supports the hypothesis that opiate antagonists in general, reduce drinking via a central action. Quaternary nalorphine, when administered IP, antagonizes opiate effects at peripheral but not central sites [20], but both forms of nalorphine are equally effective in precipitating withdrawal in rats after intraventricular administration [17]. If future studies show that quaternary nalorphine does not share all the peripheral effects of opiate antagonists, then the strength of our conclusion will have to be re-evaluated. It is also possible that higher doses of the quaternary salt might produce effects on drinking behavior, but at higher doses it may also cross the blood-brain barrier [17].

Naloxone, levo-WIN 44,441, nalorphine (Experiments 1 to 3) and naltrexone (unpublished data of our laboratory) are antagonists of both morphine and endorphins and reduce water intake. The fact that these four diverse antagonists, as well as others reported since this manuscript was originally submitted [4], each reduce water intake adds support to the idea that a function of endorphins is related to the control of ingestion. None of the antagonists came close to eliminating drinking; each merely attenuated deprivation-induced drinking. At this time, however, it seems reasonable to conclude that the antagonists have a central nervous system effect that attenuates water intake.

EXPERIMENT 4

We have thus far offered pharmacological evidence for the specificity of opioid antagonists in reducing water intake. There is, however, no information concerning the function(s) affected by the antagonists. Naloxone injections may result, for example, in amplified satiety signals, may disrupt an endorphinergic feedback loop that usually sustains appetitive behavior, may lead to increased fatigue or reduced attention. Some of these alternatives may be examined by using a procedure in which animals are given a stomach load of water before the opportunity to drink. If the naloxone-produced decrease in drinking is due to behavioral disruption and this limits the amount of water ingested in 15 min to 70% of normal intake, a stomach load of 50% of normal intake should still be followed by voluntary consumption of the other 50% of baseline intake. Since this amount is well within the behavioral capacity of naloxone-treated animals, such a result would suggest that the naloxone-produced decrease in drinking is due to behavioral disruption limiting the amount of water consumed. If behavioral incapacitation does not account of naloxone-produced reduction in drinking, the typical (about 30%) decrease in total intake produced by naloxone (2 to 10 mg/kg) might again be expected.

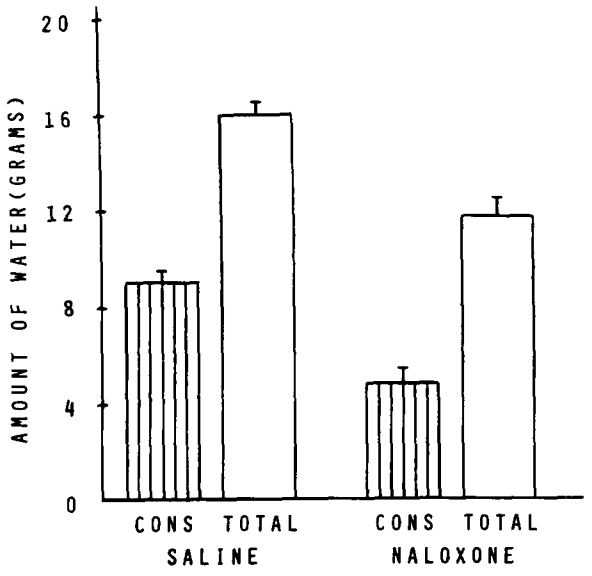

FIG. 3 Amount of water consumed after orogastric intubation with 50% of mean baseline water consumption. After gavage naloxone pre-treated (2 mg/kg) animals actively consumed (CONS) less water than saline-treated controls ($p<0.001$). The total amount of water taken by naloxone pre-treated animals (amount intubed+amount drunk) was 73.6% that of controls

METHOD

The animals were 21 male rats (Taconic Farms) housed as described in Experiment 1. Animals weighed about 212 g on the day of testing. Food was available except during daily water presentation.

Subjects were trained for 18 days to consume their daily ration of water in 15 min. On the basis of water intakes on the last two baseline days, they were divided into two matched groups; one group to receive 2 mg/kg of naloxone and the other group to receive saline, 1 ml/kg, the carrier.

On test day, the subjects received injections of naloxone or saline and after 5 min were given orogastrically 6.9 ml tap water (50% of the mean baseline intake). Ten min after the gavage (15 min after the drug), the daily 15-min drinking session was conducted as usual.

RESULTS AND DISCUSSION

The mean baseline water intakes (for the 2 days prior to treatment) were 13.8 and 13.7 g of water for the saline and naloxone groups, respectively. After the water load, saline injected rats drank a mean of 9.0 g, an amount which brought their total water (load+amount drunk; 6.9 g+9.0 g) to 15.9 g or about 2 g more than their baseline. After the stomach load, naloxone injected rats drank a mean of 4.8 g, a value that brought their total water to 11.7 g, or about 2 g less than their baseline intake. The difference in the amount of water drunk during the test by naloxone and saline treated rats were statistically significant, $t(19)=4.19$, $p<0.001$. Interestingly, the naloxone-treated animals modulated their oral intake so that their total water for the day was 73.6% of the water obtained by rats receiving saline; a percentage similar to those observed without preloading (e.g., see Experiment 1).

Rats under the influence of 2 mg/kg of naloxone are capable of drinking more than 4.8 ml of water during 15 min but the rats receiving naloxone did not. This result suggests that a number of possible explanations of antagonists' effects on water intake can be ruled out. Explanations focusing on putative nonspecific effects such as fatigue, inability to attend, or a general malaise are difficult to reconcile with these results because each of these putative capabilities must interact with gastric preloading to specifically limit total intake to about that without the gastric preload.

EXPERIMENT 5

The fact that antagonists only reduce deprivation-induced drinking and not abolish it leads to the supposition that the antagonists block only a component of a multiply determined system regulating water-balance. It is recognized that thirst may be induced by two classes of internal stimuli [35]. Hypovolemic thirst is in response to decreased extracellular fluid volume and can be induced by injections of polyethylene glycol (PG). Hyperosmolaric thirst is in response to decreased intracellular fluid volume and can be induced by injections of hypertonic solutions [35]. In pilot studies, we found that naloxone reduced drinking produced by both kinds of injections. This study was a formal attempt to confirm that naloxone reduces drinking elicited by administration of both hypertonic solutions and PG solutions.

METHOD

Subjects

The subjects were 43 experimentally naive male rats (Taconic Farms, Sprague-Dawley derived), weighing a mean of 210 g. From the time of arrival at the laboratory to the end of the procedures, the subjects were individually housed with food always available as described in Experiment 1. Water was also available except as specified.

Procedure

There were two general procedures, one involving injections of PG with appropriate control groups and one involving injections of hypertonic saline with control groups. These procedures conform to a 2 by 2 by 2 experimental design (5 or 6 subjects/cell, N=43). One main effect was associated with the two different general procedures (those associated with PG-induced thirst and those associated with salt-induced thirst). Another main effect was associated with the injection of a thirst-inducing agent compared to a placebo, while a third main effect was associated with a second injection which was either naloxone or saline.

The subjects associated with PG-induced drinking were injected 6 hr before testing. The rats were anesthetized with ether and then injected SC in the upper back with 5 ml of 30% PG (wt/vol) (molecular wt. of PG=20,000) or physiological saline. After injections rats were returned to their home cages and their water bottles were removed.

Subjects associated with salt-induced drinking were injected 1 hr before testing. The animals were anesthetized with ether and then injected. One half of the subjects received 5 ml of a 1 M NaCl solution, SC, in the back of the neck. The other half received physiological saline. After injections, rats were returned to their home cages and their water bottles were removed.

Fifteen min before testing for drinking, half of the rats in

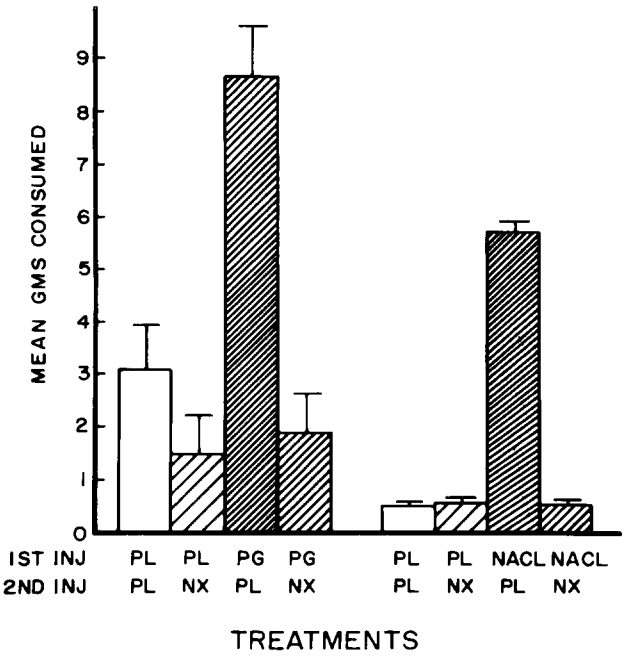

FIG 4 Mean grams of water consumed during a 1 hr test Pl =placebo, physiological saline (1st injection=5 ml; 2nd injection=1 ml/kg) Nx=naloxone HCL, 10 mg/kg. PG=5 ml of a 30% solution of polyethylene glycol NACL=5 ml of a 1M solution of sodium chloride

each subgroup were given naloxone (10 mg/kg, SC) and the other half were given physiological saline, SC, in an equivalent volume (1 ml/kg) Water intake was measured as in Experiment 1 beginning at 0400 hr, but across a 1-hr period.

RESULTS AND DISCUSSION

The amounts of water ingested (ml/hr) were submitted to a 2 by 2 by 2 ANOVA. The means±SEM for the eight groups are depicted in Fig. 4. All three main effects (the two general procedures, thirst-induction versus placebo, and saline versus naloxone) were reliable sources of variance, all $Fs>9.60$, $ps<0.005$. Since presentation of water occurred 6 hr after injections of PG, and 1 hr after injections of NaCl solution, the time between injections of the dipsogens and access to water probably contributes to the difference in drinking between animals receiving the two different general procedures (note the difference between the two groups always receiving placebo).

Both procedures for inducing drinking were effective as shown by the associated reliable main effect of the ANOVA and by comparison of the means of the groups not treated with naloxone. The PG-injections produced 5.49 g more drinking than its respective control, whereas the salt-injections produced 5.26 g more drinking than its control

The subjects receiving naloxone drank very little. Naloxone effects are also reflected in the reliable dipsogen/placebo by naloxone/saline interaction, $F(1,35)=15.63$, $p<0.001$ No other interactions, including the triple interaction, were reliable sources of variance. In a follow-up study, using naloxone as a challenge to NaCl-induced drinking, we found that doses as small as 0.5 mg/kg of naloxone significantly reduced drinking

Following our initial presentation of these findings [31] and the submission of this manuscript, it has been confirmed that naloxone suppresses drinking following hypertonic NaCl injections in mice and rats [3, 4, 6]. Further, the effect was dose-related, and stereoselective, [4] which complements our findings for water deprivation with WIN 44,441 in Experiment 1. It is worthwhile noting that the 10 mg/kg dose of naloxone used in this experiment suppressed PG- and NaCl-induced drinking by at least 80%, a far greater suppression than typically is seen with naloxone in water deprivation paradigms. This differential sensitivity, which was also noted by Brown, Blank and Holtzman [3], will be further discussed after the next experiment

EXPERIMENT 6

With the previous experiment, it was shown that two standard ways of inducing drinking were ineffective when rats were under the influence of naloxone. In this experiment, we tested the effect of naloxone on drinking induced by the hormone angiotensin II.

METHOD

The subjects were five male and five female rats (Sprague-Dawley derived, Taconic Farms) weighing from about 200 to 400 g on the day of testing. The testing procedure was similar to that described in Experiment 5. Angiotensin II (Aspl, Ileu 5 AII, Sigma, the isomer endogenous to rat) was given just before opportunity to drink at about 1200 hr. One half of the subjects received naloxone (10 mg/kg, SC) 15 min prior to angiotensin II (250 μg, SC) while the other half received saline Subjects' drinking was then measured after 1 hr and 24 hr There were no marked differences between drinking responses of males and females with respect to drug injections, therefore, data were analyzed without taking sex of subjects into account

RESULTS

Subjects given saline followed by angiotensin II drank a mean of 3.92 ml of water across the first hour of availability whereas subjects given naloxone drank only 0 58 ml, $t(8)=2.95$, $p<0.05$. Drinking across 24 hr did not differ significantly between groups. Naloxone, as it did with PG- and NaCl-induced drinking, dramatically reduced drinking from that expected following angiotensin II administration.

DISCUSSION

It is apparent that naloxone (10 mg/kg) inhibits deprivation-induced drinking with short deprivation periods [9]. When rats are deprived of water for 6 to 8 hr, subsequent water intake is reduced by approximately 50%. With longer periods of deprivation, from 24 to 48 hr, water intake is reduced by approximately 30% [24]. This difference probably reflects the fact that minimally motivated behaviors are more easily disrupted by drugs, and other classes of stimuli, than are intensely motivated behaviors In comparison, naloxone reduced PG-induced drinking by 80%, salt-induced drinking by 92%, and angiotensin II-induced drinking by 85%. These latter behaviors are typically regarded as highly motivated, since rats will work for water in these paradigms, and it is therefore unlikely that the high

suppressions are due to insufficient motivation. Neither is it likely that "nonspecific" factors such as fatigue or inattention are responsible for the reduced water intake after naloxone. One would expect only reductions of the order of the 30–50% seen after fluid deprivation. Additionally, our results from Experiment 4 argue against this type of nonspecific factor. We thus suggest, because PG-, NaCl-, and angiotensin II-induced drinking are severely attenuated by opiate antagonism, that an endogenous opioid mechanism is importantly involved in the mechanisms controlling drinking following strong homeostatic challenges.

The data additionally lead to the suggestion that some factor other than those engaged by PG, NaCl, and angiotensin II injections, that is not sensitive to naloxone, must be maintaining drinking in the water-deprived rat. It could be argued that each mechanism engaged by the respective injection cannot be, by itself, the mechanism for sustaining deprivation-induced drinking, since naloxone produces nearly complete inhibition of drinking following these homeostatic challenges but produces only an attenuation of deprivation-induced drinking. Further, because adjunctive drinking (schedule-induced polydipsia) is not attenuated by naloxone [5] it would seem that nonopioid mechanisms are controlling this type of drinking, and that they are relatively independent of opioid-dependent homeostatic controls.

There is another way of looking at the types of results generated by procedures of giving antagonists to motivated subjects. In opiate-naive animals, naloxone produces its most complete suppression of ingestive behavior when the impetus is one of the following: (a) hypovolemia induced by PG, (b) hyperosmolarity following injections of NaCl, (c) hyperangiotensinemia. On the surface, there is little commonality among these motivations except that they are goads to behavior that would rarely, if ever, be part of the phylogenetic history of the subjects [25]. What may be common to them all is their novelty as a motivational state with respect to evolutionary history. Further, the absence of drinking behavior to these stimuli has many possible interpretations [25,26]. On the other hand and as stated above, these findings are probably revealing opioid links in the mechanisms of drinking and eating that have been manifest by the various laboratory procedures.

Regardless of how one might summarize the findings, it is clear that the antagonists have significant effects on behavior associated with maintaining water balance. Furthermore, those effects are highly reliable, often dramatic, and in some ways specific (e.g., antagonists do not affect all kinds of drinking and probably not all kinds of drinking equally). Furthermore, it is reasonable to conclude that the antagonists' effects are due to their capacity to occupy opiate receptors within the central nervous system.

EXPERIMENT 7

In the previous experiments, we addressed the issues of specificity of antagonists of morphine and endorphins in reducing water intake and provided evidence leading to the suggestion that opioids play a role in regulating water balance. In the present experiment, we further examine naloxone's actions by investigating its effects on two forms of glucoprivic feeding: that induced by insulin-provoked hypoglycemia and that induced by competitive blockade of glucose metabolism with 2-deoxy-*d*-glucose (2-DG) [8].

METHOD

Subjects

The animals of the two primary procedures were 24 male Sprague-Dawley derived rats (Zivic-Miller) weighing about 300 g. They were housed individually in metal cages with food pellets on the floor of the cage and tap water available at all times. Their cages were in a room where the lights were on from 0700 to 1800 hr and testing was performed during the daytime.

Procedure

The animals were randomly assigned to four groups (6 subjects/group). The day before the first test of drug effects, they were handled, injected with physiological saline and food pellets were removed at hourly intervals to simulate test procedures. On the test day, one group of rats received naloxone HCl, 10 mg/kg, intraperitoneally (IP), followed 10 min later by injections of 2-DG (300 mg/kg dissolved in 2.5 ml/kg of saline, IP) Immediately after the 2-DG injection, subjects were returned to home cages where weighed amounts of food were available. The second group was treated similarly except that they received saline followed by 2-DG. The third group received naloxone followed by saline, and the fourth group received two saline injections. Food intakes of all subjects were measured after 1, 2, 3, and 4 hr, correcting for spillage which occurred during the entire test with the measures taken after 4 hr.

One week later, the procedures were repeated except that insulin was administered instead of 2-DG. Eighteen of the rats used previously were assigned at random to three groups. One group received naloxone (10 mg/kg, IP) followed by insulin (Iletin, Lilly, 5 U/kg, SC). The other groups received saline followed by insulin or saline followed by saline, respectively.

In an additional experiment, using 12 new rats (n=4/group), the effects of IP injections of naloxone (10 mg/kg), SC injections of naloxone (10 mg/kg) and SC injections of saline were compared with respect to 2-DG (300 mg/kg, IP) elicited eating. In yet another experiment, 12 additional rats received the procedures associated with testing the effects of naloxone on 2-DG elicited feeding, but the procedures were administered during the dark phase of the lighting cycle.

Because data lacked homogeneity of variance, Kruskal-Wallis ANOVAs were used to compare the cumulative amounts of food eaten by each group after each hour of availability of food. To compare scores between any two groups, Mann-Whitney U-tests were used. Spillage was subtracted from cumulative amounts of food taken only from the last hour of testing, so the final "cumulative" scores may be slightly depressed relative to scores from previous hourly measures

RESULTS AND DISCUSSION

Results are depicted in Fig. 5 Analyses of the data from the tests using 2-DG indicated that a reliable group effect was observed at each time period after injections ($ps<0.01$, Panel A of the figure). As can be seen from the data presented in the figure (Panel A), 2-DG was effective in eliciting eating (compare scores from vehicle plus 2-DG to scores from vehicle plus vehicle group; these differences are reliable, $ps<0.05$, at each measure after injections). Subjects receiving naloxone and 2-DG took less food at each time period

than did subjects receiving vehicle and 2-DG ($ps<0.05$) Naloxone combined with a vehicle injection also decreased food intake relative to the vehicle-vehicle control group at 1, 3, and 4 hr after injections ($ps<0.05$). The results lead to the conclusion that naloxone injections attenuate the eating elicited by injections of 2-DG as well as the spontaneous eating that occurs across the 4 hr period.

Naloxone also inhibited eating produced by injections of insulin (Fig. 5, Panel B). The groups differed reliably at each of the three hourly tests ($ps<0.05$). Naloxone was, however, only reliably effective in inhibiting insulin-induced eating at the first hour compared to the vehicle plus insulin groups ($p<0.02$).

Results of the third procedure showed that IP- and SC-injections of naloxone were both effective in reducing 2-DG induced feeding (Fig. 5, Panel C) The groups differed reliably at each time period after injections ($ps<0.05$). With measurements after the first and second hours, IP- and SC-naloxone were both effective in reducing eating compared to the scores of the vehicle plus 2-DG group ($ps<0.05$). By the third hour measurement, however, only SC-naloxone continued to reliably inhibit feeding ($p<0.02$), compared to vehicle plus 2-DG group's intake indicating that SC-naloxone was more effective than IP-naloxone. Also, there were reliable differences between the two naloxone groups (2 $ps<0.02$) at the second and third hour measurements.

With a procedure similar to the first procedure and using 12 new subjects but testing during the active phase of a reverse light/dim-light schedule, the results also indicated that naloxone (10 mg/kg, SC) was effective in attenuating eating produced by injections of 2-DG. At the end of a 3 hr test, the vehicle-control subjects ate 3.9 g of food, the vehicle plus 2-DG group ate 8.2 g of food, and the naloxone plus 2-DG group ate 4.4 g, with the differences between the two groups getting 2-DG meeting standards for statistical significance ($p<0$ 05).

In three separate procedures with three separate groups of subjects, it has been demonstrated that naloxone suppresses feeding compared to that expected following 2-DG injections. The basic effect of naloxone is independent of route of administration of naloxone and time of day of testing. Naloxone also blocks insulin induced feeding. Since this manuscript was submitted, others [19,29] were also able to demonstrate that 2-DG-induced feeding was attenuated by naloxone by doses as low as 1.0 mg/kg. In contrast, Lowy, Maickel and Yim [19] did not observe a suppression by naloxone of insulin-induced feeding. There is, however, no discrepancy between the results. They measured food intake once, 3 hr after insulin, and observed no effect which agrees with our 3 hr data point It was only by taking hourly measures that we observed a substantial early reduction in feeding. If we had only measured cumulative intake as 3 hr, we too would have missed a reliable effect of naloxone on insulin-induced feeding. The reason that insulin-treated rats eventually overcome the naloxone blockade, while 2 DG-treated rats do not, may relate to the different nature of these glucoprivic stimuli. Given insulin, a rat which does not eat will eventually die of increasing hypoglycemia; 2 DG, in contrast, does not constitute a life-threatening stimulus at the dose used.

We thus hypothesize there is an endorphinergic component to the normal total pattern of eating following glucoprivation, and this is blocked by naloxone. As with the naloxone effects on drinking, other potential explanations for the reduced intake will have to be tested. In addition, we must consider the dimension of naturalness of the hunger stimulus, as we did for thirst. Thus naloxone seems to produce larger blockade of glucoprivic feeding (above), diazepam-induced eating [32], and the "hedonic" intake of concentrated sucrose by undeprived rats [33] than of the eating after food deprivation [12].

EXPERIMENT 8

In Experiments 5 and 6 we demonstrated that naloxone produced large attenuations of drinking after acute challenges to hydrational homeostasis. In contrast, deprivation-induced drinking is less affected, and others have reported that adjunctive drinking is not at all affected by naloxone [5,24]. The results of Experiment 7 demonstrated that naloxone suppressed eating following procedures inducing acute glucoprivation. In the present experiment, we examined the effects of naloxone on another type of eating, that elicited during "stressful" tail pressure (TP) [1]. The eating resulting from TP might also be considered adjunctive since it is not in direct response to a variation in homeostasis of nutrients.

METHOD

Eight adult male Sprague-Dawley rats (Zivic Miller) were housed as in Experiment 7, with food and water freely available. The animals were screened for elicited eating and gnawing.

Testing was performed with the rat in a steel bowl with food pellets scattered on the floor. Pressure to the tail was administered with a hand held foam-padded sponge forceps applied about 3 cm from the tip of the tail. The pressure was adjusted so that the rats exhibited quiet food-directed behaviors.

On the first test day, four rats were injected with naloxone (10 mg/kg, IP) 10 min prior to the first TP-trial and four received saline vehicle. Four trials were given, each 120 sec long and they were separated by intertrial intervals of about 10 min. The time spent eating in each trial was recorded by stopwatch. The food was weighed before and after the trial (after drying, if necessary), and the amount eaten calculated. The next day, the procedure was repeated with the groups reversed with respect to drug treatment. The data from both days were similar and have been combined.

RESULTS AND DISCUSSION

Rats initiated TP-behaviors on 29 out of 32 trials (4 tests with each of 8 subjects) after vehicle injections compared to 30 out of 32 trials after naloxone. The mean durations of TP elicited oral behavior per trial were 43 ± 6 sec after saline and 44 ± 7.7 sec after naloxone. The mean amounts eaten were 0.96 ± 0 34 g after saline and 0.97 ± 0.29 g after naloxone. None of these differences was significant (paired ts, $ps>0.05$).

These results indicate that a high dose of naloxone (10 mg/kg) has no significant effect upon TP-elicited eating. These data replicate a pilot study in which we also found no effect of 3 mg/kg of naloxone. Notice that the TP-tests were finished within 60–90 min of naloxone injections. In the previous experiment, we found that IP-naloxone had a strong suppressant effect upon glucoprivic feeding for at least 2 hr. Thus, under conditions in which glucoprivic feeding is greatly attenuated (over 50%), naloxone is without effect on

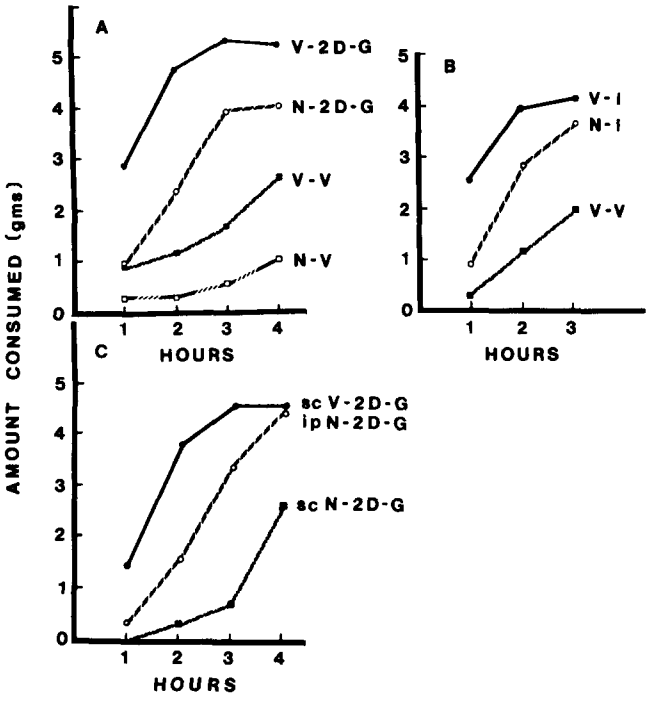

FIG. 5. Naloxone inhibits glucoprivic-induced eating. Depicted are data for Experiment 7. In Panel A the group means for cumulative grams of food eaten for each hourly test are shown for naloxone's effects on 2-DG elicited feeding Panel B shows cumulative means for naloxone's effects on insulin induced feeding. Panel C shows the cumulative amounts of food eaten after subcutaneous or intraperitoneal injections of naloxone or saline combined with 2-DG. Note that spillage was subtracted only from the cumulative amounts for the last hourly test in each experiment. V=vehicle, 2D-G=2 Deoxy-*d*-glucose (300 mg/kg); N=naloxone (10 mg/kg), I=insulin (5 U/mg), ip=intraperitoneal injection, sc=subcutaneous injection.

feeding elicited by tail pressure. In several additional experiments (Antelman and Rowland, in preparation) we have found that SC-administered naloxone in doses up to 10 mg/kg is without effect upon TP-elicited eating. Our findings contrast with very recent reports of attenuation of TP eating by naloxone [19,23], and we believe that procedural differences can account for at least some of the attenuation. Because it is always possible to get positive effects (suppressions) with ingestive tests, we feel that our negative data are potentially important in this context.

GENERAL DISCUSSION

Some of the implications and inferences that might be drawn from the results of these experiments have already been presented. Here we provide a more global discussion of our findings.

Naloxone was once thought to be inert in opioid-naive subjects. The idea that naloxone was inert became logically indefensible with the understanding that there was an extensive, endogenous, morphine-like naloxone-sensitive system having components within brain. Behavior, the sensitive index of brain functioning, should be modified in some aspects by blockade of a subsystem of brain. And, indeed, when subjects were put into special testing circumstances where motivated behavior could be observed, powerful effects from administration of naloxone were seen. To observe a reliable effect from administration of naloxone, however, is not sufficient evidence for drawing a conclusion about the functioning of an endorphinergic system; the effect may be indirect or nonspecific.

There are difficulties with interpreting the results of pharmacological manipulations especially when the result is a suppression of the measured behavior. A host of nonspecific effects, such as sickness or malaise, could account for the suppressed behavior. Consequently, there is the issue of the specificity of a drug's effects and many of the experiments reported here addressed that issue with respect to naloxone's suppression of drinking and eating.

Naloxone could reduce drinking and eating by producing a nonspecific illness or malaise, since naloxone as a putative unconditioned stimulus will sustain a CTA [18, 34, 37]. The CTA produced by naloxone, however, is not large and some rats hardly show a CTA following naloxone injections leading to the conclusion that the "illness" produced by naloxone would be rather mild. Along the same lines, it has recently been demonstrated that the ability of opioids to sustain a CTA is not related to other motivational properties of the opioids and that naloxone itself was not capable of establishing a conditioned location effect [30]. Lithium chloride, which will sustain a large CTA, and PG injections, which produce apparent discomfort, do not reduce drinking [9] suggesting that slight illness would not suppress strongly motivated drinking. Also, rather large doses of naloxone are necessary to sustain a clear CTA. Furthermore, no correlation was found between naloxone-produced CTAs and naloxone's suppression of drinking [36] or naloxone's suppression of intake of sucrose solutions [24]. Although it is clear that naloxone can sustain a CTA, that capability of naloxone cannot account for naloxone's ability to reduce intake of water and food under certain circumstances.

The results of Experiment 4 showing that naloxone reduces drinking even after an orogastric preload of water provides further evidence of the specificity of naloxone's effects. As mentioned, these data are difficult to reconcile with hypotheses stating that the reduced drinking is a function of a response limitation.

The drinking of schedule induced polydipsia and the eating induced by tail pressure, two types of adjunctive ingestion, are not reliably modified by doses of naloxone that are effective in other circumstances [5]. The finding that only certain types of drinking and eating are consistently modified by naloxone also strengthens the idea that the effects of the antagonists are specific and not due to illness, fatigue, or other response limitations.

To summarize, these results show that naloxone and other opiate antagonists lead to a reliable suppression of deprivation-induced drinking. This suppression is apparently mediated by central nervous system opiate receptors and is probably not due to general response-limiting effects. Further, our work and that of others shows that small doses of antagonists are effective in reducing drinking and only the physiologically active isomers produce this suppression [3, 4, 5]. Drinking and eating which are induced by homeostatic challenges are more sensitive to naloxone-produced disruption than are deprivation-induced drinking and eating. Schedule-induced polydipsia [5] and tail-pinch induced eating, two examples of "adjunctive" consummatory behav-

iors, are not suppressed by naloxone. These findings support the conclusions (a) that it is the specific ability of antagonists to occupy opiate receptors that is critical to their ability to suppress ingestive behaviors, and (b), that endogenous opiates are involved in the maintenance of homeostasis.

The isolated finding, in 1974 [12], that naloxone reduced food intake, by itself, did not lead to the conclusion that there was an endorphinergic mechanism involved with regulation of ingestive behavior. Currently, however, sufficient evidence has accumulated to rule out certain alternative explanations of naloxone's effects and to considerably strengthen the hypothesis that naloxone reduces certain ingestive behaviors. The inference is made, therefore, that one function of the endorphins is to maintain certain goal directed behaviors. Further, the general results have implications for theories of opiate addiction. The reinforcing capacity of opiates may accrue because exogenous opiates may mimic the reward and positive affect associated, in other circumstances, with certain motivationally relevant stimulation.

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REFERENCES

1 Antelman, S. M and A R. Caggiula. Tails of stress-related behavior A neuropharmacological model. In. *Animal Models in Psychiatry and Neurology*, edited by I Hanin and E Usdin New York, Pergamon Press, 1978, pp. 224–245
2. Brands, B., J A Thornhill, M Hirst and C W. Gowdey Suppression of food intake and body weight gain by naloxone in rats *Life Sci* **24:** 1773–1778, 1979.
3. Brown, D. R., M S Blank and S G Holtzman Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats *Life Sci* **26:** 1535–1542, 1980
4 Brown, D R and S G Holtzman Evidence that opiate receptors mediate suppression of hypertonic saline-induced drinking in the mouse by narcotic antagonists *Life Sci* **26:** 1543–1550, 1980
5 Brown, D. R and S G. Holtzman. Suppression of drinking by pure opiate antagonists in rats and monkeys. *Fedn Proc* **39:** 995, 1980
6. Czech, D A and E A Stein Naloxone suppresses osmoregulatory drinking in rats *Pharmac Biochem Behav* **12:** 987–989, 1980.
7 Dunnett, C W A multiple comparision procedure for comparing several treatments with a control *J Am statist Ass* **50:** 1096–1121, 1955
8 Epstein, A N , S Nicolaidis and R Miselis. The glucoprivic control of food intake and the glucostatic theory of feeding behavior In. *Neural Integration of Physiological Mechanisms and Behaviour*, edited by G J Mogenson and F. R. Calaresu Toronto University of Toronto Press, 1975, pp 148–168
9 Frenk, H and G H Rogers The suppressant effects of naloxone on food and water intake in the rat *Behav Neural Biol* **26:** 23–40, 1979
10. Goldstein, A Opiate receptors and opioid peptides A ten year overview In *Psychopharmacology A Generation of Progress*, edited by M A Lipton, A. Dimascio and K F. Killam New York Raven Press, 1978, pp 1557–1563
11 Grandison, L and A. Guidotti Stimulation of food intake by muscimol and beta-endorphin. *Neurophamacology* **16**:533–536, 1977
12 Holtzman, S G Behavioral effects of separate and combined administration of naloxone and *d*-amphetamine. *J Pharmac exp Ther* **189:** 51–60, 1974
13 Holtzman, S G Effects of narcotic antagonists on fluid intake in the rat *Life Sci* **16:** 1465–1470, 1975
14. Holtzman, S. G Suppression of appetitive behavior in the rat by naloxone. Lack of prior effect of morphine dependence. *Life Sci* **24:** 219–226, 1979
15 Kuhar, M. J , C B Pert and S. H. Snyder Regional distribution of opiate receptor binding in monkey and human brain *Nature* **245:** 447–450, 1973.
16 Lasagna, L and H K Beecher. The analgesic effectiveness of nalorphine and nalorphine-morphine combinations in man. *J Pharmac exp Ther* **112:** 356–363, 1954
17 Laschka, E., A. Herz and J. Blasig. Sites of action of morphine involved in the development of physical dependence in rats I Comparison of precipitated morphine withdrawal after intraperitoneal and intraventricular injection of morphine antagonists *Psychopharmacologia* **46:** 133–139, 1976.
18 LeBlanc, A. E and H Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmac Biochem. Behav* **3:** 185–188, 1975
19. Lowy, M. T., R. P Maickel and K. W. Kim Naloxone reduction of stress-related feeding *Life Sci* **26:** 2113–2118, 1980
20 Manara, L., G. Bianchi, P Ferretti, E Monferini, D. Strada and A Tavani. Local and CNS-mediated effects of morphine and narcotic antagonists on gastro-intestinal propulsion in rats In: *Endogenous and Exogenous Opiate Agonists and Antagonists*, edited by E Leong Way. New York Pergamon Press, 1980, pp 143–146.
21 Margules, D. L., B. Moisset, M J. Lewis, H Shibuya and C. B. Pert Beta-endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* **202:** 988–991, 1978
22 Michne, W F , T R. Lewis, S. J Michalec, A K. Pierson, M G C. Gillon, S. J Paterson, L E. Robson and H W Kosterlitz. Novel development of N-methylbenzomorphan narcotic antagonists In *Characteristics and Functions of Opioids*, edited by J M van Ree and L Terenius Amsterdam· Elsevier/ North Holland Biomedical Press, 1978, pp 197–206.
23. Morley, J E and A S Levine Stress-induced eating is mediated through endogenous opiates. *Science* **209:** 1259–1261, 1980
24 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:** 431–435, 1980
25. Rowland, N. Regulatory drinking. Do the physiological substrates have an ecological niche. *Biobehav Rev* **1:** 261–272, 1977
26 Rowland, N. Drinking behaviour. Physiological, neurological and environmental factors In. *Analysis of Motivational Processes*, edited by F. M Toates and T R Halliday. London Academic Press, 1980, pp 39–59
27. Sawynok, J , C Pinsky and F. S LaBella Minireview on the specificity of naloxone as an antagonists *Life Sci* **25:** 1621–1632, 1979.

28. Setler, P. E. The neuroanatomy and neuropharmacology of drinking In· *Handbook of Psychopharmacology, Vol. 18,* edited by L. L Iversen, S. D. Iversen and S. H Snyder. New York: Plenum, 1977, pp. 131–158.
29. Sewell, R. D E. and K. Jawaharlal Antagonism of 2-deoxy-D-glucose-induced hyperphagia by naloxone: possible involvement of endorphins. *J Pharm Pharmac.* **32:** 148–149, 1980.
30. Sherman, J E , C. Pickman, A. Rice, J C Liebeskind and E W. Holman Rewarding and aversive effects of morphine Temporal and pharmacological properties. *Pharmac. Biochem. Behav* **13:** 501–503, 1980.
31. Stapleton, J. M., M D. Lind, J. R Quinan, T. L. Foley, F. -F. Wu and L. D Reid Effects of naloxone on feeding and drinking in rats. *Soc. Neurosci. Abstr* **5:** 663, 1979.
32. Stapleton, J. M., M. D. Lind, V. J Merriman and L. D. Reid. Naloxone inhibits diazepam-induced feeding in rats. *Life Sci* **24:** 2421–2426, 1979.
33 Stapleton, J. M , N L. Ostrowski, V J. Merriman, M. D. Lind and L. D. Reid. Naloxone reduces fluid consumption in water-deprived and nondeprived rats *Bull. Psychon Soc* **13:** 237–239, 1979.
34. Stolerman, I. P., C. W T Pilcher and G D D'Mello Stereospecific aversive property of narcotic antagonists in morphine-free rats. *Life Sci.* **22:** 1755–1762, 1978.
35 Stricker, E M. and G. Wolf. Hypovolemic thirst in comparison with thirst induced by hyperosmolarity. *Physiol Behav* **2:** 33–37, 1967.
36. Wu, M -F., S E Cruz-Morales, J. R Quinan, J. M. Stapleton and L D. Reid Naloxone reduces fluid consumption: Relationship of this effect to conditioned taste aversion and morphine dependence *Bull. Psychon Soc* **14:** 323–325, 1979
37. Van der Kooy, D. and A. G Phillips. Temporal analysis of naloxone attentuation of morphine-induced taste aversion. *Pharmac Biochem Behav* **6:** 637–641, 1977
38 Wuster, M., R. Schulz and A Herz Opioid agonists and antagonists Action on multiple opiate receptors In: *Endogenous and Exogenous Opiate Agonists and Antagonists,* edited by E. Leong Way New York, Pergamon Press, 1980, pp. 75–78