Relative Efficacy of Buprenorphine, Nalbuphine and Morphine in Opioid-Treated Rhesus Monkeys Discriminating Naltrexone

STACY L. SELL, LANCE R. MCMAHON, and CHARLES P. FRANCE
Departments of Pharmacology (S.L.S., L.R.M., C.P.F.) and Psychiatry (C.P.F.), the University of Texas Health Science Center at San Antonio, San Antonio, Texas

Received March 7, 2003; accepted May 16, 2003

ABSTRACT
Efficacy is one determinant of whether a drug is an agonist or an antagonist under a particular set of conditions. Relative efficacy among the μ opioid receptor (MOR) ligands buprenorphine, nalbuphine, and morphine was examined in monkeys dependent on morphine (3.2 mg/kg/day) or L-α-acetylmethadol (LAAM) (1.0 mg/kg twice daily) and that discriminated naltrexone (0.0178 mg/kg) from saline. In morphine-treated monkeys, buprenorphine and not nalbuphine substituted for naltrexone. When administered before naltrexone in morphine-treated monkeys, morphine and nalbuphine shifted the naltrexone dose-effect curve to the right, while buprenorphine shifted the naltrexone dose-effect curve to the left. Under conditions of acute morphine deprivation, naltrexone-lever responding was slightly attenuated by buprenorphine and markedly attenuated by nalbuphine and morphine. In LAAM-treated monkeys, buprenorphine substituted completely for naltrexone in only one monkey, while nalbuphine and morphine failed to substitute in any monkey. When administered before naltrexone in LAAM-treated monkeys, buprenorphine, nalbuphine, and morphine dose dependently shifted the naltrexone dose-effect curve to the right, with the exception of one monkey in which buprenorphine shifted the naltrexone dose-effect curve to the left. These results demonstrate that a low efficacy MOR ligand can exert agonist or antagonist actions in the same animal depending on immediate pharmacologic history. The qualitatively different effects of buprenorphine in morphine- and LAAM-treated monkeys might be related to magnitude of dependence insofar as dependence can determine the efficacy required for agonist activity. Thus, buprenorphine has markedly different effects across different levels of opioid dependence.

Efficacy characterizes the nature of an interaction between a drug and a receptor and is one factor that determines whether a drug has agonist or antagonist effects under a particular set of conditions. The therapeutic and abuse-related effects of μ opioid receptor (MOR) ligands are related to their efficacy at MOR (for review, see Bergman et al., 2000), with high efficacy conferring agonist activity across a broader range of conditions than low efficacy at MOR. For example, high efficacy at MOR is required for antinociceptive effects at increasing levels of thermal stimulation (Gerak et al., 1994; Walker et al., 1995), and high efficacy at MOR confers greater respiratory depression than low efficacy at MOR (Liguori et al., 1996). Relative efficacy among MOR ligands also determines whether a MOR ligand exerts antagonist actions. For example, a low efficacy MOR ligand that does not exert agonist actions can, under some conditions, antagonize a high-efficacy MOR ligand (Walker et al., 1995; Liguori et al., 1996). Thus, efficacy is one characteristic of a drug-receptor interaction that determines the level of effect and whether any particular drug will appear to be an agonist or an antagonist.

Administration of a long-acting opioid agonist is a general pharmacologic strategy for treating opioid abuse (e.g., substitution therapy). This strategy relies on opioids that have sufficient efficacy both to prevent the emergence of withdrawal and to confer cross-tolerance to other opioids (e.g., methadone and LAAM; for review, see Kreek, 2000). Another strategy relies on opioids with little or no efficacy (e.g., naloxone) to prevent the actions of drugs with higher efficacy and, specifically, opioids that are likely to be abused. More recently, the low-efficacy MOR agonist buprenorphine and a combined formulation of buprenorphine and naloxone have been approved in the United States for the treatment of opioid abuse. The extent to which treatment outcome is better or worse as efficacy increases or decreases is far from clear, although the indication of buprenorphine for the treatment of heroin abuse will provide one important clinical test of this relationship.

Many bioassays, particularly in vivo, are sensitive to either agonist or antagonist actions of a drug; however, some assays

ABBREVIATIONS: MOR, μ opioid receptor; LAAM, L-α-acetylmethadol; FR, fixed ratio; CL, confidence limits; NMDA, N-methyl-D-aspartate.
can detect both agonist and antagonist actions of a drug. For example, a naltrexone discrimination procedure in morphine-treated monkeys is sensitive to both agonism and antagonism under two qualitatively distinct conditions (France and Woods, 1989). Morphine-treated monkeys are particularly sensitive to MOR antagonists, whereas monkeys acutely deprived of morphine and responding on the naltrexone-lever are sensitive to MOR agonists. Morphine treatment in this discrimination assay is predicted to increase the degree of receptor stimulation required for agonist effects relative to other drug discrimination assays in which untreated subjects are trained to discriminate moderate doses of a MOR agonist. Thus, morphine treatment might increase the probability that a low-efficacy MOR agonist will have effects that are intermediate to morphine and naltrexone. One goal of the present study was to examine whether low-efficacy MOR ligands have partial effects (agonism and antagonism) that covary with immediate pharmacologic history (morphine treatment or acute abstinence). Buprenorphine and nalbuphine were chosen for study because they have high binding affinity and low efficacy at MOR and they have behavioral effects that are mediated by MOR (Richards and Sadee, 1985; De Souza et al., 1988; Young et al., 1991; Walker et al., 1994). In addition, buprenorphine and nalbuphine precipitate withdrawal in rhesus monkeys treated with 12 mg/kg/day morphine (Woods and Gmerek, 1985), suggesting that both compounds can have antagonist actions in opioid dependent animals.

Naltrexone also has been established as a discriminative stimulus in rhesus monkeys treated with the MOR agonist LAAM (1.0 mg/kg, twice daily), and this discrimination has been used to evaluate behavioral and neuropharmacologic features of LAAM dependence and withdrawal (Brandt and France, 1998; Sell and France, 2002). It is not currently known whether the magnitude of dependence that results with LAAM or morphine differs under these particular treatment conditions. One postulate of receptor theory is that tolerance increases the magnitude of receptor activation (e.g., efficacy) required for agonist effects, e.g., tolerance inversely correlates with agonist activity. Although the relationship between dependence and agonist activity has been studied less extensively than the relationship between tolerance and agonist activity, dependence also is presumed to be inversely related to agonist activity. The present study examined whether buprenorphine and nalbuphine exert more or less agonist activity depending on the level of tolerance and dependence that might result from morphine or LAAM treatment.

Materials and Methods

Subjects. Seven adult rhesus monkeys (Macaca mulatta, two males and five females; 5.5 to 9.0 kg) were housed individually in stainless steel cages with free access to water. Monkeys received chow (high-protein monkey diet; Harlan Teklad, Madison, WI) twice daily and fresh fruit following experimental sessions. All subjects were previously trained to respond under fixed-ratio (FR) schedules (stimulus shock termination) and had received opioid agonists and antagonists in previous studies (Gauthier and France, 1999; Sell and France, 2002). Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee (The University of Texas Health Science Center, San Antonio, TX), as well as the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council; Department of Health, Education and Welfare, publication no. (NIH) 85–23, revised 1986).

Apparatus. Monkeys were seated in primate chairs (Model R001, Primate Products; Miami, FL) that provided restraint at the neck and shoulders. During experimental sessions, monkeys were placed in ventilated, sound-attenuating operant chambers containing two response levers and two red lights, one light above each lever. Each chair was equipped with a pair of shoes containing brass electrodes for delivering a brief shock (250 ms, 3 mA) from a remote A/C generator. Experimental procedures were controlled and data collected by a microprocessor and commercially available software (Med Associates, Inc., St. Albans, VT).

Behavioral Procedure. Four of the monkeys were treated twice daily with LAAM (1.0 mg/kg, s.c.) 8 to 9 h apart. This treatment has been shown to produce physical dependence (Brandt and France, 1998). The other three monkeys received morphine (3.2 mg/kg) once daily. Subsequently, both groups were trained to discriminate naltrexone (0.0178 mg/kg) from saline. Experimental sessions began 3 h after the morning injection of morphine or 7 h after the first daily injection of LAAM (France and Woods, 1989; Brandt and France, 1998). Each session consisted of two to eight 15-min cycles with each cycle beginning with a 10-min timeout during which the chamber was dark and lever presses had no programmed consequence. This was followed by a 5-min response period during which monkeys could respond under an FR5 schedule of stimulus-shock termination with shocks scheduled to occur every 15 s. The lights were illuminated at the beginning of the 15-s period, and monkeys could postpone scheduled shock for 30 s by completing five consecutive responses on the correct lever. The correct lever was determined by an injection of either saline or naltrexone (0.0178 mg/kg) administered during the first minute of the cycle. The right lever was correct following saline, and the left lever was correct following naltrexone for three monkeys (one morphine-treated and two LAAM-treated monkeys), whereas the right lever was correct following naltrexone and the left lever was correct following saline for the other four monkeys. Responses on the incorrect (injection-inappropriate) lever reset the response requirement on the correct (injection-appropriate) lever. Failure to satisfy the FR within 15 s resulted in the delivery of shock. After 5 min or four shocks, the response period ended, and the lights were extinguished. One “sham” injection cycle followed a cycle in which naltrexone was administered, and zero to six saline injection cycles preceded the naltrexone-injection cycle. On some training days, monkeys received only saline or “sham” before each of two to eight cycles.

Test drugs were administered every second or third day as long as performance during intervening training sessions satisfied the following criteria: at least 80% of responses on the injection-appropriate lever and fewer than five responses on the injection-inappropriate lever before the first reinforcer. Parameters for test sessions were the same as for training sessions except that five consecutive responses on either lever postponed scheduled shock. For substitution tests, on a day of morphine or LAAM treatment, saline was administered at the beginning of the first cycle, followed by naltrexone or a test compound at the beginning of subsequent cycles with dose increasing in 0.5 log U increments/cycle. In morphine-treated monkeys, test drugs were studied after 27 h of morphine deprivation by administering saline instead of 3.2 mg/kg morphine 3 h before a test session that consisted of one saline cycle followed by increasing doses of drug on subsequent cycles. Doses of test compounds were as follows: naltrexone (0.001–0.1 mg/kg), buprenorphine (0.0032–3.2 mg/kg), nalbuphine (0.1–32.0 mg/kg), and morphine (0.1–10.0 mg/kg). The effects of drugs were not studied after LAAM deprivation because the temporal pattern of responding obtained after discontinuation of LAAM treatment is not sufficiently homogeneous among individual monkeys for this type of study, presumably due to pharmacokinetic differences across monkeys (e.g., Brandt and France, 1998). For studies in which a test drug was combined with naltrex-
one, a dose of the test drug was administered at the beginning of the first cycle, followed by increasing doses of naltrexone (0.5 log U increments) at the beginning of subsequent cycles, up to doses that produced at least 80% responding on the naltrexone lever or to a cumulative dose of 1.0 mg/kg. Doses of test compounds studied in combination with naltrexone were as follows: buprenorphine (0.01–1.0 mg/kg), nalbuphine (0.32–10.0 mg/kg), and morphine (3.2–32.0 mg/kg).

**Drugs.** Drugs were administered s.c. in a volume of 0.1 to 3.0 ml, and doses were expressed in the forms listed below. The compounds studied were nalbuphine hydrochloride (Mallinckrodt, Inc., St. Louis, MO), buprenorphine hydrochloride, naltrexone hydrochloride, morphine sulfate, and LAAM (The Research Technology Branch, National Institute of Drug Abuse, Rockville, MD). LAAM was dissolved in a vehicle containing 77.5% sterile water, 15% Emulphor, and 7.5% ethanol and was heated and sonicated. All other drugs were dissolved in sterile saline, heated, and sonicated as needed.

**Data Analyses.** Drug discrimination data were plotted as the percentage of total responses on the drug-appropriate lever (%DR) averaged among monkeys (±S.E.M.) and plotted as a function of dose. When a test with a given compound was conducted more than once, the determinations were averaged for an individual subject for further analyses. Doses of naltrexone to occasion 50% drug lever responding (ED$_{50}$) and 95% confidence limits (95% CL) were estimated using interpolation or linear regression using the portion of the dose-effect curve spanning 50% drug-lever responding. When the smallest dose combination of buprenorphine and naltrexone did not occasion less than 50% naltrexone-lever responding in morphine-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. When the largest dose combination of buprenorphine and naltrexone did not occasion greater than 50% naltrexone-lever responding in LAAM-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. Naltrexone ED$_{50}$s after treatment with a test compound were determined using interpolation or linear regression using the portion of the dose-effect curve spanning 50% drug-lever responding. When the smallest dose combination of buprenorphine and naltrexone did not occasion less than 50% naltrexone-lever responding in morphine-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. When the largest dose combination of buprenorphine and naltrexone did not occasion greater than 50% naltrexone-lever responding in LAAM-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. Naltrexone ED$_{50}$s after treatment with a test compound were determined using interpolation or linear regression using the portion of the dose-effect curve spanning 50% drug-lever responding. When the smallest dose combination of buprenorphine and naltrexone did not occasion less than 50% naltrexone-lever responding in morphine-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. When the largest dose combination of buprenorphine and naltrexone did not occasion greater than 50% naltrexone-lever responding in LAAM-treated monkeys, that dose of naltrexone was assigned the ED$_{50}$. Naltrexone ED$_{50}$ values determined every 2 to 4 weeks in each monkey throughout the course of the experiment. Data were calculated for individual monkeys and then averaged; the magnitude of change from the control ED$_{50}$ was determined from the averaged data.

**Results**

**Effects of MOR Agonists in Morphine-Treated Monkeys.** The naltrexone dose-effect curves were highly consistent over time, with the overall average ED$_{50}$ (95% CL) for naltrexone being 0.005 mg/kg (0.002–0.009; Table 1). Monkeys responded predominantly on the naltrexone-lever at doses of 0.01 to 0.032 mg/kg naltrexone (Fig. 1, open circles). The group average control (saline) response rate was 2.39 ± 0.63 responses/s; naltrexone did not alter response rate (data not shown).

In morphine-treated monkeys, buprenorphine occasioned a maximum of 82 ± 15% naltrexone-lever responding at a dose of 1.0 mg/kg (Fig. 1, open squares). In contrast, nalbuphine (0.1–10.0 mg/kg) occasioned predominantly saline-lever responding (Fig. 1, open triangles), with the exception of 10 mg/kg nalbuphine in one monkey, which occasioned a maximum of 60% naltrexone-lever responding. Morphine (0.32–10.0 mg/kg) occasioned saline-lever responding in morphine-treated monkeys (Fig. 1, open inverted triangles). Up to the largest doses tested, buprenorphine, nalbuphine, and morphine did not alter response rate in morphine-treated monkeys (data not shown).

When saline was substituted for the daily morphine injection (i.e., 27-h morphine-deprived), monkeys responded predominantly on the naltrexone-lever (Fig. 1, closed symbols above S, all panels). Morphine dose-effect curves were determined every 4 weeks during the course of the experiment, generating four dose-effect curves per animal. In each test, morphine attenuated the naltrexone-lever responding occasioned by morphine deprivation, with all monkeys responding predominantly on the saline-lever at doses of 3.2 and 10 mg/kg morphine (Fig. 1, closed inverted triangles). The morphine dose-effect curves were stable, with the overall average ED$_{50}$ (95% CL) being 0.90 (0.21–1.93) mg/kg morphine. Response rate was not altered 27 h after morphine administration or by readministration of morphine under conditions of morphine deprivation (data not shown).

In morphine-deprived monkeys, nalbuphine dose dependently decreased naltrexone-lever responding to 18% at a dose of 10 mg/kg (Fig. 1, closed triangles). Buprenorphine also decreased naltrexone-lever responding, although to a lesser extent than nalbuphine; a dose of 0.032 mg/kg buprenorphine decreased naltrexone-lever responding to 42%.

### Table 1

<table>
<thead>
<tr>
<th>Test Dose</th>
<th>RE</th>
<th>CH</th>
<th>BU</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.009</td>
<td>0.005</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>(0.005–0.011)</td>
<td>(0.004–0.006)</td>
<td>(0.001–0.003)</td>
<td>(0.002–0.009)</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>0.009</td>
<td>N.T.</td>
<td>0.005</td>
<td>0.007</td>
</tr>
<tr>
<td>10.0</td>
<td>0.012</td>
<td>0.021</td>
<td>0.015</td>
<td>0.016</td>
</tr>
<tr>
<td>32.0</td>
<td>0.013</td>
<td>0.057</td>
<td>0.028</td>
<td>0.034</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.32</td>
<td>0.003</td>
<td>N.T.</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>1.0</td>
<td>0.008</td>
<td>0.005</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>3.2</td>
<td>0.018</td>
<td>0.021</td>
<td>0.001</td>
<td>0.013</td>
</tr>
<tr>
<td>10.0</td>
<td>0.013</td>
<td>0.061</td>
<td>0.048</td>
<td>0.041</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.006</td>
<td>0.003</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>0.032</td>
<td>0.002</td>
<td>0.003</td>
<td>&lt;0.00032</td>
<td>0.0008</td>
</tr>
<tr>
<td>0.1</td>
<td>0.005</td>
<td>0.001</td>
<td>&lt;0.00032</td>
<td>0.001</td>
</tr>
</tbody>
</table>

N.T., not tested.
and larger doses, up to 1.0 mg/kg, did not further attenuate naltrexone-lever responding (Fig. 1, closed squares). In morphine-deprived monkeys, responding occurred predominantly on the naltrexone-lever after administration of naltrexone (0.001–0.032 mg/kg; Fig. 1, closed circles). At doses attenuating naltrexone-lever responding in morphine-deprived monkeys, nalbuphine slightly increased response rate, whereas buprenorphine and naltrexone did not alter response rate (data not shown).

Various doses of morphine, nalbuphine, and buprenorphine were administered before redetermination of a naltrexone dose-effect curve under conditions of morphine treatment. Pretreatment with morphine attenuated the naltrexone discriminative stimulus as evidenced by an increase in the naltrexone ED\textsubscript{50}. Thus, 10.0 and 32.0 mg/kg morphine shifted the naltrexone dose-effect curve 3.2- and 6.8-fold to the right (Table 1; Fig. 2, top). Pretreatment with nalbuphine also attenuated the naltrexone discriminative stimulus. Doses of 3.2 and 10.0 mg/kg nalbuphine increased the naltrexone ED\textsubscript{50} by 2.6- and 8.2-fold, respectively (Table 1; Fig. 2, middle); smaller doses (0.32 and 1.0 mg/kg) of nalbuphine did not substantially modify the naltrexone discriminative stimulus. In contrast, pretreatment with buprenorphine enhanced the naltrexone discriminative stimulus. Thus, doses of 0.032 and 0.1 mg/kg buprenorphine shifted the naltrexone dose-effect curve 6.3- and 5.0-fold to the right as evidenced by a decrease in the ED\textsubscript{50} of naltrexone (Table 1; Fig. 2, bottom).

Effects of MOR Agonists in LAAM-Treated Monkeys.

Naltrexone dose-effect curves (four per monkey) were determined every 2 to 4 weeks throughout the course of the experiment. The naltrexone dose-effect curves were stable, with the overall average ED\textsubscript{50} (95% CL) for naltrexone being 0.006 mg/kg (0.004–0.008; Table 2), a value similar to that in morphine-treated monkeys. Monkeys responded predominantly on the naltrexone-lever at doses of 0.01 to 0.1 mg/kg naltrexone (Fig. 3, circles). The group average control response rate was 1.77 ± 0.10 responses/s; naltrexone did not alter response rate in LAAM-treated monkeys (data not shown).

Buprenorphine (0.032–3.2 mg/kg) occasioned predominantly saline-lever responding in LAAM-treated monkeys, with the exception of one monkey (OP) for which a dose of 3.2 mg/kg buprenorphine occasioned 100% responding on the naltrexone-lever (Fig. 3, squares). Nalbuphine also occasioned predominantly saline-lever responding in LAAM-treated monkeys (Fig. 3, triangles). Buprenorphine and nalbuphine did not alter response rate in LAAM-treated monkeys (data not shown).

Various doses of morphine, nalbuphine, and buprenorphine were administered before redetermination of a naltrexone dose-effect curve in LAAM-treated monkeys. Pretreatment with morphine attenuated the naltrexone discriminative stimulus as evidenced by an increase in the naltrexone ED\textsubscript{50}. Thus, 3.2, 10.0, and 32.0 mg/kg morphine dose dependently shifted the naltrexone dose-effect curve 2.7-, 2.7-, and 9.8-fold to the right (Table 2; Fig. 4, top). Pretreatment with nalbuphine also attenuated the naltrexone discriminative stimulus as evidenced by an increase in the naltrexone ED\textsubscript{50}. Thus, 1.0, 3.2, and 10.0 mg/kg nalbuphine dose dependently shifted the naltrexone dose-effect curve 2.7-, 6.5-, and 23.2-fold to the right (Table 2; Fig. 4, top). Pretreatment with buprenorphine also attenuated the naltrexone discriminative stimulus in three of four monkeys; however, in a fourth LAAM-treated monkey (OP), buprenorphine enhanced the naltrexone discriminative stimulus. When the data were averaged for the entire group, doses of 0.032, 0.1, 0.32, and 1.0 mg/kg buprenorphine shifted the naltrexone dose effect

![Fig. 1. Effects of naltrexone, buprenorphine, nalbuphine, and morphine in morphine-treated monkeys discriminating naltrexone from saline. Abscissae: dose in milligrams per kilogram body weight; saline (S) was administered in the first cycle. Ordinates: mean (±S.E.M.) percentage of responding on the naltrexone-lever (%DR = drug responding).](image-url)
The relationship between therapeutic utility in the treatment of opioid abuse and efficacy at MOR is not well understood. Long-acting MOR agonists such as methadone and LAAM can decrease heroin abuse (for review, see Kreek, 2000), and their effectiveness might be related to substitution for some subjective effects of heroin and to attenuation of withdrawal (Dole and Nyswander, 1965; Schuster et al., 1995; Houtsomuller et al., 1998). Drugs with lower efficacy than methadone and LAAM might be alternative treatments with less abuse and dependence liability. For example, buprenorphine is a low efficacy agonist that attenuates some abuse-related effects of heroin and other opioids (Greenwald et al., 2002; Strain et al., 2002); however, it is not clear whether buprenorphine has less abuse and dependence liability than other MOR agonists (Woods and Gmerek, 1985; Eissenberg et al., 1996; Winger and Woods, 2001; Comer and Collins, 2002).

The results of this study demonstrate the utility of drug discrimination for obtaining highly quantitative data on the relative efficacy of drugs under conditions of chronic opioid treatment and acute abstinence in nonhuman primates. Whereas many assays are sensitive to only agonism or antagonism, the naltrexone discriminative stimulus in morphine-treated monkeys was sensitive to both agonism and antagonism under two qualitatively distinct conditions. Morphine treatment was sensitive to antagonism as evidenced by substitution of buprenorphine for naltrexone. In contrast, nalbuphine and morphine did not have naltrexone-like effects in morphine-treated monkeys. When the same monkeys were acutely deprived of morphine, naltrexone-lever responding was slightly attenuated by buprenorphine and markedly attenuated by nalbuphine and morphine. Thus, depending on the immediate pharmacologic history of morphine-treated monkeys, buprenorphine was an antagonist or an agonist, whereas nalbuphine was only an agonist, suggesting that buprenorphine has lower efficacy than nalbuphine. While these results are consistent with previous studies in rhesus monkeys (Woods and Gmerek, 1985; Walker et al., 1995), they differ from studies reporting that buprenorphine has higher efficacy than nalbuphine in rats (Zimmerman et al., 1987; Morgan and Picker, 1998; Walker and Young, 2002). It is not clear whether these apparent differences in rank order efficacy are species specific or due to methodological differences across studies.

In contrast to morphine-treated monkeys, buprenorphine did not substitute for naltrexone in LAAM-treated monkeys, perhaps reflecting differences in the dependence that develops under these particular conditions of morphine and LAAM treatment. Chronic treatment with a MOR agonist can increase the MOR efficacy required for agonist activity in a particular assay (e.g., tolerance and cross-tolerance). The agonist effects of buprenorphine in LAAM- and not morphine-treated monkeys suggest that morphine treatment is a condition that requires higher efficacy than LAAM treatment and further suggest that this particular condition of morphine treatment confers greater dependence than this particular condition of LAAM treatment. These results are not likely due to morphine having higher efficacy than LAAM; on the contrary, LAAM and morphine appear to have similar MOR efficacy because LAAM attenuates naltrexone-lever responding and enhances the effects of morphine in morphine-abstinent monkeys (e.g., Brandt et al., 1997). Alternatively, these results might be due to morphine treatment (3.2 mg/kg/day) conferring greater stimulation of MOR than LAAM treatment (1.0 mg/kg twice daily), as morphine is 3-fold more potent than LAAM in attenuating naltrexone-lever responding and further suggest that this particular condition of morphine treatment confers greater dependence than this particular condition of LAAM treatment. These results are not likely due to morphine having higher efficacy than LAAM; on the contrary, LAAM and morphine appear to have similar MOR efficacy because LAAM attenuates naltrexone-lever responding and enhances the effects of morphine in morphine-abstinent monkeys (e.g., Brandt et al., 1997). In addition, morphine has a shorter duration of action (approximately 8 h) than LAAM (approximately 48 h), suggesting that with once daily dosing (morphine) withdrawal might occur daily, whereas with twice daily dosing (long-acting LAAM) withdrawal might not occur daily. If the frequency of withdrawal from a particular drug positively correlates with dependence on that drug (e.g., Mhatre and Gonzalez, 1999), then more frequent withdrawal might contribute to the apparently greater dependence in morphine-treated compared with LAAM-treated monkeys.

**Discussion**

The relationship between therapeutic utility in the treatment of opioid abuse and efficacy at MOR is not well understood. Long-acting MOR agonists such as methadone and LAAM can decrease heroin abuse (for review, see Kreek, 2000), and their effectiveness might be related to substitution for some subjective effects of heroin and to attenuation of withdrawal (Dole and Nyswander, 1965; Schuster et al., 1995; Houtsomuller et al., 1998). Drugs with lower efficacy than methadone and LAAM might be alternative treatments with less abuse and dependence liability. For example, buprenorphine is a low efficacy agonist that attenuates some abuse-related effects of heroin and other opioids (Greenwald et al., 2002; Strain et al., 2002); however, it is not clear whether buprenorphine has less abuse and dependence liability than other MOR agonists (Woods and Gmerek, 1985; Eissenberg et al., 1996; Winger and Woods, 2001; Comer and Collins, 2002).

The results of this study demonstrate the utility of drug discrimination for obtaining highly quantitative data on the relative efficacy of drugs under conditions of chronic opioid treatment and acute abstinence in nonhuman primates. Whereas many assays are sensitive to only agonism or antagonism, the naltrexone discriminative stimulus in morphine-treated monkeys was sensitive to both agonism and antagonism under two qualitatively distinct conditions. Morphine treatment was sensitive to antagonism as evidenced by substitution of buprenorphine for naltrexone. In contrast, nalbuphine and morphine did not have naltrexone-like effects in morphine-treated monkeys. When the same monkeys were acutely deprived of morphine, naltrexone-lever responding was slightly attenuated by buprenorphine and markedly attenuated by nalbuphine and morphine. Thus, depending on the immediate pharmacologic history of morphine-treated monkeys, buprenorphine was an antagonist or an agonist, whereas nalbuphine was only an agonist, suggesting that buprenorphine has lower efficacy than nalbuphine. While these results are consistent with previous studies in rhesus monkeys (Woods and Gmerek, 1985; Walker et al., 1995), they differ from studies reporting that buprenorphine has higher efficacy than nalbuphine in rats (Zimmerman et al., 1987; Morgan and Picker, 1998; Walker and Young, 2002). It is not clear whether these apparent differences in rank order efficacy are species specific or due to methodological differences across studies.

In contrast to morphine-treated monkeys, buprenorphine did not substitute for naltrexone in LAAM-treated monkeys, perhaps reflecting differences in the dependence that develops under these particular conditions of morphine and LAAM treatment. Chronic treatment with a MOR agonist can increase the MOR efficacy required for agonist activity in a particular assay (e.g., tolerance and cross-tolerance). The agonist effects of buprenorphine in LAAM- and not morphine-treated monkeys suggest that morphine treatment is a condition that requires higher efficacy than LAAM treatment and further suggest that this particular condition of morphine treatment confers greater dependence than this particular condition of LAAM treatment. These results are not likely due to morphine having higher efficacy than LAAM; on the contrary, LAAM and morphine appear to have similar MOR efficacy because LAAM attenuates naltrexone-lever responding and enhances the effects of morphine in morphine-abstinent monkeys (e.g., Brandt et al., 1997). Alternatively, these results might be due to morphine treatment (3.2 mg/kg/day) conferring greater stimulation of MOR than LAAM treatment (1.0 mg/kg twice daily), as morphine is 3-fold more potent than LAAM in attenuating naltrexone-lever responding and further suggest that this particular condition of morphine treatment confers greater dependence than this particular condition of LAAM treatment. These results are not likely due to morphine having higher efficacy than LAAM; on the contrary, LAAM and morphine appear to have similar MOR efficacy because LAAM attenuates naltrexone-lever responding and enhances the effects of morphine in morphine-abstinent monkeys (e.g., Brandt et al., 1997). In addition, morphine has a shorter duration of action (approximately 8 h) than LAAM (approximately 48 h), suggesting that with once daily dosing (morphine) withdrawal might occur daily, whereas with twice daily dosing (long-acting LAAM) withdrawal might not occur daily. If the frequency of withdrawal from a particular drug positively correlates with dependence on that drug (e.g., Mhatre and Gonzalez, 1999), then more frequent withdrawal might contribute to the apparently greater dependence in morphine-treated compared with LAAM-treated monkeys.
Qualitative differences in the effects of buprenorphine between morphine- and LAAM-treated monkeys were confirmed through drug combination studies. Thus, while buprenorphine enhanced the naltrexone discriminative stimulus in morphine-treated monkeys, buprenorphine markedly attenuated the naltrexone discriminative stimulus in LAAM-treated monkeys. For example, monkeys responded only 66% on the naltrexone-lever when buprenorphine (1.0 mg/kg) was combined with a dose (1.0 mg/kg) of naltrexone 100-fold greater than the smallest dose (0.01 mg/kg) of naltrexone that occasioned predominantly naltrexone-lever responding under control conditions. That buprenorphine (1.0 mg/kg) occasioned predominantly naltrexone-lever responding in morphine-treated monkeys underscores the different consequences of morphine and LAAM treatment under these conditions. Although nalbuphine and morphine attenuated the naltrexone discriminative stimulus in both morphine- and LAAM-treated monkeys (had qualitatively similar effects), the effects of nalbuphine and morphine were quantitatively different between the two groups of monkeys. For example, nalbuphine (10.0 mg/kg) shifted the naltrexone dose-effect curve 24.0-fold to the right in LAAM-treated monkeys and only 8.2-fold to the right in morphine-treated monkeys. Morphine (32.0 mg/kg) also was more potent in LAAM-treated (10.0-fold rightward shift in the naltrexone dose-

### Table 2

The ED$_{50}$ (milligrams per kilogram) for naltrexone determined for each LAAM-treated monkey following pretreatment with saline (control), morphine, nalbuphine, or buprenorphine.

<table>
<thead>
<tr>
<th>Test Dose</th>
<th>OP</th>
<th>CL</th>
<th>XE</th>
<th>KA</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Control</td>
<td>0.005 (0.004–0.006)</td>
<td>0.004 (0.003–0.006)</td>
<td>0.008 (0.006–0.010)</td>
<td>0.007 (0.006–0.009)</td>
<td>0.006 (0.004–0.008)</td>
</tr>
<tr>
<td>Morphine</td>
<td>3.2 0.010</td>
<td>0.015</td>
<td>0.020</td>
<td>0.018</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>10.0 0.010</td>
<td>0.019</td>
<td>0.018</td>
<td>0.018</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>32.0 0.018</td>
<td>0.065</td>
<td>0.029</td>
<td>0.123</td>
<td>0.059</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>1.0 0.006</td>
<td>0.008</td>
<td>0.008</td>
<td>0.018</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>3.2 0.036</td>
<td>0.019</td>
<td>0.046</td>
<td>0.057</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>10.0 0.175</td>
<td>0.050</td>
<td>0.145</td>
<td>0.187</td>
<td>0.139</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.032 0.003</td>
<td>0.145</td>
<td>0.137</td>
<td>0.024</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>0.1 &lt;0.001</td>
<td>0.272</td>
<td>&gt;1.000</td>
<td>0.127</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>0.32 N.T.</td>
<td>0.274</td>
<td>N.T.</td>
<td>0.186</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>1.0 N.T.</td>
<td>0.272</td>
<td>&gt;1.000</td>
<td>0.578</td>
<td>0.630</td>
</tr>
</tbody>
</table>

N.T., not tested.

**Fig. 3.** Effects of naltrexone, buprenorphine, and nalbuphine in LAAM-treated monkeys discriminating naltrexone from saline. See Fig. 1 for details.

**Fig. 4.** Effects of naltrexone alone and in combination with morphine, nalbuphine, and buprenorphine in LAAM-treated monkeys discriminating naltrexone from saline. See Figs. 1 and 2 for details.
different effects of buprenorphine and other low efficacy ligands. Combination studies with morphine, nalbuphine, and naltnexone, therefore, suggest that morphine treatment conferred greater MOR tolerance than LAAM treatment and are consistent with the qualitatively different effects of buprenorphine between these two groups of monkeys.

While low efficacy at MOR appears to be responsible for the intermediate levels of naltrexone-lever responding in morphine-treated and -abstinent monkeys, another interpretation of these results is that buprenorphine nonselectively disrupted stimulus control. N-Methyl-D-aspartate (NMDA) antagonists can occasion intermediate levels of drug-appropriate responding in animals trained to discriminate MOR agonists (Koek, 1999); however, these effects of NMDA antagonists are not limited to low efficacy at MOR because the same doses of NMDA antagonists that occasion intermediate levels of drug-appropriate responding do not antagonize MOR agonists. In the present study, antagonism of morphine by buprenorphine was not only evidenced by intermediate-levels of naltrexone-like responding but also by the ability of buprenorphine to shift the naltrexone-dose effect curve to the left in morphine-treated monkeys. NMDA antagonists also occasion MOR agonist-lever responding at doses that decrease response rate, whereas buprenorphine did not alter response rate at doses that occasioned intermediate levels of naltrexone-lever responding. Thus, the intermediate naltrexone-lever responding occasioned by buprenorphine in morphine-treated and -abstinent monkeys appears to be related to low efficacy at MOR and not to performance-disrupting or other effects of buprenorphine unrelated to discriminative stimulus effects.

In summary, the effects of morphine, nalbuphine, and buprenorphine in morphine-treated monkeys varied according to their purported efficacy at MOR. Morphine and nalbuphine produced antagonistic-like effects under all conditions, whereas buprenorphine had naltrexone-like effects in morphine- and not LAAM-treated monkeys. These results with buprenorphine suggest that 3.2 mg/kg/day of morphine confers greater tolerance and dependence than 2.0 mg/kg/day of LAAM, perhaps due to neuroadaptive changes that occur differentially under the two dosing conditions. Moreover, this study suggests that buprenorphine has markedly different effects that vary according to immediate pharmacologic history, with buprenorphine attenuating withdrawal in morphine-abstinent monkeys and precipitating withdrawal in morphine-treated monkeys. It is possible that markedly different effects of buprenorphine and other low efficacy ligands across different levels of dependence will have a significant impact on treatment outcome in opioid abusers.

Acknowledgments
We thank A. Gaylor, H. Jasso, R. Jimenez, and K. Stone for providing technical assistance.

References

Address correspondence to: Dr. Charles P. France, Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900. E-mail: france@uthscsa.edu