Discriminative Stimulus Effects of Acute Morphine Followed by Naltrexone in the Squirrel Monkey: A Further Characterization

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ABSTRACT

The discriminative stimulus effects of acute morphine followed by naltrexone have been described previously in nonhuman primates. The purposes of this study were to 1) extend the pharmacological characterization of the discrimination by testing μ-opioid agonists other than morphine and opioid-like compounds other than naltrexone and 2) to examine further the relationship between agonist pretreatment time and manifestation of the cue produced by morphine followed by naltrexone. Subjects were trained to discriminate 1.7 mg/kg morphine → 0.1 mg/kg naltrexone (MOR → NTX) versus saline followed by 0.1 mg/kg naltrexone. When combined with 0.1 mg/kg naltrexone, all agonists tested, save buprenorphine, meperidine, and nalbuphine, produced dose-dependent increases in MOR → NTX-appropriate responding, culminating in criterion levels of responding. Comparing agonist ED₅₀ values revealed a rank order of potency of etorphine ≫ fentanyl ≫ levorphanol ≫ heroin ≳ methadone ≳ nalbuphine ≳ morphine. ED₅₀ values for buprenorphine and meperidine could not be calculated. MOR → NTX-appropriate responding after doses of agonist that produced criterion or near criterion levels of responding was also a function of naltrexone dose. After morphine pretreatment, diprenorphine and nalorphine, but not buprenorphine, dose-dependently substituted for naltrexone. The MOR → NTX discrimination also depended upon the interval between morphine and NTX administration. Finally, 1-h pretreatment with morphine and etorphine, but not buprenorphine, followed by naltrexone generalized to 4-h MOR → NTX. These results suggest a minimum efficacy requirement of acutely administered agonists together with the naltrexone training dose for stimulus control of behavior. However, in some cases this requirement can be overcome with higher doses of naltrexone.

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ABBREVIATIONS: MOR, morphine; NTX, naltrexone; SAL, saline; MOR → NTX, morphine followed by naltrexone; SAL → NTX, saline followed by naltrexone; U69,593, (5α,7α,8β)-(−)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)benzeneacetamide; ANOVA, analysis of variance.
nist U69,593 did not. These data are consistent with previous findings with rats trained to discriminate 10 mg/kg morphine (4-h pretreatment) followed by 0.3 mg/kg naltrexone (15-min pretreatment) versus saline followed by naltrexone (Easterling and Holtzman, 1999; Holtzman, 2003). Together, these data suggest that the interoceptive stimuli associated with the MOR → NTX cue reflect antagonist-precipitated withdrawal from acute physical dependence upon morphine.

Our first objective was to extend the pharmacological characterization of the MOR → NTX discrimination by squirrel monkeys by testing μ-opioid agonists other than morphine and levorphanol and antagonists other than naltrexone. We initially determined the “optimal” doses to produce full MOR → NTX responding when followed by the training dose of naltrexone (0.1 mg/kg), for a variety of morphine-like agonists. These agonists represented a range of intrinsic efficacies (e.g., buprenorphine, nalbuphine, and meperidine: relatively low; morphine and heroin: intermediate; and fentanyl, methadone, and etorphine: relatively high) (Schmidt et al., 1985; Emmerson et al., 1996; Selley et al., 1998). Intrinsic efficacy is a determinant of the magnitude of tolerance development to the antinoceptive effects of morphine-like drugs (Paronis and Holtzman, 1991). However, despite evidence that common mechanisms mediate tolerance and physical dependence (Way, 1993), until recently (Holtzman, 2003), there has been relatively little research on the role of intrinsic efficacy in the manifestation of acute opioid dependence or its associated interoceptive stimuli.

We then tested the optimal doses of the morphine-like agonists, which were estimated to be equipotent with 1.7 mg/kg morphine (i.e., the training dose), and a range of naltrexone doses for stimulus generalization. With buprenorphine, meperidine, and nalbuphine, criterion levels of responding could not be reached at any dose. Therefore, stimulus generalization was assessed after pretreatment with several doses of buprenorphine and meperidine and 3.0 mg/kg nalbuphine followed by naltrexone. Naloxone substituted fully for naltrexone in squirrel monkeys and rats that were pretreated with a single dose of morphine (Easterling and Holtzman, 1999; White and Holtzman, 2003). We extended observations to two more opioid antagonists: diprenorphine and nalorphine.

The second objective of the current study was to examine further the functional relationship between agonist pretreatment interval and stimulus control of behavior. We extended our previous observations (White and Holtzman, 2003) to include stimulus generalization curves for naltrexone after 1- and 2-h morphine pretreatment. Finally, we sought to determine whether MOR → NTX-appropriate responding could be elicited at an earlier time point (1 h) after buprenorphine pretreatment. A stimulus generalization curve for 1-h pretreatment with etorphine followed by NTX was also constructed for comparison. This last set of experiments was based on the finding that potentiation of naltrexone-induced suppression of water consumption in water-deprived rats was an inverse function of the interval between agonist and antagonist administration (White and Holtzman, 2001).

Materials and Methods

Subjects. Six male squirrel monkeys (Saimiri sciureus), weighing between 700 and 1100 g at the beginning of experimentation, were pair-housed with unlimited access to food and water. Monkeys were provided with fresh fruit, peanuts, or a vitamin supplement mixture each day in the home cage. All of the monkeys were opioid drug-experienced and had previously been trained to discriminate 1.7 mg/kg morphine followed by 0.1 mg/kg naltrexone versus saline followed by naltrexone (White and Holtzman, 2003). Animals were maintained according to the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996), and all procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

Apparatus. During experimental sessions, monkeys were seated in small primate chairs housed in ventilated and sound-attenuated chambers (BRS/LVE Inc., Laurel, MD). The chairs were equipped with a small stock and two brass electrodes through which electric current was delivered to a shaved portion of the monkey’s tail. Two response levers were mounted 9.5 cm apart on the front panel and 3 cm from the side walls. A Plexiglas partition extended from the ceiling to the waist-plate of the chair, creating a wall 6 cm out from the front panel. Two slots approximately 10 cm apart, measuring 4 × 5 cm each were cut out of this partition just in front of each lever with approximately 10 cm between the slots to prevent the monkey from reaching and pressing both levers simultaneously. A red stimulus light was mounted at eye level and centered between the two response levers on the front panel. A white house light was positioned above the red stimulus light or on the rear wall of the chamber, depending on the chamber used. The chambers were equipped with white noise to mask extraneous sounds.

Drug Discrimination Procedure. This procedure has been described previously (White and Holtzman, 2003). In short, experimental sessions were conducted 3 to 5 days a week, Monday through Friday, and consisted of 25 trials. Subjects did not receive drug on nonexperimental days. Monkeys were trained to press the response levers under a fixed-ratio 1 schedule of stimulus termination/avoidance. At the beginning of each trial, the house light was illuminated, and the monkey had 5 s to press the lever appropriate for the injection combination received before the session to avoid a 2 to 4-mA electrical stimulus to the tail. If the monkey failed to press the correct lever within 5 s, the electrical stimulus was delivered in 1-s pulses every 2 s until the monkey responded on the correct lever or until 15 stimuli were delivered. At the end of the trial the house light was turned off, and the red stimulus light was turned on for a 30-s time-out period. Each response on the incorrect lever resulted in an electrical stimulus and a 3-s change over delay, during which responses on the correct lever did not end the trial. Each response during the 30-s time-out period also resulted in the delivery of an electrical stimulus to the tail to discourage responding between trials.

For training sessions, monkeys received an i.m. injection of either saline or 1.7 mg/kg morphine 4 h before each daily session, followed by 0.1 mg/kg naltrexone 3.75 h later (SAL → NTX and MOR → NTX, respectively). Lever assignments for each training-drug combination were balanced across subjects. Drug discrimination training continued for each monkey until it achieved a criterion of emitting the first response on the injection-appropriate lever in ≥88% (i.e., ≥22 of the trials in a session for four consecutive daily sessions. Monkeys then underwent two test sessions, one for each condition. Test sessions were similar to training sessions, except there was not a “correct” lever, so that a trial was terminated by a response on either lever. However, subjects were still required to respond within 5 s to avoid an electrical stimulus. If monkeys responded on the injection-appropriate lever in ≥88% of the trials on both test days, they were considered to have met criteria for acquisition of the discrimination. After acquisition of the discrimination, novel doses and/or drugs were tested one or two times per week with at least 3 days between successive tests. Between test sessions, monkeys were required to perform at criterion (≥88% injection-appropriate responding) in at least two consecutive training sessions.
The first experiment involved varying the dose of the opioid agonists buprenorphine (0.01–0.1 mg/kg), etorphine (0.56–1.78 μg/kg), fentanyl (0.01–0.03 mg/kg), heroin (0.1–0.56 mg/kg), levoephonal (0.03–0.3 mg/kg), meperidine (3.0–17.8 mg/kg), methadone (0.1–1.78 mg/kg), morphine (0.56–1.7 mg/kg), and nalbuphine (0.1–17.8 mg/kg), while holding the pretreatment interval (3.75 h) and naltrexone dose constant (0.1 mg/kg, 0.25 h). This experiment was performed to determine the optimal dose of agonist required to elicit criterion levels of MOR → NTX-appropriate responding when followed by the training dose of naltrexone. Drugs were tested up to doses that either substituted completely for MOR → NTX or produced observable side effects (e.g., muscle tremors, sedation, and/or respiratory depression) that suggested that higher doses could be a threat to the well being of the monkeys. For the next experiment, the optimal dose and pretreatment time of the agonists were held constant and the dose of naltrexone was varied to determine whether the interoceptive effects of withdrawal from acute opioid dependence upon the different agonist are a function of the naltrexone dose. The only exceptions were after buprenorphine, meperidine, and nalbuphine, where criterion levels of MOR → NTX-appropriate responding were not achieved. For buprenorphine and meperidine, naltrexone dose-response curves were constructed after all the doses of agonist tested in the initial experiment to determine whether criterion levels of responding could be reached. For nalbuphine, the dose at which maximal (80%) responding occurred (i.e., 3.0 mg/kg) was used before naltrexone to determine whether criterion levels of responding could be reached. After this experiment, other drugs, including buprenorphine (0.1 and 1.0 mg/kg), diprenorphine (0.001–1.0 mg/kg), and nalorphine (0.03–10 mg/kg), were then tested in place of naltrexone after morphine pretreatment. We also examined the effects of varying agonist pretreatment time on MOR → NTX-appropriate responding by first constructing a time course for 1.7 mg/kg morphine given 1 to 24 h after testing and with the naltrexone dose and pretreatment time held constant. We then constructed naltrexone dose-response curves after morphine pretreatment at 1, 2, and 4 h before testing. In the last set of experiments, we wanted to determine whether we could elicit greater MOR → NTX-appropriate responding after a shorter buprenorphine pretreatment time. Therefore, a naltrexone dose-response curve was constructed after 1-h pretreatment with a maximal dose of buprenorphine (0.1 mg/kg). Naltrexone dose-response curves after 1-h pretreatment with the determined optimal doses of morphine and etorphine at 4 h were also constructed for comparative purposes. Naltrexone was administered 0.25 h before testing. All experiments were performed on two to six subjects.

Drugs. The drugs used and their sources are as follows: buprenorphine hydrochloride, etorphine hydrochloride, fentanyl hydrochloride, heroin hydrochloride, and meperidine hydrochloride (National Institute on Drug Abuse, Bethesda, MD); morphine sulfate (Penick, Newark, NJ); naltrexone hydrochloride and nalbuphine hydrochloride (Sigma-Aldrich, St, Louis, MO); levophanol tartrate (Hoffman-La Roche, Nutley, NJ); methadone hydrochloride (Mallinkrodt, St, Louis, MO); and nalorphine hydrochloride (Merck Research Labs, West Point, PA). Buprenorphine was dissolved in distilled water, and all of the other drugs were dissolved in normal (0.9%) saline. All drugs except nalbuphine were injected i.m. in a volume of 0.25 ml/kg body weight to minimize irritation. Doses of nalbuphine up to 3.0 mg/kg were prepared in a volume of 0.25 ml/kg body weight; higher doses were administered in a volume of 0.5 to 1.0 ml/kg due to the limited solubility of the drug. Drug doses are expressed as the free base.

Data Analysis. Stimulus-generalization data are expressed as the mean (n = 2–6) number of trials completed on the response lever appropriate for the MOR → NTX condition; the remaining trials of the session were completed on the lever appropriate for SAL → NTX. Complete substitution (generalization) for MOR → NTX was defined as completion of ≥88% of the trials in the test session on the MOR → NTX-appropriate lever. Agonist and antagonist ED50 values (i.e., the dose at which 50% of the trials are completed on the MOR → NTX-appropriate lever) were estimated for each monkey by log-linear interpolation of ascending or descending portions of the dose-response curve; group means and S.E.M. were calculated from those data. ED50 data were analyzed with either a one-factor ANOVA and Newman-Keuls test post hoc or with Student’s t test, as appropriate. The time until responding on the MOR → NTX-appropriate lever reached and returned to half its maximal value (t1/2) was calculated by linear regression of the ascending or descending part of the time-course curve. These served as estimates of the onset and offset rates of acute morphine dependence.

The latency to emit the first response was recorded and averaged over the 25 trial session. Mean group (saline or drug pretreatment) response latencies were calculated and analyzed using one-factor repeated measures ANOVA and Dunnett’s test post hoc. For control sessions in which an injection of either a drug or saline preceded an injection of saline, mean response latencies were compared with either a one-factor ANOVA followed by Newman-Keuls test or by Student’s t test, as appropriate. P values equal to or less than 0.05 were accepted as statistically significant.

Results

All of the monkeys responded within 5 s of the start of a trial, thereby avoiding the electrical stimulus to the tail. This performance was not altered within the dose ranges tested; therefore, avoidance data are not presented.

Determination of Optimal Agonist Doses. Six of the nine drugs tested dose-dependently and completely substituted for morphine when administered 4 h before testing and 3.75 h before the 0.1 mg/kg training dose of naltrexone (Fig. 1). Buprenorphine, meperidine, and nalbuphine were the only exceptions. After meperidine pretreatment, MOR → NTX-appropriate responding dose-dependently increased, but only to a maximum average of 6.5 trials (26%) at a dose
of 10 mg/kg. However, this effect was biphasic: pretreatment with 17.8 mg/kg meperidine occasioned responding exclusively on the SAL \(\rightarrow\) NTX-appropriate lever (Fig. 1) and produced seizures in one monkey (data excluded from group average). Regardless of the pretreatment dose, buprenorphine occasioned virtually no MOR \(\rightarrow\) NTX-appropriate responding. Because 0.1 mg/kg buprenorphine clearly produced observable side effects (e.g., behavioral and respiratory depression), higher doses were not tested. Pretreatment with nalbuphine dose-dependently increased MOR \(\rightarrow\) NTX-appropriate responding to a maximum average of 19.5 trials (78%) at a dose of 3.0 mg/kg; at higher doses (10 and 17.8 mg/kg), slightly fewer trials were completed on the MOR \(\rightarrow\) NTX-appropriate lever (18.5 and 18.3 trials, respectively). Comparison of agonist ED\(_{50}\) values revealed a significant difference among them (Table 1; \(F(6,30) = 87.8\)), with a resultant rank order of potency of etorphine \(\gg\) fentanyl \(\gg\) levorphanol \(\gg\) heroin \(\gg\) methadone \(\gg\) nalbuphine \(\approx\) morphine. The average latency to respond after various doses of each agonist and 0.1 mg/kg naltrexone ranged between 0.51 ± 0.07 and 1.64 ± 0.25 s and did not adversely affect responding.

**Stimulus-Generalization Curves after Different Agonist or Antagonist Pretreatment.** After pretreatment with optimal doses of the agonists etorphine, fentanyl, heroin, levorphanol, methadone, and morphine, increasing doses of naltrexone produced dose-dependent increases in MOR \(\rightarrow\) NTX-appropriate responding (Fig. 2A). The curve for 0.3 mg/kg levorphanol naltrexone was biphasic: full MOR \(\rightarrow\) NTX-appropriate responding occurred after 0.1 mg/kg naltrexone, but 40% fewer trials were completed on this lever after 1.0 mg/kg naltrexone. A similar result was seen after pretreatment with 0.03 mg/kg fentanyl, albeit to a lesser degree, because fentanyl pretreatment followed by 1.0 mg/kg naltrexone resulted in an average of 21.2 responses on the MOR \(\rightarrow\) NTX lever, just below criterion. The ED\(_{50}\) values for naltrexone after 3.75-h pretreatment with an agonist (including nalbuphine; see below) spanned only a 6.4-fold range, which nevertheless was statistically reliable (\(F(6,29) = 3.72\); Table 2). Presumably, this variance would have been even less with a finer titration of the agonist doses (i.e., finer than one-fourth log unit) in the optimal agonist doses study (see above). Regardless, only the naltrexone ED\(_{50}\) after fentanyl pretreatment differed significantly from the naltrexone ED\(_{50}\) after morphine pretreatment (Table 2).

To determine how much MOR \(\rightarrow\) NTX-appropriate responding could be attained with buprenorphine, meperidine, and nalbuphine, naltrexone dose-response curves were constructed after 0.01, 0.03, and 0.1 mg/kg buprenorphine, all the doses of meperidine used in the optimal agonist dose study, and 3.0 mg/kg nalbuphine—the dose at which the greatest MOR \(\rightarrow\) NTX-appropriate responding occurred. Me-

### TABLE 1

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Doses</th>
<th>Agonist ED(_{50}) and 95% CI</th>
<th>Potency Relative to Morphine</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etorphine</td>
<td>0.00056–0.00178</td>
<td>0.001 (0.00033, 0.0024)*</td>
<td>1600</td>
<td>4</td>
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<tr>
<td>Fentanyl</td>
<td>0.01–0.03</td>
<td>0.02 (0.017, 0.023)*</td>
<td>48</td>
<td>5</td>
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<td>Levorphanol</td>
<td>0.03–0.3</td>
<td>0.10 (0.04, 0.29)*</td>
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<tr>
<td>Heroin</td>
<td>0.1–0.56</td>
<td>0.31 (0.22, 0.44)*</td>
<td>3.0</td>
<td>4</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.1–1.78</td>
<td>0.57 (0.37, 0.89)</td>
<td>1.6</td>
<td>5</td>
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<tr>
<td>Nalbuphine</td>
<td>0.1–1.78</td>
<td>0.82 (0.14, 4.68)</td>
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<tr>
<td>Morphine</td>
<td>0.56–1.7</td>
<td>0.94 (0.60, 1.47)</td>
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<tr>
<td>Buprenorphine</td>
<td>0.01–0.1</td>
<td>Ineffective</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Meperidine</td>
<td>3.0–17.8</td>
<td>Ineffective</td>
<td></td>
<td>3*</td>
</tr>
</tbody>
</table>

CI, confidence interval.

* Significantly different from ED\(_{50}\) of morphine; one-factor ANOVA, Dunnnett’s \(t\) test post hoc, \(P < 0.05\).

\(n = 2\) at 17.8 mg/kg.
peridine substituted for morphine dose-dependently but partially, a maximum average of 11.75 trials to the MOR → NTX-appropriate lever after 3.0 mg/kg meperidine and 10 mg/kg naltrexone (Fig. 2B). Similar to the findings with meperidine, buprenorphine in combination with naltrexone failed to substitute fully for MOR → NTX (Fig. 2B). When naltrexone was preceded by 0.1 mg/kg buprenorphine, there was a dose-dependent increase in responding, reaching a maximum average of six responses at 10 mg/kg. However, pretreatment with lower doses of buprenorphine, resulted in virtually no responding on the MOR → NTX lever regardless of naltrexone dose. Unlike the findings with meperidine and buprenorphine, pretreatment with 3.0 mg/kg nalbuphine in combination with naltrexone completely substituted for MOR → NTX (Fig. 2B). Responding was naltrexone dose-dependent, reaching criterion at a dose of 1.0 mg/kg. Four-hour agonist pretreatment followed by 15-min saline pretreatment did not occasion significant MOR → NTX responding (Fig. 2, A and B).

After 4-h agonist and 15-min saline pretreatment, the average response latency ranged between 1.21 ± 0.27 and 1.93 ± 0.66 s and did not differ among agonists (data not shown). There was a significant effect of naltrexone after pretreatment with 0.01 and 0.03 buprenorphine (F[3,23] = 3.95 and F[3,23] = 6.59, respectively); etorphine (F[5,35] = 14.10); fentanyl (F[5,35] = 12.36); and 5.6, 10, and 17.8 mg/kg meperidine (F[3,23] = 4.04; F[3,23] = 28.55; and F[3,23] = 130.0, respectively). Latencies were usually lower, often significantly, after agonist followed by naltrexone pretreatment versus agonist followed by saline pretreatment. For example, the average latency to respond after etorphine followed by naltrexone (0.1 and 1.0 mg/kg) was 50 to 60% shorter compared with etorphine followed by saline (saline: 1.93 ± 0.66 s versus 0.03 mg/kg: 0.67 ± 0.01 s and 0.1 mg/kg: 0.99 ± 0.33 s).

Morphine followed by diprenorphine or nalorphine substituted for MOR → NTX fully and dose-dependently, but morphine followed by buprenorphine did not (Fig. 3). The ED50 of diprenorphine was comparable with that of naltrexone, whereas the ED50 of nalorphine was almost 50-fold higher (t[1,9] = −15.50; Table 2).

The combination of saline followed by diprenorphine or buprenorphine occasioned little or no MOR → NTX-appropriate responding (Fig. 3), even, in the case of diprenorphine, at doses up to 80-fold higher than the ED50 after morphine pretreatment (Table 2). Similarly, no MOR → NTX-appropriate responding occurred after pretreatment with saline fol-

**TABLE 2**

The effects of opioid agonists 1 to 4 h prior to testing on the ED50 of opioid antagonists administered 15 min prior to testing.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Dose (mg/kg)</th>
<th>Pretreatment Time</th>
<th>Antagonist</th>
<th>Antagonist ED50 (mg/kg) and 95% CI</th>
<th>Potency Relative to 4 h MOR → NTX</th>
<th>n</th>
</tr>
</thead>
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<tr>
<td>Buprenorphine</td>
<td>0.1</td>
<td>1</td>
<td>Naltrexone</td>
<td>0.042 (0.006, 0.28)</td>
<td>0.54</td>
<td>4</td>
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<tr>
<td></td>
<td>0.01</td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.03</td>
<td>4</td>
<td></td>
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<td></td>
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<td>0.1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Etorphine</td>
<td>0.00178</td>
<td>1</td>
<td>Naltrexone</td>
<td>0.012 (0.002, 0.061)</td>
<td>1.88</td>
<td>4</td>
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<tr>
<td></td>
<td>0.00178</td>
<td>4</td>
<td>Naltrexone</td>
<td>0.077 (0.030, 0.19)</td>
<td>0.30</td>
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<tr>
<td>Fentanyl</td>
<td>0.03</td>
<td>4</td>
<td>Naltrexone</td>
<td>0.012 (0.007, 0.022)</td>
<td>1.85</td>
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<tr>
<td>Heroin</td>
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<td>4</td>
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<td>0.036 (0.012, 0.11)</td>
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<tr>
<td></td>
<td>5.6</td>
<td>4</td>
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<tr>
<td></td>
<td>10</td>
<td>4</td>
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<td>17.8</td>
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<tr>
<td>Methadone</td>
<td>1.0</td>
<td>4</td>
<td>Naltrexone</td>
<td>0.024 (0.003, 0.22)</td>
<td>0.97</td>
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<tr>
<td>Morphine</td>
<td>1.7</td>
<td>1</td>
<td>Naltrexone</td>
<td>0.17 (0.094, 0.303)*</td>
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<td>Naltrexone</td>
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<td></td>
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<td>4</td>
<td>Naltrexone</td>
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<tr>
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<td>Diprenorphine</td>
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<td>Nalorphine</td>
<td>1.09 (0.25, 4.65)*</td>
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<tr>
<td></td>
<td>1.7</td>
<td>4</td>
<td>Buprenorphine</td>
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<tr>
<td>Nalbuphine</td>
<td>3.0</td>
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<td>Naltrexone</td>
<td>0.06 (0.019, 0.22)</td>
<td>0.34</td>
<td>4</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>4</td>
<td>Diprenorphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>Nalorphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>Buprenorphine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from ED50 of 4 h morphine pretreatment group; one-factor ANOVA, Dunnett’s t test post hoc, P < 0.05.
† Significantly different from ED50 of 4 h morphine pretreatment group followed by naltrexone; Student’s t test, P < 0.05.

Fig. 3. Diprenorphine and nalorphine but not buprenorphine substitute for naltrexone after 4-h pretreatment with morphine. The discriminative stimulus effects of 1.7 mg/kg MOR or saline followed by BUP (0.01 and 0.1 mg/kg s.c.; 0.25-h pretreatment), diprenorphine (DIP; 0.001–1.0 mg/kg s.c.; 0.25-h pretreatment), or nalorphine (NAL; 0.03–10 mg/kg s.c.; 0.25-h pretreatment) were determined. MOR → NTX curve is reproduced from ASPET Journals on October 17, 2017 jpet.aspetjournals.org Downloaded from
allowed 3.75 h later by 1.0 mg/kg nalorphine (Fig. 3), a dose equivalent to the ED$_{50}$ of nalorphine after morphine pretreatment (Table 2). However, a 10-fold higher dose of nalorphine elicited an average of 11.25 trials to the MOR treatment (Table 2). A 10-fold higher dose of nalorphine, diprenorphine, or buprenorphine after saline pretreatment on MOR latency to respond (data not shown). Neither diprenorphine (F[1,11] = 0.17) or nalorphine (F[1,7] = 0.001) altered average response latency after saline pretreatment (data not shown). The average latency to respond after saline and 0.1 mg/kg buprenorphine was 1.53 s.

**Temporal Dependence of MOR → NTX-Appropriate Responding.** To determine the temporal dependence of morphine pretreatment on MOR → NTX-appropriate responding, pretreatment with 1.7 mg/kg morphine was systematically varied from 0.5 to 24 h, whereas pretreatment with 0.1 mg/g naltrexone remained constant at 0.25 h (Fig. 4). At 1 h after the morphine injection, an average of 5.7 (approximately 23% of maximum) responses occurred on the MOR → NTX lever. However, MOR → NTX-appropriate responding reached criterion levels when pretreatment was lengthened to 2 h and remained there as the pretreatment intervals was lengthened further to 4, 6, or 8 h (Fig. 4). Responding occurred exclusively on the SAL → NTX-appropriate lever when the morphine pretreatment was 24 h. The onset and offset of MOR → NTX-appropriate responding occurred with $t_{1/2}$ of 1.68 ± 0.07 and 12.74 ± 1.47 h, respectively. A number of studies have reported signs of spontaneous withdrawal after acutely administered morphine (for review, see Harris and Gewirtz, 2005), including subjective signs in humans (Kirby and Stitzer, 1993). Morphine followed 16 h later by saline resulted only in responding on the SAL → NTX-appropriate lever (Fig. 4), eliminating the possibility that interoceptive cues associated with spontaneous withdrawal after morphine administration at longer pretreatment intervals contributed to MOR → NTX-appropriate responding.

Given that the interoceptive effects of withdrawal from acute dependence are a function of morphine dose and duration of morphine exposure, a shorter pretreatment interval should produce less dependence and smaller interoceptive effects. As a result, a larger antagonist dose would be required to produce discriminative stimulus effects equivalent to those occurring during training. Therefore, we assessed MOR → NTX-appropriate responding at 1, 2, and 4 h after morphine pretreatment (Fig. 5A). Compared with pretreatment with 1.7 mg/kg morphine at 4 h, shorter pretreatment times of 2 and 1 h resulted in rightward shifts of the morphine-naltrexone stimulus-generalization curve. There was a significant effect of morphine pretreatment time on the ED$_{50}$ values of naltrexone (Table 2; F[2,13] = 19.83), with approximately 2- and 7-fold higher ED$_{50}$ values after 2- and 1-h pretreatment, respectively.

Four-hour pretreatment with buprenorphine resulted in very little MOR → NTX-appropriate responding. To determine whether greater responding could be achieved at an earlier time point, we assessed the effects of 1-h buprenorphine (0.1 mg/kg) pretreatment on MOR → NTX-appropriate responding. For comparative purposes, we included a naltrexone dose-response curve after 1-h pretreatment with the optimal dose etorphine (0.00178 mg/kg; Fig. 5B).

One-hour etorphine pretreatment followed by naltrexone dose-dependently produced MOR → NTX-appropriate responding, which reached maximum at a dose of 0.3 mg/kg naltrexone (Fig. 5B). Consistent with the outcome after 1 and 4 h morphine pretreatment (Fig. 5A), the etorphine → naltrexone curve 1 h after etorphine pretreatment was shifted to the right of the 4 h curve, and naltrexone had a 3.5-fold higher ED$_{50}$ (Table 2). The dose-response curves for 0.1 mg/kg buprenorphine at 1- and 4-h pretreatment followed by naltrexone were very similar to each other (Fig. 5B); there was little MOR → NTX-appropriate responding, even at doses as high as 3.0 and 10 mg/kg naltrexone.
pretreatment with 0.1 mg/kg buprenorphine (BUP) or 1.78 mg/kg etorphine but not buprenorphine pretreatment time and naltrexone dose. A, Buprenorphine and meperidine paired with naltrexone occasionally elicited criterion levels of responding in combination with the NTX-appropriate lever, even at doses that were behaviorally active and were well within the range of doses shown to be effective in other behavioral models using squirrel monkeys (Schaefer and Holtzman, 1977; Dykstra, 1983, 1985; DeRossett and Holtzman, 1984; Negus et al., 1991; Dykstra et al., 2002; Allen et al., 2003). Likewise, pretreatment with the partial μ-opioid receptor agonist nalbuphine in combination with 0.1 mg/kg naltrexone failed to elicit criterion levels of responding, even at a dose 10 times higher than that of the training dose of morphine. These results might be a consequence of the training doses used. Perhaps greater responding would have occurred if the monkeys had been trained with a lower dose of morphine and/or naltrexone, resulting in a lower degree of dependence and less intense discriminative stimuli with less stringent efficacy requirements. The degree of opioid dependence can alter efficacy requirements and subsequently impact the qualitative effects of morphine-like drugs. For example, in opioid-dependent rhesus monkeys trained to discriminate 0.0178 mg/kg naltrexone from saline, buprenorphine exhibited agonistic or antagonistic properties, depending on the drug (morphine or L-α-acetylmethadol) upon which the monkeys were dependent (Sell et al., 2003). There are also examples of lower efficacy agonists substituting completely for higher efficacy agonists when the training dose of the latter was low and substituting only partially or not at all when the training dose was high (Shannon and Holtzman, 1979; Picker et al., 1993; Holtzman, 1997). When we used nalbuphine in combination with a higher dose of naltrexone, criterion MOR → NTX responding was attained, demonstrating agonist efficacy requirements can be overcome with higher doses of naltrexone.

Buprenorphine, which binds the μ-opioid receptor with very high affinity and has slow pharmacokinetics (for a recent review, see Tzschentke, 2002), produces long-lasting behavioral effects (Dykstra, 1983; DeRossett and Holtzman, 1984). This could account for the fact that there was not an increase in MOR → NTX responding even at the highest dose of naltrexone. Our inability to precipitate withdrawal from buprenorphine is consistent with the clinical literature (Heel et al., 1979; Lewis, 1985; Lange et al., 1990; Bickel and Amass, 1995; Ling et al., 1998). However, the opioid antagonist naloxone administered almost 2 h after buprenorphine completely reversed the effect of buprenorphine on squirrel monkeys responding in a shock-titration procedure (Dykstra, 1985).

High agonist affinity for the receptor and slow dissociation from it cannot account for the failure of meperidine followed by naltrexone to substitute completely for MOR → NTX. It is well documented that one of the metabolites of meperidine, normeperidine, is a convulsant (Dykstra and Leander, 1978; Leander and Carter, 1982), effectively limiting the dose range at which meperidine can be tested. However, in the dose range tested this study, meperidine was dose-dependently and fully generalized to morphine by squirrel monkeys trained to discriminate morphine (3.0 mg/kg) from saline (Schaefer and Holtzman, 1977). Meperidine also elicits morphine-like analgesia and suppresses scheduled controlled responding (Leander, 1980; Witkin et al., 1983). The latter effect is antagonized by naloxone and exhibits cross-tolerance with morphine (Witkin et al., 1983), indicating that morphine and meperidine share a common site of action.

Our data suggest there is a minimum agonist efficacy requirement for MOR → NTX-like stimulus control of behavior when the training dose of naltrexone is administered. Buprenorphine and meperidine paired with naltrexone occasioned little or no responding on the MOR → NTX-appropri-
that the discriminative effects associated with MOR \rightarrow NTX are similar in rats and squirrel monkeys (Easterling and Holtzman, 1999; White and Holtzman, 2003). However, broadening the pharmacological characterization of the phenomenon has revealed differences between the two species. In rats trained to discriminate 4-h pretreatment with 10 mg/kg morphine and 15-min pretreatment with 0.3 mg/kg naltrexone from pretreatment with saline and naltrexone, only heroin and levorphanol substituted completely for morphine when administered acutely in combination with naltrexone (Holtzman, 2003). Other agonists substituted for morphine only partially, regardless of whether their intrinsic efficacy was higher (e.g., etorphine, fentanyl, and methadone) or lower (e.g., buprenorphine and meperidine) than that of morphine. In the present study, only lower intrinsic efficacy drugs failed to substitute for morphine when the training dose of naltrexone was administered, suggesting a minimum efficacy requirement. It seems that once this efficacy requirement has been satisfied or overcome with a higher dose of naltrexone, as with nalbuphine, \( \mu \)-opioid agonists combined with naltrexone more uniformly produce the MOR \rightarrow NTX-associated cues in the squirrel monkey than they do in the rat. The results of our study are consistent with the clinical literature on acute opioid dependence (Wright et al., 1991; Greenwald et al., 1996) and provide one more example of interspecies differences in opioid pharmacology.

The pure antagonist diprenorphine, but not the partial \( \mu \)-opioid agonist nalorphine, fully substituted for naltrexone in rats (Holtzman, 2003). This discrepancy was attributed to the fact that diprenorphine, like naltrexone, is devoid of intrinsic efficacy, whereas nalorphine is not (Holtzman, 2003). In our study, full dose-dependent stimulus generalization occurred after both diprenorphine and naltrexone in monkeys pretreated with morphine. Together with data from our previous report (White and Holtzman, 2003), the relative antagonist potency of naltrexone = diprenorphine \( \geq \) naloxone = nalorphine reflects the affinity of those drugs for the \( \mu \)-opioid receptor. Thus, in this case it was the action of an antagonist that differed between rats and squirrel monkeys.

Our results revealed a significant effect of morphine pretreatment time on MOR \rightarrow NTX responding. Monkeys responded at criterion levels of MOR \rightarrow NTX-appropriate responding as early as 2 h after morphine, and significant MOR \rightarrow NTX responding persisted as long as 16 h after morphine administration. This is in contrast to our previous report, where criterion MOR \rightarrow NTX responding only occurred at 4 h after morphine administration (White and Holtzman, 2003). It is possible that classic conditioning occurred to the unconditioned stimulus effects of naltrexone after morphine or perhaps to naltrexone alone. However, reassessment of the naltrexone dose-response curve after morphine pretreatment revealed that the stimulus-generalization curve was unchanged from one study to the next: the naltrexone \( ED_{50} \) values were virtually identical between the two studies. Additionally, in our previous study (White and Holtzman, 2003), sensitization to the stimulus effects of naltrexone did not occur after repeated training or testing; the combination of saline pretreatment and as much as 1.0 mg/kg NTX exclusively elicited selection of the SAL \rightarrow NTX-appropriate lever. The reason(s) why the time course of discriminative effects changed between studies, whereas the morphine \rightarrow naltrexone stimulus-generalization curve did not, is obscure.

References


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