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Discriminative Stimulus Effects of Naltrexone After a Single Dose of Morphine in the Rat[1]

KEITH W. EASTERLING and STEPHEN G. HOLTZMAN

Emory University School of Medicine, Department of Pharmacology, Atlanta, Georgia

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ABSTRACT

The discriminative stimulus effects of an acute morphine (MOR) → naltrexone (NTX) combination were characterized and compared with the stimulus effects of NTX-precipitated and spontaneous withdrawal from chronic MOR administration. Adult male Sprague-Dawley rats (n = 6–8) were trained to discriminate between two drug treatments in a discrete-trial avoidance/escape procedure: MOR (10 mg./kg, s.c., 4 h) → NTX (0.3 mg/kg, s.c., 0.25 h) versus saline (SAL, 1 ml/kg, s.c., 4 h) → NTX (0.3 mg/kg, s.c., 0.25 h). Subjects responded only on the SAL → NTX-appropriate lever when SAL was given 3.75 h after MOR or 3.75 h before any dose of NTX (0.3–100 mg/kg). Responding was dose dependent and MOR → NTX-appropriate when NTX (0.01–0.1 mg/kg) followed MOR. Full MOR → NTX-appropriate responding was dependent on the pretreatment dose and time of MOR, with full effects observed only when MOR (10 mg/kg) was given 3 to 4 h before NTX. While subjects were maintained on either 20- or 40 mg/kg/day of MOR via osmotic pump, NTX produced full dose-dependent, MOR → NTX-appropriate responding. When the MOR-filled pumps were removed, partial MOR → NTX-appropriate responding occurred, peaking at 6 to 12 h. The physical withdrawal signs produced by NTX after acute or during chronic MOR exposure were of smaller magnitude compared with the ones that occurred during abrupt withdrawal from chronic MOR. A qualitatively unique "withdrawal" stimulus that is dose- and time-dependent appears to be the basis of this MOR → NTX discrimination.

Morphine (MOR) is widely used in the clinical management of pain. However, prolonged MOR administration may be limited by the high abuse liability of the drug, which is also true of other analgesics that produce MOR-like subjective effects (Jasinski, 1977). Abrupt termination of chronic opioid treatment or the administration of an opioid antagonist during treatment produces the physical withdrawal/abstinence signs and subjective symptoms that define physical dependence in humans.

Schedule-controlled behaviors maintained by food or brain stimulation reinforcement are sensitive indicators of both spontaneous and antagonist-precipitated opioid withdrawal in animals. Without prior MOR exposure, opioid antagonists (30–100 mg/kg), such as naloxone (NX) and naltrexone (NTX), have behavioral effects only at significantly higher (10- to 100-fold) doses than those required to antagonize the acute behavioral effects of even large doses of MOR (Adams and Holtzman, 1990). In contrast, after prolonged MOR exposure, opioid antagonists significantly suppress schedule-controlled behaviors at 100- to 1000-fold lower doses than those having effects in animals not treated with MOR (Valentino et al., 1983).

In humans given a single dose of MOR, NX precipitates a syndrome comprising both physical signs and dysphoric subjective effects that are qualitatively similar to those experienced after withdrawal from chronic MOR treatment (Bickel et al., 1988; Heishman et al., 1989). In rats, increased behavioral sensitivity to antagonists also occurs after exposure to a single dose of MOR (Meyer and Sparber, 1977; Young, 1986). Indeed, as little as 2 to 4 h after an acute MOR (3–10 mg/kg) pretreatment, low doses of NTX (≤0.1 mg/kg) decrease rates of responding by as much as 75% (Easterling and Holtzman, 1997), and slightly higher doses produce somatic signs that are qualitatively similar to but less intense than those seen during spontaneous withdrawal from chronic MOR administration (Schulteis et al., 1997). These data support the conclusion that antagonist-induced disruptions in operant responding, in part, operationally define withdrawal and an acute dependence syndrome in the rodent.

Drug discrimination affords an animal model for studying subjective drug effects, including those associated with MOR withdrawal (Holtzman, 1990). Rats that are maintained chronically on MOR can be trained to discriminate saline (SAL) from as little as 0.1 mg/kg of NTX (Gellert and Holtz-

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ABBREVIATIONS: MOR, morphine; NX, naloxone; NTX, naltrexone.

man, 1979; Holtzman, 1985) orders of magnitude lower than the discriminable dose in animals not maintained on MOR (France and Woods, 1985). This potent antagonist drug cue generalizes dose dependently to other opioid antagonists but not to opioid agonists or to nonopioid drugs. Most important, when rats are abruptly withdrawn from the chronic MOR regimen, they respond on the NTX-appropriate lever, indicating similarities in the interoceptive states produced by NTX-precipitated and abrupt/spontaneous opioid withdrawal.

As with chronic MOR administration, a single dose of MOR (0.1–1 mg/kg) can also produce a subsequent increase in the potency of the interoceptive effects of NTX in pigeons trained on a discrimination task (France and Woods, 1985). In rats responding on an FR10 schedule of food reinforcement, a single MOR pretreatment (40 mg/kg, 8 h) increases the stimulus potency of a lower dose of NX (1.25 mg/kg), making it effective as a dose-dependent discriminative stimulus (versus SAL; Miksic et al., 1981).

The studies reviewed above suggest that when opioid antagonists are given after a single or prolonged exposure to MOR, a common and distinctive interoceptive stimulus is produced that coincides with physical withdrawal signs and, in part, defines opioid dependence. However, the antagonist-precipitated interoceptive effects that follow a single MOR exposure remain incompletely characterized. Therefore, the present experiments were designed to provide data on the acute NTX-precipitated withdrawal stimulus produced after a single dose of MOR. First, we sought to determine whether or not subjects could be trained to discriminate NTX (0.3 mg/kg, 0.25 h) alone from a MOR (10 mg/kg, 4 h) + NTX (0.3 mg/kg, 0.25 h) combination. Once it was determined that they could be, we generated antagonist stimulus-generalization curves after various MOR pretreatment doses (1–10 mg/kg) or times (0.5–6 h), and before, during, and after chronic MOR administration. Specifically, we sought to determine whether the acute NTX-induced stimulus that follows a single dose of MOR is 1) dose and time dependent, 2) mediated by opioid receptors, 3) generalizable to another opioid antagonist, or 4) to abrupt/spontaneous withdrawal following the termination of chronic MOR treatment.

Materials and Methods

Subjects. The subjects were male Sprague-Dawley-derived rats (Charles-River; Raleigh, NC) initially weighing 250 to 350 g. The rats were housed individually in polycarbonate cages with continuous access to food and water. The colony room was maintained on a 12:12 h light/dark cycle with lights on at 7:00 AM.

Drugs. The following drugs were dissolved in normal SAL (0.9%): MOR sulfate (Penick Corp., Newark NJ), NTX hydrochloride and NX hydrochloride (Research Biochemicals Inc., Natick, MA). All doses are expressed as the free base. MOR was administered in a volume of 1 ml/kg s.c. or by osmotic minipump before training or testing. When given s.c., NX and NTX were always administered in a volume of 1 ml/kg.

Osmotic Pump Implantation. During chronic MOR administration experiments, two osmotic pumps (Models 2 ML1 or 2 ML2; Alza Corp., Palo Alto, CA) were implanted in each 500 to 700 g rat while it was under light methoxyflurane anesthesia. A small incision was made in the mid-scapular region, and pumps were inserted in a rostral-caudal direction, with their flow-moderator entering first. Wounds were closed with 9-mm stainless steel wound clips. These wound clips were removed when the pumps were removed while the rats were under light methoxyflurane anesthesia. At this time, a second set of wound clips was installed and then removed 14 days later.

In the "Pump MOR 20" condition, the concentration of MOR in each 2-week pump (model 2 ML2) was individually adjusted so that each rat received a total of 20 mg/kg/day. In the "Pump MOR 40" condition, two 7-day pumps (model 2 ML1) delivered a total of 40 mg/kg/day. At the same time, beginning the day after implantation, the rats in the Pump MOR 40 condition received supplemental daily MOR injections (s.c.). The concentration of the supplemental injection was incremented daily on days 2 to 5 (1, 3, 5.6, and 10 mg/kg, respectively), and on days 6 and 7, 10 mg/kg MOR was given at 9:00 AM and again at 5:00 PM. Therefore, the rats in the Pump MOR 40 condition received a total of 339.6 mg/kg of MOR over 7 days, whereas the Pump MOR 20 rats received 82% (280 mg/kg) of that total dose over 14 days.

Discrimination Testing Apparatus. The apparatus has been described in detail previously (Shannon and Holtzman, 1976). Briefly, a standard two-lever operant test chamber was modified by adding one lever (the "observing" lever) to the wall opposite the two original levers (the "choice" levers). The choice levers were separated by a 5-cm-wide clear polycarbonate partition that extended from floor to ceiling of the chamber. A constant current generator delivered a scrambled electric current to the grid floor of the chamber, which was housed in a ventilated, light- and sound-attenuating outer enclosure. All contingencies of the behavioral schedule were controlled via a desktop computer.

Drug Discrimination Training. Rats were trained to discriminate between s.c. injections of SAL [(1 ml/kg, 4 h) and NTX (0.3 mg/kg, 0.25 h)] and MOR [(10 mg/kg, 4 h) and NTX (0.3 mg/kg, 0.25 h)] in a discrete-trial avoidance/escape procedure in which a two-lever-press response chain terminated a trial. The doses of MOR and NTX and the pretreatment duration (4 h) were chosen on the basis of published data characterizing antagonist-induced operant response-rate disruptions following an acute MOR injection (Young, 1986; Adams and Holtzman, 1990). In this training procedure, the observing lever and two choice levers were always available. The beginning of each of the 20 trials composing a session was signaled by the simultaneous illumination of the house light and the onset of white noise. Unless the appropriate two-response chain (observing-choice) occurred beforehand, 5 s after a trial began, a 1-s, 1- to 3-mA current was delivered to the grid floor of the chamber every 3 s for 300 s or until terminated by the appropriate response chain. The first observing response made in a trial terminated the white noise, and a response on the correct choice lever extinguished the house light and ended the trial. If a response on the incorrect choice lever followed an observing response, the white noise resumed and another observing response and correct choice response was required to end the trial. Because NTX was always given 0.25 h before testing, the correct choice response was determined by what the animal was injected with 4 h before the session, i.e., SAL or 10 mg/kg MOR. An incorrect choice response was the alternative for that session. The intertrial interval was 50 s, and during this time the chamber was dark. Each session ended after 20 trials or 30 min.

Training sessions were conducted once daily 5 days a week (Monday-Friday). Either SAL or MOR (10 mg/kg) was administered 4 h before the session, usually in an alternating pattern, and NTX (0.3 mg/kg) was administered 0.25 h before the session. Half the rats were trained to press the right choice lever after MOR followed by NTX (MOR → NTX) and the left lever after SAL followed by NTX (SAL → NTX); the other half were trained with the choice lever assignments reversed. Training continued until rats distributed 90% (18 of 20 trials) of their responses on the correct lever over 4 consecutive training days, 2 SAL → NTX and 2 MOR → NTX.

Drug Discrimination Testing. Following training, individual rats were tested twice weekly with doses of drug administered according to the sequence shown in Table 1. In test sessions, a response on either choice lever after a response on the observing lever termi-

TABLE 1
Summary of experimental treatments

Agonist Dose	Agonist Pretreatment	Antagonist Dose	Antagonist Pretreatment	Antagonist AD_{50} or ED_{50}	Potency Relative to Training Condition
mg/kg	*h*	*mg/kg*	*hr*	± *S.E.M.*	
SAL	4.0	NTX 3.0–100	0.25	>100	
MOR, 10	4.0	NTX 0.003–3.0	0.25	0.05 ± 0.02	
MOR, 10	0.25–8.0	NTX 0.3	0.25		
MOR, 10	3.5	NTX 0.3	0.25–2.0		
MOR, 10	4.0	NTX 0.001–1.0	4.25	0.11 ± 0.04	
		NTX 0.3	0.25		
MOR, 1.0	4.0	NTX 0.3–10	0.25	2.1 ± 0.62	0.024[a,b]
MOR, 3.0	4.0	NTX 0.3	0.25		
MOR, 10	0.5	NTX 1.0–100	0.25	>100	
MOR, 10	1.0	NTX 1.0–30	0.25	3.66 ± 1.02	0.014[a,b]
SAL	4.0	NX 30	0.25		
MOR, 10	4.0	NX 0.03–30	0.25	0.35 ± 0.09	0.14[a,b]
Pump MOR 20, 280	14days	NTX 0.001–1.0 (days 7–13)	0.25	0.04 ± 0.02	1.25[a]
Pump MOR 40, 340	7days	NTX 0.001–0.01 (days 5–6)			
MOR, 10	4.0	NTX 0.003–3.0	0.25	0.03 ± 0.01	1.67[a]
Pump MOR 40, 340	7days	NTX 0.001–0.01 (days 5–6)	0.25	0.005 ± 0.0002	10[a,b]

[a] Significantly different from SAL-pretreated control $p \leq 0.05$.
[b] A significant potency difference relative to training dose or time ($p \leq 0.05$).

nated the trial. As with training sessions, all test sessions consisted of 20 trials. As each subject met the training criterion for stable discrimination performance (90% correct responding), it was randomly assigned to a test drug series until each drug series included six to eight subjects.

Some experiments required that subjects be given several consecutive testing sessions within 1 day. When this occurred, a maximum of one session was preceded by a drug injection and the rest of the sessions were preceded by SAL injections. For instance, the duration of the stimulus effects of the training dose of NTX was examined by giving an acute MOR pretreatment (10 mg/kg), followed by SAL and then the training dose of NTX (0.3 mg/kg, 0.25 h) and repeatedly testing the animals in consecutive sessions until responding became primarily SAL → NTX appropriate. In this case, a SAL injection preceded (0.25 h) the first of the consecutive test sessions (before NTX). NTX (0.3 mg/kg) was then given 0.25 h before the second session (4 h after MOR). Each subsequent test session was preceded (0.25 h) by another SAL injection.

Once the experiments involving a single (acute) injection of MOR had been completed, subjects ($n = 10$) were given 2 weeks of continued training, and then received two MOR-filled 14-day osmotic pumps, which delivered a total of 20 mg/kg/day. Seven, 9, 11, and 13 days after the pumps were implanted, they were tested following (0.25 h) an injection of SAL or NTX (0.001–1 mg/kg) given in a randomized sequence. On day 14, the pumps were removed and multiple test sessions, preceded by a SAL injection, were given at 6, 24, and 48 h. After the first set of osmotic pumps was removed and the 48-h testing was completed, subjects were not trained or tested for 14 days.

The rats received 2 to 4 weeks of training after the 14-day rest period. If they met the 90% testing criteria, a second set of MOR-filled (40 mg/kg/day for 7 days) osmotic pumps was implanted. SAL or NTX (0.001–0.01) challenges were given during pump operation (days 5 and 6), and then subjects were given SAL injections and tested 3, 6, 12, 24, 48, and 72 h after the pumps were removed (on day 7). Because SAL challenges did not produce MOR → NTX-appropriate responding during pump operation, these subjects received their SAL challenge session 4 h before a NTX-challenge session on either day 5 or 6 (randomly chosen), allowing three tests in each subject while the pumps were implanted. Seventy-two hours after the second set of osmotic pumps was removed, subjects were given another 14-day rest period and then trained until they again reached the criteria for stable discrimination performance. At this point, an acute MOR (10 mg/kg, 4 h) → NTX (0.003–3 mg/kg, 0.25 h) curve was redetermined. Finally, a third set of MOR-filled (40 mg/kg/day) osmotic pumps was implanted in the surviving rats. As before, they received SAL or NTX challenges on days 5 and 6, and were tested for stimulus generalization and signs of physical withdrawal 3 to 72 h after the pumps were removed.

Global Rating of Physical Signs. Signs of physical dependence were assessed by visual observation (Gellert and Holtzman, 1978) of rats in the home cage over the 0.25 h pretreatment period that preceded discrimination training or testing. Before this visual scoring, subjects were weighed and given an injection of SAL or NTX (0.03–30 mg/kg), and then weighed again following discrimination testing an hour after the initial weighing. In the acute dependence experiments, SAL or MOR (1–10 mg/kg) was given 3.75 h before scoring. While subjects were receiving MOR (20–40 mg/kg day) through osmotic pumps, they were given a SAL or a NTX (0.001–1 mg/kg) injection and then scored (0.25 h). To assess spontaneous withdrawal signs, visual scoring and discrimination testing continued at intervals for 3 to 72 h after pump removal. In the Pump MOR 20 group, physical signs were scored 24 h after pumps were removed, whereas in the group that followed, the Pump MOR 40 group, subjects were scored at 3, 6, 9, 12, and 24 h.

Data Analysis. Discrimination data are presented as an average number of trials to the MOR → NTX-appropriate choice lever. The remaining trials of the session were completed on the choice lever appropriate for SAL → NTX. Discrimination data were excluded from analysis if subjects did not complete the entire range of doses within a drug series. The discrimination data were used to calculate ED_{50} or antagonist-dose 50 (AD_{50}, NTX) values by linear regression of the ascending or descending portion of each individual dose-response curve. The time until discrimination responding reached or returned to one-half its maximal value ($T_{1/2}$) was calculated by linear regression of the ascending or descending part of the time course curve. Subsequently, group means for these data were calculated and t tests (protected for planned multiple comparisons) were used for comparison among drug conditions. The α level chosen for all analyses was .05. Subjects received osmotic pumps delivering 40 mg/kg/day on two different occasions. During each replication, one SAL and two NTX challenges were given (days 5 and 6). These

discrimination data were combined for the purposes of generating ED_{50}s and figures based on four drug doses.

After individual withdrawal signs were scored, a global withdrawal score was calculated for each subject under each condition, using a previously validated procedure (Gellert and Holtzman, 1978; Schulteis et al., 1994; Espejo et al., 1995). In the Gellert-Holtzman scoring system, nine "checked" signs are marked as present or absent during observation. These signs include diarrhea, facial fasciculations or teeth chatter, swallowing movements, salivation, chromodacryorrhea, ptosis, abnormal posture, erection or ejaculation, and irritability on handling. Each checked sign that is present is weighted by a constant factor. The number of occurrences of three additional "graded" signs (escape attempts, abdominal constrictions, and wet-dog shakes) is recorded and each incidence is similarly weighted. The sum of the checked and graded signs is added to the percent of weight lost (above control), and represents the global withdrawal score. Mean global withdrawal scores for each condition were compared using planned t tests. As with the discrimination data, the global withdrawal scores from the two Pump MOR 40 replications were combined for analysis.

Results

Stimulus Generalization Curves

Subjects (n = 20) reached the discrimination testing criterion of 90% correct responding in an average (±S.E.M.) of 43 ± 6 training sessions. Initially, stimulus generalization curves for NTX were determined after both of the pretreatments used in training (Fig. 1). After SAL pretreatment, NTX (≤100 mg/kg) did not produce substantial MOR → NTX-like responding. Consequently, no ED_{50} was calculated for this condition. Likewise, MOR (10 mg/kg)-pretreated rats responded on the SAL → NTX-appropriate lever when they were injected with SAL 0.25 h before a session. When graded doses of NTX were given 3.75 h after 10 mg/kg MOR, the number of trials completed on the MOR → NTX-appropriate lever increased in a dose-dependent manner. The ED_{50} of NTX was 0.05 ± 0.02 mg/kg. The group completed an average of ≥90% of the trails on the MOR → NTX-appropriate choice lever when MOR pretreatment was followed by either 0.3 (training dose) or 3 mg/kg NTX (Fig. 1).

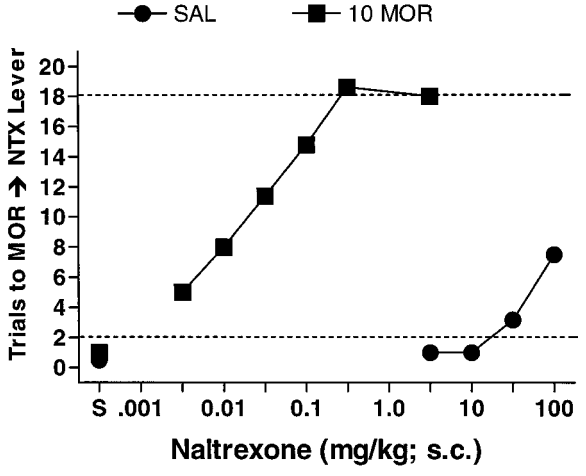

Fig. 1. Comparison of discriminative stimulus effects of NTX (s.c., 0.25 h) following SAL (4 h) or MOR (10 mg/kg, s.c., 4 h) administration. Each point represents mean of six to eight rats. Broken line at top represents criteria for full MOR → NTX-appropriate responding, and broken line at bottom represents full SAL → NTX-appropriate responding. Data indicate that NTX is a potent training stimulus in acutely MOR-treated rats (ED_{50} = 0.05 mg/kg), and that this stimulus is distinct from that produced by MOR alone or up to 2000-fold higher doses of NTX alone.

Temporal Dependency

To assess the temporal dependencies of the discrimination, the duration of the MOR (10 mg/kg) pretreatment was systematically varied around the training duration of 4 h (0.25–8 h), while holding the NTX dose (0.3 mg/kg) and pretreatment time (0.25 h) constant (Fig. 2A). When the duration of the MOR pretreatment was less than 1 h, responding was fully SAL → NTX appropriate. Three hours after MOR, responding was fully MOR → NTX-like, but by 8 h, responding had returned to less than 50% of its peak value. Thus, the onset of MOR → NTX-appropriate responding occurred with a $T_{1/2}$ = 1.75 ± 0.2 h.

Next, the duration of the stimulus effects of the training dose of NTX was examined by giving an acute MOR pretreatment (10 mg/kg), followed (3.75 h) by the training dose of NTX (0.3 mg/kg, 0.25 h) and repeatedly testing the animals in consecutive sessions until responding was SAL → NTX

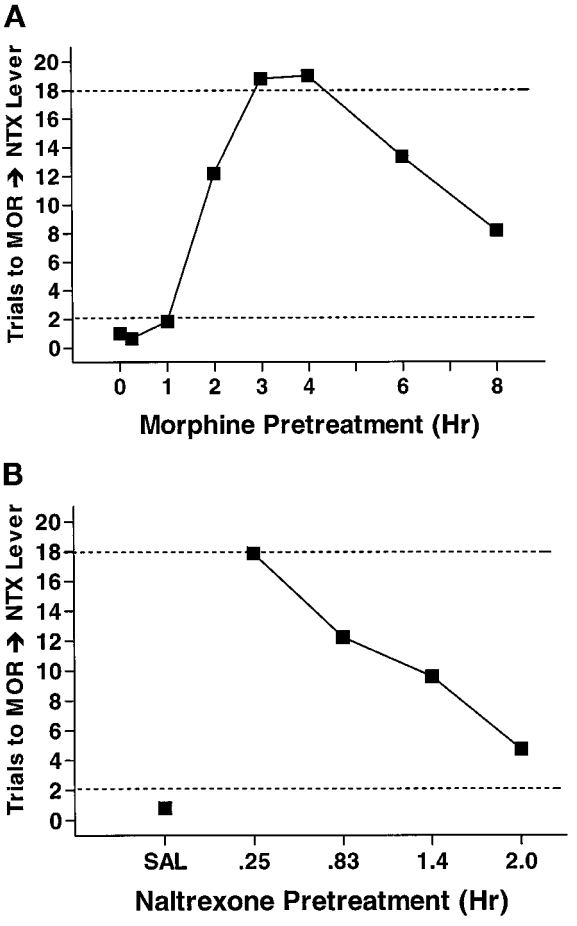

Fig. 2. Temporal dependence of MOR → NTX discrimination. A, MOR → NTX-appropriate responding as a function of time between MOR and NTX injections. Discriminative effects were produced by NTX (0.3 mg/kg, 0.25 h) when a single MOR (10 mg/kg) pretreatment was given 0.25 to 8 h before testing. Although 0.3 mg/kg NTX produced full stimulus effects 3 to 4 h after MOR, partial (50%) effects developed in as little as 1.75 h ($T_{1/2}$ = 1.75 ± 0.2 h). B, MOR → NTX-appropriate responding in MOR-pretreated (10 mg/kg, 3.5 h) rats as a function of time after injection of NTX. When a SAL injection preceded (0.25 h) the first, third, fourth, and fifth of the consecutive test trials and NTX (0.3 mg/kg) was given before the second, stimulus effects of NTX were short-lived ($T_{1/2}$ = 1 ± 0.25 h). Other details are as in Fig. 1.

appropriate (Fig. 2B). As it did in training sessions, NTX had full stimulus effects 0.25 h after injection, but these effects were almost completely absent by 2 h. Thus stimulus control of behavior by MOR → NTX declined with a $T_{1/2}$ of 1 ± 0.25 h after the injection of NTX.

Antagonism of Stimulus Effects

If the interoceptive effects of the MOR pretreatment are indeed mediated by opioid receptors, they should be attenuated by prior opioid antagonist administration. We tested this hypothesis by giving SAL or an additional NTX injection (0.001–1 mg/kg) 4.25 h before the test session, which was 0.25 h before the MOR injection (10 mg/kg, 4 h); NTX (0.3 mg/kg) was administered a second time 0.25 h before the test session, as usual.

SAL given 4.25 h before testing and before the training drug combination, did not significantly affect the full MOR → NTX-like stimulus effects of the MOR → NTX combination (Fig. 3). However, the increasing doses of NTX that were given (4.25 h) dose dependently attenuated MOR → NTX-appropriate responding, with a dose of 1 mg/kg completely blocking the stimulus effects of the combination. The AD_{50} of NTX for blocking the discriminative effects of MOR → NTX was 0.11 ± 0.04 mg/kg.

Reductions in MOR Pretreatment Time or Dose

If the interoceptive effects of acute dependence are a function of the dose and duration of MOR exposure, a lower dose of MOR or a shorter pretreatment interval might produce less dependence and smaller interoceptive effects. Consequently, a larger antagonist dose would be required to produce discriminative stimulus effects equivalent to those occurring during training. Pretreatment with 1 mg/kg of MOR compared with the training dose (10 mg/kg) produced a significantly ($p < .05$) different NTX stimulus-generalization curve (Fig. 4A). The ED_{50} of NTX after pretreatment with 1 mg/kg of MOR (2.1 ± 0.62 mg/kg) was 42 times greater than the ED_{50} of NTX after pretreatment with 10 mg/kg MOR, and trials completed on the MOR → NTX-appropriate lever did not reach 90%, even after 10 mg/kg of NTX. Only one dose of NTX (0.3 mg/kg) was given following a 3-mg/kg MOR pretreatment (4 h); its effects were intermediate between those of the same dose of NTX given after the lower (1 mg/kg) and higher (10 mg/kg) doses of MOR.

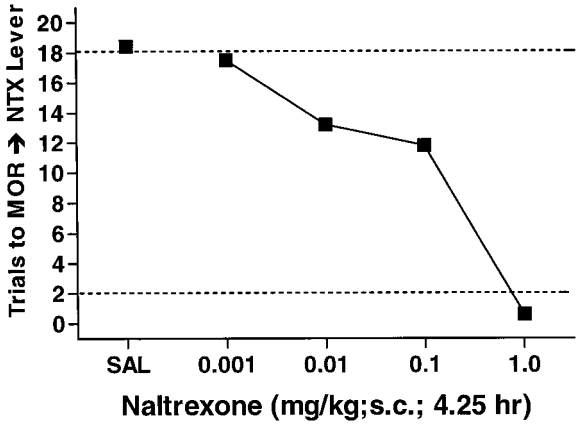

Fig. 3. Administration of NTX (0.001–1 mg/kg) 0.25 h before 4-h pretreatment with 10 mg/kg MOR dose dependently blocks discriminative effects of MOR → NTX. Discrimination dose-response curve for NTX given 4.25 h before testing, and before training drug combination MOR (10 mg/kg, 4 h) → NTX (0.3 mg/kg, 0.25 h). NTX potently antagonized full stimulus effects of MOR → NTX combination (AD_{50} = 0.11 mg/kg). Other details are as in Fig. 1.

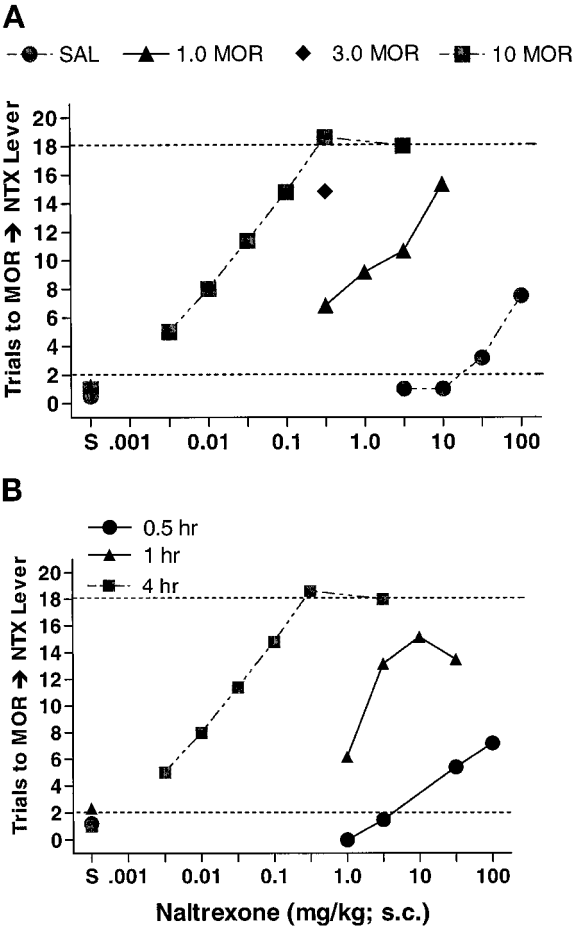

Fig. 4. Reducing acute MOR pretreatment dose or duration decreases potency of NTX for occasioning MOR → NTX-appropriate responding. A, Discrimination dose-response curves for NTX (s.c., 0.25 h) given after a 4-h pretreatment with SAL or various doses of MOR (1, 3, or 10 mg/kg). Only one dose of NTX was tested after 3 mg/kg of MOR. B, Discrimination dose-response curves for NTX (s.c., 0.25 h) given following MOR (10 mg/kg) pretreatment of varied durations (0.5–4 h). Compared with training conditions, a decrease in MOR pretreatment dose or duration of pretreatment (10 mg/kg) produced a significantly smaller increase in potency of NTX. SAL and 10 mg/kg MOR curves (gray symbols and dashed lines) are reproduced from Fig. 1. Other details are as in Fig. 1.

When MOR (10 mg/kg) pretreatments were shorter (0.5 and 1 h) than the training pretreatment (4 h), the stimulus-generalization curves for NTX (s.c., 0.25 h) changed (Fig. 4B). After the 0.5-h MOR pretreatment, the NTX dose-effect curve was not different from the one determined after SAL pretreatment. Following the 1-h MOR pretreatment and compared with the SAL pretreatment NTX produced substantial MOR → NTX-like responding; comparing ED_{50}s, it was only 0.014 times as potent as it was after the 4-h MOR pretreatment (ED_{50} = 3.66 ± 1). Peak (80%) MOR → NTX-appropriate responding was produced by 10 mg/kg NTX, and a higher dose of NTX (30 mg/kg) resulted in less responding on the MOR → NTX-appropriate lever than occurred after the lower dose. Therefore, reducing the pretreatment dose of MOR to 1 mg/kg or reducing the pretreatment time to 1 h resulted in approximately equivalent ($p > .05$) decreases in

the potency of NTX relative to its potency under the conditions used for training (Table 1).

Generalization to NX

The dose-response curves for the discriminative stimulus effects of NTX and NX were compared following a 4-h pretreatment with SAL or MOR (Fig. 5). As with NTX, after SAL pretreatment, NX (30 mg/kg) did not produce MOR → NTX-like responding. When MOR (10 mg/kg) was given 4 h before the antagonists, NX (ED_{50} = 0.35 ± 0.09 mg/kg) fully and dose dependently generalized to the MOR → NTX cue, but was significantly (7-fold) less potent than NTX.

Chronic MOR Administration

Drug Discrimination. In the next set of experiments, the discriminative stimulus effects (Fig. 6) and physical withdrawal signs (Table 2) produced by NTX were compared following acute pretreatment with SAL or MOR (1–10 mg/kg) or during the chronic infusion of either of two doses (Pump MOR 20 or Pump MOR 40) of MOR by osmotic pump. Between the implantation of the first set of osmotic pumps (n = 10, Pump MOR 20) and the second set of pumps (n = 7, Pump MOR 40), two rats died of causes unrelated to the experiment, and one rat failed to meet the training criteria. Before the implantation of the third set of pumps (n = 5), two more rats died. The data from these rats were excluded from the analysis.

SAL did not produce MOR → NTX-like stimulus effects in rats in any of the experiments involving MOR infusion by osmotic pump (Figs. 6 and 7). NTX produced dose-dependent increases in MOR → NTX-like responding in animals infused with 20 mg/kg/day of MOR (ED_{50} = 0.04 ± 0.02 mg/kg). When the acute MOR (10 mg/kg, 4 h) → NTX (0.003–3 mg/kg, 0.25 h) curve was redetermined after pump removal, it was not significantly different from either the initial (acute) determination or the Pump MOR 20 curve that preceded it (ED_{50} = 0.03 ± 0.01 mg/kg); data not shown. NTX was approximately 10-fold more potent (ED_{50} = 0.005 ± 0.0002 mg/kg) during infusion with the highest dose of MOR (Pump MOR 40) than during infusion with the lower dose of MOR

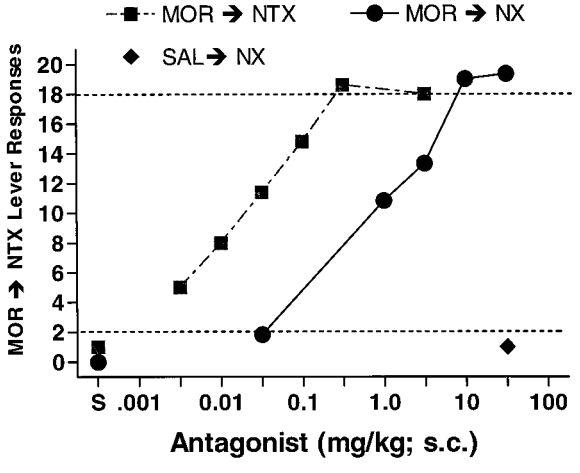

Fig. 5. A comparison of dose-response curves for discriminative stimulus effects of NTX and NX following a 4-h pretreatment with MOR (10 mg/kg). MOR→ NTX curve (gray symbols and dashed line) is reproduced from Fig. 1. Under these conditions, NX (ED_{50} = 0.35 mg/kg) was significantly (7-fold) less potent than NTX. Other details are as in Fig. 1.

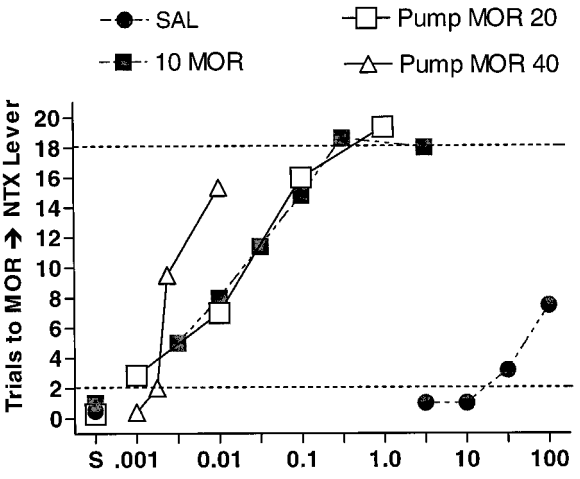

Fig. 6. A comparison of discriminative stimulus effects produced by SAL (S) or NTX (0.001–100 mg/kg) following either acute pretreatment with SAL or MOR or during chronic infusion of MOR by osmotic minipump (open symbols). Subjects received NTX 0.25 h before discrimination testing and 4 h after an acute MOR injection or after a minimum of 5 days of continuous MOR infusion. SAL and 10 MOR curves (gray symbols and dashed lines) are reproduced from Fig. 1. Highest infused dose of MOR (Pump MOR 40; 40 mg/kg/day) produced a significantly greater increase in stimulus potency of NTX than either lower infused dose (20 mg/kg/day) or acutely administered MOR (10 mg/kg, 4 h). Other details are as in Fig. 1.

TABLE 2
Mean Gellert-Holtzman (G-H) scores

Morphine Pretreatment	Naltrexone	G-H Score	n
	mg/kg	± *SEM*	
Acute, 1.0 mg/kg (4 h)	1.0	1.2 ± 0.3	6
	3.0	3.3 ± 1.9	6
	10	1.2 ± 0.2	6
Acute, 3.0 mg/kg (4 h)	3.0	7.0 ± 1.5	6
	10	12.2 ± 1.5[a]	6
	30	12.5 ± 1.3[a]	6
Acute, 10 mg/kg (4 h)	SAL	2.6 ± 0.3	8
	0.03	0.5 ± 0.2	8
	0.1	7.1 ± 1.5	8
	0.3	11.2 ± 0.4[a]	8
	1.0	11.0 ± 2.4[a]	8
	3.0	12.5 ± 0.9[a]	8
	30	11.8 ± 1.6[a]	8
Pump MOR 20	SAL	2.6 ± 0.3	10
	0.1	26.4 ± 1.9[a,b]	10
	1.0	26.7 ± 2.9[a,b]	10
Pump MOR 40	SAL	3.6 ± 0.4	5
	0.001	12.5 ± 2.4[a]	5
	0.003	10.8 ± 1.0[a]	5
	0.01	26.2 ± 6.2[a,b]	5

[a] Significantly greater than SAL-pretreated control ($p \leq 0.05$).
[b] Significantly greater than largest score (12.5) under acute conditions ($p \leq 0.05$).

(Pump MOR 20; ED_{50} = 0.04 ± 0.02 mg/kg) or after acutely administered MOR (10 mg/kg, 4 h).

After the pumps delivering 20 mg/kg/day of MOR were removed, partial MOR → NTX-appropriate responding occurred, and it peaked (40%) 6 h after pump removal but had become primarily SAL → NTX-like by 48 h (Fig. 7A). In the Pump MOR 40 group, more (50%) MOR → NTX-appropriate responding was seen 6 h postpump. This effect disappeared completely by 72 h postpump.

Physical Withdrawal Signs. As determined by our scoring procedure, no significant physical signs of withdrawal were produced by NTX (≤10 mg/kg) 4 h after an acute injection of SAL or 1 mg/kg of MOR (Table 2). A significant

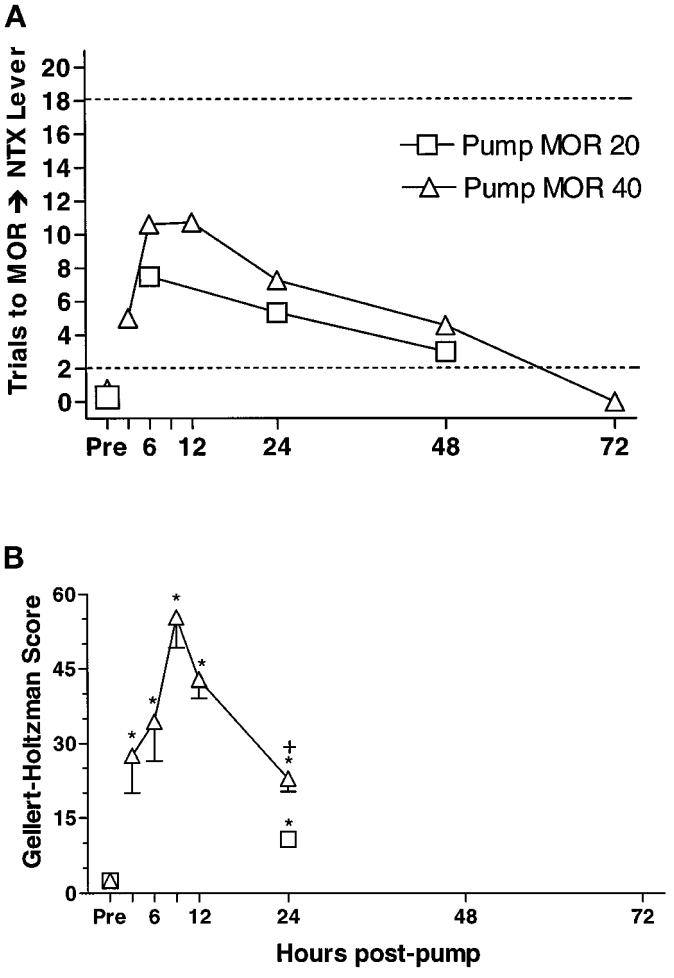

Fig. 7. A time course of discriminative stimulus effects (A) and physical withdrawal signs (B) seen while MOR-filled (Pump MOR 20 or Pump MOR 40) osmotic pumps were in operation and in first 3 to 72 h after their removal. Subjects were not tested in each procedure at all time points. MOR-filled pumps were removed after 7 to 14 days of operation, and a SAL injection preceded each discrimination session or 0.25-h scoring period. A, MOR → NTX-appropriate responding was not seen while pumps were in operation (Pre). After pumps were removed, responding was partially MOR → NTX appropriate and dose dependent and in both experimental groups it peaked 6 to 12 h after pumps were removed. *Significantly greater than Pre ($p \leq .05$). $^{+}$Significantly greater than Pump MOR 20 ($p \leq .05$). Other details are as in Figs. 1 and 6.

number of physical withdrawal signs were produced by NTX (0.03–30 mg/kg) after an acute 4-h pretreatment with 3 or 10 mg/kg of MOR. These effects were dependent on both the MOR and NTX doses, and regardless of MOR or NTX dose, appeared to plateau with a global score of 10 to 12. The specific signs seen included profuse salivation, ptosis, facial fasciculations/teeth chattering, and genital grooming; weight loss (above a 1.6% control loss) and other physical signs, were almost completely absent.

While subjects were receiving MOR via osmotic pump, handling and SAL injection did not produce any significant physical withdrawal signs (Fig. 7, Pre). Compared with acute MOR pretreatment (≤10 mg/kg), lower doses of NTX (0.01–1 mg/kg) produced a significantly greater (200%) increase in global withdrawal scores in subjects receiving MOR via osmotic pump; smaller doses of NTX were required to produce equivalent elevations in global scores as the amount of MOR infused increased. In addition to the signs seen after acute MOR pretreatment, NTX induced other physical signs of withdrawal, such as weight loss (2.5%), abdominal constriction, and abnormal posture.

In the Pump MOR 40 group, 3 to 9 h following osmotic pump removal there was a progressive increase in the number of physical withdrawal signs (Fig. 7B). These signs peaked 9 h after pump removal, at approximately 200% of the peak NTX-induced value seen during pump operation, or 500% of the peak NTX-induced values that followed acute MOR (10 mg/kg) administration. This dramatic increase in global scores was accounted for, in part, by the appearance of previously unseen signs, such as irritability, wet-dog shakes, and swallowing movements, and a dramatic increase in the occurrence of abdominal contractions. Escape attempts were also seen during this period. These signs declined significantly by 12 h, and, by 24 h, the only time at which the Pump MOR 20 group was also scored, physical signs were only 36% of their peak global score of 55. This 24-h value was significantly above that seen in the Pump MOR 20 subjects at the same time point and, although some minor checked signs were seen, primarily reflecting continued irritability on handling, abdominal contraction, and wet-dog shakes. Although profuse salivation was consistently produced by NTX after a single dose of MOR (10 mg/kg), it was not observed in subjects given NTX during osmotic pump operation or after the MOR-filled osmotic pumps were removed.

Discussion

In the current two-choice drug discrimination training procedure, rats were able to discriminate between daily doses of NTX (0.3 mg/kg) that were either preceded by a single dose of MOR or the alternative (SAL). Up to 100 mg/kg of NTX alone produced relatively little MOR → NTX-appropriate responding. However, when MOR preceded NTX, stimulus control of behavior was an orderly function of both the MOR and NTX doses and of the time between those doses. During chronic MOR treatment, NTX also produced full MOR → NTX-like stimulus effects, with its stimulus potency being dependent on the concentration of the MOR infusion. Thus, the discriminative stimulus effects of acute MOR pretreatment followed by NTX appear to be qualitatively similar to the discriminative effects of NTX in rats made physically dependent by chronic treatment with MOR.

In rats responding on an FR10 schedule of food reinforcement, stimulus control of behavior can be established (versus SAL) by a single MOR pretreatment (40 mg/kg, 8 h) that is followed by a lower dose of NX (1.25 mg/kg; Miksic et al., 1981) than that used in the present study. In this case, the discriminative effects of NX are not evident 1 h after MOR pretreatment but peak at the training interval (8 h), and then disappear progressively over the next 24 to 96 h. The stimulus effects that followed acute MOR treatment in the present study appeared earlier, also at the training interval (3–4 h), and were only half-maximal by 8 h. In this respect, they corresponded with the previously reported time course of MOR-induced sensitization to antagonist-induced disruption of food-reinforced operant responding (Young, 1986). These results suggest that training parameters are important determinants of the time course of stimulus generalization. Consistent with the studies reviewed above, NX shared full stimulus properties with NTX in MOR-pretreated rats.

The 7-fold potency difference between NTX and NX in this study is virtually identical with their potency difference in producing NTX-appropriate responding in MOR-treated rhesus monkeys (France et al., 1990).

Although both MOR and NTX can easily be discriminated from SAL and each other (France and Woods, 1985), animals cannot readily be trained to discriminate (from SAL) a dose of NTX that is coadministered with MOR (Jarbe et al., 1979). Therefore, under the proper temporal contingencies, NTX can competitively block the development of MOR-like stimulus effects by an opioid-dependent mechanism. Consistent with this finding, in the present study NTX given shortly (0.25 h) before MOR completely blocked the development of MOR → NTX-like stimulus effects. Because later MOR → NTX-appropriate responding was completely attenuated by this prior NTX treatment, it is clear that MOR must occupy opioid receptors for a period of time before NTX is given to produce the MOR → NTX stimulus.

In spite of the fact that MOR pretreatment is a prerequisite for NTX-induced stimulus effects, the mere presence or absence of MOR during testing is not the primary determinant of MOR → NTX-appropriate responding. When SAL was given 4 h after the training dose of MOR alone or during MOR infusion via osmotic pump, no MOR → NTX-appropriate responding occurred. Therefore, the discrimination was not simply based on the presence versus absence of MOR (agonist)-like effects at the time of testing. NTX pretreatment blocked the MOR → NTX discrimination, also ruling out the possibility that MOR → NTX-appropriate responding was based on a nonopioid effect of MOR that was simply "unmasked" by the administration of an opioid antagonist. Rather, it indicates the discrimination was dependent upon MOR occupying opioid receptors, presumably μ, for a finite period of time.

It is possible that acute MOR pretreatment simply enhances some opioid-specific stimulus effect of a lower (0.3 mg/kg) NTX dose, effectively producing a high-dose stimulus. However, because doses of NTX (≥100 mg/kg) that were 2000-fold higher than the NTX ED_{50} after MOR pretreatment produced less than 50% MOR → NTX-appropriate responding, this explanation appears unlikely. Similarly, a lower dose of NTX (0.3 mg/kg) preceded by SAL was the alternative choice during training, and it clearly did not share significant MOR → NTX-like stimulus effects.

Repeated opioid antagonist administration sometimes results in progressive sensitization to the behavioral effects of the antagonist (Goldberg et al., 1981; Dykstra, 1983; Adams and Holtzman, 1990; Schindler et al., 1993). Therefore, NTX might become a more potent stimulus, either alone or after MOR, over the course of a lengthy study such as the present one. However, there was little change in the effects of NTX in MOR-pretreated rats over the course of this year-long study (Table 1, first and second determinations). Moreover, the continued efficacy of SAL → NTX as an alternative training stimulus argues against any significant increase in the stimulus potency of NTX alone over the course of the experiments.

Previous drug discrimination studies have yielded generalization profiles for opioid antagonists that were dependent on previous MOR exposure, and it has been suggested that compared with antagonists alone, a novel and presumably opioid-mediated stimulus state results when opioid antagonists are given following MOR (Frey and Winter, 1978; France and Woods, 1985). For example, naive animals can easily discriminate among MOR (0.1–10 mg/kg), SAL, and NTX (10–100 mg/kg; France and Woods, 1985). However, at least in naive pigeons, opioid antagonists such as nalorphine, diprenorphine, or cyclazocine do not generalize to the interoceptive cue produced by a high training dose of NX or NTX, suggesting that the interoceptive effects of high doses of NTX or NX are not opioid specific (Valentino et al., 1983). In contrast, rats that are maintained chronically on MOR can be trained to discriminate SAL from as little as 0.1 mg/kg of NTX, a cue that generalizes dose dependently to other opioid antagonists (Gellert and Holtzman, 1979; Holtzman, 1985).

The present data add further support to the theory that acute MOR pretreatment does not simply enhance the existing stimulus effects of NTX but instead induces a state of physical dependence that is unmasked by an antagonist that also produces attendant interoceptive stimuli (Meyer and Sparber, 1977). In humans, physical dependence is assessed by the emergence of a withdrawal syndrome upon removal of chronic MOR treatment or by administration of an opioid antagonist after chronic or acute (single-dose) MOR treatment (Jaffe, 1990). There are qualitative similarities in the negative interoceptive states associated with abrupt/spontaneous withdrawal of chronic MOR treatment or with antagonist administration in acutely MOR-treated subjects (Bickel et al., 1988; Kanof et al., 1992). In this study, NTX produced signs of physical opioid withdrawal, in conjunction with full MOR → NTX-like stimulus effects after acute or during chronic MOR administration, providing further support for the existence of an acute opioid dependence syndrome with interoceptive and somatic components.

Dissociations between the physical and interoceptive signs of opioid dependence have been reported, and this fact must be considered in interpreting the dependence-predictive relevance of the physical signs reported herein (Higgins and Sellers, 1994). Compared with the NTX-induced signs seen after acute MOR pretreatment (Wei et al., 1973; Schulteis et al., 1994), a larger number of signs (i.e., wet-dog shakes and abdominal contractions) were seen 6 to 12 h following the removal of the MOR-filled osmotic pumps. At these same time points, only partial MOR → NTX-appropriate responding occurred. The dose- and time-dependence of the physical signs accompanying abrupt/spontaneous withdrawal are consistent with previous literature on withdrawal from chronic MOR treatment (Wei et al., 1973). In fact, only when comparing among MOR pretreatment conditions is it evident that full MOR → NTX-appropriate responding, although dependent on the presence of NTX, is seen (Fig. 1, training curve) when physical withdrawal signs are comparatively minimal (Table 2).

The potency of NTX to occasion MOR → NTX-appropriate responding was comparable in rats pretreated with a single dose of MOR (10 mg/kg) and in rats treated with MOR chronically (20 mg/kg/day). The present surprising results are consistent with previous findings that the potency of NTX in producing effects on behaviors maintained by food or i.c. self-stimulation is comparable after acute MOR injection or during chronic MOR infusion (Adams and Holtzman, 1990; Easterling and Holtzman, 1997). The generality of these previous observations is now extended to interoceptive effects. In all of the experiments reported herein, antagonist

injection produced full MOR → NTX-like interoceptive effects in MOR-treated rats. Following either acute or chronic MOR treatment, the similarity in the stimulus potency of NTX (Fig. 6) suggests that acute MOR pretreatment probably results in a relatively high-intensity interoceptive stimulus. The fact that antagonist-precipitated abstinence syndromes are generally more intense and of shorter duration than the spontaneous withdrawal syndrome (Wei et al., 1973) might explain the failure of spontaneous withdrawal to generalize completely with MOR → NTX. Regardless, our results suggest that acute MOR treatment produces changes in the cellular substrates for these antagonist-induced effects that are comparable with those changes produced by chronic MOR treatment.

One possible mechanism that might account for the effects of NTX that follow acute or prolonged MOR treatment is MOR-induced conversion of opioid receptors to a constitutively active state (Cruz et al., 1996). The existence of constitutively active opioid receptors, which couple to G proteins in the absence of an agonist, has been proposed (Bilsky et al., 1996). In a system with a large number of constitutively active opioid receptors (a dependent rat) an antagonist might have negative intrinsic efficacy (Wang et al., 1994). In such a system, an antagonist, by rapidly binding constitutively active receptors, might produce quantitatively more intense or qualitatively different effects than those that would be expected to follow the abrupt withdrawal of chronic MOR treatment. Although the model requires experimental validation, it would provide an explanation of how low doses of opioid antagonists can have such profound effects on behavior hours after a single dose of MOR.

In humans who are physically dependent upon a MOR-like drug, the desire to prevent the emergence of aversive withdrawal symptomatology is an important factor in the perpetuation of drug self-administration (Jaffe, 1990). Animal models are often criticized for failing to provide a subjective measure of the motivational changes occurring during opioid withdrawal that have been documented in humans. In the procedure described herein, changes in MOR → NTX-appropriate responding were noted when subjects were undergoing NTX-precipitated withdrawal from acute or chronic MOR and when MOR-filled pumps were abruptly removed. Therefore, this drug discrimination procedure should afford a valuable animal model for studying behavioral, and ultimately, cellular events that reflect the early drug-receptor interactions underlying the development of physical dependence upon MOR-like drugs.

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Send reprint requests to: Dr. Keith W. Easterling, Emory University, Department of Pharmacology, Atlanta, GA 30322. E-mail: keaster@emory.edu