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

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ABSTRACT

Rats were trained to discriminate 4-h 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 seems to derive from 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 μ-opioid agonists tested in place of morphine, only two (heroin and 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, and methadone) or lower (buprenorphine and meperidine) than that of morphine substituted only partially. However, when naltrexone was administered during continuous infusion of fentanyl or methadone via s.c. 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 dextrorotatory isomer of levorphanol, or κ-opioid agonists (5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzeneacetamide (U69,593) and spiradoline. Antagonists with no intrinsic efficacy at μ-opioid receptors (naloxone and diprenorphine) substituted completely for naltrexone, whereas those with some efficacy (nalorphine and levallorphan) substituted partially. Thus, morphine—naltrexone-like stimulus control of behavior by drugs administered acutely requires pretreatment with certain μ-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 ED₅₀ of the opioid antagonist naloxone to elicit withdrawal jumping in mice that had received an s.c. morphine pellet decreased steadily from 12 mg/kg immediately after pellet implantation to 0.045 mg/kg 72 h 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 to 3 orders of magnitude in rats that had been pretreated 4 h 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 μ-opioid receptors (Adams and Holtzman, 1990, 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

ABBREVIATIONS: ANOVA, analysis of variance.
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-h 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 h before a session, half-maximal when morphine was administered 8 h 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 an s.c. 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., etorphine and fentanyl; Emmerson et al., 1996; Selsey et al., 1998), to relatively high (e.g., morphine and meperidine; Emmerson et al., 1996; Selsey et al., 1998), to be equieffective 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 κ-opioid agonists induces much less sensitization to response rate-decreasing effects of naltrexone than does acute administration of μ-opioid agonists (Adams and Holtzman, 1990; Easterling and Holtzman, 1997). In this study, a relatively high dose of each of two κ-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 μ-opioid receptors (Lee et al., 1999); and nalorphine and levallorphan, drugs that are weak partial μ-agonists (Emmerson et al., 1996; Selsey et al., 1998).

Materials and Methods

Subjects. Adult male rats of Sprague-Dawley descent were obtained from Charles River Laboratories, Inc. (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 naive. 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 s.c. 4 h before a session on alternate days; naltrexone, 0.3 mg/kg, was injected s.c. 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 lightproof 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 seconds later, a constant current of 1.0 to 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 and 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-h 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 3 days of each week, with morphine and saline pretreatment alternating. Test sessions were held on the other 2 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 canceled 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 h 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. To determine whether 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 to 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 an s.c. osmotic pump (model 2 ML2; Alza, 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 implantation, 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 h later, saline was injected and a test session was held 15 min after each injection. Normal training and testing resumed 1 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 fasciulation or teeth chatter, swallowing movements, salivation, chromatocoryorrhea, ptosis, abnormal posture, erection or ejaculation, and irritability to handling. “Graded” signs were number of escape attempts, “watery” shakes, and abdominal contractions.

Each rat was weighed just before the injection and again at the conclusion of the session, 40 to 45 min later, 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 non-parametric ANOVA for repeated measures, yielding the statistic $t$. If the data were statistically reliable, the Wilcoxon 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$_{90}$) 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$_{90}$ was estimated by simple interpolation. The ED$_{90}$ values 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$_{90}$ was derived from the group mean 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 forms, and their sources were as follows: naltrexone hydrochloride, naloxone hydrochloride, and dextrophan tartrate (Sigma/RBI, Natick, MA); buprenorphine, diprenorphine, etorphine, fentanyl, heroin, meperidine, all as hydrochlorides, and (5α,7α,8β)-(−)-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, levalorphan tartrate (Hoffmann-La Roche, Nutley, NJ); methadone hydrochloride (Mallinkrodt, St. Louis, MO); nalorphine hydrochloride (Merck Research Labs, West Point, PA); and 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 of 8.5% lactic acid and 2 parts of 1.0 N sodium hydroxide. Except when they were administered via SC osmotic pumps, the drugs were injected s.c. in a volume of 1.0 ml (2.0 ml for high meperidine doses) per kilogram of body weight. Doses represent the free-base form of the drug.

Results

Baseline. The 22 rats that were experimentally naive 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 h 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 h 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 versus 19.2 trials, $p < 0.001$). The ED$_{90}$ of naltrexone, derived from the ascending portion of the stimulus-generalization curve, was 0.051 (0.034–0.077) mg/kg. In contrast, 4-h 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 s in test sessions held 4 h after 10 mg/kg morphine and 15 min after saline and 148 ± 4 s in sessions held after two injections of saline (t$_{139}$).
after 1.0 mg/kg levorphanol and 3.0 mg/kg naltrexone, and to

3
naltrexone-appropriate lever decreased to 14.8
morphine
one. The maximum number of trials completed on the
dose dependently (Fig. 2, bottom). The animals completed an
dose of 10 mg/kg morphine (\( \leq \)). All animals remained on the

3
maining trials were completed on the choice lever appropriate for
3
mg/kg) also substituted for morphine
3
saline. The ordinate shows the number
3
saline or saline
3
naltrexone, respectively.

= 2.47, \( p = 0.018 \)). Cumulative response latencies were un-

affected by either 0.01 to 1.0 mg/kg naltrexone administered
after 10 mg/kg morphine \( (F_{1,24} = 1.00, p = 0.430) \) or by 0.3
to 30 mg/kg naltrexone administered after saline \( (F_{1,15} =
1.27, p = 0.859) \). 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 h before a ses-
tion. When combined with 0.3 mg/kg naltrexone adminis-
tered 15 min before a session, 3.0 mg/kg heroin occasioned
completion of an average of more than 18 trials on the choice
levers of each session. The morphine—naltrexone curve \( (n = 25) \) was determined in test sessions at the begin-
ning 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.

1.0 mg/kg heroin and naltrexone was biphasic: doses of naltrex-
one higher than 0.3 mg/kg resulted in progressively fewer
trials being completed on the morphine—naltrexone-appropri-
ate 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-appropri-
ate 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
0.255 (0.008–0.079) and 0.286 mg/kg, respectively. The

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-appropri-
ate lever after 3.0 mg/kg levorphanol and 3.0 mg/kg naltrex-
one. 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 nal-
trexone. The ED\(_{50}\) value of naltrexone after 3.0, 1.0, and 0.3
mg/kg levorphanol was 0.148 (0.085–0.256), 0.193, and 0.243
mg/kg, respectively. The former ED\(_{50}\) value was significantly
higher than the ED\(_{50}\) value of naltrexone after pretreatment
with either 10 mg/kg morphine \( (p < 0.05) \) or 3.0 mg/kg heroin
\( (p < 0.01; F_{[2,15]} = 5.53, p = 0.008) \). However, combinations
of 4-h pretreatment with dextrorphan, the nonopioid stereo-

isomer 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-hour 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 oc-
asioned 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 h 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-h pre-
treatment did.

Neither 3- nor 4-h pretreatment with 3.0 mg/kg metha-
done and 15-min pretreatment with naltrexone sub-
tituted completely for morphine—naltrexone, although 4-h
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
whether the failure of methadone and naltrexone pretreatments to substitute completely for morphine-naltrexone was due to a peculiarity of the antagonist. Three milligram per kilogram methadone was administered 4 h 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-naltrexone was an orderly and biphasic function of the naloxone dose, not unlike the naltrexone curve after 3-h 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 shown).

The pairing of 4-h 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 ($F_{r/11005} = 14.72, p = 0.012$) and meperidine ($F_{r/11005} = 14.16, p = 0.007$), and not quite significant for buprenorphine ($F_{r/11005} = 8.65, p = 0.070$). 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 shown).

A high dose of a $\mu$-opioid agonist, 3.0 mg/kg of either spiradoline or U69,593 (4-h 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 ($F_{r/11005} = 6.31, p = 0.177$).

**Fentanyl and Methadone Administered Continuously.** Saline was injected 15 min before a test session into rats that had been getting a continuous s.c. 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 $ED_{50}$ values for naltrexone were 0.019 (0.011–
than in those with methadone pumps (approximately 3 times more potent in rats with fentanyl 0.033) and 0.059 (0.030 – 0.245) mg/kg, making naltrexone 24 h postpump (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 (t110) = 6.40, p < 0.001).

**Other Antagonists.** The combination of 4-h 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 [ED50 values: 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 (t110) = 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). ED50 values 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-h 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 dia

![Fig. 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 s.c. osmotic pump, and 15 min after pretreatment with saline 6 or 24 h 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 ± S.E.M. and are absent if the S.E.M. 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.

0.033) and 0.059 (0.030–0.114) mg/kg, making naltrexone approximately 3 times more potent in rats with fentanyl pumps than in those with methadone pumps (t171 = 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 h postpump, and responded only on the lever appropriate for saline—naltrexone 24 h postpump (Fig. 5, top). The group that had received methadone completed more than 90% of the trials on the saline—naltrexone-appropriate lever in both postpump 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 0.1 mg/kg naltrexone compared with 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 h postpump and for the group that had received fentanyl at 24 h postpump (Fig. 5, bottom).
morphine in occasioning morphine
levorphanol, substituted completely for acutely administered
well above the ED$_{50}$ values for suppressing food-maintained
doses that are at least equivalent to 10 mg/kg morphine, and
potent than morphine (Young et al., 1991; Walker et al.,
hand, buprenorphine, fentanyl, and etorphine are approxi-
100 mg/kg would be needed for complete substitution. How-
potent as morphine in producing morphine-like discrimina-
tive signs. However, the concordance of the two variables
completing on the morphine
3 naltrexone-appropriate
completed on the morphine
3 naltrexone-appropriate lever corre-
determined in rats that had pumps releasing morphine (0.04
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.

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$_{50}$ value
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 mul-
tiple. In some cases, it was not possible to test a dose that was
equieffective with 10 mg/kg morphine. Meperidine is 1/10 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 to 100, 80 to 100, and 2000 to 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$_{50}$ values for suppressing food-maintained
responding (Young et al., 1991; Walker et al., 1994), but did
not substitute completely. Some of the agonists have dura-
tions of action shorter than that of morphine. However,
shortening the pretreatment interval to 3 h for methadone
and to 3 or 2 h for fentanyl did not increase the number of
trials completed on the morphine→naltrexone-appropriate
lever compared with 4-h pretreatment.

Naloxone precipitated signs and symptoms of opioid with-
drawal 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 administration, they were administered by con-
tinuous s.c. 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 with those en-
gendered by acute pretreatment with the combination of
morphine and naltrexone. Furthermore, the ED$_{50}$ value 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 discrimi-
native effects associated with naltrexone-precipitated with-
drawal 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 h 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 nalt-
rexone 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 biphase
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 mor-
phine→naltrexone derives from interoceptive stimuli associ-
ated with antagonist-precipitated withdrawal from acute
morphine dependence. In addition, the number of trials com-
pleted on the morphine→naltrexone-appropriate lever corre-
lated significantly with the global withdrawal score of so-
matic signs. However, the concordance of the two variables
was far from perfect quantitatively. “Behavioral/motiva-
tional” signs of morphine withdrawal, such as decreased
food-maintained operant responding and conditioned place
aversion, manifest 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, 1999). More-
over, 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 depen-
dence, they are not necessarily predictive of nor do they
account for behavioral/motivational manifestations of with-
drawal, 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 revers-
ible; 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
(Eisenberg 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 adenyl cyclase and other components of the cAMP pathway while they activate μ-opioid receptors (Sharma et al., 1975; Finn and Whistler, 2001). This up-regulated second-messenger system seems to contribute to the withdrawal syndrome that emerges when morphine is displaced from the receptor by an antagonist (Nestler and Aghajanian, 1997). High-efficiency agonists (e.g., etorphine), on the other hand, cause desensitization and endocytosis of μ-opioid receptors, which prevents up-regulation of adenyl 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 a μ-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 such as 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 μ-opioid receptors.

Another possible mechanism for acute opioid dependence is agonist-induced constitutive activation of μ-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 μ-opioid receptor; naloxone and naltrexone are inverse agonists in those expression systems, increasing intracellular levels of cAMP (Wang et al., 1994, 2001). Only those drugs that were inverse agonists in vitro precipitated withdrawal jumping in mice given a single dose of morphine 4 h earlier (Wang et al., 2001). There is little information on whether μ-opioid agonists other than morphine induce constitutive activity of μ-opioid receptors.

Given the disparate results obtained with the μ-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 from 0.3 to 3.0 mg/kg followed by naltrexone. However, the combination of 3.0 mg/kg dextrorphan, the nonopioid dextrorotatory 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 κ-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 ED50 value for suppressing food-maintained responding (Smith and Picker, 1995; Holtzman, 2000). Thus, if κ-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): naltrexone and diprenorphine substituted completely for naltrexone and levallorphan and nalorphine substituted partially. Diprenorphine and naloxone, like naltrexone, are essentially devoid of intrinsic efficacy at μ-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 seems that even limited intrinsic efficacy at μ-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.

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