0022-3565/98/2873-1029$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 287:1029–1037, 1998

Vol. 287, No. 3
Printed in U.S.A.

Chronic *l-Alpha* Acetylmethadol in Rhesus Monkeys: Discriminative Stimulus and Other Behavioral Measures of Dependence and Withdrawal[1]

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Accepted for publication July 13, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

This study characterized discriminative stimulus and other effects of naltrexone in rhesus monkeys treated daily with the long-acting opioid *l-alpha* acetylmethadol (LAAM). An initial dose-finding study assessed the rate-decreasing effects of naltrexone in three monkeys receiving LAAM daily (0.32–1.78 mg/kg); subsequently, these monkeys and a fourth received 1.0 mg/kg/12 hr of LAAM although discriminating between naltrexone and saline. Responding occurred on the saline lever after the administration of LAAM, whereas >90% drug-lever responding occurred after the administration of 0.1 mg/kg of naltrexone that also elicited signs of withdrawal. Naloxone and quadazocine, but not morphine, nalbuphine or ketamine, substituted for naltrexone. Morphine and nalbuphine shifted the naltrexone dose-effect curve to the right. Compared to precipitated withdrawal, deprivation-induced withdrawal occasioned less naltrexone-lever responding and fewer observable signs of withdrawal. Maximal naltrexone-level responding occurred 24 to 48 hr after the discontinuation of LAAM treatment; the frequency of other withdrawal signs also peaked 24 to 48 hr after the discontinuation of LAAM. Partial naltrexone-lever responding occurred for up to 10 days after discontinuation of LAAM treatment; 4 and 8 days after the discontinuation of LAAM treatment, 0.1 mg/kg of naltrexone did no further increase naltrexone-lever responding or withdrawal signs suggesting that less-then-maximal naltrexone-lever responding was not due to long-lasting effects of LAAM or its metabolites. The discriminative stimuli that are associated with LAAM deprivation might be different from the stimuli associated with either training condition. This study is the first antagonist discrimination in non-humans primates treated chronically with LAAM and the results indicate that the naltrexone stimulus is related to opioid withdrawal.

The frequent administration of some drugs often results in the development of dependence that can be quantified by the severity of withdrawal that occurs after either the discontinuation of drug treatment or the administration of a pharmacologic antagonist. Of particular clinical relevance are the adverse signs and symptoms (*i.e.*, withdrawal) that often emerge on the discontinuation of drug use and that can contribute to the continued drug use. Moreover, individuals often report an increased motivation to obtain and use drugs during periods of withdrawal (Schuster *et al.*, 1995) that could contribute significantly to the maintenance or re-initiation of drug use.

LAAM is a *mu* opioid agonist that is used as a maintenance therapy in opioid-dependent patients. In human (Kreek, 1992; Ling *et al.*, 1994) as well as non-human primates (Aceto *et al.*, 1992), LAAM attenuates opioid withdrawal and has a long duration of action that effectively prevents the emergence of withdrawal when it is administered as infrequently as thrice weekly. Two active metabolites of LAAM, *l-alpha* acetylnormethadol (*i.e.*, nor-LAAM) and *l-alpha* acetyldinormethadol (*i.e.*, dinor-LAAM), appear to contribute to its long duration of action (Finkle *et al.*, 1982; Henderson *et al.*, 1977) and, in many species, these metabolites have behavioral effects that are similar to the effects of other *mu* agonists and potencies that are equal to or greater than the parent compound, LAAM (Bertalmio *et al.*, 1992; Holtzman, 1979; McGivney and McMillan, 1981). The combination of *mu* agonist activity and a long duration of action appears to account for the relative success of LAAM as a maintenance therapy for opioid dependence.

This study assessed the behavioral effects of chronic LAAM administration in rhesus monkeys, a species that has been used extensively to study opioid dependence and withdrawal

Received for publication January 14, 1998.
[1] This work was supported by United States Public Health Service Grant DA05018. C.P.F. is the recipient of a Research Scientist Development Award (DA00211). Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, Louisiana State University Medical Center, and guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council (Department of Health, Education and Welfare, Publication No. (NIH) 85-23, revised 1983).

ABBREVIATIONS: % DR, percent drug responding; FR, fixed ratio; LAAM, *l-alpha* acetylmethadol; nor-LAAM, *l-alpha* acetylnormethadol; dinor-LAAM, *l-alpha* acetyldinormethadol.

(Aceto *et al.*, 1992; Downs *et al.*, 1977; Holtzman and Villarreal, 1973). In non-human primates, opioid dependence and withdrawal are typically assessed using three general approaches. The first approach consists of observational procedures where unconditioned behavioral signs of withdrawal are rated after either the discontinuation of chronic drug treatment (*i.e.*, deprivation-induced withdrawal; Aceto *et al.*, 1992) or the administration of a pharmacologic antagonist (*i.e.*, precipitated withdrawal; Gmerek and Woods, 1985; Gmerek *et al.*, 1987). Generally, withdrawal signs are pharmacologically specific for a class of drugs and are typically reversed only by drugs that have pharmacologic actions similar to the treatment drug. For example, the discontinuation of chronic morphine treatment produces signs of withdrawal that are reversed by other *mu* opioid agonists and not by *kappa* opioid agonists (Gmerek and Woods, 1985; Gmerek *et al.*, 1987).

A second approach for studying the behavioral effects of withdrawal uses simple schedules of reinforcement. For example, in agonist-treated subjects, the termination of drug treatment or the administration of an antagonist can reliably disrupt conditioned behavior (*e.g.*, responding maintained by a schedule of food presentation). Such disruptions in behavior have been observed after the termination of chronic treatment with various different drugs including phencyclidine (Slifer *et al.*, 1984), tetrahydrocannabinol (Beardsley *et al.*, 1986), cocaine (Woolverton and Kleven, 1988) and opioids (Holtzman and Villarreal, 1973; Thompson and Schuster, 1964). Moreover, reinitiation of responding occurs after several days or, more immediately, after the administration of the treatment drug or a pharmacological equivalent; these findings support the view that the disruptive effects of drug deprivation are related to withdrawal.

A third approach for studying withdrawal involves drug discrimination procedures in which agonist-treated subjects are trained to discriminate between an antagonist and vehicle. Because antagonist-lever responding increases in a dose-dependent manner after the administration of an antagonist, or in a time-dependent manner after the termination of agonist treatment, it is thought that responding on the antagonist-associated lever represents a withdrawal condition, whereas responding on the vehicle-associated lever represents a dependent condition (for reviews, see Emmett-Oglesby *et al.*, 1990; France 1994b). In general, drugs with pharmacological actions similar to the treatment drug reverse antagonist-associated responding, whereas drugs with dissimilar pharmacologic actions do not reverse antagonist-associated responding (France and Woods, 1989; Holtzman, 1985).

This study examined the rate-altering effects, discriminative stimulus effects and other behavioral effects that occur on administration of an opioid antagonist in LAAM-treated monkeys and those effects that occur on discontinuation of twice daily injections of LAAM. Because of the long duration of LAAM, our study used multiple dependent variables to assess whether precipitated withdrawal was different from deprivation-induced withdrawal. These studies on opioid withdrawal in LAAM-treated subjects could be especially relevant to the long term use of LAAM as a maintenance therapy in humans. To date, no published study has systematically assessed the relationship between the discriminative stimulus and other behavioral manifestations of withdrawal in LAAM-dependent primates.

Methods

Subjects. Two male and two female adult rhesus monkeys (*Macaca mulatta*) were individually housed in stainless-steel cages with free access to water. Monkeys received fresh fruit and peanuts several times each week and were maintained under a 14-hr light/10-hr dark schedule. Access to food (Teklad monkey food) in the home cage was unrestricted during stimulus-shock termination procedures and restricted during food-maintained responding procedures; in the latter case, monkeys were maintained at no less than 90% of their free-feeding weights. All monkeys were experimentally naive at the initiation of these studies.

Apparatus. Monkeys were seated in either aluminum or Lexan chairs within ventilated, sound-attenuating chambers. Each chamber contained three response levers; the center lever could be extended (available) into the operant chamber or retracted out of the reach of the monkey (unavailable). Located above each lever were red and green stimulus lights. An externally mounted pellet dispenser could deliver 300 mg banana-flavored pellets (product F0179 Bio-serve, Frenchtown NJ) to a food cup located below the center lever. Each chair was equipped with a pair of shoes containing brass electrodes to which brief (250 msec) electric shock (3 mA) could be delivered from a remote a.c. shock generator. The feet of the monkeys were placed in shoes during all phases of these studies. Control of experiments and data recording were accomplished with a microprocessor as well as a commercially available interface and software.

Behavioral studies. Initially, three monkeys were trained to respond under a FR-10 schedule of food presentation and the antinociceptive, ventilatory and rate-decreasing effects of acutely administered opioids and nonopioids were assessed. After the completion of these acute drug studies (data from these studies will be presented in a subsequent report), sensitivity to the rate-decreasing effects of naltrexone was examined when the same three monkeys received LAAM daily.

LAAM was administered daily to three monkeys in their home cages 2 hr before sessions consisting of four or five discrete, 15-min cycles. Each cycle comprised a 10-min timeout, during which the chamber was dark and responses had no programmed consequence, followed by a 2-min response period, during which the center light was illuminated green and monkeys could respond on that lever under a FR-10 schedule of food presentation (two pellets delivered for each ratio completed). A maximum of 20 pellets could be received per cycle (*i.e.*, completion of 10 ratios) and responding on the left or right lever had no programed consequence. The response period (for food) was followed by a second timeout (3-min), during which the chamber was dark, the center lever was unavailable (*i.e.*, retracted from the chamber), and responses had no scheduled consequence. The dose of LAAM was increased weekly with monkeys receiving 0.32, 1.0 and 1.78 mg/kg/day of LAAM for 7 days each. On the seventh day of treatment with each dose of LAAM, sensitivity to the rate-decreasing effects of naltrexone was assessed. Saline was administered on the first cycle and cumulative doses of naltrexone were administered on subsequent cycles, up to a maximum dose of 0.1 mg/kg. Next, conditions were changed to a FR-5 schedule of stimulus-shock termination and monkeys were trained to discriminate naltrexone (see below).

A pilot study in a fourth monkey (this monkey did not participate in the studies described above) identified an appropriate dosing condition for establishing naltrexone as a discriminative stimulus in LAAM-treated monkeys. While receiving 3.2 mg/kg/day of morphine, this monkey was trained to discriminate between 0.01 mg/kg of naltrexone and saline (France and Woods, 1989). Subsequently, various doses of LAAM were substituted for the daily dose of morphine to determine whether the discriminative-stimulus effects of naltrexone (0.01 mg/kg) could be maintained during LAAM administration.

Dosing conditions for LAAM and naltrexone were systematically altered until the monkey responded at least 90% on the injection-appropriate lever (saline or naltrexone) in six consecutive or seven of eight consecutive sessions. After these conditions were identified, three additional monkeys were trained to discriminate between naltrexone and saline under those conditions.

Terminal dosing conditions consisted of twice daily injections of 1.0 mg/kg of LAAM (0700 and 1900 hr) and once daily experimental sessions (1700 hr), during which monkeys ($n = 4$) discriminated between saline and 0.0178 mg/kg of naltrexone. Daily sessions comprised multiple (2–6), discrete 20-min cycles with each cycle consisting of a 15-min time out, during which the chamber was dark and responses had no programmed consequence, followed by a 5-min response period, during which monkeys could respond under a FR-5 schedule of stimulus-shock termination. Under these conditions, shocks were scheduled to occur every 15 sec in the presence of red stimulus lights that were located above the left and right levers (the center lever was retracted from the chamber). Monkeys could extinguish the stimulus lights and postpone the scheduled shock by responding five times consecutively on the lever designated correct by the injection (saline or 0.0178 mg/kg of naltrexone) administered at the beginning of the cycle. The order of stimulus presentation varied nonsystematically over training sessions with the stipulation that a given condition (drug or saline) was not presented consecutively for more than 2 days. Responses on the injection-inappropriate lever reset the response requirement on the injection-appropriate lever.

Testing began after the establishment of adequate stimulus control that was defined as: fewer than five responses on the injection-inappropriate lever before the first shock avoidance and at least 90% of the total responses on the injection-appropriate lever. Test sessions were parametrically identical to training sessions except that monkeys could extinguish the stimulus lights and postpone scheduled shocks by making five consecutive responses on either the left or right lever.

During the course of these studies, schedule-controlled performance deteriorated in two of the monkeys, apparently due to the rate-decreasing (*e.g.*, withdrawal-precipitating) effects of 0.0178 mg/kg of naltrexone. Stable performance was quickly re-established by decreasing the training dose of naltrexone to 0.01 mg/kg in one monkey and to 0.0133 mg/kg in the other monkey.

The *mu* agonists nalbuphine, morphine and alfentanil, the selective *mu* antagonists naloxone and quadazocine and the N-methyl-D-aspartate antagonist ketamine were studied to determine whether they substitute for the naltrexone discriminative stimulus in LAAM-treated monkeys. Saline was administered on the first cycle and cumulative doses of drug were administered on subsequent cycles. Drugs that did not substitute for naltrexone (*i.e.*, ketamine, nalbuphine and morphine) were administered acutely before cumulative doses of naltrexone to determine whether they modify the discriminative stimulus effects of naltrexone. For ketamine, the largest dose that did not modify rates of responding when administered alone was administered one cycle before the beginning of the naltrexone dose-effect curve. Similarly, doses of nalbuphine were administered one cycle before the beginning of the naltrexone dose-effect curve, whereas, morphine was administered 1 hr before the first cycle. Previous studies have demonstrated that the peak discriminative stimulus effects of nalbuphine and morphine (s.c.) occur during these times (Brandt *et al.*, 1997; Gerak and France, 1996).

Other studies determined whether naltrexone-lever responding or other withdrawal-associated behavioral signs occur on discontinuation of LAAM treatment. Vehicle was substituted for twice daily injections of LAAM for 10 days. Immediately before daily sessions, monkeys received an acute injection of either saline (days 1, 2, 3, 5, 6, 7 and 9) or 0.1 mg/kg of naltrexone (days 4 and 8) in the home cage. The behavior of the monkeys was video taped for 25 min. Monkeys were then immediately (*i.e.*, within 5 min) transported to the operant chambers where they received two discrimination cycles that were conducted under test conditions (*i.e.*, both levers active). On the 10th day of LAAM deprivation, monkeys received an injection of saline on the first cycle and cumulative doses of morphine on subsequent cycles to assess whether stimulus control had been maintained over the extended period without explicit discrimination training. After LAAM deprivation studies, twice daily LAAM treatment and discrimination training were resumed and further testing was suspended for a minimum of 2 wk.

On a separate occasion, morphine was administered daily to determine whether it modified the discriminative stimulus effects of LAAM deprivation. A dose of 3.2 mg/kg of morphine was administered every 6 hr (*i.e.*, 0100, 0700, 1300, and 1900 hr) to monkeys, beginning 12 hr after the last dose of LAAM. Discrimination test sessions, comprising two saline test cycles, were conducted daily at 1700 hr for 4 days. The times and doses for the administration of morphine were identical to other studies on opioid dependence that were also conducted in rhesus monkeys (Gmerek *et al.*, 1987; Katz, 1986).

Behavioral signs. Video tapes were evaluated by an experienced observer who was blind to experimental conditions. Behavioral signs [*e.g.*, grimacing, vocalization, retching, vomiting, holding abdomen, lying on side, wet dog shakes, yawning, coughing and masturbation (male monkeys only)] that are commonly observed during *mu* opioid withdrawal (*e.g.*, Katz, 1986) were scored for the 25-min session. Each period was divided into twenty-five 1-min bins and behavioral signs were recorded as either present or absent during each bin [*i.e.*, a maximum frequency of 25 occurred when the sign was observed during every min (bin) of an observation period].

Data analyses. The rate-decreasing effects of naltrexone under the simple FR schedule in monkeys receiving LAAM daily are presented as a percentage of the average rate of responding during the 10 cycles immediately preceding LAAM treatment. Results of drug discrimination studies are presented as the average percentage of responses on the naltrexone lever (*i.e.*, percent drug responding; %DR) ± 1 S.E.M. plotted as a function of either dose or time. The %DR was not included for an individual monkey either when the response rate was less than 20% of the control rate (*i.e.*, average response rate for that monkey for the five previous saline training cycles in which the testing criteria had been attained) or when more than two shocks were delivered. Response rates are presented as a mean percentage ± 1 S.E.M. of control response rates. Naltrexone dose-effect curves were determined approximately monthly throughout these studies; although there was very little variation among naltrexone dose-effect curves (see "Results"), in figures the dose-effect curves for other drugs are compared to the naltrexone dose-effect curve that was determined at a similar time. In LAAM-treated monkeys, test compounds were considered to have substituted for the naltrexone discriminative stimulus when they produced at least 90% naltrexone-appropriate responding. Substitution studies typically ended in a particular subject when that subject responded at least 90% on the naltrexone lever or when more than two shocks were delivered; thus, the largest dose studied was not necessarily the same among all subjects. Nalbuphine and morphine were studied up to a maximum dose of 56.0 and 32.0 mg/kg, respectively. ED_{50} values were calculated for individual subjects by linear regression when three or more data points were available and by interpolation when two data points (one above and one below 50% DR) were available; these ED_{50} values were averaged among subjects. Differences between dose-effect curves (potency) were identified with two-way repeated measures analyses of variance using ED_{50} values ($P < .05$). For behavioral signs, the frequency with which a particular sign was observed was averaged among subjects (± 1 S.E.M.) and was plotted as a function of either dose or time. Data that are presented for unconditioned behavioral signs of withdrawal were obtained in all monkeys with the exception of masturbation which represents data for only the two male monkeys.

Drugs. The compounds studied were: LAAM, morphine sulfate, naloxone hydrochloride and naltrexone hydrochloride (the Research Technology Branch, National Institute on Drug Abuse, Rockville,

MD), ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, IA), nalbuphine hydrochloride (Mallinckrodt Inc., St. Louis, MO) and quadazocine methanesulfonate (Sterling-Winthrop Research Institute, Rensselaer, NY). With the exceptions of LAAM and ketamine, all compounds were dissolved in sterile water and injected s.c. in a volume of 0.1 to 4.0 ml. LAAM was dissolved in 85% H_2O, 10% emulphor and 5% EtOH; a small quantity of 5 molar NaOH was added to increase the pH to 6. Ketamine was diluted, as needed with saline, from a commercially available solution. Doses are expressed in mg/kg of body weight in terms of the salt forms.

Results

In the dose-finding study, the average rate of food-maintained responding for three monkeys before daily treatment was 1.22 ± 0.22 responses/sec. Rates of lever pressing were not altered during a week of treatment with 0.32 mg/kg/day of LAAM or during a subsequent week of treatment with 1.0 mg/kg/day (fig. 1, circles and squares, respectively, above days 1 and 6). After an increase in the daily dose of LAAM to 1.78 mg/kg, rates of responding were slightly decreased (triangles above days 1 and 6), primarily because one monkey responded at 75% or less of the control rate throughout treatment with 1.78 mg/kg/day of LAAM.

After 7 days of treatment with 0.32 mg/kg of LAAM, doses of naltrexone up to 0.1 mg/kg did not modify rates of responding (fig. 1). In contrast, a dose of 0.1 mg/kg of naltrexone decreased the average rate of responding to 51% of the control rate after 1 wk of treatment with 1.0 mg/kg of LAAM. Still smaller doses of naltrexone decreased rates of responding when the daily dose of LAAM was increased to 1.78 mg/kg (fig. 1, triangles).

Discrimination training commenced after the terminal dosing conditions were established (1.0 mg/kg/12 hr of LAAM) with monkeys satisfying the criteria for adequate stimulus control after an average of 46 training sessions (range = 20–76). The mean rate of responding (control) in four monkeys receiving LAAM was 2.3 ± 0.1 responses/sec. Naltrexone dose-dependently increased responding on the drug lever with a dose of 0.01 mg/kg producing more than 90% DR (fig. 2, circles, top); doses of naltrexone that occasioned drug-lever responding also decreased rates of responding (circles, bottom).

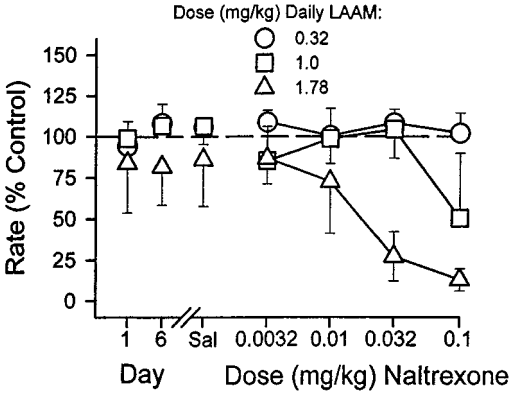

Fig. 1. Rates of responding engendered under a FR schedule of food presentation in three monkeys that were treated with increasing doses of LAAM. LAAM was administered daily 2 hr before experimental sessions with each dose administered for 7 consecutive days. On the 7th day of treatment for each dose of LAAM, saline was administered during with first cycle followed by cumulative doses of naltrexone during subsequent cycles. Ordinate: average rate of responding calculated as a percentage (±1 S.E.M.) of the average rate of responding before LAAM treatment. Abscissa: left, effects of saline on days 1, 6 and 7 (Sal) each dose of LAAM treatment; right, dose of naltrexone in mg/kg body weight administered on day 7 of each week of treatment.

Other opioid antagonists substituted completely for the naltrexone discriminative stimulus in LAAM-treated monkeys. Naloxone had a similar potency to naltrexone, both as a discriminative stimulus and in decreasing rates of responding (fig. 2, squares, top and bottom), and quadazocine was 10-fold less potent than naltrexone and naloxone. A dose of 0.1 mg/kg of quadazocine produced at least 90% drug-lever responding in two monkeys, whereas doses of 0.32 or 1.0 mg/kg of quadazocine were required to produce at least 90% drug-lever responding in the other monkeys.

Morphine, nalbuphine and ketamine failed to substitute for naltrexone (data not shown), with each compound producing <10% DR at all doses. Moreover, up to doses of 32.0 and 56.0 mg/kg, respectively, morphine and nalbuphine did not modify rates of responding. In contrast, a dose of 3.2 mg/kg of ketamine eliminated responding in all monkeys.

Morphine, nalbuphine and ketamine also were studied in combination with naltrexone to determine whether they modified the discriminative stimulus effects of naltrexone. Morphine shifted the naltrexone discrimination dose-effect curve to the right (fig. 3, top) as evidenced by significant ($P <$ .05 for 3.2 and 10.0 mg/kg) increases in the naltrexone ED_{50}:

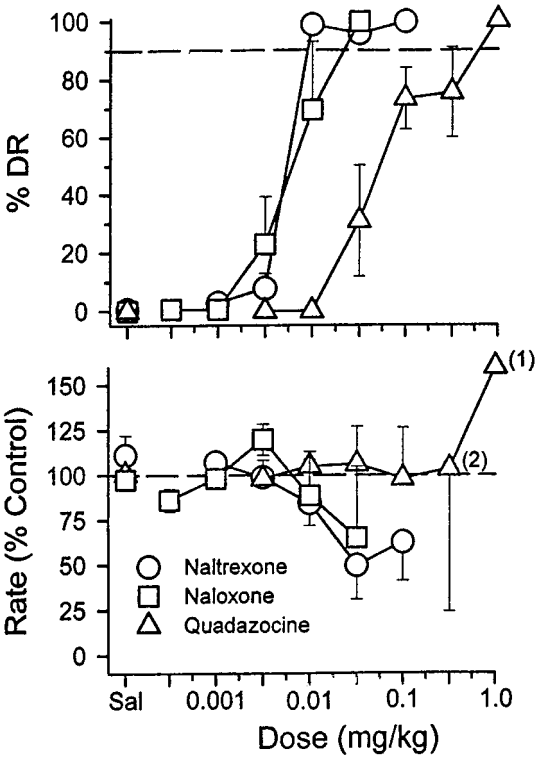

Fig. 2. Discriminative stimulus effects of opioid antagonists in four LAAM-treated monkeys discriminating between naltrexone and saline and responding under a FR schedule of stimulus shock termination. Drugs were studied in an individual subject up to a dose that occasioned at least 90% drug-lever responding and numbers in parentheses represent the number of monkeys contributing to the respective data points (*i.e.*, doses of quadazocine larger than 0.1 mg/kg were studied only in two monkeys). Ordinate: top, average percentage of responses on the naltrexone lever (%DR) ± 1 S.E.M.; bottom, average rate of responding calculated as a percentage (±1 S.E.M.) of the control rate of responding. Abscissa: dose in mg/kg body weight; saline (Sal) was administered on the first test cycle.

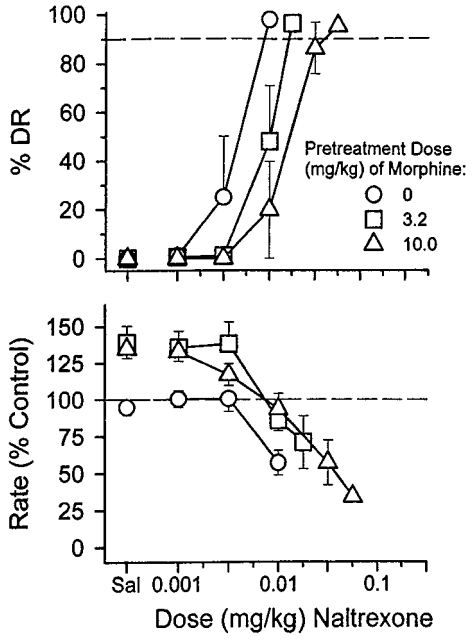

Fig. 3. Discriminative stimulus effects of naltrexone alone and in combination with 3.2 or 10.0 mg/kg of morphine (n = 4). Morphine was administered 1 hr before experimental sessions and saline (Sal) was administered on the first cycle. See figure 2 for additional details.

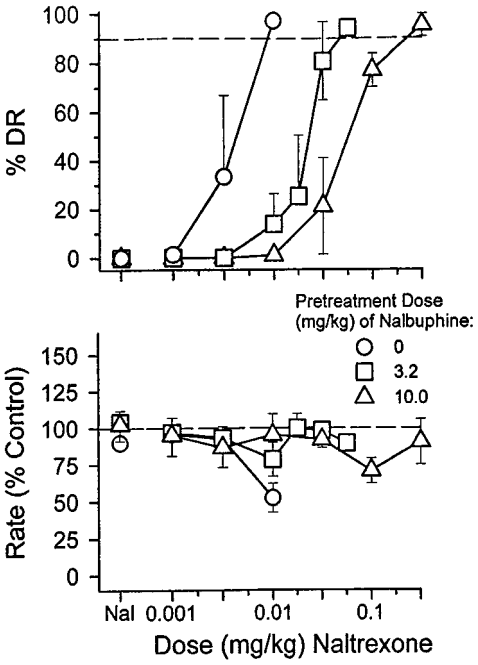

Fig. 4. Discriminative stimulus effects of naltrexone alone and in combination with 3.2 or 10.0 mg/kg of nalbuphine (n = 4). Nalbuphine was administered at the beginning of the first cycle (*i.e.*, 20-min pretreatment; data above Nal). See figure 2 for additional details.

a 2-fold increase with a dose of 3.2 mg/kg of morphine and a 4-fold increase with a dose of 10.0 mg/kg. When administered alone, doses of 3.2 and 10.0 mg/kg of morphine increased rates of responding to more than 135% of the control rate (data above Sal); these doses of morphine also shifted the dose-effect curve for rate-decreasing effects of naltrexone to the right (fig. 3, bottom panel).

At similar doses, nalbuphine was more effective than morphine in modifying the discriminative stimulus effects of naltrexone. Doses of 3.2 and 10.0 mg/kg of nalbuphine also shifted the naltrexone dose-effect curve to the right with the naltrexone ED_{50} increasing ($P < .05$) 5- and 14-fold, respectively, in the presence of nalbuphine (fig. 4, top). When administered alone, neither dose of nalbuphine modified rates of responding; however, each dose appeared to attenuate the rate-decreasing effects of naltrexone (bottom).

In contrast to morphine and nalbuphine, each of which significantly increased the naltrexone ED_{50} for discriminative stimulus effects, a dose of 0.32 mg/kg of ketamine failed to significantly alter the potency of naltrexone as a discriminative stimulus ($P > .05$; fig. 5, top). This dose of ketamine also failed to modify the rate-decreasing effects of naltrexone (bottom).

LAAM treatment was discontinued in order to compare the discriminative stimulus effects of naltrexone to the discriminative stimulus effects of LAAM deprivation. Monkeys varied markedly in their response to LAAM deprivation and results from individual subjects are presented in fig. 6. Ten hr after the last injection of 1.0 mg/kg of LAAM, all four monkeys responded exclusively on the saline lever (fig. 6, leftmost open symbols, all panels). For monkey O (upper left), responding was predominantly on the naltrexone lever for 5 days after discontinuation of LAAM treatment. This monkey responded predominantly on the saline lever 6 and 7 days after discontinuation of LAAM treatment. Monkey C responded on the saline lever 2 and 3 days after discontinuation of LAAM treatment; at times less than 2 days and longer than 3 days, this monkey responded predominantly on the naltrexone lever (upper right). Monkeys K and T each responded predominantly on the naltrexone lever either 1 or 1.5 days after discontinuation of LAAM treatment and, thereafter, on the saline lever (lower panels).

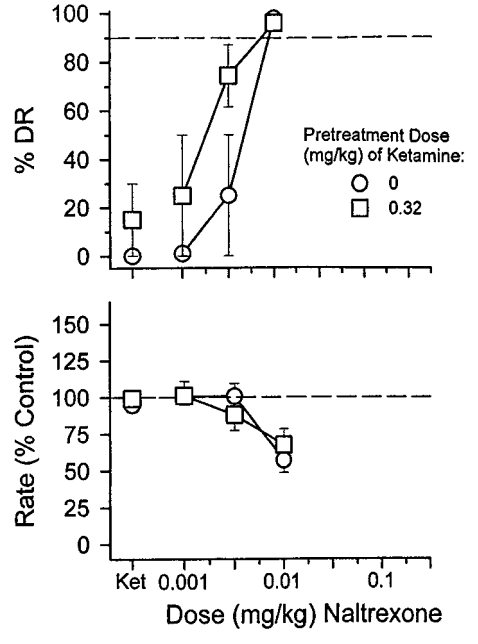

Fig. 5. Discriminative stimulus effects of naltrexone alone and in combination with 0.32 mg/kg of ketamine (n = 4). Ketamine was administered at the beginning of the first cycle (*i.e.*, 20-min pretreatment; data above Ket). See figure 2 for additional details.

When administered 10 hr after an injection of 1.0 mg/kg of LAAM, a dose of 0.1 mg/kg of naltrexone occasioned more

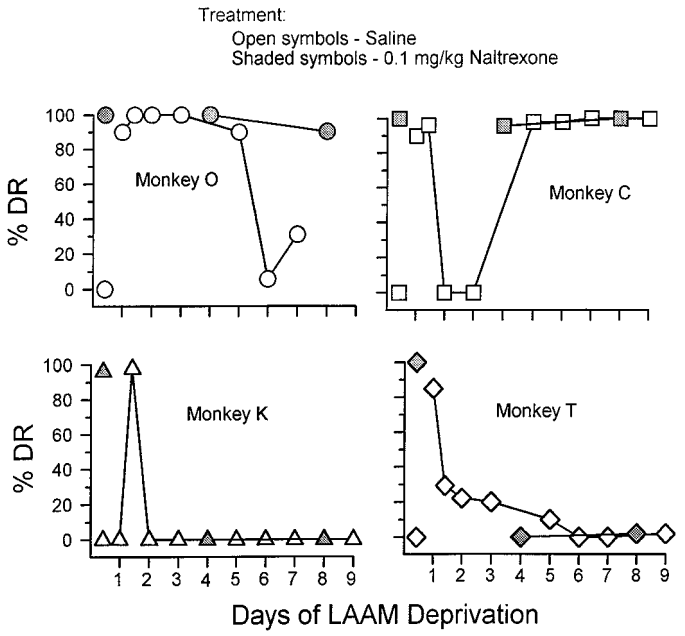

Fig. 6. Discriminative stimulus effects of LAAM deprivation in four monkeys (different panel for each subject). Twice daily injections of saline were substituted for LAAM. In addition, acute injections of naltrexone (0.1 mg/kg) were tested 10 hr, 4 and 8 days after discontinuation of LAAM treatment. Abscissa: days after the termination of LAAM treatment. See figure 2 and "Methods" for additional details.

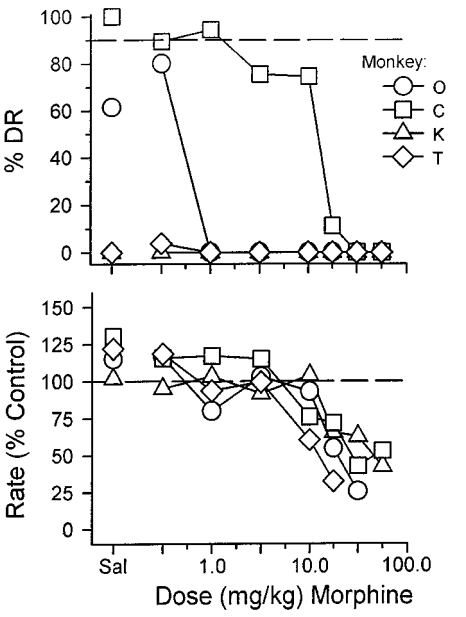

Fig. 7. Discriminative stimulus and rate-decreasing effects of saline (Sal) and cumulative doses of morphine 10 days after the discontinuation of LAAM treatment. See figure 2 for additional details.

than 95% naltrexone-lever responding in all four monkeys (fig. 6, leftmost closed symbols, all panels) and decreased average rates of responding to 69% of control rates (data not shown). When administered 4 or 8 days after the discontinuation of LAAM treatment, the same dose of naltrexone occasioned predominantly naltrexone-lever responding in two monkeys (O and C) and saline-lever responding in the remaining two monkeys (K and T). In contrast to the marked rate-decreasing effects that were observed with this dose of naltrexone 10 hr after LAAM, response rates were not altered by naltrexone 4 or 8 days after discontinuation of LAAM treatment (data not shown).

On a second occasion, morphine was substituted for LAAM to determine whether another *mu* agonist would attenuate the naltrexone lever responding that occurred when LAAM treatment had been discontinued. All four monkeys responding exclusively on the saline lever when 3.2 mg/kg/6 hr of morphine was substituted for 1.0 mg/kg/12 hr of LAAM (data not shown). Rates of lever pressing were not changed when morphine was substituted for LAAM (not shown).

A third study further examined reversal by morphine of deprivation-induced naltrexone-lever responding. Figure 7 shows the individual data obtained with cumulative doses of morphine 10 days after discontinuation of twice daily LAAM treatment. A cumulative dose of 1.0 mg/kg fully reversed naltrexone-lever responding in monkey O and a dose of 32.0 mg/kg fully reversed naltrexone-lever responding in monkey C. Subjects K and T responded exclusively on the saline lever prior administration of any morphine (points above Sal, fig. 7) and continued to do so throughout the morphine dose-effect determination. Morphine decreased rates of responding in a dose-related fashion in 10-day LAAM-deprived monkeys (lower panel) with a cumulative dose of 10.0 mg/kg decreasing the average rate to 56% of control rates. In contrast, up to a dose of 32.0 mg/kg, morphine had no rate-decreasing effect when monkeys were receiving LAAM twice daily (data not shown).

The frequency with which other behavioral signs were observed when saline was administered 10 hr after LAAM are shown by the shaded circles in figure 8; with the exception of "holds abdomen," behavioral signs that often occur during opioid withdrawal were not present under this condition. In contrast, acute administration of 0.1 mg/kg of naltrexone 10 hr after LAAM (fig. 8, shaded triangles) elicited a variety of behaviors including grimacing, wet-dog shakes and vomiting.

Similarly, withdrawal-associated behavioral signs were also observed when twice daily LAAM injections were discontinued (fig. 8, open circles) with a peak in signs occurring 1 to 3 days after the last injection of LAAM. For example, lying on side, grimacing and masturbation occurred during the first 3 days of LAAM deprivation and not thereafter. Vocalizations, holding abdomen and wet-dog shakes also occurred with greater frequency during the 3 days immediately after the discontinuation of LAAM treatment. In contrast, retching, vomiting and coughing were not observed during 9 days of LAAM deprivation. With the exception of an apparent increase in vocalization 4 days after discontinuation of LAAM, acute administration of 0.1 mg/kg of naltrexone (open triangles) did not further increase the frequency of withdrawal-associated behavioral signs (compare days 4 and 8 for naltrexone to 6 and 8 for saline). Finally, some behavioral signs (*e.g.*, retching, vomiting and coughing) were observed when naltrexone was administered 10 hr but not 4 or 8 days after the discontinuation of LAAM treatment.

Discussion

The magnitude of dependence and withdrawal that develop after repeated drug treatment can be quantified by changes in behavior after either the discontinuation of drug

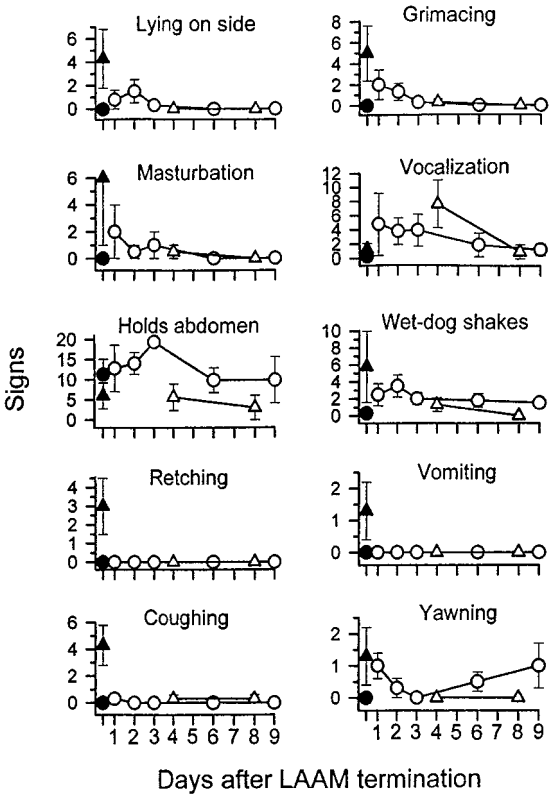

Fig. 8. Behavioral signs observed during 25-min periods at various times after the discontinuation of LAAM treatment. In addition, acute injections of 0.1 mg/kg of naltrexone were assessed 10 hr, 4 and 8 days after discontinuation of treatment. Ordinates: the average (±1 S.E.M.) number of signs. See figure 7 and "Methods" for additional details.

treatment or by the administration of a pharmacological antagonist. In monkeys receiving morphine chronically, behavioral signs such as wet-dog shakes, agitation and yawning occur after the discontinuation of morphine treatment or the administration of an opioid antagonist (Gmerek *et al.*, 1987; Katz, 1986). Similarly, in this study, the discontinuation of LAAM treatment or the administration of naltrexone resulted in the expression of some behavioral signs of *mu* opioid withdrawal, although in the former case the magnitude and frequency of these signs were less than typically observed after discontinuation of treatment with morphine. For example, coughing, retching and vomiting that are often observed after discontinuation of morphine (*e.g.*, 3.2 mg/kg/6 hr) treatment (*e.g.*, Gmerek *et al.*, 1987) were not observed when LAAM treatment was discontinued. Nevertheless, several qualitatively similar effects emerge after discontinuation of treatment with either LAAM or morphine, thereby implicating a common mechanism (*mu* receptor) for dependence on each of these opioids.

It is likely that the development of dependence on LAAM was important for establishing naltrexone as a discriminative stimulus in this study. A discrimination has been established with an opioid antagonist in monkeys treated subchronically with a *mu* agonist (France, 1994a), although most often opioid antagonists are studied in subjects receiving agonist chronically (*e.g.*, France and Woods, 1989; Holtzman, 1985). In one study, the dose of naloxone that was established as a discriminative stimulus in morphine-treated rats could not be established as a discriminative stimulus in untreated rats (Weissman, 1978). When a large dose of naltrexone was used as the training stimulus in untreated subjects, the discriminative stimulus effects of naltrexone appeared to be unrelated to opioid actions (Valentino *et al.*, 1983). In contrast, the discriminative stimulus effects of naltrexone clearly were mediated by *mu* opioid receptors in monkeys receiving LAAM; the relative potencies of naltrexone, naloxone and quadazocine for producing drug-lever responding were similar to their potencies for producing (other) signs of withdrawal (Gmerek *et al.*, 1987), as well as for producing naltrexone-like discriminative stimulus effects in monkeys receiving morphine chronically (France and Woods, 1989). Acute injections of the *mu*-selective agonists nalbuphine and morphine (France and Woods, 1989; Gerak and France, 1996) shifted the naltrexone dose-effect curve to the right, further supporting the view that the discriminative stimulus effects of naltrexone in LAAM-treated monkeys involve *mu* opioid receptors. The larger shifts to the right in the naltrexone dose-effect curve that were obtained with nalbuphine, as compared to the same doses of morphine, might be due to the higher affinity that nalbuphine has for *mu* binding sites, as compared to morphine (Emmerson *et al.*, 1996). Collectively, these results indicate that the discriminative stimulus effects of naltrexone are mediated by *mu* opioid receptors in LAAM-treated monkeys.

Two-choice discrimination procedures in drug-treated subjects, like the one used in our study, cannot easily identify the qualitative nature of the training stimuli. For example, the stimulus condition that is associated with an antagonist could represent withdrawal or could simply indicate the absence of agonist. The notion that monkeys in this study were discriminating between withdrawal and nonwithdrawal stimuli, and not simply between the presence and absence of agonist, is supported by the observance of certain behavioral effects upon discontinuation of LAAM treatment. Upon discontinuation of LAAM treatment, naltrexone-lever responding was maximal when other behavioral signs of withdrawal were also maximal (*i.e.*, 1–2 days after discontinuation of treatment). Thereafter, responding was distributed between the saline and the naltrexone levers, among the various monkeys. It is unlikely that variations in response patterns among subjects (2–10 days after the discontinuation of LAAM treatment) simply represent a loss of stimulus control because morphine completely reversed naltrexone-lever responding 10 days after the discontinuation of LAAM treatment. It is also unlikely that the lack of sustained naltrexone-lever responding in some monkeys, after discontinuation of LAAM treatment, was due to the long duration of LAAM or one of its metabolites, because naltrexone did not increase naltrexone-lever responding 4 or 8 days after the discontinuation of LAAM. Indeed, variations in response patterns were consistent within, but not between, individuals, perhaps reflecting individual differences in sensitivity to and expression of opioid withdrawal.

Compared to the effects obtained when LAAM treatment was discontinuing, a greater number and magnitude of withdrawal-associated behavioral effects were observed after the administration of naltrexone. In rats, a less severe withdrawal was observed after the termination of LAAM treatment as compared to the withdrawal signs that were evident after the administration of naltrexone (Young *et al.*, 1979a). Similarly, in monkeys, the frequency of withdrawal signs,

decreases in response rates and the proportion of responses made on the naltrexone lever were less after the discontinuation of LAAM treatment as compared to the administration of naltrexone. These results suggest that differences in the magnitude of withdrawal between deprivation-induced and precipitated withdrawal might be related to the duration of action of LAAM and its metabolites. This notion is further supported by other studies showing that the magnitude of withdrawal is less after the termination of LAAM treatment as compared to the magnitude of withdrawal observed after the termination of treatment with shorter acting opioids (*e.g.*, morphine and methadone; Young *et al.*, 1977, 1979a,b).

Early studies in humans reported numerous toxic effects with LAAM (*e.g.*, respiratory depression; Fraser and Isbell, 1952; Keats and Beecher, 1952) which might have resulted from the use of small inter-injection intervals and the accumulation of LAAM and its metabolites. More recent studies have varied dosing conditions and demonstrated LAAM to be a safe and effective treatment for opioid dependence (Blaine *et al.*, 1981; Tennant *et al.*, 1986); however, the rate of metabolite formation and the peak levels of metabolites in plasma after either a single dose or multiple doses of LAAM can be highly variable among individuals (Billings *et al.*, 1974; Henderson *et al.*, 1977; Kaiko and Inturrisi, 1975). Similarly, previous studies in non-human primates have reported marked individual differences in the behavioral effects of LAAM, even among subjects receiving the same dose of LAAM over long periods of time. Chronic dosing with 2.0 mg/kg of LAAM, administered orally three times per week for periods of up to 1 yr, was reported to produce mild to severe signs of opioid intoxication and, occasionally, lethal effects, but only in some monkeys (Downs *et al.*, 1977; Misra and Mule, 1977, 1978). In another study, variations in the response to LAAM among subjects necessitated an adjustment in dose to avoid toxic effects (Crowley *et al.*, 1985). In our study, 1.0 mg/kg of LAAM, administered s.c. every 12 hr, was not associated with any behavioral signs of toxicity over a 2-yr study period. Notably, the total dose of LAAM administered in the current study (14.0 mg/kg per week, s.c.) was more than twice the total dose of LAAM administered by others (p.o. 6.0 mg/kg per week; Downs *et al.*, 1977; Misra and Mule, 1977, 1978). Differences between our study and other studies with regard to toxicity, might be related to the dosing intervals; in our study, LAAM was administered every 12 hr, whereas LAAM was administered every 24 or 48 hr in other studies. A shorter dosing interval with smaller doses might more closely approximate steady-state conditions or, at least, a condition whereby plasma concentrations of LAAM and its metabolites change relatively little across days. Because the formation of active metabolites appears to be important for the behavioral effects of LAAM, the effectiveness of LAAM in treating opioid dependence could vary markedly among individuals, thereby complicating empirical assessment of its relative utility for this indication.

The rate at which LAAM is metabolized to nor-LAAM and dinor-LAAM might also account for differences among studies. When drugs are administered orally they are more susceptible to rapid hepatic metabolism (*i.e.*, first-pass effect) than when they are administered by other routes (*e.g.*, s.c.). Because hepatic metabolism is primarily responsible for the conversion of LAAM to its active metabolites, nor-LAAM and dinor-LAAM (*e.g.*, Billings *et al.*, 1974), differences among studies could be related to variations in the rate of metabolism of LAAM by different routes. Early studies in humans reported that orally administered LAAM had a faster onset on action, with effects observed within 1.5 hr, whereas s.c. administered LAAM had effects only after 14 hr (Fraser and Isbell, 1952). The homogeneity in response to twice daily injections of LAAM among the four monkeys in our study, as well as the general lack of toxic effects obtained with dependence-producing doses of LAAM, suggest that the variability in effects reported by others might be related to dosing interval and route of administration. It is clear that oral dosing with LAAM should be carefully tailored to individuals to maximize therapeutic effects without generating toxicity.

Acknowledgments

The authors thank R. Fortier and C. Scheuermann for their excellent technical assistance.

References

Aceto MD, Bowman ER, Harris LS and May EL (1992) Dependence studies of new compounds in the rhesus monkey and mouse, in *Proceedings, The Committee on Problems of Drug Dependence, National Institute on Drug Abuse Research Monograph 119* (Harris LS, ed) pp 513–558, US Govt Printing Office, Washington, DC.

Beardsley PM, Balster RL and Harris LS (1986) Dependence on tetrahydrocannabinol in rhesus monkeys. *J Pharmacol Exp Ther* **239:**311–319.

Bertalmio AJ, Medzihradsky F, Winger G and Woods JH (1992) Differential influence of N-dealkylation on the stimulus properties of some opioid agonists. *J Pharmacol Exp Ther* **261:**278–284.

Billings RE, McMahon RE and Blake DA (1974) l-acetylmethadol (LAM) treatment of opiate dependence: Plasma and urine levels of two pharmacologically active metabolites. *Life Sci* **14:**1437–1446.

Blaine JD, Renault PR, Thomas DB and Whysner JA (1981) Clinical status of methadyl acetate (LAAM). *Ann NY Acad Sci* **362:**101–115.

Brandt MR, Cabansag SR and France CP (1997) Discriminative stimulus effects of l-α-acetylmethadol (LAAM), buprenorphine and methadone in morphine-treated rhesus monkeys. *J Pharmacol Exp Ther* **282:**574–584.

Crowley TJ, Macdonald MJ and Zerbe G (1985) Variability in simian motor and social behavior with alternating-day acetylmethadol. *Psychopharmacology* **85:** 353–360.

Downs DA, Thomas DJ and Braude MC (1977) Chronic effects of acetylmethadol and methadone in rhesus monkeys. *Life Sci* **21:**719–726.

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

Emmett-Oglesby MW, Mathis DA, Moon RTY and Lal H (1990) Animal models of drug withdrawal symptoms. *Psychopharmacology* **101:**292–309.

Finkle BS, Jennison TA and Chinn DM, Ling W and Holmes ED (1982) Plasma and urine disposition of l-α-acetylmethadol and its principal metabolites in man. *J Anal Toxicol* **6:**100–105.

France CP (1994a) Discrimination among morphine, saline and naltrexone in rhesus monkeys receiving morphine subchronically. *Behav Pharmacol* **5:**15–20.

France CP (1994b) Drug discrimination as a model for studying opioid dependence and withdrawal in animals, in *Strategies for Studying Brain Disorders, Vol. 1* (Palomo T and Archer T eds) pp 429–445, Farrand Press, London.

France CP and Woods JH (1989) Discriminative stimulus effects of naltrexone in morphine-treated rhesus monkeys. *J Pharmacol Exp Ther* **250:**937–943.

Fraser HF and Isbell H (1952) Actions and addiction liabilities of alpha-acetylmethadols in man. *J Pharmacol Exp Ther* **105:**458–465.

Gerak LR and France CP (1996) Discriminative stimulus effects of nalbuphine in rhesus monkeys. *J Pharmacol Exp Ther* **276:**523–531.

Gmerek DE and Woods JH (1985) Effects of β-funaltrexamine in normal and morphine-dependent rhesus monkeys: observational studies. *J Pharmacol Exp Ther* **235:**296–301.

Gmerek DE, Dykstra LA and Woods JH (1987) *Kappa* opioids in rhesus monkeys. III. Dependence associated with chronic administration. *J Pharmacol Exp Ther* **242:** 428–436.

Henderson GL, Weinberg JA, Hargreaves WA, Lau DHM, Tyler J and Baker B (1977) Accumulation of l-α-acetylmethadol [LAAM] and active metabolites in plasma following chronic administration. *J Anal Toxicol* **1:**1–5.

Holtzman SG (1979) Discriminative stimulus properties of *levo-alpha*-acetylmethadol and its metabolites. *Pharmacol Biochem Behav* **10:**565–568.

Holtzman SG (1985) Discriminative stimulus effects of morphine withdrawal in the dependent rat: Suppression by opiate and nonopiate drugs. *J Pharmacol Exp Ther* **233:**80–86.

Holtzman SG and Villarreal JE (1973) Operant behavior in the morphine-dependent rhesus monkey. *J Pharmacol Exp Ther* **184:**528–541.

Kaiko RF and Inturrisi CE (1975) Disposition of acetylmethadol in relation to pharmacologic action. *Clin Pharmacol Ther* **18:**96–103.

Katz JL (1986) Effects of clonidine and morphine on opioid withdrawal in rhesus monkeys. *Psychopharmacology* **88:**392–397.
Keats AS and Beecher HK (1952) Analgesic activity and toxic effects of acetylmethadol isomers in man. *J Pharmacol Exp Ther* **18:**92–103.
Kreek MJ (1992) Rationale for maintenance pharmacotherapy of opiate dependence, in *Addictive States* (O'Brien CP and Jaffe JH eds) pp 205–230, Raven Press, Ltd., New York.
Ling W, Rawson RA and Compton MA (1994) Substitution pharmacotherapies for opioid addiction: From methadone to LAAM and buprenorphine. *J Psychoactive Drugs* **26:**119–128.
McGivney WT and McMillan DE (1981) The effects of levo-α-acetylmethadol and its metabolites on schedule-controlled behavior in the pigeon. *J Pharmacol Exp Ther* **216:**299–305.
Misra AL and Mule SJ (1977) Severe toxicity and lethality in some monkeys following chronic administration of l-α-acetylmethadol (LAAM). *Am J Drug Alcohol Abuse* **4:** 431–440.
Mule SJ and Misra AL (1978) Pharmacology of l-α-acetylmethadol (LAAM) in subhuman primates. *Ann NY Acad Sci* **311:**199–213.
Schuster CR, Greenwald MK, Johanson C-E and Heishman SJ (1995) Measurement of drug craving during naloxone-precipitated withdrawal in methadone-maintained volunteers. *Exp Clin Psychopharmocol* **3:**424–431.
Slifer BL, Balster RL and Woolverton WL (1984) Behavioral dependence produced by continuous phencyclidine infusion in rhesus monkeys. *J Pharmacol Exp Ther* **230:**399–406.
Tennant FS, Rawson RA, Pumphrey E and Seecof R (1986) Clinical experiences with 959 opioid-dependent patients treated with levo-alpha-acetylmethadol (LAAM). *J Substance Abuse Treat* **3:**195–202.
Thompson T and Schuster CR (1964) Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. *Psychopharmacologia* **5:**87–94.
Valentino RJ, Herling S and Woods JH (1983) Discriminative stimulus effects of naltrexone in narcotic-naive and morphine-treated pigeons. *J Pharmacol Exp Ther* **224:**307–313.
Weissman A (1978) The discriminability of naloxone in rats depends on concomitant morphine treatment, in *Stimulus Properties of Drugs: Ten Years of Progress* (Colpaert FC and Rosecrans JA eds) pp 209–214, Elsevier/North-Holland Biomedical Press, Amsterdam.
Woolverton WL and Kleven MS (1988) Evidence for cocaine dependence in monkeys following a prolonged period of exposure. *Psychopharmacology* **94:**288–291.
Young GA, Steinfels GF and Khazan N (1979a) Spontaneous vs. naloxone-induced abstinence in dependent rats self-administering l-alpha-acetylmethadol (LAAM) or morphine. *Pharmacol Biochem Behav* **10:**585–589.
Young GA, Steinfels GF and Khazan N (1979b) Pharmacodynamic profiles of l-α-acetylmethadol (LAAM) and its N-demethylated metabolites, nor-LAAM and dinor-LAAM, during self-administration in the dependent rat. *J Pharmacol Exp Ther* **210:**453–457.
Young GA, Moreton JE, Meltzer LT and Khazan N (1977) l-Alpha-acetylmethadol (LAAM), methadone and morphine abstinence in dependent rats: EEG and behavioral correlates. *Drug Alcohol Depend* **2:**141–148.

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