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Cross-tolerance and μ agonist efficacy in pigeons treated with LAAM or buprenorphine☆

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Abstract

The mechanism responsible for decreased opioid use during opioid substitution therapy is not fully understood. To examine whether L-α-acetylmethadol (LAAM) or buprenorphine attenuate behavioral effects of opioids through cross-tolerance, discriminative stimulus effects of high and low efficacy μ agonists were examined following 3- or 7-day treatment with LAAM or buprenorphine in pigeons discriminating between saline and heroin or between saline and buprenorphine, respectively. Heroin, buprenorphine and nalbuphine occasioned high levels of drug-appropriate responding in both groups; κ opioids and non-opioids occasioned predominantly saline-appropriate responding. Administration of LAAM (3.2 mg/kg) or buprenorphine (3.2 mg/kg) occasioned predominantly heroin- or buprenorphine-appropriate responding, respectively. After discontinuation of LAAM treatment, the potency in occasioning heroin-key responding was markedly decreased for nalbuphine, slightly decreased for buprenorphine, and unchanged for heroin. Following discontinuation of buprenorphine treatment, the potency in occasioning buprenorphine-key responding was decreased for nalbuphine and unchanged for buprenorphine and heroin. Thus, greater cross-tolerance developed from LAAM and buprenorphine to low efficacy μ agonists as compared to a higher efficacy agonist. Failure of LAAM and buprenorphine treatment to modify the effects of heroin, under conditions that attenuate the effects of lower efficacy μ opioids, provides a possible rationale for why heroin abuse persists in some patients receiving large doses of agonists in substitution therapy.

Keywords: Opioid; Tolerance; LAAM; Buprenorphine; Heroin; Drug discrimination

Methadone was developed nearly 40 years ago and has been used widely for treating opioid abuse and dependence (Kreek, 2000). Subsequently, other μ agonists, including L-α-acetylmethadol (LAAM) and buprenorphine, were developed and approved for the treatment of opioid dependence and abuse. The mechanism(s) by which these compounds decrease opioid use appears to be related to their ability to prevent opioid withdrawal and to their ability to mimic at least some of the effects of opioids (Kreek and Vocci, 2002). However, despite the therapeutic utility of these medications, some patients continue to use heroin during treatment (Best et al., 1999; Ling and Wesson, 2003), suggesting these drugs might not adequately prevent withdrawal or mimic some effects of opioids in some patients. Further development of pharmacotherapies for opioid dependence and abuse will be accelerated by a better understanding of the factors that determine whether certain medications alter the effects of opioids that contribute to abuse.

Repeated treatment with a μ agonist can attenuate the effects of that agonist (tolerance) as well as the effects of other μ agonists (cross-tolerance). One potentially important mechanism underlying the therapeutic utility of methadone, LAAM, and buprenorphine is attenuation of the effects of

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abused opioids through the development of cross-tolerance. Early clinical studies demonstrated that repeated treatment with methadone or LAAM attenuated the physiologic (e.g. pupillary dilation) and subjective effects of heroin (Zaks et al., 1971; Levine et al., 1973; Volavka et al., 1978). A similar decrease in the subjective effects of opioids (e.g. morphine and hydromorphone) was reported in a majority of subjects receiving buprenorphine daily (Bickel et al., 1988; Teoh et al., 1994) suggesting that buprenorphine either induced cross-tolerance to other μ agonists or that it antagonized the effects of higher-efficacy μ agonists (e.g., Greenwald et al., 2003). While many studies indicate that agonist replacement therapy confers cross-tolerance to the subjective effects of opioids, a decreased subjective effect of heroin is not unanimously reported in subjects receiving long-acting agonists such as buprenorphine (e.g. Teoh et al., 1994). Failure of agonist replacement therapy to confer cross-tolerance to the subjective effects of high efficacy μ agonists such as heroin might contribute to continued drug abuse during treatment.

Pre-clinical studies have helped to identify factors controlling the development of tolerance and cross-tolerance resulting from μ agonist treatment. Studies on the antinociceptive effects of μ agonists, for example, have shown that cross-tolerance varies as a function of μ efficacy (Paronis and Holtzman, 1992; Walker and Young, 2001). Thus, under identical conditions of agonist treatment, greater cross-tolerance developed to low efficacy than to high efficacy agonists, presumably due to a greater receptor reserve for high-efficacy agonists. The relationship between efficacy of the agonist used for treatment and the development of cross-tolerance has proven more difficult to characterize because of pharmacokinetic (i.e. onset and duration of action) differences among μ agonists (Emmett-Oglesby et al., 1988). Nevertheless, some data indicate that treatment with low efficacy agonists confers greater cross-tolerance than treatment with high efficacy agonists (Paronis and Holtzman, 1992; Walker and Young, 2001).

Drug discrimination also has been used to assess tolerance and cross-tolerance to μ agonists and the results of these studies are generally consistent with the notion that chronic μ agonist treatment confers cross-tolerance among μ agonists (Emmett-Oglesby et al., 1988; Young et al., 1991; but see Colpaert, 1995). However, it is not clear whether daily treatment with LAAM or buprenorphine modifies the discriminative stimulus effects of heroin. Drug discrimination was used in the current study to examine the relationship between LAAM or buprenorphine treatment and the discriminative stimulus effects of heroin, buprenorphine and nalbuphine, three opioids that vary markedly in their relative efficacy at μ opioid receptors (Chen et al., 1993; Selley et al., 2000, 2001). Specifically, this study tested the hypothesis that cross-tolerance from daily treatment with LAAM or buprenorphine is inversely related to μ agonist efficacy.

1. Materials and methods

1.1. Subjects

Fifteen adult white Carneau pigeons (*Columbia livia*; Palmetto, Sumter, SC) were maintained at 90% of their free-feeding weight and individually housed in aluminum cages; pigeons had free access to water and to 10–20 g of mixed grain per day. Six pigeons were trained previously to discriminate saline and buprenorphine (0.178 mg/kg; Galici et al., 2002) and nine pigeons were trained in the current study to discriminate between saline and 0.32 mg/kg heroin. Pigeons had received opioids and other drugs acutely in previous studies (Galici et al., 2002). The animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

1.2. Apparatus

Experiments were conducted in sound-attenuating, ventilated chambers (BRS/LVE, Laurel, MD) equipped with two keys that could be transilluminated red and a food hopper that could be illuminated white. An interface (Med Associate, Inc., East Fairfield, VT) connected the chambers to a computer that controlled and recorded experimental events.

1.3. Discrimination procedure

Separate groups of pigeons discriminated between saline and buprenorphine (0.178 mg/kg) or between saline and heroin (0.32 mg/kg) while responding under a fixed ratio (FR) 20 schedule of food presentation (Purina Pigeon Checkers, Purina Mills, St. Louis, MO). Experimental sessions consisted of 2–8, 15-min cycles; each cycle consisted of a 10-min timeout, during which responses had no programmed consequence, and a 5-min response period, during which both response keys were illuminated. Twenty consecutive responses (FR20) on the key designated correct by the injection (saline or training drug) administered during the first minute of the cycle resulted in illumination of the food hopper and access to food. A maximum of 10 food presentations was available during a cycle; when the maximum number of food presentations was obtained in less than 5 min, the remainder of the response period was a timeout.

Saline training comprised administration of saline or a sham injection during the first minute of each of no more than eight cycles. Drug training comprised administration of buprenorphine or heroin during the first minute of a cycle followed by saline or sham during the first minute of a second cycle; completion of the FR on the drug-key

was required for a reinforcer during both of these cycles. For some training sessions, 2–6 saline/sham cycles preceded the cycle during which drug was administered. Test sessions were conducted after two consecutive training sessions in which at least 80% of the total responses occurred on the correct key and fewer than 20 responses (one FR) occurred on the incorrect key before the first food presentation for all cycles. For pigeons discriminating heroin, the first test was conducted when performance over 4 consecutive training days (including 2 drug and 2 saline training days) satisfied these criteria; pigeons discriminating buprenorphine previously satisfied these criteria (Galici et al., 2002). Collectively, the current and the prior (Galici et al., 2002) studies suggest that the two training stimuli were qualitatively similar (substituting for each other) in heroin-discriminating (current study) and in buprenorphine-discriminating (prior study) pigeons. Test sessions were identical to training sessions except that 20 consecutive responses on either key resulted in food presentation.

1.4. Testing before daily opioid treatment

Pigeons discriminating between saline and heroin or between saline and buprenorphine received the μ opioid agonists nalbuphine (0.01–1.0 m/kg), buprenorphine (0.01–0.32 mg/kg) and heroin (0.01–1.0 mg/kg). The pharmacologic specificity of the discriminative stimulus effects of heroin was assessed with the indirect-acting monoamine agonists amphetamine (0.032–1 mg/kg) and cocaine (0.1–10 mg/kg) and the κ opioid agonists enadoline (0.01–1.0 mg/kg) and spiradoline (0.32–3.2 mg/kg). The pharmacologic specificity of the discriminative stimulus effects of buprenorphine has been described previously (Galici et al., 2002). Cumulative dose–effect tests comprised administration of vehicle during the first minute of the first cycle followed by doses of a test drug, increasing by 0.5–1.0 log unit, during the first minute of subsequent cycles. Test sessions ended when greater than 80% of the total responses occurred on the drug-appropriate lever or when response rate was less than 20% of the control response rate.

The parameters for testing after discontinuation of drug treatment were established by assessing the duration of action of LAAM (3.2 mg/kg) and buprenorphine (3.2 mg/kg) in pigeons discriminating between saline and heroin or between saline and buprenorphine, respectively. Tests were conducted at 30 min, 24 and 48 h after administration of a dose of each compound by administering saline at the beginning of a first cycle and sham at the beginning of a second cycle at each time.

1.5. Testing during and after daily opioid treatment

On different occasions, pigeons discriminating between saline and heroin were treated with 3.2 mg/kg/day of LAAM for 3 or 7 days. Similarly, pigeons discriminating between saline and buprenorphine were treated with 3.2 mg/kg/day of buprenorphine for 3 or 7 days. On each day of the 3-day treatments and on days 1, 3, 5 and 7 of the 7-day treatments, buprenorphine or LAAM was administered 4 or 8 h, respectively, prior to the administration of saline and two test cycles (i.e., no discrimination training occurred during drug treatments). Drugs were administered at the same time of day on days 2, 4 and 6 of the 7-day treatments, although no test or training sessions were conducted on those days. Pretreatment times were selected based on prior studies with buprenorphine and LAAM (unpublished data as well as Galici et al., 2002). Dose–effect curves for a particular test agonist (heroin, buprenorphine and nalbuphine) were multiply determined on days 1, 3, 5 and 7 after discontinuation of treatment; pigeons were tested with saline on days 2, 4 and 6 after discontinuation of treatment. Each 3- or 7-day treatment was repeated for each test drug, and a particular treatment was initiated only when the ED_{50} of a particular test drug after discontinuation of treatment was not different from the control ED_{50} determined prior to that treatment; in every case, a minimum of 2 weeks separated the end of testing with one compound and the beginning of testing with another.

1.6. Data analysis

Discrimination data are expressed as the percentage of total responses on the drug-appropriate key averaged among pigeons (±S.E.M.) and plotted as a function of dose or time. Discrimination data determined at various times following acute administration of LAAM or buprenorphine represent an average of responding from two cycles. Drug discrimination data determined during the 3- and 7-day LAAM and buprenorphine treatments and 1 day after discontinuation of those treatments represent an average of all determinations for a particular treatment. The dose of a compound to occasion 50% drug-appropriate responding (ED_{50}) was estimated by linear regression on more than two data points. ED_{50}s were determined for individual subjects and the 95% confidence limits (CL) were calculated from the group-averaged ED_{50}. The ED_{50} for a particular compound (heroin, buprenorphine and nalbuphine) determined after discontinuation of LAAM or buprenorphine treatment was compared to the control ED_{50} determined just prior to that treatment. To compare changes in potency across treatments, a potency ratio was calculated for each pigeon by dividing the post-treatment ED_{50} by the control ED_{50}; when the 95% CL of the dose ratio did not include 1, treatments were considered significantly different.

Control response rate represents an average of the five vehicle training sessions before a test. Discrimination data were not included for analysis when response rate for a particular pigeon was less than 20% of control for that pigeon; however, response rate data were included in the group average.

1.7. Drugs

The drugs used in this study were heroin hydrochloride, buprenorphine hydrochloride, D-amphetamine sulfate, cocaine hydrochloride, and LAAM (National Institute on Drug Abuse, Research Technology Branch, Rockville, MD, USA), nalbuphine hydrochloride (Mallinckrodt Inc., St. Louis, MO, USA), enadoline hydrochloride (Warner Lambert/Parke Davis, Ann Arbor, MI, USA), and spiradoline mesylate (Pharmacia/Upjohn, Kalamazoo, MI, USA). 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 0.9% saline. Compounds were injected i.m. in a volume of 0.1–1.0 ml.

2. Results

2.1. Effects of μ opioids before LAAM or buprenorphine treatment

Heroin dose-dependently increased drug-appropriate responding in pigeons discriminating between saline and heroin with responding occurring predominantly on the heroin key at doses of 0.32 mg/kg and larger (Fig. 1, left, squares). The largest dose (1.0 mg/kg) of heroin decreased response rate to 55% of control, whereas smaller doses of heroin did not alter response rate (data not shown). Nalbuphine and buprenorphine also dose-dependently increased heroin-appropriate responding, e.g., pigeons responded predominantly on the heroin key at doses of 0.1 mg/kg and larger of buprenorphine (Fig. 1, middle, squares) and doses of 1.0 mg/kg and larger of nalbuphine (Fig. 1, right, squares). The largest doses of buprenorphine (0.32 mg/kg) and nalbuphine (3.2 mg/kg) decreased response rate to 83% and 56% of control, respectively (data not shown). Administration of vehicle during the first cycle of these tests occasioned predominantly saline-appropriate responding (Fig. 1, all panels, squares above S) and did not modify response rate (data not shown). In contrast to μ opioids, the indirect-acting monoamine agonists cocaine and amphetamine and the κ opioid agonists spiradoline and enadoline occasioned predominantly saline-appropriate responding in pigeons discriminating heroin, up to doses that decreased response rate to less than 20% of control (Table 1).

Buprenorphine dose-dependently increased drug-appropriate responding in pigeons discriminating between saline and buprenorphine with responding occurring predomi-

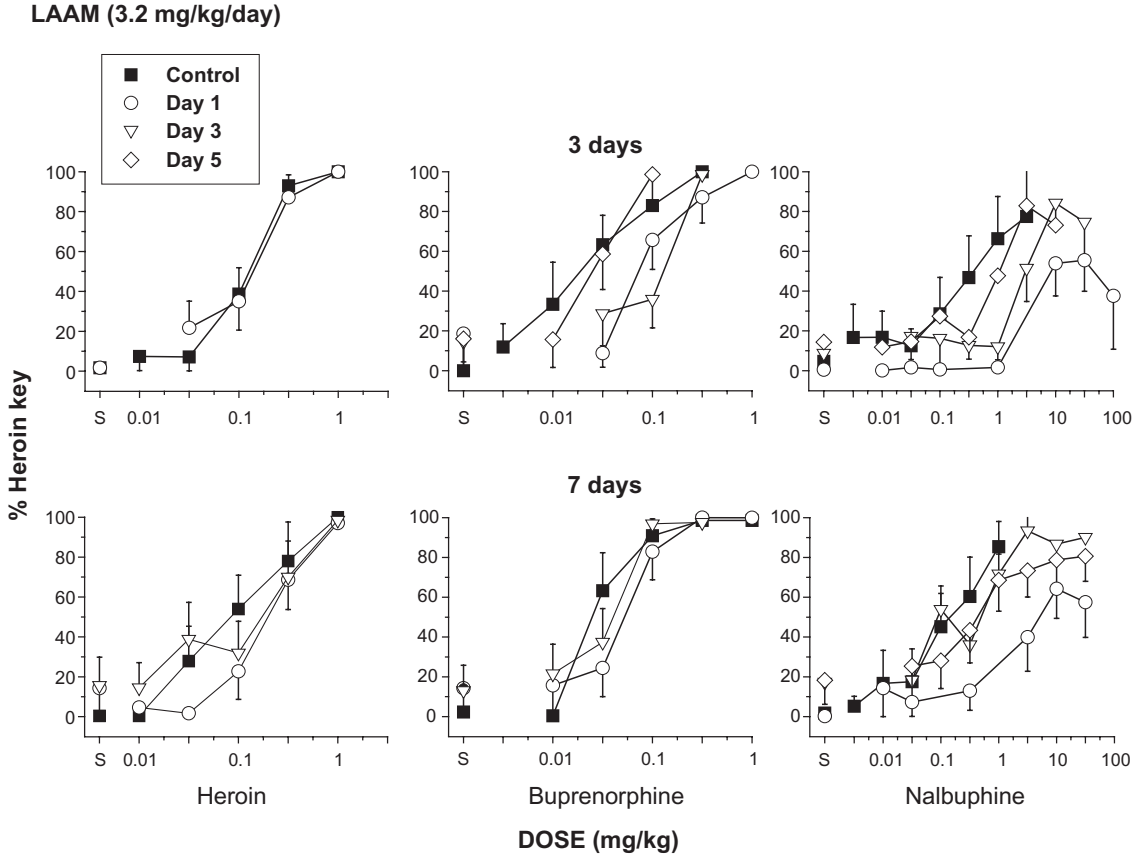

Fig. 1. Discriminative stimulus effects of heroin (left), buprenorphine (middle) and nalbuphine (right) before and after treatment with LAAM (3.2 mg/kg) for 3 (top) or 7 days (bottom) in pigeons discriminating heroin. Abscissae: dose in mg/kg body weight; S, saline. Ordinates: mean (±S.E.M.) percentage of responding on the drug (heroin)-appropriate key. Dose–effect curves were determined before (Control) and after discontinuation of treatment (Days 1–7).

Table 1
Maximum percentage (mean±S.E.M.) of drug-appropriate responding (Max % DR) across a range of doses of cocaine, amphetamine, spiradoline and enadoline in pigeons discriminating heroin

Drug	Dose range (mg/kg)	Max % DR
Cocaine	0.1–10	5.7±3.3
Amphetamine	0.032–10	39.7±24.4
Spiradoline	0.32–3.2	16.7±16.7
Enadoline	0.01–1.0	19.4±15.5

All drugs were studied up to doses that decreased rate of responding to less than 20% of control.

nantly on the buprenorphine key after doses of 0.1 mg/kg and larger (Fig. 2, middle, squares); up to a dose of 0.1 mg/kg, buprenorphine did not alter response rate (data not shown). Heroin and nalbuphine also dose-dependently increased buprenorphine-appropriate responding, e.g., pigeons responded predominantly on the buprenorphine key after receiving doses of 0.32 mg/kg and larger of heroin (Fig. 2, left, squares) and doses of 3.2 mg/kg and larger of nalbuphine (Fig. 2, right, squares). Heroin and nalbuphine did not alter response rate at the largest doses studied (data not shown). Administration of vehicle during the first cycle of these tests occasioned predominantly saline-appropriate responding (Fig. 2, all panels, squares above S) and did not modify response rate (data not shown).

2.2. Acute and chronic effects of LAAM or buprenorphine

In pigeons discriminating heroin, acute administration of 3.2 mg/kg of LAAM occasioned a mean (±S.E.M.) of 95 (±2)% drug-appropriate responding at 30 min; drug-appropriate responding decreased to 35 (±21)% and 13 (±8)% at 24 and 48 h, respectively. In pigeons discriminating buprenorphine, acute administration of 3.2 mg/kg of buprenorphine occasioned 100% drug-appropriate responding at 30 min; drug-appropriate responding decreased to 36 (±20)% and 1 (±0.2)% at 24 and 48 h, respectively.

When administered for three or seven consecutive days, LAAM and buprenorphine occasioned predominantly heroin- or buprenorphine-key responding, respectively (Fig. 3). Rate of responding was not significantly different from control on any day of treatment with LAAM or buprenorphine (data not shown). Twenty-four h after administration of LAAM, pigeons discriminating heroin responded predominantly on the saline key after receiving saline (Fig. 3, top, days 4 or 8) and rate of responding was not different from control on these days (data not shown). Similarly, 24 h after administration of buprenorphine, responding on the buprenorphine key was 35% and 38% on days 4 and 8, respectively (Fig. 3, bottom); rate of responding was not different from control on these days (data not shown).

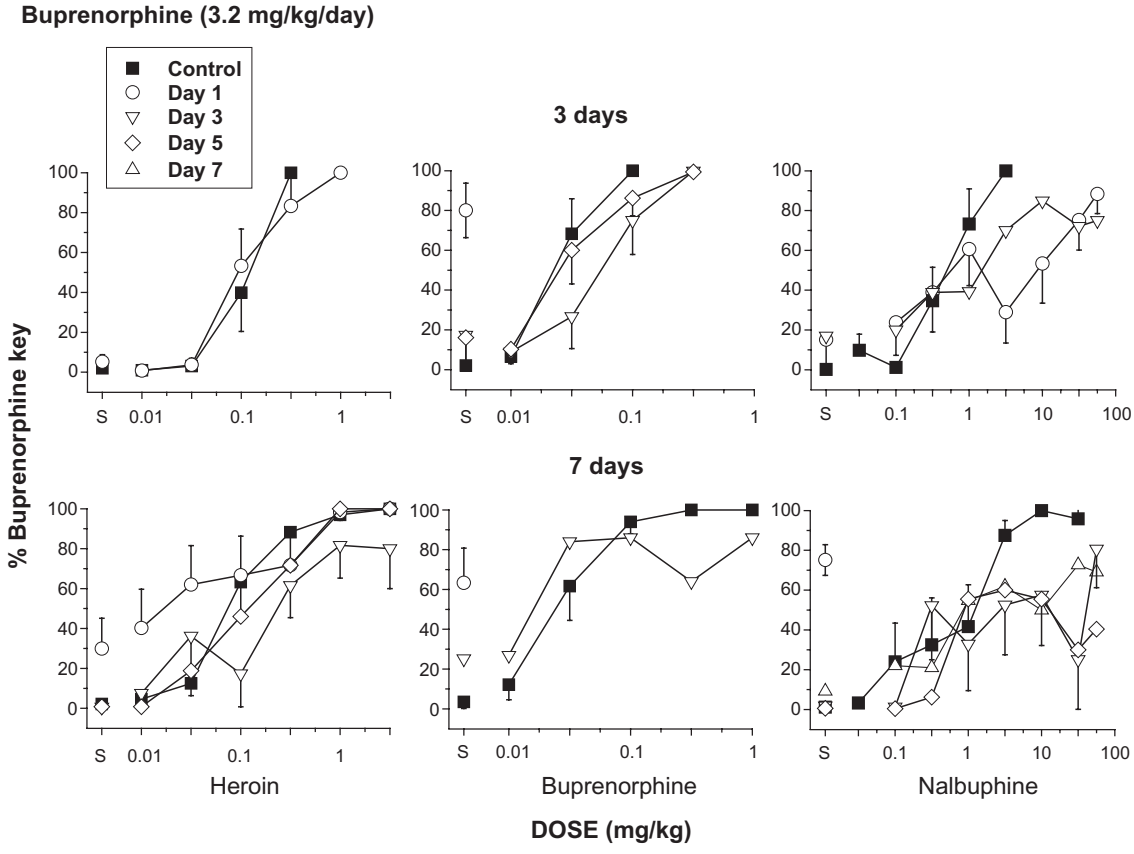

Fig. 2. Discriminative stimulus effects of heroin (left), buprenorphine (middle) and nalbuphine (right) before and after treatment with buprenorphine (3.2 mg/kg) for 3 (top) or 7 days (bottom) in pigeons discriminating buprenorphine. See Fig. 1 for details.

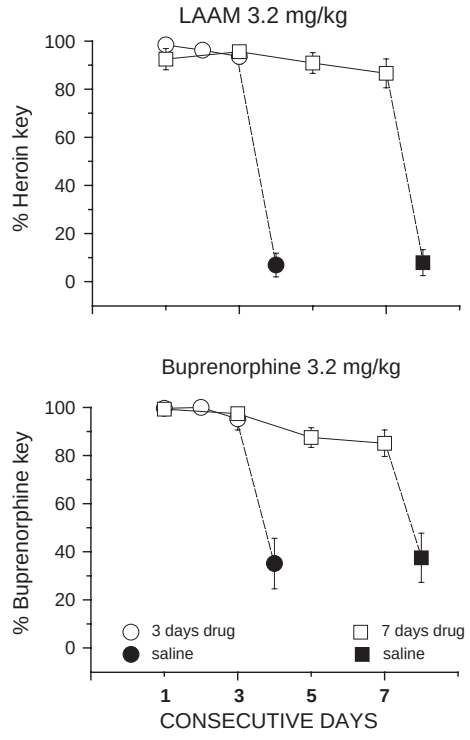

Fig. 3. Discriminative stimulus effects of LAAM (top) or buprenorphine (bottom) during daily treatment in pigeons discriminating heroin or buprenorphine, respectively. Abscissae: consecutive days of treatment. See Fig. 1 for other details.

2.3. Effects of μ opioids after discontinuation of LAAM or buprenorphine treatment

Administration of LAAM (3.2 mg/kg/day) or buprenorphine (3.2 mg/kg/day) for 3 or 7 days did not modify the discriminative stimulus effects of heroin either in pigeons discriminating between saline and heroin or in pigeons discriminating between saline and buprenorphine (Figs. 1 and 2, left panels and Tables 2 and 3, respectively). The ED_{50} of heroin determined prior to treatment was not significantly modified on any day following discontinuation of treatment with either agonist, although there was a trend for decreased sensitivity to heroin 3 days after discontinuation of the 7-day buprenorphine treatment (Fig. 2, bottom left, triangles; Table 3).

Treatment with LAAM (3.2 mg/kg/day) for 3 or 7 days significantly increased the ED_{50} of buprenorphine in pigeons discriminating heroin (Fig. 1, middle top and bottom, respectively). On days 1 and 3 following discontinuation of the 3-day LAAM treatment, the ED_{50} of buprenorphine was increased 3- and 4-fold, respectively (Table 2); sensitivity to buprenorphine was not significantly different from control 5 days after discontinuation of the 3-day LAAM treatment. The day following discontinuation of the 7-day LAAM treatment, the ED_{50} of buprenorphine was increased 3-fold; the ED_{50} of buprenorphine was not different from control by day 3 following discontinuation of the 7-day LAAM treatment. In contrast to LAAM, treatment with buprenorphine for 3 or 7 days did not significantly modify sensitivity to buprenorphine in pigeons discriminating buprenorphine (Fig. 2, middle top and bottom, respectively; Table 3, day 3 vs. control). The day after discontinuation of 3 or 7 days of buprenorphine treatment, sensitivity to buprenorphine could not be determined because pigeons responded predominantly on the buprenorphine key at the beginning of these tests (Fig. 2, middle, circles).

Sensitivity to nalbuphine was markedly decreased by treatment with LAAM or buprenorphine (Figs. 1 and 2, respectively, right). One day after discontinuation of LAAM treatment, the ED_{50} of nalbuphine was increased 32-fold (3-day treatment) and 102-fold (7-day treatment; Table 2). The ED_{50} of nalbuphine was not significantly different from control by day 5 (3-day treatment) and by day 3 (7-day

Table 2
Mean ED_{50} values, dose ratios and respective 95% confidence limits (CL) for discriminative stimulus effects of μ opioids before and after LAAM (3.2 mg/kg/day) treatment in pigeons discriminating heroin

Drug	3-Day treatment				7-Day treatment			
	ED_{50}	95% CL	Dose ratio vs. before	95% CL	ED_{50}	95% CL	Dose ratio vs. before	95% CL
Heroin								
Before	0.11	0.06–0.20			0.09	0.05–0.21		
Day 1	0.12	0.06–0.23	1.03	0.46–2.30	0.21	0.12–0.37	2.10	0.73–6.09
Day 3	–	–	–	–	0.13	0.15–0.38	1.35	0.42–4.39
Buprenorphine								
Before	0.03	0.01–0.06			0.02	0.01–0.03		
Day 1	0.09*	0.05–0.18	3.32	1.14–9.69	0.05*	0.03–0.09	2.64	1.18–5.87
Day 3	0.11*	0.07–0.17	3.95	1.72–9.06	0.03	0.02–0.06	1.80	0.86–3.77
Day 5	0.03	0.02–0.04	0.95	0.40–2.26	–	–	–	–
Nalbuphine								
Before	0.15	0.04–0.56			0.11	0.04–0.36		
Day 1	3.19*	1.22–8.31	31.55	4.90–203.29	8.05*	2.99–21.63	101.60	62.30–165.71
Day 3	0.73*	0.18–3.04	7.22	1.61–32.45	0.25	0.09–0.68	2.19	0.41–11.77
Day 5	0.15	0.03–0.73	1.47	0.23–9.26	–	–	–	–

* Indicates a significant difference from ED_{50} determined before daily treatment.

Table 3
Mean ED_{50} values, dose ratios and respective 95% confidence limits (CL) for discriminative stimulus effects of μ opioids before and after buprenorphine (3.2 mg/kg/day) treatment in pigeons discriminating buprenorphine

Drug	3-Day treatment				7-Day treatment			
	ED_{50}	95% CL	Dose ratio vs. before	95% CL	ED_{50}	95% CL	Dose ratio vs. before	95% CL
Heroin								
Before	0.16	0.13–0.19			0.07	0.03–0.16		
Day 1	0.10	0.06–0.15	0.61	0.33–1.14	0.04	0.01–0.21	0.64	0.09–4.36
Day 3	–	–	–	–	0.22	0.07–0.73	3.16	0.67–14.84
Day 5	–	–	–	–	0.15	0.05–0.44	2.22	0.39–12.53
Buprenorphine								
Before	0.03	0.02–0.05			0.03	0.02–0.05		
Day 1	ND	–	–	–	ND	–	–	–
Day 3	0.06	0.03–0.11	1.87	0.80–4.40	0.04	0.01–0.25	1.62	0.39–6.77
Day 5	–	–	–	–	–	–	–	–
Nalbuphine								
Before	0.59	0.27–1.29			0.90	0.40–2.04		
Day 1	6.77*	1.15–40.02	14.24	2.44–83.07	ND	–	–	–
Day 3	2.39	0.60–9.41	2.00	0.35–11.56	7.46*	0.84–66.11	11.86	1.09–129.39
Day 5	–	–	–	–	5.60	0.67–47.00	8.90	0.72–109.71
Day 7	–	–	–	–	1.54	0.18–13.05	2.44	0.22–26.67

ND, not determined; responding was predominantly on the buprenorphine-key 1 day after discontinuation of buprenorphine treatment.
* Indicates a significant difference from ED_{50} determined before daily treatment.

treatment). One day after discontinuation of the 3-day buprenorphine treatment, the ED_{50} of nalbuphine was increased 14-fold (Table 3); the ED_{50} of nalbuphine was not significantly different from control 3 days after discontinuation of this treatment. Sensitivity to nalbuphine could not be determined 1 day after discontinuation of the 7-day buprenorphine treatment because pigeons responded predominantly on the buprenorphine key at the beginning of this test (Fig. 2, bottom right, circle above S). The ED_{50} of nalbuphine increased 12-fold 3 days after discontinuation of the 7-day buprenorphine treatment (Table 3). Although the ED_{50} of nalbuphine was not significantly different from control 5 and 7 days after discontinuation of this treatment, the maximum level of buprenorphine key responding occasioned by nalbuphine was less than obtained before this treatment (Fig. 2, bottom right).

3. Discussion

LAAM and buprenorphine decrease opioid abuse in some, although not all, patients receiving these substitution treatments. Identifying the factors responsible for the therapeutic utility of LAAM and buprenorphine will facilitate the development of more effective treatments. To address the possibility that LAAM and buprenorphine treatment confer cross-tolerance to the behavioral effects of heroin, the discriminative stimulus effects of heroin and the low efficacy μ agonists buprenorphine and nalbuphine were assessed before and after daily treatment (i.e., 3 or 7 days) with LAAM or buprenorphine in pigeons. All three μ agonists (nalbuphine, buprenorphine and heroin) occasioned high levels of drug-appropriate responding in pigeons discriminating either between saline and heroin or between saline and buprenorphine; in contrast, compounds with little or no μ agonist activity (cocaine, amphetamine, spiradoline and enadoline) occasioned predominantly saline-appropriate responding. Overall, the pharmacologic profile of the heroin (current study) and buprenorphine (Galici et al., 2002) discriminative stimuli appear to be qualitatively similar and consistent with the well-characterized μ agonist actions of both compounds. Following discontinuation of once daily treatment with LAAM or buprenorphine for 3 or 7 days, at doses that occasioned high levels of drug-appropriate responding throughout treatment, the potency of nalbuphine was markedly decreased, the potency of buprenorphine was slightly decreased, and the potency of heroin was unchanged. Collectively, these data are consistent with the notion that the magnitude of cross-tolerance that develops to a μ agonist is inversely related to agonist efficacy and they extend this general finding to the long-acting therapeutic LAAM and to the commonly-abused μ agonist heroin. These results further emphasize that, under conditions of LAAM and buprenorphine treatment that markedly attenuate the discriminative stimulus effects of some μ agonists, the discriminative stimulus effects of heroin are unchanged. Thus, continued use of heroin by some individuals that are receiving LAAM or buprenorphine might be due to the failure of these drugs to modify the effects of high efficacy agonists.

The mechanism(s) responsible for tolerance and cross-tolerance to repeated administration of μ opioids might involve down-regulation (Belcheva et al., 1993) or internalization of μ receptors (Marvizon et al., 1999) or the uncoupling of G-proteins from μ receptors (Parolaro et al., 1993). Thus, treatment with LAAM or buprenorphine might decrease the population of available (active) μ receptors that

mediate the discriminative stimulus effects of μ agonists. Consequently, such treatments more effectively attenuated the effects of low efficacy agonists that require greater receptor occupancy to exert their effects as compared to high efficacy agonists. That the discriminative stimulus effects of heroin were not modified by the same treatments indicates that daily administration of LAAM or buprenorphine for 3 or 7 days is not sufficient to decrease the number or activation state of μ receptors below a threshold that is necessary for discriminative stimulus effects of a high efficacy μ agonist such as heroin. Indeed, parallel differences to those obtained herein by the induction of tolerance are obtained when receptor reserve is reduced by administration of an irreversible antagonist, such that the effects of low efficacy agonists are attenuated (e.g., reduced) by doses of irreversible antagonists that have little or no effect on higher efficacy agonists (e.g., Walker and Young, 2002).

Cross-tolerance to other opioids can develop from repeated LAAM treatment for measures of rate-decreasing effects (McMillan and Brocco, 1984; Gerak and France, 1997), antinociception (Brandt and France, 2000) and subjective effects (Levine et al., 1973; Houtsmuller et al., 1998) and cross-tolerance develops from repeated buprenorphine treatment for measures of antinociception (Walker and Young, 2001) and subjective effects (Bickel et al., 1988). In the current study, treatment with either LAAM or buprenorphine conferred similar, although not identical, cross-tolerance to the discriminative stimulus effects of μ agonists. The somewhat greater cross-tolerance that developed from LAAM, as compared to buprenorphine, could be due to the particular doses used of each drug or to the overall higher efficacy of LAAM as compared to buprenorphine (e.g. Brandt et al., 1997). In addition to differences in efficacy, LAAM and buprenorphine bind differentially to other opioid receptors and buprenorphine dissociates more slowly than LAAM from μ receptors (Hambrook and Rance, 1976). Slow dissociation from μ receptors might have contributed to the long duration of discriminative stimulus effects following discontinuation of buprenorphine treatment that occasionally precluded direct comparisons between buprenorphine and LAAM 1 day after discontinuation of treatment.

Although buprenorphine can have antagonist activity under some conditions (Martin et al., 1976; Cowan et al., 1977; Tallarida and Cowan, 1982; Walker et al., 1995; Amass et al., 1998), including attenuation of the subjective effects of other (higher efficacy) agonists in humans (Greenwald et al., 2003), there was no evidence in the current study that buprenorphine attenuated the discriminative stimulus effects of nalbuphine through competitive antagonism at μ receptors. Similarly, in pigeons not treated chronically with an agonist, buprenorphine has long-lasting agonist actions in the absence of any evidence for antagonist actions (France et al., 1984). The slow dissociation of buprenorphine from μ receptors can result in pseudo-irreversible antagonist actions (Hambrook and Rance, 1976), and the behavioral consequences of both irreversible antagonism and cross-tolerance from repeated agonist treatment can be similar, e.g., both conditions can decrease the effects of μ ligands in a manner inversely related to agonist efficacy. While a role for antagonist activity of buprenorphine or one of its metabolites cannot be rejected, given that parallel data that were obtained with LAAM and buprenorphine in these studies and that the effects of heroin were not altered by doses of buprenorphine that markedly attenuated the effects of nalbuphine, it appears likely that attenuation of the discriminative stimulus effects of lower efficacy agonists by buprenorphine was the result of cross-tolerance and not competitive antagonism.

In summary, treatment with LAAM or buprenorphine markedly attenuated the discriminative stimulus effects of the low efficacy μ agonist nalbuphine without modifying the discriminative stimulus effects of the higher efficacy μ agonist heroin. These results are consistent with previous studies in rats (Young et al., 1991; Walker and Young, 2001) showing that, under identical conditions of agonist treatment, greater cross-tolerance occurs to low than to high efficacy μ agonists. Regardless of the mechanism by which buprenorphine modifies the discriminative stimulus effects of lower efficacy agonists, these results clearly demonstrate that repeated treatment with LAAM or buprenorphine fails to modify the discriminative stimulus effects of heroin under conditions where sensitivity to other μ opioids is markedly reduced. Together with other studies that examined the relationship among tolerance, cross-tolerance and efficacy, these data provide a rationale for why heroin abuse persists in some patients receiving large doses of agonists in substitution therapy.

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