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Antagonist-Precipitated Opioid Withdrawal in Rats: Evidence for Dissociations Between Physical and Motivational Signs

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HIGGINS, G. A. AND E. M. SELLERS. *Antagonist precipitated opioid withdrawal in rats: Evidence for dissociations between physical and motivational signs.* PHARMACOL BIOCHEM BEHAV **48**(1) 1-8, 1994.—In rats made opioid dependant by the implantation of a single morphine 75 mg base pellet, an attempt was made to determine whether any correlation existed between physical and motivational withdrawal signs by adjusting the dose of naloxone used to precipitate withdrawal. The models used to study motivational signs were taste (one- and two-bottle) conditioning and operant responding for food under an FR15 schedule of reinforcement. Naloxone at doses of 0.01 mg/kg and above produced both a conditioned taste aversion (two-bottle test only) and reduced food responding in morphine pellet, but not placebo pellet, implanted animals. No physical withdrawal signs, e.g., wet dog shakes, diarrhoea, were noted until naloxone doses of 0.05 mg/kg and above were used. It is concluded that the difference in naloxone doses required to elicit physical and motivational withdrawal components provides further support for their dissociation.

Naloxone-precipitated opioid withdrawal Taste conditioning Operant responding Rat
Physical withdrawal signs Motivational withdrawal signs

EARLY views that physical dependence served as the primary impetus for substance abuse behavior has been largely superseded by an acknowledgement that the positive reinforcing properties of the abused drugs themselves serve a critical role in this process [e.g., see (20,37,38) for reviews]. Indeed, by implication, adaptive processes such as physical dependence are subsequent to chronic drug use and, therefore, offer no explanation as to why this behavior becomes established in the first place. Nevertheless, within certain drug classes, particularly the opiates, the induction of physical dependence and consequent avoidance of an aversive withdrawal state, represents one aspect of the motivational impetus maintaining drug self-administration behavior (18,37). It may be hypothesized then, that drugs that relieve withdrawal could serve as useful therapies for the treatment of substance, including opiate, abusers.

Before attempting to identify such agents it is important to have some knowledge as to the clinical manifestations of a drug withdrawal state. Opioid withdrawal is characterized by a wide range of symptoms including nausea, gastrointestinal disturbance, various sympathomimetic reactions, agitation/anxiety, and marked dysphoria (18,19,39). The time course and severity of these various symptoms are variable between patients and dependent on a variety of factors such as the particular drug, daily dosage, duration of use, environmental context, and method of withdrawal (i.e., spontaneous or antagonist-precipitated withdrawal) (18). However, irrespective of this, it seems to be a common observation that this phenomenon has profound behavioral, psychological, and physiological components.

In preclinical research there has been a greater emphasis towards the delineation of these aspects of opioid withdrawal, for it seems that multiple systems are implicated. The periaqueductal grey and locus coeruleus appear to be particularly important for the expression of many physical opioid withdrawal signs (4,24,25) although other brain/spinal areas have been implicated (23-25) and a peripheral component may be responsible for secretory signs such as diarrhoea (23,25,27).

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Perhaps of greater interest have been the recent observations implicating the amygdala and the nucleus accumbens as critical sites for the expression of the negative motivational consequences of opioid withdrawal (21,33). Models presumably reflective of this withdrawal aspect include place and taste conditioning procedures and disruption of operant responding for food reward (8,13,26–28). Thus, in morphine-dependent rats, microinjections of methylnaloxone into the nucleus accumbens has been reported to elicit a clear place aversion (33) and marked disruption of operant responding for food (21) at doses that produce few, if any, physical withdrawal signs (25,33). Also, rats undergoing either spontaneous or antagonist-precipitated opioid withdrawal show a marked suppression of accumbens DA release that may be related to the induction of these aversive behaviors (1,30). The amygdala also appears to be a particularly sensitive site for the expression of place aversion conditioning to opioid withdrawal (33).

In a previous report (15), we examined the effect of varying naloxone doses on the induction of place aversion conditioning in morphine-dependent rats. From these studies it seemed that place aversions could be established by naloxone doses that did not produce any overt (physical) signs of withdrawal. However, because these behaviors were not continuously monitored, it was the purpose of the present study to more formally measure the incidence of opioid withdrawal signs across the same naloxone dose range as that used in the place conditioning studies. Also, we extended this work to examine taste aversion conditioning and disruption of fixed ratio operant responding for food reinforcement. The ultimate aim of these studies was to assess whether physical and motivational withdrawal signs could be dissociated by varying the withdrawal severity.

METHOD

Animals and Housing

Male, Wistar rats (Charles River, Quebec, Canada), weighing 250–300 g at the time of study, were used throughout. Rats were usually housed in groups of four, with the exception of the animals used in the operant and feeding studies, which were individually housed. The animal holding room was controlled for constant temperature (22 ± 1°C) and humidity (50%) and all rats were allowed at least 6 days acclimatization to this environment before behavioral testing. Food (Lab Diet, Richmond, IN) and water were constantly available except: a) during behavioral conditioning and testing; and b) for the rats used in the taste conditioning and feeding studies, which had limited access to water and food, respectively (see appropriate Method section). The light cycle was 0700–1900 h and all experiments were conducted during this period.

Drugs

The morphine pellets consisted of 75 mg morphine base (Ward Robertson Chemicals, Scarborough, Canada) formulated with an equivalent quantity of microcrystalline cellulose. The placebo pellets were prepared in the same way except morphine base was replaced by cellulose. Naloxone hydrochloride (RBI, Natick, MA) was dissolved in 0.9% sodium chloride solution immediately before use and injected subcutaneously in a dose volume of 2 ml/kg. Final drug concentration refers to that of the base.

Induction of Morphine Dependence and Withdrawal

Under methoxyflurane anesthesia each animal was implanted with a single morphine or placebo pellet subcutaneously in the nape of the neck. The incision was sealed with surgical clips and antiseptic applied to the wound area. Implantation of a single 75 mg morphine pellet has been reported to produce plasma concentrations between 70–126 ng/ml morphine 1–9 days postimplantation (29). Following a defined time period that varied according to the procedure used, withdrawal was precipitated by naloxone injection.

Assessment of Physical Signs to Morphine Withdrawal

Forty-three rats were each implanted with morphine pellets. Seventy-two hours later the rats were treated with either saline control or naloxone (0.002–5 mg/kg) and then immediately transferred to single plastic observation chambers (20 × 38 × 48 cm) containing woodchips bedding. The following checked and counted signs were monitored over the subsequent 30-min period: diarrhoea, lacrimation, ptosis, rhinorrhoea, teeth chattering (each recorded as present/absent), salivation (4-point rating scale: 0 = absent, 1 = slight, 2 = moderate, 3 = marked), mouth movements, paw shakes, wet dog shakes, penile grooming (each recorded as total number of occurrences during session), startle (5-point rating scale; 0 = no response, 1 = mild with slight vocalization, 2 = moderate response with vocalization, 3 = marked response/jump with vocalization, 4 = intense with marked vocalization), weight loss (body weight change in the animal recorded immediately prior to, and 30 min after, naloxone injection). Secretory signs and startle were assessed at the end of the test session. All behavioral measures were made by a trained observer who was unaware of drug pretreatment. Each animal received only one treatment. Further detail concerning this behavioral assessment may be found in Higgins et al. (15).

Assessment of Taste Conditioning to Morphine Withdrawal

For this part of the study two procedures were used: a one- and two-bottle choice test.

In the one-bottle choice test, rats were presented with fluid for limited periods of the day: a 20-min exposure between 0900 and 1030 h in a wire mesh cage (18 × 30 × 18 cm; W × L × H) and a 40 min exposure between 1500–1600 h in the home cage. Intakes were measured in the morning session only. For a 5–7-day period to allow acclimatization to this procedure, the rats were presented with water only during both access periods. Immediately after the last morning fluid access period, each rat was implanted with either a morphine or placebo pellet. For the following 3 days the rats received fluid as before and a SC saline injection immediately followed each morning access period. On the fourth day postimplant the rats were presented with a novel 0.1% w/v saccharin solution instead of water during the 20-min schedule. This was followed by a SC injection of naloxone or saline vehicle according to group designation. During the next 3 morning sessions the rats were only allowed access to saccharin solution. No further injections were given during this period and the rats continued to receive 40-min water access in the afternoon.

In the two-bottle choice experiment, the procedure was virtually identical, the only exception being that on the 6 days after conditioning the rats were presented with two fluids, one water and the other 0.1% w/v saccharin during the morning session. Tube positions were alternated daily to control for position preferences. No injections were given during this period.

Assessment of Operant Responding for Food Reinforcement to Morphine Withdrawal

The operant chambers used for this study measured 22 × 22 × 28 cm (L × W × H) (Med Associates Inc., East Fairfield, VT). Food (45 mg Noyes pellet) was delivered by depression of one of two levers positioned on either side of the dispenser 7 cm above the chamber floor. Reinforcement delivery was controlled by microcomputer interface (Med Associates) linked to a 386SX computer.

Following initial shaping, rats were trained to lever press for food reinforcement made available under a fixed ratio 15 (FR15) schedule (i.e., 15 presses on the designated lever resulted in the delivery of one food pellet). For half of the rats the right lever was active, for the remainder the left lever was active. Two 20-min sessions were run per day, each spaced 8–10 h apart. At midday and after the afternoon operant session, the rats were given a further supplement of lab chow in the home cage (i.e., total daily allowance of pellets and chow was 16 g). Following stable rates of responding over at least four consecutive sessions, the rats were implanted with either a morphine (n = 8) or placebo (n = 5) pellet. Implantation was conducted after the afternoon session, just prior to the lights out period. Over the next 3 days the rats were run as previously described, and by the third day response rates in all animals had begun to stabilize. On the fourth and fifth days the rats were pretreated with either saline or naloxone (0.002, 0.01, 0.05 mg/kg) immediately prior to the operant session. Each rat received all treatments according to a randomized design.

Assessment of Feeding Following 20-h Food Deprivation in Morphine-Withdrawn Rats

As an adjunct to the operant study described above, a separate group of rats (n = 5) were trained to consume their food (standard lab chow) during a 2-h period each day between 1100–1300 h. Following a period of 6 days to allow acclimatization to this procedure, each rat was implanted with a morphine pellet. By the third day, postimplant daily intakes had returned to baseline levels and on days 3 and 5 the rats received saline SC immediately prior to food access. Intake

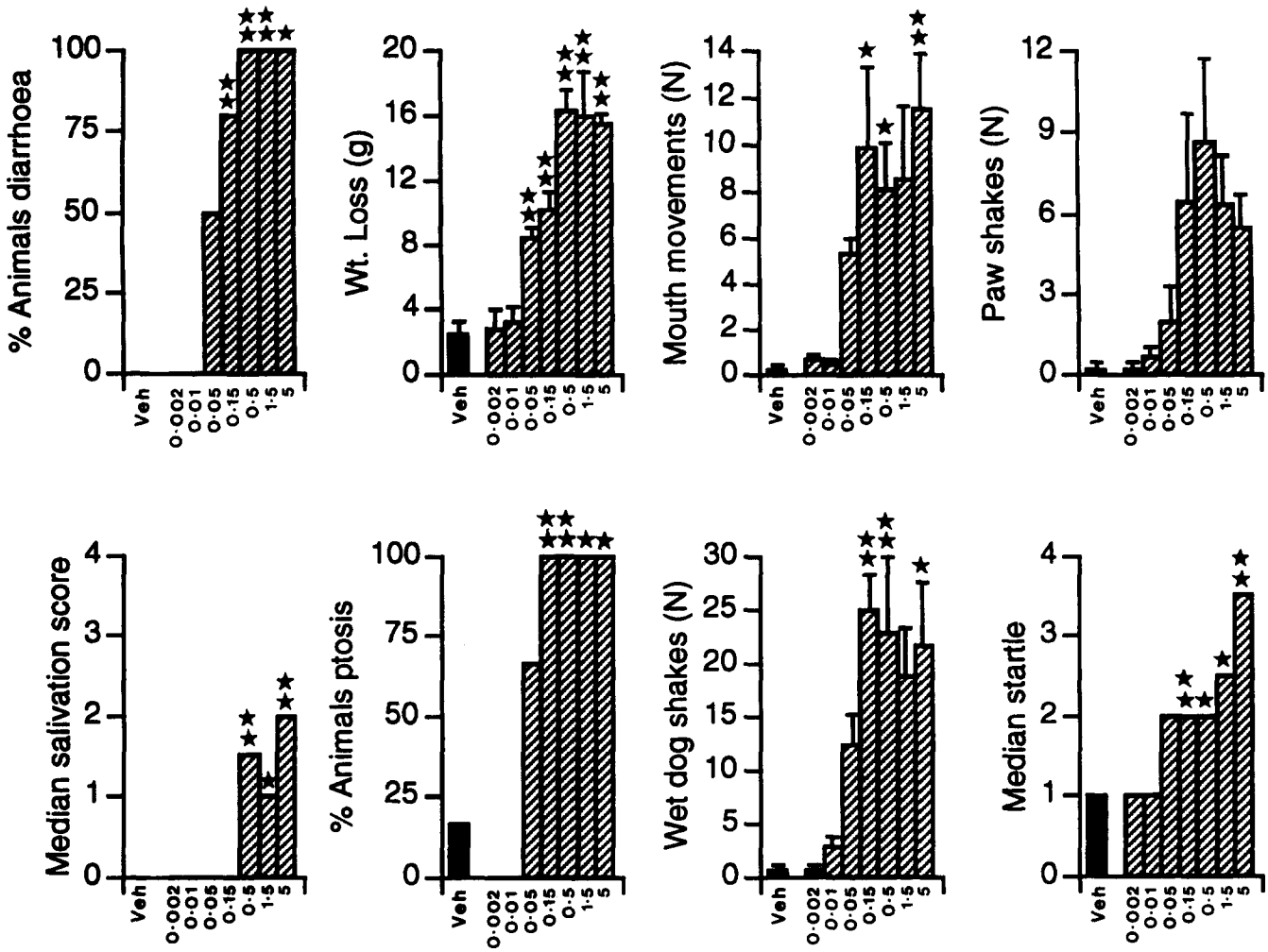

FIG. 1. Effect of naloxone dose on the incidence of various checked and counted opioid withdrawal signs. All rats implanted with a single morphine pellet 3 days before test. n = 6 (Veh), 5 (0.002 mg/kg), 5 (0.01 mg/kg), 6 (0.05 mg/kg), 5 (0.15 mg/kg), 8 (0.5 mg/kg), 4 (1.5 mg/kg), 4 (5 mg/kg). *$p < 0.05$, **$p < 0.01$ vs. vehicle pretreatment. See text for description of scoring procedure and statistical analysis.

was measured at 20, 60, and 120 min. On days 4 and 6 the rats were treated with naloxone (0.01 or 0.05 mg/kg) instead of saline. All rats received both treatments in a random sequence. Total food intakes were corrected for spillage.

Analysis of Data

Data are presented as means ± SEM except diarrhoea and ptosis, where the percentage number of animals responding per group was shown, and salivation and startle measures where the median value is presented. To analyze the effect of naloxone dose on a particular withdrawal behavior, the data were analyzed by one-way ANOVA with post hoc Neuman–Keuls test except for the diarrhoea, salivation, ptosis, and startle measures, where the data were analyzed by Kruskal–Wallis ANOVA and post hoc Wilcoxon test.

In the taste conditioning studies, for the one-bottle test saccharin intakes were compared between groups across test days by two-way ANOVA (treatment and days as factors) with days as the repeated measure. For the two-bottle test, the percentage saccharin preference scores (ml saccharin/ml saccharin and water × 100) were subjected to arcsine transformation to normalize the data followed by two-way ANOVA (treatment and days as factors). In both the one- and two-bottle tests, within-day comparisons between treatment and control groups was made by post hoc Dunnetts *t*-test.

For the operant and feeding studies all data were analyzed by one (treatment)- or two (treatment and time)-way repeated measures ANOVA where appropriate with post hoc testing using Dunnetts *t*-test. In all experiments the accepted level of significance was $p < 0.05$.

RESULTS

Effect of Naloxone Dose on the Incidence of Physical Opioid Withdrawal Signs

In morphine pellet-implanted rats, naloxone produced a dose-related incidence of weight loss, $F(7, 35) = 2.8$, $p < 0.05$, diarrhoea ($p < 0.01$), mouth movements, $F(7, 35) = 5.6$, $p < 0.01$, ptosis ($p < 0.01$), wet dog shakes, $F(7, 35) = 5.3$, $p < 0.01$, and paw shakes, $F(7, 35) = 2.8$, $p < 0.05$. Salivation, teeth chatter, jumping (interpreted as escape attempts), chin rubbing, penile grooming, lacrimation, and rhinorrhea were also seen in some rats but were not clearly dose related. The startle response was also enhanced in morphine-withdrawn rats ($p < 0.01$) (see Fig. 1). The threshold dose of naloxone for the induction of most of these behaviors was 0.05 mg/kg, although secretory signs (salivation, lacrimation, rhinorrhoea) were usually only evident at naloxone doses in excess of 0.5 mg/kg.

Effect of Naloxone Dose on the Development of a Conditioned Taste Aversion in Morphine-Dependent Rats: One-Bottle Test

In an initial experiment, naloxone (0.5 mg/kg) produced a significant aversion for a 0.1% w/v saccharin solution following one pairing in morphine but not placebo pellet implanted rats. Thus, a main effect of treatment, $F(3, 28) = 18.4$, $p < 0.01$, but not days, $F(3, 84) = 1.8$, NS, was found. Post hoc tests indicated that the volume of saccharin consumed in the morphine/naloxone conditioned group was significantly lower than all other groups during test days 1–3 (see Table 1).

A second experiment then examined the effect of varying the naloxone dose in this model. Main effects of dose, $F(5, 52) = 7.2$, $p < 0.01$, days, $F(3, 156) = 4.6$, $p < 0.01$, and a naloxone dose × days interaction, $F(15, 156) = 3.3$, $p < 0.01$, were found (see Fig. 2A). Subsequent post hoc tests showed that morphine-dependent rats treated with naloxone doses of 0.05 mg/kg and above drank significantly less saccharin solution between test days 1–3 by comparison to vehicle controls. By the third test day this effect was only seen in the naloxone 0.5 mg/kg group.

Effect of Naloxone Dose on the Development of a Conditioned Taste Aversion in Morphine-Dependent Rats: Two-Bottle Test

In the two-bottle choice test naloxone pretreatment (0.002–0.05 mg/kg) produced a significant taste aversion in morphine pellet, but not placebo pellet-implanted rats (Fig. 2B). Thus, analysis of the saccharin preference scores over postconditioning days 1–6 revealed significant main effects of treatment, $F(4, 25) = 9.7$, $p < 0.01$, and days, $F(5, 125) = 4.3$, $p < 0.01$, but not treatment × days interaction, $F(20, 125) = 1.1$, NS. Subsequent within-day analysis showed significant differences in preference scores between the naloxone 0.05 mg/kg/morphine group and the vehicle/morphine group. At the 0.01 mg/kg dose, naloxone also produced a significant reduction in saccharin preference in morphine pellet-implanted rats; however, this effect was not consistently seen across days. At no time did naloxone 0.05 mg/kg produce any reduction in saccharin preference in rats implanted with a placebo pellet compared to the vehicle/morphine pellet group.

Effect of Naloxone Dose on Fixed Ratio Responding for Food Reinforcement in Morphine-Dependent Rats

Following implantation of a single morphine pellet, all rats showed a marked decline in responding for food reinforce-

TABLE 1

EFFECT OF NALOXONE (0.5 mg/kg) ON SACCHARIN INTAKE IN A ONE-BOTTLE TASTE CONDITIONING PROCEDURE

		Conditioning Day	Test Day 1	Test Day 2	Test Day 3
Placebo pellet	Vehicle	16.1 ± 1.3	17.7 ± 1.1	17.1 ± 1.2	19.7 ± 1.6
	Naloxone	14.2 ± 1.3	15.6 ± 1.1	19.5 ± 0.8	19.0 ± 0.8
Morphine pellet	Vehicle	13.8 ± 1.1	17.3 ± 0.8	16.9 ± 0.8	12.6 ± 1.9
	Naloxone	13.2 ± 0.4	8.2 ± 0.9*†	10.2 ± 1.1*†	9.3 ± 1.4*†

$n = 8$ per group.
*$p < 0.01$ vs. placebo/vehicle group.
†$p < 0.01$ vs. placebo/naloxone group (Dunnetts *t*-test).

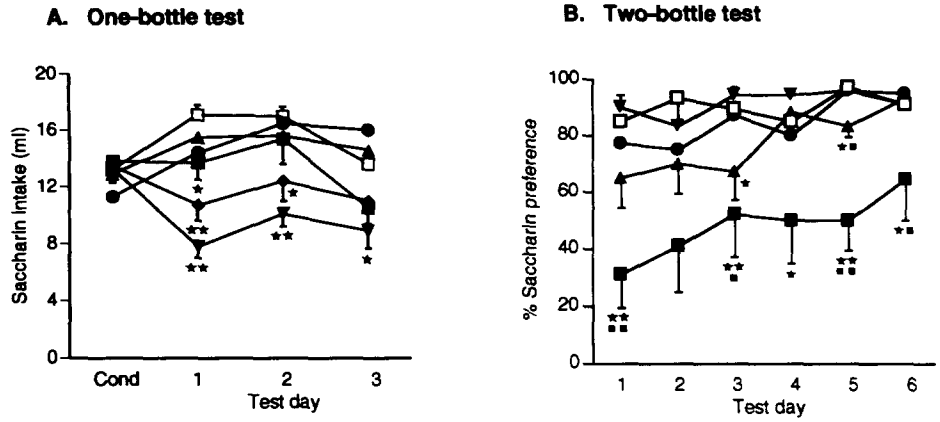

FIG. 2. Effect of naloxone dose on the incidence of conditioned taste aversion in (A) a one-bottle and (B) two-bottle test. (A) (□) vehicle, (●) naloxone 0.002 mg/kg, (▲) naloxone 0.01 mg/kg, (■) naloxone 0.05 mg/kg, (♦) naloxone 0.15 mg/kg,(▼) naloxone 0.5 mg/kg—all rats received morphine pellets. Cond = conditioning day. $*p < 0.05$, $**p < 0.01$ vs. vehicle pretreatment on respective test day (Dunnetts t-test). $n = 10$ per group except, veh $n = 12$, nal 0.002 mg/kg $n = 6$. (B) (▼) vehicle, (●) naloxone 0.002 mg/kg, (▲) naloxone 0.01 mg/kg, (■) naloxone 0.05 mg/kg—all rats received morphine pellets, (□) naloxone 0.05 mg/kg/placebo pellet. $*p < 0.05$, $**p < 0.01$ vs. vehicle/morphine group on respective test day. $■p < 0.05$, $■■p < 0.01$ vs. naloxone 0.05 mg/kg/placebo pellet group on respective test day (Dunnetts t-test). $n = 6$ per group.

ment under an FR15 schedule (i.e., preimplantation response: 88 ± 5 pellets; day 1 postimplantation: 27 ± 6 pellets). Such an effect was not seen in placebo pellet-implanted rats. However, by the third day response rates began to stabilize in the morphine pellet-implanted rats and had returned to preimplantation levels (see veh group; Fig. 3A). Although not significant, $t(11) = 1.6$, NS, at the time of testing (days 4 and 5 postimplantation), the rates of responding in the morphine rats was slightly lower than their placebo counterparts.

Naloxone (0.002–0.05 mg/kg) failed to influence operant responding in placebo pellet-implanted rats, $F(3, 12) = 0.3$, NS. However, in morphine-implanted rats a marked disruption was recorded, $F(3, 21) = 29.2$, $p < 0.01$, with post hoc testing showing a significant decrease at the 0.01 mg/kg and 0.05 mg/kg doses of naloxone compared to vehicle controls (Fig. 3A). Analysis of the temporal data (Fig. 3B) from the morphine/naloxone group again showed a main effect of treatment, $F(3, 21) = 22.2$, $p < 0.01$, and of time, $F(3, 21) = 293.3$, $p < 0.01$, and treatment × time interaction, $F(9, 63) = 36.7$, $p < 0.01$. Post hoc analysis showed a significant suppression of responding at each time point following naloxone 0.05 mg/kg; but at the 0.01 mg/kg dose this suppression was only significant at the 20-min period.

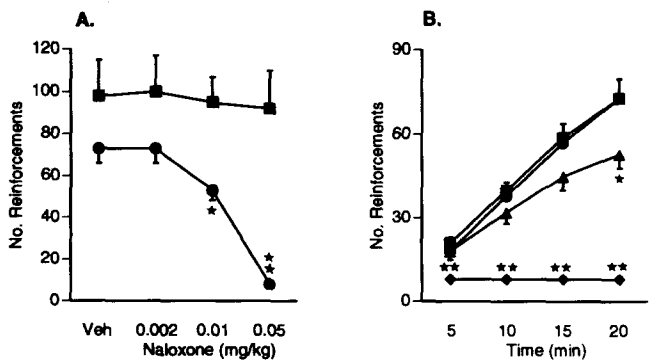

FIG. 3. Effect of naloxone dose on operant responding for food reinforcement under FR15 schedule. (A) Comparison between (■) placebo ($n = 5$) and (●) morphine ($n = 8$) pellet-implanted rats. (B) Time course of responding in the morphine-dependent group following (■) vehicle, (●) naloxone 0.002 mg/kg), (▲) naloxone 0.01 mg/kg, (♦) naloxone 0.05 mg/kg treatment. $*p < 0.05$. $**p < 0.01$ vs. vehicle pretreatment (Dunnetts t-test).

Effect of Naloxone Dose on Deprivation-Induced Feeding in Morphine-Dependent Rats

Analysis of the feeding data showed main effects of treatment, $F(2, 8) = 20.3$, $p < 0.01$, time, $F(2, 8) = 73.6$, $p < 0.01$, and treatment × time, $F(4, 16) = 9.2$, $p < 0.01$ (see Table 2). Post hoc testing showed a significant decrease in food intake following naloxone 0.05 mg/kg pretreatment at 20 and 60 min, but not 120 min. At no time period did naloxone 0.01 mg/kg affect this measure.

Despite the fact that total daily food intakes between the operant and 2 h food access models were similar (i.e., 14–16 g), it seemed that the operant procedure was the more sensitive to naloxone-precipitated opioid withdrawal. Thus, at the 0.01 mg/kg dose, naloxone significantly affected behavior in the operant test but not in the free-feeding test. Also, at the 0.05 mg/kg dose, a greater suppression relative to vehicle pretreatment was seen in the operant feeding model (i.e., 20 min FR15 model −89%, 20 min free-feed model −61%).

DISCUSSION

In the present study we attempted to precipitate opioid withdrawal reactions of varying severity by adjusting the dose of naloxone administered to rats implanted 3–5 days earlier with a single morphine base pellet. The influence of opioid withdrawal on subsequent behavior in a variety of tests was then examined. This was to determine whether any dissocia-

TABLE 2

EFFECT OF NALOXONE-PRECIPITATED OPIOID WITHDRAWAL ON FOOD INTAKE IN 22 h FOOD DEPRIVED RATS

	Food Consumed (g)		
	20 min	60 min	120 min
Vehicle	7.0 ± 0.6	11.4 ± 1.2	14.3 ± 1.1
Naloxone 0.01 mg/kg	6.3 ± 0.4	10.3 ± 0.8	13.8 ± 0.9
Naloxone 0.05 mg/kg	2.7 ± 0.5**	4.3 ± 0.8**	11.9 ± 1.0

n = 5 rats.

*$p < 0.01$ vs. vehicle pretreatment at respective time period (Dunnett's t-test). Please note that vehicle intake measure represents the mean value for each animal at days 3 and 5 (see appropriate Method section).

tion between physical and motivational (aversive) behaviors could be distinguished.

Regarding the physical signs of opioid withdrawal, it seemed that under our experimental conditions the threshold naloxone dose to induce most of these components was 0.05 mg/kg. Secretory signs such as salivation and rhinorrhoea were generally not evident until naloxone doses of 0.5 mg/kg and above were used. Even at a dose of 5 mg/kg naloxone, we failed to consistently observe such well-documented withdrawal signs as teeth chattering, jumping, and lacrimation (2, 5,23). This is probably a reflection that the rats used in the present study were moderately opioid dependent at the time of testing.

It was interesting to note the incidence of paw shaking, salivation, ptosis, chin rubbing, and mouth movements in many of the experimental subjects during withdrawal. These have been previously described as unconditioned behaviors to aversive stimuli that are emetogenic in vomiting species such as lithium chloride and 5-HT_3 receptor agonists (12,14). As such, they may represent behavioral responses in rats to a withdrawal-induced malaise, a commonly reported feature of opioid withdrawal in man [e.g., see 18,39]. It would be interesting to test this hypothesis by examining a variety of antiemetics against these behaviors. Interestingly, in a previous study we found that the NMDA antagonist dizocilpine seemed to selectively attenuate some of these signs (16). However, the efficacy of such drugs against clinical forms of nausea/emesis, especially induced by opioid withdrawal is, to our knowledge, unknown.

In the taste-conditioning experiments, the two-bottle choice procedure was the more sensitive method, for significant taste aversions were detected with this model in morphine-dependent rats using naloxone doses five times lower compared to the one-bottle procedure (0.01 mg/kg compared to 0.05 mg/kg). Also, the aversions were sustained for longer time periods. This difference between the two procedures was not unexpected (3,34) and is probably because, to a fluid-deprived rat, the motivation to consume the saccharin solution is greatest in the one-bottle test because there is no fluid alternative. In a previous study (15) we found the lowest dose of naloxone that produced a significant place aversion in depen-

TABLE 3

SUMMARY OF VARYING NALOXONE DOSE ON BEHAVIOUR IN MORPHINE-DEPENDENT RATS

		Naloxone Dose (mg/kg)						
		0.002	0.01	0.05	0.15	0.5	1.5	5
A. Physical Withdrawal signs–	WDS	0	0	+	++	++	++	++
	Wt. loss	0	0	+	+	++	++	++
	Mouth Movements	0	0	+	++	++	++	++
	Salivation	0	0	0	0	+	+	+
B. Place aversion conditioning		0	+	++	++	++	++	++
C. Taste aversion conditioning–	One bottle test	0	0	+	+	++	NT	NT
	Two bottle test	0	+	++	NT	NT	NT	NT
D. Suppression of operant responding food reinforcement (FR15)		0	+	++	NT	NT	NT	NT
E. Suppression of deprivation-induced feeding		NT	0	+	NT	NT	NT	NT
F. Suppression of exploration–	Open field	NT	NT	o/+	NT	++	NT	NT
	Elevated plus maze	NT	NT	o/+	NT	++	NT	NT

0 = absent, 0/+ = equivocal, + = present, ++ = marked, NT = not tested. All data presented in this table are summarised from the present report, with the exception of the place aversion conditioning data (15), and the suppression of exploratory behaviour (17).

dent animals was 0.01 mg/kg. This was identical to the findings from the two-bottle taste conditioning test suggesting some equivalence between each model to detect the aversive consequences of opioid withdrawal.

Measurement of operant responding for food reinforcement has been widely used to investigate the motivational consequences of drug withdrawal, including opioid (8,21,31). Unlike the place and taste aversion paradigms, in this model the influence of the withdrawal state is examined in animals performing a learned operant response as opposed to the conditioning of environmental cues to this state. In morphine-dependant rats only, naloxone (0.01–0.05 mg/kg) administration produced a profound suppression of fixed ratio responding. The duration of this suppression was brief, for responding had returned to baseline levels within 5 h of treatment. This rate of recovery was similar to that previously reported by Gellert and Sparber (8). In the food deprivation study, a naloxone-precipitated withdrawal of similar intensity (i.e., induced by naloxone dose of 0.05 mg/kg), reduced food intake 20 min and 1 h following onset, but not at 2 h. While this feeding study clearly indicated that an anorectic effect contributed to the deficits in operant responding produced by naloxone, the operant task was a more sensitive procedure, which might imply that other interoceptive stimuli associated with opioid withdrawal are involved. Such stimuli could concievably include dysphoria, anxiety, and malaise (6,21), and it is also very likely that similar processes are involved in the conditioned aversions seen to this phenomena (13,15,27,33). It is, however, difficult at present to ascertain the relative contributions of these interoceptive cues to each behavior; although, for example, it is known that the taste conditioning paradigm may be particularly sensitive to emetogenic or malaise-inducing stimuli (11) and that a number of anxiogenic drugs produce marked place aversions (7,32).

Taking the results from these studies as a whole, distinctions between somatic and motivational states were clearly evident because there seemed to be no correlation between the onset of physical withdrawal symptoms, with measures obtained from the operant and conditioning tests. Thus, at a dose of 0.01 mg/kg, naloxone did not induce any overt withdrawal signs and yet place (15) and taste (two-bottle test only) aversions were seen, as well as reductions in fixed ratio responding for food reinforcement (see Table 3 for summary). Failure to observe physical signs at the lower naloxone doses would not appear to be due to the measurement procedure, because the animals were continuously monitored for these behaviors. The present observations are of interest for a number of reasons. Firstly, they confirm previous studies identifying each of these procedures as sensitive measures of opioid withdrawal (8,13,26–28). Secondly, by showing that aversions can develop in the absence of any physical signs, the present data provide further evidence for a distinction between physical and motivational withdrawal components (see the introductiory paragraphs). These results essentially confirm and extend the findings of Mucha (27), who reported no apparent correlation between the onset and magnitude of physical withdrawal signs and the incidence of place aversion conditioning. Finally, because of this distinction, it also highlights the importance of examining potential treatments for opioid physical dependence against a variety of withdrawal components (19). It is noteworthy that the α_2 agonist clonidine, a clinically effective treatment for opioid withdrawal states (9,10), has been reported to attenuate withdrawal-induced suppressions in operant food responding (31) and place aversion conditioning (22) as well as physical withdrawal signs (35,36) in rats. It may be a useful practice if in future, before drugs could be considered as novel therapies for opioid dependence, their efficacy across a similarly diverse range of models is first evaluated.

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