JPET Fast Forward. Published on November 25, 2002 as DOI:10.1124/jpet.102.044875

JPET/2002/44875

Discrimination of a Single Dose of Morphine Followed by Naltrexone: Substitution of Other Agonists for Morphine and Other Antagonists for Naltrexone in a Rat Model of Acute Dependence

Stephen G. Holtzman

Department of Pharmacology

Emory University School of Medicine

Atlanta, GA 30322

Running title: Discriminative Effects of Acute Morphine and Naltrexone

Address correspondence to: Stephen G. Holtzman, Ph.D.

Department of Pharmacology

Emory University School of Medicine

1510 Clifton Road

Atlanta, GA 30322

Tel: 404-727-5990

Fax: 404-727-0365

E-mail: sholtzm@emory.edu

Text	30 pages
Figures	7
References	40
Abstract	248 words
Introduction	711 words (including references)
Discussion	1523 words (including references)

ABBREVIATIONS: ANOVA, analysis of variance, cAMP; cyclic adenosine monophosphate; C.L., confidence limits; ED_{50}, effective dose-50 percent

Recommended section assignment: Behavioral Pharmacology

Abstract

Rats were trained to discriminate 4-hr pretreatment with 10 mg/kg morphine and 15-min pretreatment with 0.3 mg/kg naltrexone (morphine→naltrexone) from pretreatment with saline and 0.3 mg/kg naltrexone (saline→naltrexone). The discrimination appears to derive from upon interoceptive stimuli from antagonist-precipitated withdrawal from acute morphine dependence. The purpose of this study was to extend pharmacological characterization of the discrimination by testing opioid agonists other than morphine and antagonists other than naltrexone. Of seven *mu*-opioid agonists tested in place of morphine, only two (heroin, levorphanol) substituted completely for it; trials completed on the morphine→naltrexone-appropriate lever increased as a function of agonist and naltrexone dose. Agonists with intrinsic efficacy higher (etorphine, fentanyl, methadone) or lower (buprenorphine, meperidine) than that of morphine substituted only partially. However, when naltrexone was administered during continuous infusion of fentanyl or methadone via SC osmotic pump, rats responded as if they had received morphine→naltrexone; discriminative responding correlated with global withdrawal scores. Rats responded primarily on the saline→naltrexone-appropriate lever when naltrexone was administered after pretreatment with dextrorphan, the *dextro*rotatory isomer of levorphanol, or *kappa*-opioid agonists (U69,593, spiradoline). Antagonists with no intrinsic efficacy at *mu*-opioid receptors (naloxone, diprenorphine) substituted completely for naltrexone, whereas those with some efficacy (nalorphine, levellorphan) substituted partially. Thus, morphine→naltrexone-like stimulus control of behavior by drugs administered acutely requires pretreatment with certain *mu*-opioid agonists and a

pure antagonist, is independent of agonist efficacy, and is stereoselective. Interoceptive stimuli from naltrexone-precipitated opioid withdrawal are more similar across morphine-like agonists during chronic dependence than they are during acute dependence.

A hallmark of physical dependence upon morphine-like opioids is an increase in sensitivity to effects of opioid antagonists. For example, the ED_{50} of the opioid antagonist naloxone to elicit withdrawal jumping in mice that had received a SC morphine pellet decreased steadily from 12 mg/kg immediately after pellet implantation to 0.045 mg/kg 72 hr after implantation (Way et al., 1969).

Sensitivity to opioid antagonists increases not only during prolonged exposure to an opioid agonist but also after pretreatment with only a single dose of morphine or a related drug. The potency of naloxone and other opioid antagonists (*e.g.*, naltrexone) to decrease the rate of schedule-controlled responding maintained by food or brain stimulation increased by as much as 2-3 orders of magnitude in rats that had been pretreated 4 hr earlier with a single dose of a morphine-like drug (Young, 1986;Adams and Holtzman, 1990;Easterling and Holtzman, 1997). The sensitizing effect of opioid agonists was reversible, stereoselective for the *levo*rotatory isomer, and mediated centrally, primarily by *mu*-opioid receptors (Adams and Holtzman, 1990;Adams and Holtzman, 1991;Easterling and Holtzman, 1997) These and other examples of acute agonist-induced sensitization to effects of opioid antagonists have been viewed as evidence of a state of acute opioid dependence (Meyer and Sparber, 1976;Eisenberg and Sparber, 1979;White and Holtzman, 2001). The results of clinical studies support this conclusion. Naloxone induced many of the physiological manifestations and subjective symptoms of withdrawal when it was administered several hours after a single dose of a morphine-like drug to otherwise drug-free volunteers (Bickel et al., 1987;Wright et al., 1991;Greenwald et al., 1996).

Drug discrimination affords an approach for studying in animals drug effects that have relevancy to the subjective effects of the drug in humans (Holtzman, 1990). We trained rats to discriminate 4-hr pretreatment with 10 mg/kg morphine and 15-min pretreatment with 0.3 mg/kg naltrexone (morphine→naltrexone) from pretreatment with saline and 0.3 mg/kg naltrexone (saline→naltrexone; (Easterling and Holtzman, 1999). The discriminative effects of morphine→naltrexone were an orderly function of the dose of morphine, the dose of naltrexone, and the morphine pretreatment interval. They were maximal when morphine was administered 3 or 4 hr before a session, half maximal when morphine was administered 8 hr before a session, and virtually absent when morphine was administered only 30 min before a session. When training was suspended and a continuous infusion of morphine was administered via a SC osmotic pump (20 mg/kg/day), naltrexone engendered dose-dependent increases in morphine→naltrexone-appropriate responding and substituted completely for morphine→naltrexone. These results suggested that stimulus control of behavior by morphine→naltrexone derived from interoceptive stimuli associated with antagonist-precipitated withdrawal from acute physical dependence upon morphine (Easterling and Holtzman, 1999).

The purpose of this study was to extend pharmacological characterization of the morphine→naltrexone discrimination by testing opioid agonists other than morphine and antagonists other than naltrexone. The morphine-like agonists examined represented a range of intrinsic efficacies, from relatively low (*e.g.*, buprenorphine, meperidine) to relatively high (*e.g.*, etorphine, fentanyl; (Emmerson et al., 1996;Selley et al., 1998), in order to assess the contribution of intrinsic efficacy to stimulus control of behavior. Two

mu-opioid agonists that did not substitute completely for morphine when administered acutely also were administered continuously via SC osmotic pump while discrimination training was suspended; stimulus control of behavior was assessed following the administration of naltrexone. Insofar as possible, the *mu*-opioid agonists were tested at doses estimated to be equi-effective with 10 mg/kg morphine, based upon discriminative effects in rats trained to discriminate morphine (Shannon and Holtzman, 1976;Young et al., 1991;Walker et al., 1994;Holtzman, 1997).

Acute administration of *kappa*-opioid agonists induces much less sensitization to response-rate-decreasing effects of naltrexone than does acute administration of *mu*-opioid agonists (Adams and Holtzman, 1990;Easterling and Holtzman, 1997). In this study, a relatively high dose of each of two *kappa*-opioid agonists, spiradoline and U69,593, was tested in place of morphine.

Naloxone substituted fully for naltrexone in rats that were pretreated with the combination of a single dose of morphine (Easterling and Holtzman, 1999). We replicated these results and extended observations to three more opioid antagonists: diprenorphine, which has negligible intrinsic efficacy at *mu*-opioid receptors (Lee et al., 1999), and nalorphine and levallorphan, drugs that are weak partial *mu* agonists (Emmerson et al., 1996;Selley et al., 1998).

Methods

Subjects

Adult male rats of Sprague-Dawley descent were obtained from Charles River Laboratories (Wilmington, MA), and were housed in pairs in a colony room that was maintained on a 12-h light/dark cycle. Food and water always were available in the home cage. Twenty-five rats met the criterion for acquisition of the discrimination (see below) and were used in the study. Three had undergone approximately 3 months of discrimination training with 5.6 mg/kg morphine and 0.3 mg/kg naltrexone versus saline and 0.3 mg/kg naltrexone; the other 22 were experimentally naïve. Experiments were conducted according to a protocol approved by the Institutional Animal Care and Use Committee of Emory University and were in keeping with the 1996 Guide for the Care and Use of Laboratory Animals (National Academy of Sciences).

Training Procedure

Discrimination performance was maintained by a two-choice discrete-trial avoidance/escape procedure (Easterling and Holtzman, 1999). During the acquisition phase, daily training sessions were conducted Monday through Friday. Either saline or morphine, 10 mg/kg, was injected SC 4 hr before a session on alternate days; naltrexone, 0.3 mg/kg, was injected SC 15 min before every training session. At the end of the pretreatment interval the rats were placed in a testing chamber that was inside of a ventilated cubicle that was light-proof and sound-attenuating. A single lever was mounted in one wall of the chamber and two “choice” levers were mounted in the

opposite wall. The choice levers were separated by a Plexiglas partition that extended 5.0 cm into the chamber and ran from the grid floor to the ceiling. Illumination of the house light and onset of a white noise signaled the start of a trial. Five sec later, a constant current of 1.0-1.5 mA was distributed to the grid floor of the chamber in pulses of 1.0 s every 3.0 s. A rat could end the trial at any time by completing a two-response chain: pressing the single lever in one wall of the chamber, then pressing the choice lever that was correct for the substance injected before the session (*i.e.*, morphine→naltrexone or saline→naltrexone). For half of the animals the right choice lever was correct on days that morphine was injected and the left choice lever was correct on days that saline was injected; lever assignments were reversed for the other half of the rats. A response on the first lever turned off the white noise and a response on the correct choice lever extinguished the house light and ended the trial. The next trial began 50 s later. In the absence of a correct response sequence, a trial was terminated after 30 s. Each session consisted of 21 trials; the first "warm-up" trial was excluded from data analyses. A trial was scored as correct if the rat pressed the first lever and then pressed the correct choice lever; a trial was scored as incorrect if the rat pressed the first lever and then pressed the incorrect choice lever before pressing the correct choice lever.

When a rat completed correctly at least 18 of 20 trials (*i.e.*, 90%) in four consecutive training sessions (two with 4-hr saline pretreatment and two with morphine pretreatment), the next two sessions were conducted as tests: one with saline pretreatment and the other with morphine pretreatment. Test sessions were similar to training sessions, with the important exception that a trial ended after the rat pressed the

first lever and then pressed either of the two choice levers. A rat met the criterion for acquisition of the discrimination if it completed at least 18 trials in each test session on the injection-appropriate choice lever. Thereafter, training sessions were conducted on three days of each week, with morphine and saline pretreatment alternating. Test sessions were held on the other two days, usually Tuesday and Friday, provided the rat had completed correctly at least 18 trials in the two most recent training sessions. If it did not, the test session was cancelled and training sessions were held until the rat met the criterion of completing correctly 90% of the trials in two consecutive training sessions.

Stimulus-Generalization Tests

All rats were tested first with a range of naltrexone doses after pretreatment with 10 mg/kg morphine. Other drugs were then tested in an unsystematic order. In some cases the drugs were administered in place of morphine, 4 hr before the session; in other cases they were administered in place of naltrexone, 15 min before the session. In most of the drug series, three doses of naltrexone (or other antagonist) and saline were tested in a random sequence and then, depending upon the results, additional drug doses were tested. In order to determine if repeated exposure to morphine and other opioid agonists sensitized the rats to naltrexone, a large subgroup (16) of the rats was tested with naltrexone after saline pretreatment at various times during the study. In each animal, doses of naltrexone (0.3, 3.0, and 30 mg/kg) and saline were tested once in a random sequence over a period of 3-6 months

In two sets of experiments, naltrexone was tested over a range of doses in rats that were receiving either fentanyl (0.25 mg/kg/day) or methadone (10 mg/kg/day) by continuous infusion via a SC osmotic pump (Model 2 ML2, Alza Corp., Palo Alto, CA). Rats were anesthetized with halothane and the pumps were inserted through a small incision (Easterling and Holtzman, 1999). No training sessions were conducted while the pump was in the animal. On day 5 after pump implanationt, the rats were tested 15 min after an injection of saline. Different doses of naltrexone were tested (15-min pretreatment) in ascending order on days 7, 9, 11, and 13. The pump was removed after 14 days, again while the rat was anesthetized with halothane. Six and 24 hr later saline was injected and a test session was held 15 min after each injection. Normal training and testing resumed one week after the pump had been removed.

Opioid Withdrawal Syndrome

Physical signs of opioid withdrawal were assessed with the Gellert-Holtzman Global Withdrawal Rating Scale (Gellert and Holtzman, 1978). Beginning 5 min after an injection of either saline or naltrexone, individual rats were observed for 10 min while they were in a polycarbonate holding cage. Signs marked as either present or absent (*i.e.*, "checked" signs) were: diarrhea, facial fasciculation or teeth chatter, swallowing movements, salivation, chromodacryorrhea, ptosis, abnormal posture, erection or ejaculation, and irritability to handling. "Graded" signs were number of escape attempts, "wet-dog" shakes, and abdominal constrictions. Each rat was weighed just before the

injection and again at the conclusion of the session, 40-45 min later, in order to determine loss of body weight.

Data Analysis

Discrimination data are presented as the average number of trials completed on the choice lever appropriate for the morphine→naltrexone condition; the remaining trials of the session were completed on the choice lever appropriate for saline→naltrexone. Means were compared with Friedman's nonparametric ANOVA for repeated measures. If the data were statistically reliable, the Wilcoxin matched-pairs signed-ranks test was used to compare two means. Both tests were corrected for ties. During test sessions that followed pretreatment with 10 mg/kg morphine and 0.3 mg/kg, rats completed an average of at least 18 trials on the choice lever appropriate for morphine→naltrexone. Therefore, naltrexone or the combination of an agonist other than morphine and an antagonist was considered to have substituted completely for morphine→naltrexone if the group of rats completed an average of at least 18 trials on that choice lever.

The dose of naltrexone or other antagonist that would occasion selection of the morphine→naltrexone-appropriate choice lever in 10 trials (ED_{50}) was estimated for individual rats by linear regression of the ascending portion of the stimulus-generalization curve, using $logarithm_{10}$ dose and at least three points. In cases where only two points defined the ascending portion of the curve, the ED_{50} was estimated by simple interpolation. The ED_{50}s were averaged to obtain a group mean and 95% confidence limits, and were compared by ANOVA or Student's t test, as appropriate. In

cases where all of the animals in a drug series did not complete at least 10 trials on the morphine→naltrexone-appropriate lever, the ED_{50} was derived from the group means instead of from individual animals and is shown without confidence limits.

The time from the start of a trial until the first lever was pressed (observing-response latency) was summed over the 20 trials of the session for each rat. The data for individual rats were averaged and the means were compared by ANOVA for repeated measures.

A global withdrawal score was calculated for each rat by assigning a weighting factor to the various physical signs of withdrawal (Gellert and Holtzman, 1978) and adding one point for each 1 % decrease in body weight. Scores of individual rats were averaged and means were compared either with ANOVA for repeated measures, followed by Dunnett's t test, or by Student's paired t test, as appropriate.

P values of ≤ 0.05 were considered to be statistically significant.

Drugs

The drugs used, their salt form, and their source were: naltrexone hydrochloride, naloxone hydrochloride, and dextrorphan tartrate (RBI, Natick, MA); buprenorphine, diprenorphine, etorphine, fentanyl, heroin, meperidine, all as hydrochlorides, and (5-α, 7-α, 8-β)-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzeneacetamide (U69,593; National Institute on Drug Abuse, Bethesda, MD); morphine sulfate (Penick Co., Nutley, NJ); levorphanol tartrate, levallorphan tartrate (Roche Laboratories, Nutley, NJ); methadone hydrochloride (Mallinkrodt, St. Louis, MO); nalorphine hydrochloride

(Merck & Co., West Point, PA); spiradoline methane sulfonate (Pharmacia, Kalamazoo, MI). All drugs were dissolved in either physiological (0.9 %) saline or distilled water, except U69,593, which was dissolved in 3 parts 8.5 % lactic acid and 2 parts 1.0 N sodium hydroxide. Except when they were administered via SC osmotic pumps, the drugs were injected SC in a volume of 1.0 ml (2.0 ml for high meperidine doses) per kg of body weight. Doses represent the free-base form of the drug.

Results

Baseline

The 22 rats that were experimentally naïve at the beginning of the study met the criteria for acquisition of the discrimination in an average of 53 sessions (range: 23-84). When 10 mg/kg morphine was administered 4 hr before a test session and saline was administered 15 min before, the rats responded almost exclusively on the choice lever appropriate for saline→naltrexone. However, the combination of morphine 4 hr before a session and naltrexone (0.01-1.0 mg/kg) occasioned dose-dependent responding on the morphine→naltrexone-appropriate lever that peaked at the 0.3 mg/kg training dose of naltrexone (Fig. 1). The highest dose of naltrexone, 1.0 mg/kg, resulted in slightly but significantly fewer trials being completed on the morphine→naltrexone-appropriate lever than 0.3 mg/kg did (16.8 vs. 19.2 trials, $p < 0.001$). The ED_{50} of naltrexone, derived from the ascending portion of the stimulus-generalization curve, was 0.051 (0.034 – 0.077) mg/kg. In contrast, 4-hr pretreatment with saline and 15-min pretreatment with naltrexone (0.3-30 mg/kg) occasioned relatively little responding on the morphine→naltrexone-appropriate lever, with a maximum of 2.9 trials after 30 mg/kg, 100 times the training dose (Fig. 1). Nevertheless, there was a significant effect of dose when responding after saline→saline was included in the analysis ($Fr = 14.61$, $p = 0.002$).

The latency from trial onset to response on the first lever over the 20 trials of a session was 180 ± 10 sec in test sessions held 4 hr after 10 mg/kg morphine and 15 min after saline and 148 ± 4 sec in sessions held after two injections of saline ($t_{[39]} = 2.47$; $p =$

0.018). Cumulative response latencies were unaffected by either 0.01-1.0 mg/kg naltrexone administered after 10 mg/kg morphine ($F_{[6,24]}$ = 1.00, p = 0.430) or by 0.3-30 mg/kg naltrexone administered after saline ($F_{[3,15]}$ = 1.27, p = 0.859). None of the other combinations of drugs examined subsequently had a statistically reliable affect on cumulative response latencies; therefore, no further response latency data are presented.

Other agonists administered acutely

Only two of seven morphine-like agonists substituted completely for morphine when administered in a single dose 4 hr before a session. When combined with 0.3 mg/kg naltrexone administered 15 min before a session, 3.0 mg/kg heroin occasioned completion of an average of more than 18 trials on the choice lever appropriate for morphine→naltrexone (Fig. 2, top). Like the morphine→naltrexone curve, the curve for 3.0 mg/kg heroin and naltrexone was biphasic: doses of naltrexone higher than 0.3 mg/kg resulted in progressively fewer trials being completed on the morphine→naltrexone-appropriate lever than did 0.3 mg/kg. A lower dose of heroin (1.0 mg/kg) followed by naltrexone occasioned completion of a maximum of 12.5 trials on the morphine→naltrexone-appropriate lever, this at 3.0 mg/kg naltrexone (Fig. 2, top). The ED_{50} of naltrexone after 3.0 and 1.0 mg/kg heroin was 0.025 (0.008 – 0.079) and 0.286 mg/kg, respectively.

Levorphanol (0.3-3.0 mg/kg) also substituted for morphine dose-dependently (Fig. 2, bottom). The animals completed an average of 18.3 trials on the morphine→naltrexone-appropriate lever after 3.0 mg/kg levorphanol and 3.0 mg/kg

naltrexone. The maximum number of trials completed on the morphine→naltrexone-appropriate lever decreased to 14.8 after 1.0 mg/kg levorphanol and 3.0 mg/kg naltrexone, and to 10.7 trials after 0.3 mg/kg levorphanol and 0.3 mg/kg naltrexone. The ED_{50} of naltrexone after 3.0, 1.0, and 0.3 mg/kg levorphanol was, respectively, 0.148 (0.085 – 0.256), 0.193, and 0.243 mg/kg. The former ED_{50} was significantly higher than the ED_{50} of naltrexone after pretreatment with either 10 mg/kg morphine ($p < 0.05$) or 3.0 mg/kg heroin ($p < 0.01$; $F_{[2,34]} = 5.53$, $p = 0.008$). However, combinations of 4-hr pretreatment with dextrorphan, the non-opioid stereoisomer of levorphanol, and 15-min pretreatment with doses of naltrexone as low as 0.03 mg/kg and as high as 30 mg/kg occasioned responding only on the lever appropriate for saline→naltrexone (Fig. 2, bottom).

Four-hr pretreatment with fentanyl (0.056 or 0.1 mg/kg) and 15-min pretreatment with naltrexone occasioned a dose-dependent increase in responding on the morphine→naltrexone-appropriate lever that fell short of the level of responding occasioned by the two training drugs: a maximum of 9.2 trials after 0.056 mg/kg fentanyl and 3.0 mg/kg naltrexone and 15.3 trials after 0.1 mg/kg fentanyl and 3.0 mg/kg naltrexone (Fig. 3, top). Shortening the pretreatment time for 0.1 mg/kg fentanyl to either 3 or 2 hr while holding the naltrexone pretreatment time at 15 min did not result in completion of more trials on the morphine→naltrexone-appropriate lever than the 4-hr pretreatment did.

Neither 3- nor 4-hr pretreatment with 3.0 mg/kg methadone and 15-min pretreatment with naltrexone substituted completely for morphine→naltrexone, although

4-hr pretreatment resulted in an average of 16.7 trials to the morphine→naltrexone-appropriate lever (Fig. 3, bottom).

Another set of experiments was performed to determine if the failure of methadone and naltrexone pretreatments to substitute completely for morphine→naltrexone was due to a peculiarity of the antagonist. Three mg/kg methadone was administered 4 hr before a session and naloxone (0.003-30 mg/kg) was given as a 15-min pretreatment in place of naltrexone. The stimulus-generalization curve for morphine→naloxone was an orderly and biphasic function of the naloxone dose, not unlike the naltrexone curve after 3-hr pretreatment with methadone. The animals completed an average of 0.5, 4.3, 11.8, 11.3, and 11.0 trials on the morphine→naltrexone-appropriate lever after naloxone doses of 0.003, 0.03, 0.3, 3.0, and 30 mg/kg, respectively (data not graphed).

The pairing of 4-hr pretreatment with either 0.01 mg/kg etorphine, 1.0 mg/kg buprenorphine, or 30 mg/kg meperidine with 15-min naltrexone pretreatment resulted in only intermediate levels of responding appropriate for the morphine→naltrexone state (Fig. 4, top). The maximum effect for any of the drug combinations was an average of 10.8 trials to the morphine→naltrexone-appropriate lever after 0.01 mg/kg etorphine and 3.0 mg/kg naltrexone (based upon 0, 7, 10, 13, 16, and 19 trials by the individual rats). The peak effect of meperidine pretreatment occurred at 3.0 mg/kg naltrexone (mean of 9.8 trials, from individual responses of 1, 4, 10, 12, 13, and 19 trials); that of buprenorphine pretreatment occurred at 0.3 mg/kg naltrexone (mean of 6.7 trials, from individual responses of 0, 3, 3, 7, 8, and 19 trials) The main effect of dose was

significant for etorphine (Fr = 14.72, p = 0.012) and meperidine (Fr = 14.16, p = 0.007), and not quite significant for buprenorphine (Fr = 8.65, p = 0.070). Four-hour pretreatment with 0.3 or 3.0 mg/kg buprenorphine and 15-min pretreatment with 0.3 mg/kg naltrexone resulted in an average of 0.6 and 5.2 trials, respectively, being completed on the morphine→naltrexone-appropriate lever (data not graphed).

A high dose of a *kappa*-opioid agonist, 3.0 mg/kg of either spiradoline or U69,593 (4-hr pretreatment), with a broad range of naltrexone doses (15-min pretreatment), occasioned relatively little responding on the morphine→naltrexone-appropriate lever; the most responding occurred with spiradoline and 3.0 mg/kg naltrexone (Fig 4, bottom). However, there was not a significant main effect of spiradoline dose (Fr = 6.31, p = 0.177).

Fentanyl and methadone administered continuously

Saline was injected 15 min before a test session into rats that had been getting a continuous SC infusion of either fentanyl (0.25 mg/kg/day) or methadone (10 mg/kg/day) for 5 days in the absence of training sessions. Almost all of the trials were completed on the lever appropriate for saline→naltrexone (Fig. 5, top). In contrast, naltrexone administered in incremental doses across days 7, 9, 11, and 13 of pump implantation occasioned dose-dependent increases in trials to the morphine→naltrexone-appropriate lever, substituting completely for morphine→naltrexone at either 0.1 (fentanyl) or 0.175 mg/kg (methadone). The respective ED_{50}s for naltrexone were 0.019 (0.011 - 0.033) and

0.059 (0.030 - 0.114) mg/kg, making naltrexone approximately three times more potent in rats with fentanyl pumps than in those with methadone pumps ($t_{[7]} = 3.64$, $p = 0.008$).

There was little responding on the morphine→naltrexone-appropriate lever after the pumps were removed. The group that had received fentanyl completed an average of 8.3 trials on the morphine→naltrexone-appropriate lever in sessions that followed a saline injection (15-min pretreatment) 6 hr post pump, and responded only on the lever appropriate for saline→naltrexone 24 hr post pump (Fig. 5, top). The group that had received methadone completed more than 90 % of the trials on the saline→naltrexone-appropriate lever in both post pump test sessions.

Naltrexone also produced dose-dependent increases in withdrawal scores in animals with either of the two pumps (Fig. 5, bottom). However, the maximum score was almost twice as high in the group with the fentanyl pump as it was in the one with the methadone pump even though the dose of naltrexone was lower in the former group: 27.8 ± 1.5 at 0.1mg/kg naltrexone compared to 15.4 ± 1.0 at 0.56 mg/kg. The size of the withdrawal scores after naltrexone correlated significantly with the number of trials completed on the morphine→naltrexone-appropriate lever: $r = 0.825$, $p = 0.003$ (Fig. 6). Withdrawal scores abated after the pumps were removed. Nevertheless, they remained significantly elevated (relative to those recorded after a saline injection while the pumps were implanted) for both groups at 6 hr post pump and for the group that had received fentanyl at 24 hr post pump (Fig. 5, bottom). Despite those elevated withdrawal scores, the rats responded almost exclusively on the choice lever appropriate for saline→naltrexone at two of the three time points.

For purposes of comparison, withdrawal scores were determined for 20 rats undergoing standard training sessions with either 10 mg/kg morphine and 0.3 mg/kg naltrexone or saline and 0.3 mg/kg naltrexone. The scores averaged 15.1 ± 0.8 when the rats were pretreated with morphine and naltrexone and 8.0 ± 0.7 when they were pretreated with saline and naltrexone ($t_{[19]} = 6.40$, $p << 0.001$).

Other antagonists

The combination of 4-hr pretreatment with 10 mg/kg morphine and 15-min pretreatment with either naloxone or diprenorphine occasioned dose-dependent increases in trials completed on the morphine→naltrexone-appropriate lever and substituted completely for the stimulus effects of morphine→naltrexone (Fig. 7). Naloxone was twice as potent as diprenorphine [ED_{50}s: 0.059 (0.014 - 0.245) and 0.141 (0.086 - 0.231) mg/kg, respectively] but the difference between the drugs was not statistically reliable ($t_{[10]} = 1.49$, $p = 0.168$).

When combined with morphine pretreatment, levallorphan and nalorphine also occasioned orderly increases in morphine→naltrexone-appropriate responses that peaked at an average of just over 14 trials. The stimulus generalization curves were biphasic, so that the highest dose of each drug occasioned fewer responses on the morphine→naltrexone-appropriate lever than the next-to-highest dose did (Fig. 7). ED_{50}s derived from group data were 0.57 (levallorphan) and 2.03 mg/kg (nalorphine).

As a control, the highest dose of each antagonist was tested with 4-hr saline pretreatment. The rats completed an average of not more than 0.5 trials on the

morphine→naltrexone-appropriate lever after 10 mg/kg naloxone, 10 mg/kg diprenorphine, or 3.0 mg/kg levallorphan, and 3.1 trials after 30 mg/kg nalorphine (Fig. 7).

Discussion

Of the seven *mu*-opioid agonists tested, only two, heroin and levorphanol, substituted completely for acutely-administered morphine in occasioning morphine→naltrexone-appropriate responding. The maximum number of trials completed on the morphine→naltrexone-appropriate lever and the ED_{50} of naltrexone were graded functions of the pretreatment dose of the agonist. The reasons that the other *mu*-opioid agonists did not substitute for morphine completely are probably multiple. In some cases it was not possible to test a dose that was equi-effective with 10 mg/kg morphine. Meperidine is one-tenth as potent as morphine in producing morphine-like discriminative effects (Shannon and Holtzman, 1976), implying that 100 mg/kg would be needed for complete substitution. However, a dose that high could not be tested safely. On the other hand, buprenorphine, fentanyl, and etorphine are approximately 70-100, 80-100 and 2,000-3000 times more potent than morphine (Young et al., 1991;Walker et al., 1994;Holtzman, 1997), respectively. They were tested at doses that are at least equivalent to 10 mg/kg morphine, and well above the ED_{50} for suppressing food-maintained responding (Young et al., 1991;Walker et al., 1994), but did not substitute completely. Some of the agonists have durations of action shorter than that of morphine. However, shortening the pretreatment interval to 3 hr for methadone and to 3 or 2 hr for fentanyl did not increase the number of trials completed on the morphine→naltrexone-appropriate lever compared to 4-hr pretreatment.

Naloxone precipitated signs and symptoms of opioid withdrawal in volunteers

pretreated with a single dose of fentanyl or methadone (Wright et al., 1991;Greenwald et al., 1996). When those drugs did not substitute for morphine completely after acute admnistration, they were administered by continuous SC infusion at a daily dose approximately equivalent to the morphine dose infused in a previous study (Easterling and Holtzman, 1999). Under these conditions, naltrexone occasioned discriminative effects comparable to those engendered by acute pretreatment with the combination of morphine and naltrexone. Furthermore, the ED_{50} of naltrexone in the rats receiving infusions of fentanyl (0.02 mg/kg) or methadone (0.06 mg/kg) was similar to the one determined in rats that had pumps releasing morphine (0.04 mg/kg; (Easterling and Holtzman, 1999). Thus, the discriminative effects associated with naltrexone-precipitated withdrawal from *chronic* fentanyl or methadone administration were equivalent to those engendered by acute pretreatment with morphine→naltrexone, whereas discriminative effects engendered by *acute* pretreatment with either fentanyl or methadone were not.

The specific signs of antagonist-precipitated withdrawal from morphine in rats can change qualitatively as well as quantitatively as a function of degree of physical dependence, dose of antagonist, and time after antagonist administration (Blasig et al., 1973). In this study, rats were trained with interoceptive cues that occur 4 hr after 10 mg/kg morphine and 15 min after 0.3 mg/kg naltrexone. Apparently, this same cluster of interoceptive cues does not occur when naltrexone is administered after acute pretreatment with some *mu*-opioid agonists or at doses of naltrexone different from the training dose. The latter point would account for the biphasic nature of most of the

stimulus-generalization curves.

That rats receiving a continuous infusion of either fentanyl of methadone generalized completely to naltrexone provides further evidence that stimulus control of behavior by morphine→naltrexone derives from interoceptive stimuli associated with antagonist-precipitated withdrawal from acute morphine dependence. In addition, the number of trials completed on the morphine→naltrexone-appropriate lever correlated significantly with the global withdrawal score of somatic signs. However, the concordance of the two variables was far from perfect quantitatively. "Behavioral/motivational" signs of morphine withdrawal, such as decreased food-maintained operant responding and conditioned place aversion, appear at antagonist doses lower than those that precipitate many of the somatic signs of withdrawal, such as weight loss and diarrhea (Schulteis et al., 1994;Schulteis et al., 1999). Moreover, some somatic signs of withdrawal are mediated in the periphery (Maldonado et al., 1992) and can be blocked by drugs that do not affect behavioral/motivational signs of withdrawal (Shippenberg et al., 2000), suggesting the two classes of withdrawal signs are mediated by separate neural substrates. Therefore, although somatic withdrawal signs provide a quantitative index of severity of physical dependence, they are not necessarily predictive of nor do they account for behavioral/motivational manifestations of withdrawal, such as stimulus control of behavior by morphine→naltrexone.

The fact that rats pretreated with saline and naltrexone at doses as high as 30 mg/kg responded almost exclusively on the lever appropriate for saline→naltrexone in test sessions scattered throughout the study supports two conclusions drawn previously

(Easterling and Holtzman, 1999). First, the state of acute physical dependence upon morphine is reversible; there were no detectable residual effects of the morphine training dose on days when morphine was not administered. Acute physical dependence in humans also is reversible, with a duration that reflects the half-life of the opioid agonist (Eissenberg et al., 1996;Greenwald et al., 1996). Second, morphine did not merely potentiate an existing effect of naltrexone; rather, morphine→naltrexone gives rise to a unique set of interoceptive stimuli that are absent when naltrexone is administered without morphine pretreatment.

The cellular events that underlie acute physical dependence upon opioid drugs are not known. Agonists that lack high efficacy (*e.g.*, morphine) induce up-regulation of adenylyl cyclase and other components of the cAMP pathway while they activate *mu*-opioid receptors (Sharma et al., 1975;Finn and Whistler, 2001). This up-regulated second-messenger system appears to contribute to the withdrawal syndrome that emerges when morphine is displaced from the receptor by an antagonist (Nestler and Aghajanian, 1997). High efficacy agonists (*e.g.*, etorphine), on the other hand, cause desensitization and endocytosis of *mu*-opioid receptors, which prevents up-regulation of adenylyl cyclase (Sternini et al., 1996;Finn and Whistler, 2001). Therefore, lower-efficacy agonists might be more likely to produce acute dependence than higher efficacy agonists, if up-regulation of the cAMP pathway is an important factor. However, intrinsic efficacy did not seem to be a determinant of whether or not a *mu*-opioid agonist substituted for morphine after acute administration. The lower-efficacy agonists buprenorphine and meperidine administered before naltrexone were no more effective than etorphine was in

occasioning morphine→naltrexone-appropriate responding. On the other hand, the balance between efficacy and promotion of receptor endocytosis might be the critical determinant of dependence development, with dependence induced quickest by drugs like morphine that have reasonably high efficacy but do not cause receptor internalization (Whistler et al., 1999). Heroin is rapidly converted to monoacetylmorphine and morphine (Way, 1967). Little is known about the intrinsic efficacy of levorphanol or on the propensity of that drug to promote endocytosis of *mu*-opioid receptors.

Another possible mechanism for acute opioid dependence is agonist-induced constitutive activation of *mu*-opioid receptors, where the receptor remains coupled to G protein and intracellular signaling pathways after the agonist has dissociated from it (Chavkin et al., 2001). Prior exposure to morphine increases basal signaling activity in cell lines expressing the *mu*-opioid receptor; naloxone and naltrexone are inverse agonists in those expression systems, increasing intracellular levels of cAMP (Wang et al., 1994;Wang et al., 2001). Only those drugs that were inverse agonists *in vitro* precipitated withdrawal jumping in mice given a single dose of morphine 4 hr earlier (Wang et al., 2001). There is little information on whether *mu*-opioid agonists other than morphine induce constitutive activity of *mu*-opioid receptors.

Given the disparate results obtained with the *mu*-opioid agonists, it is difficult to address the pharmacological selectivity of the morphine→naltrexone discrimination. Nevertheless, the results of this study permit some conclusions. First, stimulus control of behavior was produced stereoselectively. Rats generalized from partially to completely to levorphanol doses of 0.3 to 3.0 mg/kg followed by naltrexone. However, the

combination of 3.0 mg/kg dextrorphan, the non-opioid *dextro*rotatory isomer of levorphanol, and a broad range of naltrexone doses occasioned responding only on the lever appropriate for saline→naltrexone. Stimulus control of behavior by morphine alone exhibits similar stereoselectivity (Shannon and Holtzman, 1976). Second, the results with U69,593 and spiradoline indicate that the combination of a *kappa*-opioid agonist and naltrexone does not result in the same discriminative effects as morphine→naltrexone does. The dose tested, 3.0 mg/kg, is readily discriminated by rats and far exceeds the ED_{50} for suppressing food-maintained responding (Smith and Picker, 1995;Holtzman, 2000). Thus, if *kappa*-opioid agonists induce acute physical dependence, the interoceptive cues associated with antagonist-precipitated withdrawal from that state are different from those arising from precipitated withdrawal from the morphine-dependent state.

The effects of the antagonists paralleled those in rats chronically dependent upon morphine and discriminating between 0.1 mg/kg naltrexone and saline (Gellert and Holtzman, 1979): naloxone and diprenorphine substituted completely for naltrexone and levellorphan and nalorphine substituted partially. Diprenorphine and naloxone, like naltrexone, are essentially devoid of intrinsic efficacy at *mu*-opioid receptors (Lee et al., 1999). Levallorphan and nalorphine, on the other hand, have intrinsic efficacy estimated to range from 5 to 15% that of morphine (Emmerson et al., 1996;Selley et al., 1998), and substitute partially for morphine in rats discriminating between it and saline (Shannon and Holtzman, 1977). Thus, it appears that even limited intrinsic efficacy at *mu*-opioid receptors is sufficient to prevent a drug from substituting completely for naltrexone in

morphine-pretreated rats. The similarity of the effects of the five antagonists in rats acutely or chronically dependent upon morphine is further evidence of the commonalities shared by these two states.

Acknowldegements

Christine Engels and Kimberly Zdrojewski provided expert technical assistance that included training and testing the animals. Keith W. Easterling, Ph.D. provided advice on numerous aspects of the study and insightful comments about the manuscript. Drugs were contributed generously by Roche Laboratories, Merck & Company, and Pharmacia.

References

Adams JU and Holtzman SG (1990) Pharmacologic characterization of the sensitization to the rate-decreasing effects of naltrexone induced by acute opioid pretreatment in rats. *J.Pharmacol.Exp.Ther.* **253**:483-489.

Adams JU and Holtzman SG (1991) Naltrexone-sensitizing effects of centrally administered morphine and opioid peptides. *Eur.J.Pharmacol.* **193**:67-73.

Bickel WK, Stitzer ML, Liebson IA, and Bigelow GE (1987) Acute physical dependence in man: effects of naloxone after brief morphine exposure. *J.Pharmacol.Exp.Ther.* **244**:126-132.

Blasig J, Herz A, Reinhold K, and Zieglgansberger S (1973) Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. *Psychopharmacologia.* **33**:19-38.

Chavkin C, McLaughlin JP, and Celver JP (2001) Regulation of opioid receptor function by chronic agonist exposure: Constitutive activity and desensitization. *Mol.Pharmacol.* **60**:20-25.

Easterling KW and Holtzman SG (1997) Intracranial self-stimulation in rats: sensitization to an opioid antagonist following acute or chronic treatment with mu opioid agonists. *J.Pharmacol.Exp.Ther.* **281**:188-199.

Easterling KW and Holtzman SG (1999) Discriminative stimulus effects of naltrexone after a single dose of morphine in the rat. *J.Pharmacol.Exp.Ther.* **288**:1269-1277.

Eisenberg RM and Sparber SB (1979) Changes in plasma corticosterone levels as a measure of acute dependence upon levorphanol in rats. *J.Pharmacol.Exp.Ther.* **211**:364-369.

Eissenberg T, Greenwald MK, Johnson RE, Liebson IA, Bigelow GE, and Stitzer ML (1996) Buprenorphine's physical dependence potential: Antagonist- precipitated withdrawal in humans. *J.Pharmacol.Exp.Ther.* **276**:449-459.

Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH, and Medzihradsky F (1996) Characterization of opioid agonist efficacy in a C_6 glioma cell line expressing the μ opioid receptor. *J.Pharmacol.Exp.Ther.* **278**:1121-1127.

Finn AK and Whistler JL (2001) Endocytosis of the mu opioid receptor reduces tolerance and a cellular hallmark of opiate withdrawal. *Neuron* **32**:829-839.

Gellert VF and Holtzman SG (1978) Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J.Pharmacol.Exp.Ther.* **205**:536-546.

Gellert VF and Holtzman SG (1979) Discriminative stimulus effects of naltrexone in the morphine-dependent rat. *J.Pharmacol.Exp.Ther.* **211**:596-605.

Greenwald MK, June HL, Stitzer ML, and Marco AP (1996) Comparative clinical pharmacology of short-acting *mu* opioids in drug abusers. *J.Pharmacol.Exp.Ther.* **277**:1228-1236.

Holtzman SG (1990) Discriminative stimulus effects of drugs: relationship to potential for abuse, in *Testing and Evaluation of Drugs of Abuse* (Adler MW and Cowan A eds) pp 195-212, Wiley-Liss, Inc., New York.

Holtzman SG (1997) Antagonism of morphine-like discriminative effects by β-funaltrexamine. *Pharmacol.Biochem.Behav.* **57**:771-777.

Holtzman SG (2000) Further characterization of the discriminative stimulus effects of spiradoline. *Pharmacol.Biochem.Behav.* **66**:517-522.

Lee KO, Akil H, Woods JH, and Traynor JR (1999) Differential binding properties of oripavines at cloned μ- and δ-opioid receptors. *Eur.J.Pharmacol.* **378**:323-330.

Maldonado R, Stinus L, Gold LH, and Koob GF (1992) Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *J.Pharmacol.Exp.Ther.* **261**:669-677.

Meyer DR and Sparber SB (1976) Evidence of possible opiate dependence during the behavioral depressant action of a single dose of morphine. *Life Sci.* **21**:1087-1094.

Nestler EJ and Aghajanian GK (1997) Molecular and cellular basis of addiction. *Science* **278**:58-63.

Schulteis G, Heyser CJ, and Koob GF (1999) Differential expression of response-disruptive and somatic indices of opiate withdrawal during the initiation and development of opiate dependence. *Behav.Pharmacol.* **10**:235-242.

Schulteis G, Markou A, Gold LH, Stinus L, and Koob GF (1994) Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. *J.Pharmacol.Exp.Ther.* **271**:1391-1398.

Selley DE, Liu QX, and Childers SR (1998) Signal transduction correlates of *Mu* opioid agonist intrinsic efficacy: Receptor-stimulated [^{35}S]GTPgammaS binding in mMOR-CHO cells and rat thalamus. *J.Pharmacol.Exp.Ther.* **285**:496-505.

Shannon HE and Holtzman SG (1976) Evaluation of the discriminative effects of morphine in the rat. *J.Pharmacol.Exp.Ther.* **198**:54-65.

Shannon HE and Holtzman SG (1977) Further evaluation of the discriminative effects of morphine in the rat. *J.Pharmacol.Exp.Ther.* **201**:55-66.

Sharma SK, Klee WA, and Nirenberg M (1975) Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc.Natl.Acad.Sci.U.S.A* **72**:3092-3096.

Shippenberg TS, Funada M, and Schutz CG (2000) Dynorphin A (2-17) attenuates the unconditioned but not the conditioned effects of opiate withdrawal in the rat. *Psychopharmacology (Berl)* **151**:351-358.

Smith MA and Picker MJ (1995) Examination of the kappa agonist and antagonist properties of opioids in the rat drug discrimination procedure: Influence of training dose and intrinsic efficacy. *Behav.Pharmacol.* **6**:703-717.

Sternini C, Spann M, Anton B, Keith DE, Jr., Bunnett NW, Von Zastrow M, Evans C, and Brecha NC (1996) Agonist-selective endocytosis of μ opioid receptor by neurons *in vivo*. *Proc.Natl.Acad.Sci.USA* **93**:9241-9246.

Walker EA, Makhay MM, House JD, and Young AM (1994) In vivo apparent pA2 analysis for naltrexone antagonism of discriminative stimulus and analgesic effects of opiate agonists in rats. *J.Pharmacol.Exp.Ther.* **271**:959-968.

Wang DX, Raehal KM, Bilsky EJ, and Sadée W (2001) Inverse agonists and neutral antagonists at μ opioid receptor (MOR): possible role of basal receptor signaling in narcotic dependence. *J.Neurochem.* **77**:1590-1600.

Wang Z, Bilsky EJ, Porreca F, and Sadee W (1994) Constitutive mu opioid receptor activation as a regulatory mechanism underlying narcotic tolerance and dependence. *Life Sci.* **54**:L339-L350.

Way EL (1967) Brain uptake of morphine: pharmacologic implications. *Fed.Proc.* **26**:1115-1118.

Way EL, Loh HH, and Shen FH (1969) Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J.Pharmacol.Exp.Ther.* **167**:1-8.

Whistler JL, Chuang HH, Chu P, Jan LY, and Von Zastrow M (1999) Functional dissociation of μ opioid receptor signaling and endocytosis: Implications for the biology of opiate tolerance and addiction. *Neuron* **23**:737-746.

White DA and Holtzman SG (2001) Acute opioid pretreatment potentiates naltrexone-induced drinking suppression in water-deprived rats. *J.Pharmacol.Exp.Ther.* **298**:156-164.

Wright C, Bigelow GE, Stitzer ML, and Liebson IA (1991) Acute physical dependence in humans: repeated naloxone-precipitated withdrawal after a single dose of methadone. *Drug Alcohol Depend.* **27**:139-148.

Young AM (1986) Effects of acute morphine pretreatment on the rate-decreasing and antagonist activity of naloxone. *Psychopharmacology (Berl)* **88**:201-208.

Young AM, Kapitsopoulos G, and Makhay MM (1991) Tolerance to morphine-like stimulus effects of mu opioid agonists. *J.Pharmacol.Exp.Ther.* **257**:795-805.

Footnote

This research was supported by grant DA00541 and by Senior Scientist Award KO5 DA00008, both from the National Institute on Drug Abuse, National Institutes of Health.

Figure Legends

Figure 1. Comparison of stimulus generalization curves for 4-hr pretreatment with 10 mg/kg morphine (4 hr) and 15-min pretreatment with graded doses of naltrexone and for 4-hr pretreatment with saline and 15-min pretreatment with graded doses of naltrexone. The morphine→naltrexone curve (n=25) was determined in test sessions at the beginning of the study and the saline→naltrexone curve (n=16) was determined in test sessions scattered throughout the study. Points above Sal represent the results of test sessions in which animals received a 15-min pretreatment with saline in place of naltrexone (*i.e.*, morphine→saline or saline→saline). The ordinate shows the number of trials completed on the choice lever appropriate for morphine(10 mg//kg)→naltrexone(0.3 mg/kg; NTX) in a 20-trial session; the remaining trials were completed on the choice lever appropriate for saline→naltrexone(0.3 mg/kg). The upper and lower horizontal dashed lines indicate

the performance level at which behavior was maintained during training sessions that followed pretreatment with morphine→naltrexone and saline→naltrexone, respectively.

Figure 2. Stimulus-generalization curves for 4-hr pretreatment with the indicated dose of heroin (top), levorphanol, or dextrorphan (bottom) and 15-min pretreatment with graded doses of naltrexone. Each point is a mean based upon one observation in each of six rats. Other details are the same as in Fig. 1.

Figure 3. Stimulus-generalization curves for different pretreatment times and doses of fentanyl (top) or methadone (bottom) and 15-min pretreatment with graded doses of naltrexone. Fentanyl was administered as a single dose of 0.056 mg/kg (4-hr pretreatment) or 0.1 mg/kg (2-, 3-, 4-hr pretreatment). A single dose of methadone (3.0 mg/kg) was administered either 3 or 4 hr before a session. Each point is a mean based upon one observation in each of six rats. Other details are the same as in Fig. 1.

Figure 4. Stimulus-generalization curves for 4-hr pretreatment with a *mu*-opioid agonist (0.01 mg/kg etorphine, 1.0 mg/kg buprenorphine, 30 mg/kg meperidine) and 15-min pretreatment with graded doses of naltrexone (top) and for 4-hr pretreatment with a *kappa*-opioid agonist (3.0 mg/kg spiradoline, 3.0 mg/kg U69,593) and 15-min pretreatment with graded doses of naltrexone (bottom). Each point is a mean based upon one observation in each of six rats. Other details are the same as in Fig. 1.

Figure 5. Trials completed on the morphine→naltrexone-appropriate choice lever (top) and corresponding Gellert-Holtzman global withdrawal scores (bottom) in rats tested with saline or naltrexone (15-min pretreatment) while receiving a continuous infusion of either fentanyl (0.25 mg/kg/day) or methadone (10 mg/kg/day) via SC osmotic pump, and 15 min after pretreatment with saline 6 or 24 hr after pumps had been removed (points to right of axis break). Saline was tested on day 5 of pump implantation and naltrexone was tested on days 7, 9, 11, and 13. Pumps were removed on day 14. Each point is a mean based upon one observation in each of four (fentanyl pump) or five (methadone pump) rats. Vertical lines in the bottom panel represent ±1 SEM, and are absent if the SEM was less than the radius of the point. *Significantly different from corresponding withdrawal score after saline administration while pumps were implanted (points above Sal), $p < 0.05$

Figure 6. Trials completed on the morphine-naltrexone-appropriate choice lever correlate significantly with global withdrawal score in rats receiving a continuous infusion of either fentanyl (n=4) or methadone (n=5) by SC osmotic pump and tested after 15-min pretreatment with either saline or graded doses of naltrexone. Points were derived from data to the left of the axis break in Fig. 5.

Figure 7. Stimulus-generalization curves for 4-hr pretreatment with 10 mg/kg morphine and 15-min pretreatment with graded doses of one of the indicated opioid antagonists in place of naltrexone. Points above Sal represent the results of test sessions that followed 4-hr pretreatment with saline in place of morphine and 15-min pretreatment with the

highest dose of each antagonist that had been tested as part of the stimulus-generalization curve. Each point is a mean based upon one observation in each of six or seven (nalorphine) rats. Other details are the same as in Fig. 1.

Figure 1

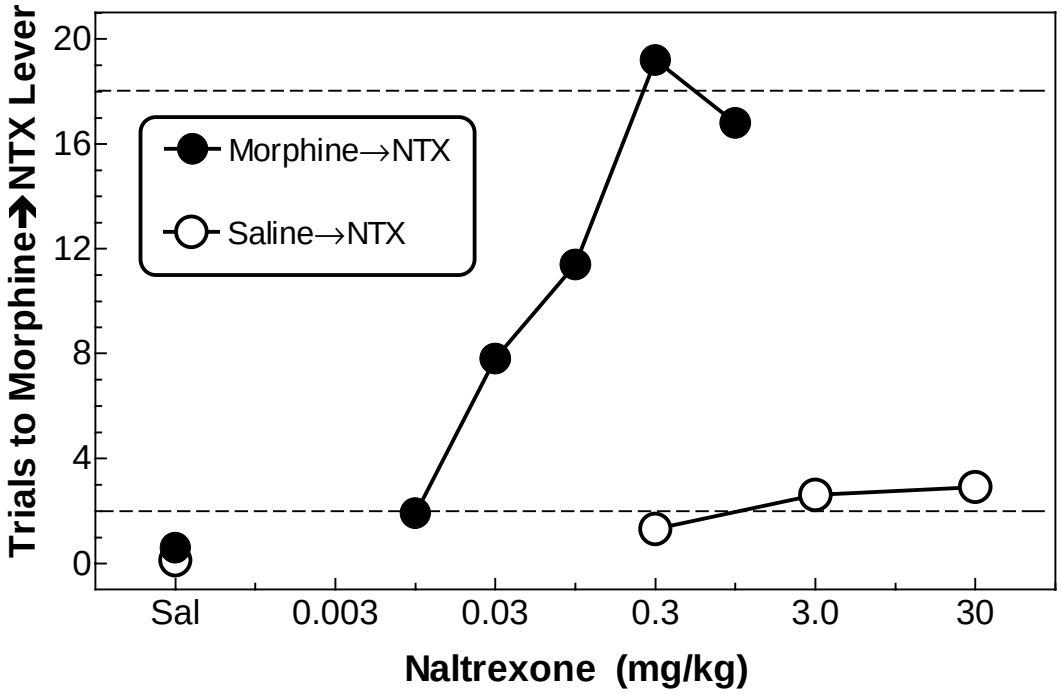
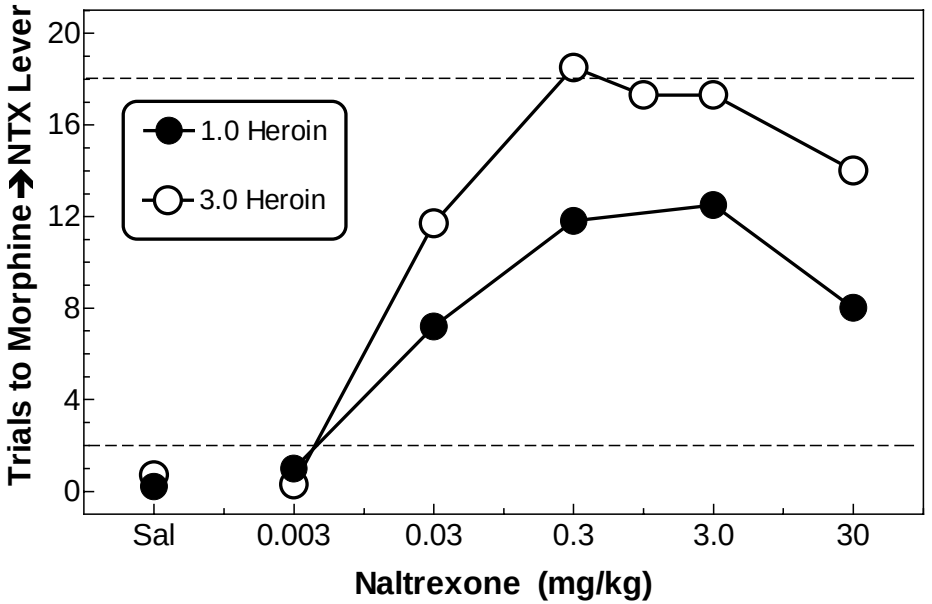


Figure 2



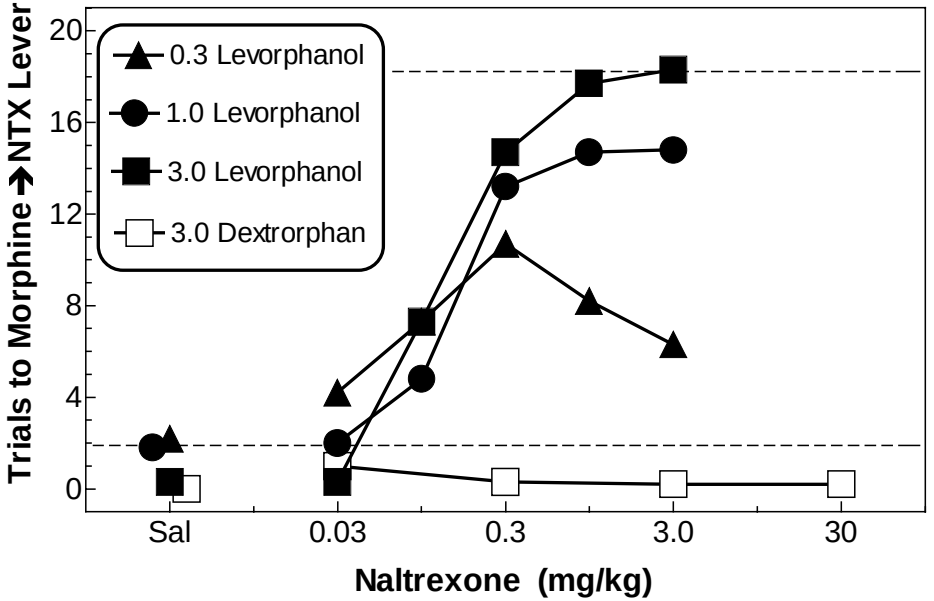
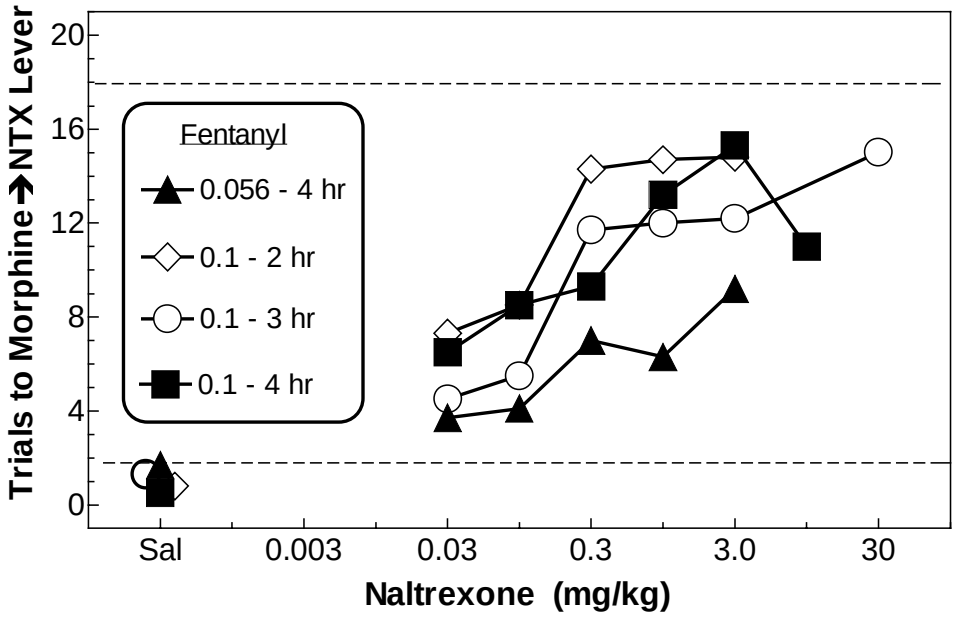


Figure 3



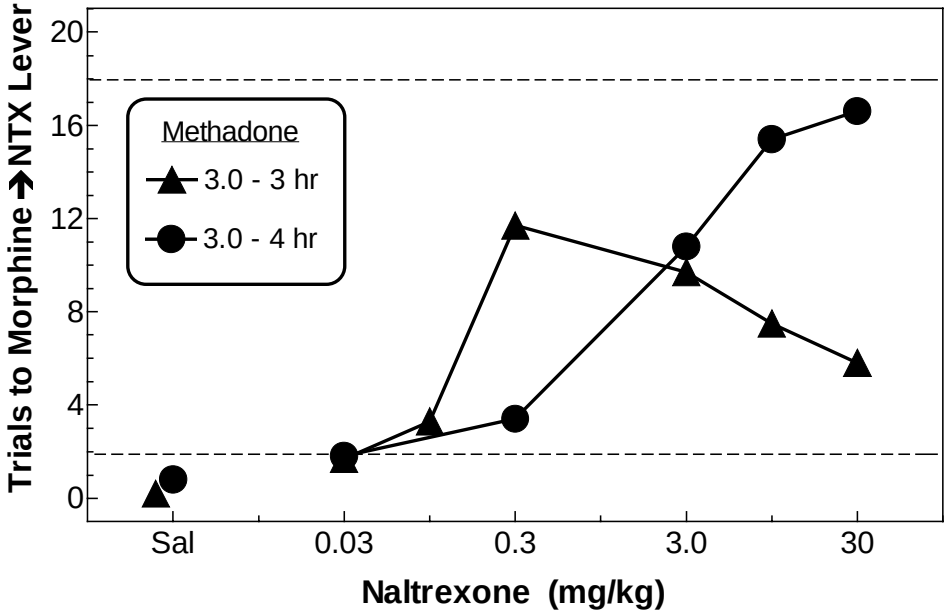
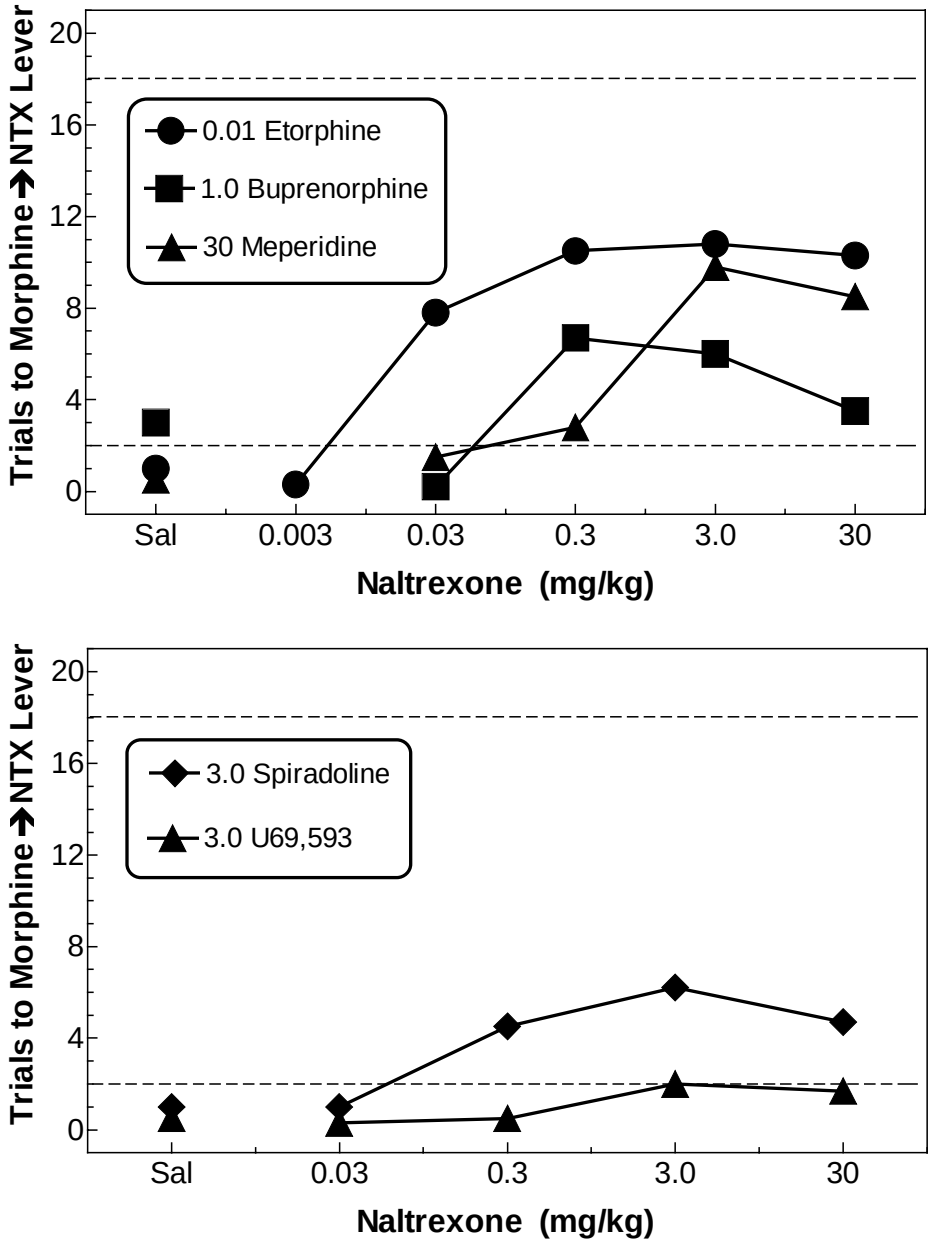


Figure 4



JPET Fast Forward. Published on November 25, 2002 as DOI: 10.1124/jpet.102.044875
This article has not been copyedited and formatted. The final version may differ from this version.

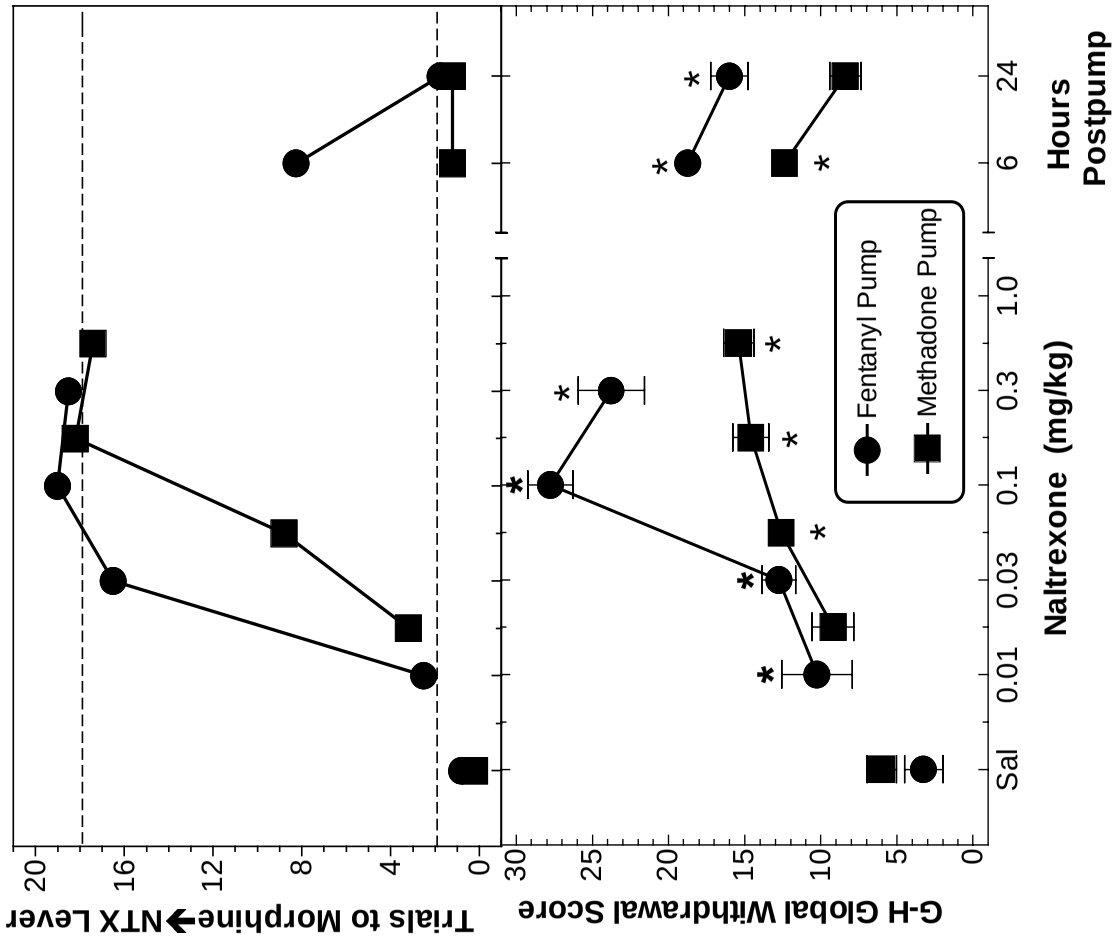


Figure 5

Figure 6

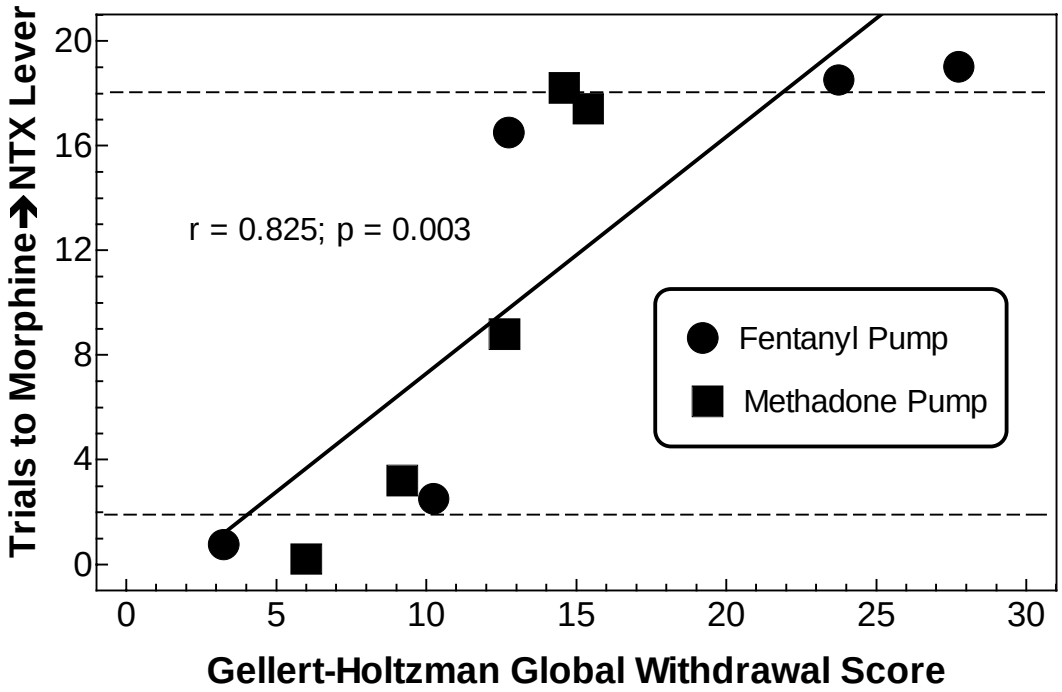


Figure 7