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Chronic *l*-α-Acetylmethadol (LAAM) in Rhesus Monkeys: Tolerance and Cross-Tolerance to the Antinociceptive, Ventilatory, and Rate-Decreasing Effects of Opioids[1]

MICHAEL R. BRANDT and CHARLES P. FRANCE

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana

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ABSTRACT

Although *l*-α-acetylmethadol (LAAM) is a maintenance treatment for opioid dependence, few studies have systematically assessed the behavioral effects of LAAM and other drugs in LAAM-treated subjects. In the current study, we assessed the ventilatory, antinociceptive, and rate-decreasing effects of drugs (s.c. except dynorphin, which was administered i.v.) in rhesus monkeys (n = 3 or 4) before and during chronic treatment with 1.0 mg/kg/12 h LAAM (s.c.). Minute volume (V_E) was reduced to 62% of baseline during LAAM treatment and remained depressed after more than 10 months of LAAM treatment. A cumulative dose of 10.0 mg/kg morphine decreased V_E to similar values under baseline (53%) and LAAM-treated (52%) conditions; however, larger doses of morphine (up to 56.0 mg/kg) could be administered safely to LAAM-treated monkeys. LAAM treatment produced dependence as evidenced by a 220% increase in V_E after a dose of naltrexone (0.032 mg/kg) that did not modify ventilation under baseline conditions. Compared with baseline, LAAM treatment increased the ED_{50} values for the rate-decreasing effects of nalbuphine, morphine, and alfentanil by 7-, 7-, and 2-fold, respectively, in monkeys responding under a fixed ratio 10 schedule of food presentation. Similarly, LAAM treatment increased ED_{50} values for the antinociceptive effects of morphine and alfentanil by 5- and 3-fold, respectively. LAAM treatment also increased the ED_{50} values for the antinociceptive effects of the κ-agonist enadoline by 5-fold and not those of U-50,488. That tolerance developed differentially to the ventilatory, rate, and antinociceptive effects of μ-agonists in LAAM-treated monkeys suggests that cross-tolerance might not be a safe therapeutic approach for the treatment of some opioid abusers.

There are inconsistencies in the literature as to whether tolerance develops to the analgesic and respiratory effect of opioids. The analgesic effects of μ-opioids make them clinically useful for alleviating moderate to severe pain, and they are often administered for long periods of time to patients with chronic pain. In some chronic-pain patients receiving at least 50 mg of morphine daily, postoperative pain relief was obtained only with doses of morphine that were much larger than those required to relieve pain in chronic-pain patients who were not receiving morphine daily (De Leon-Casasola et al., 1993). In other chronic-pain patients, the analgesic effects of morphine did not change despite long periods of treatment (Gourlay et al., 1986; Pfeifer et al., 1989). Apparent discrepancies among studies can be in part attributed to the difficulties in studying tolerance in humans, in whom disease progression and intensifying pain can necessitate an increase in the dose of opioid agonist (Portenoy and Foley, 1986).

An important adverse effect of μ-opioids is their ability to depress respiration. The few studies that have systematically evaluated tolerance to the respiratory effects of opioids in humans reported mixed results. For example, in humans receiving 60 mg of morphine four times daily, ventilation was decreased by 15 to 20% after more than 34 weeks of treatment, which suggests that tolerance did not develop (Martin and Jasinski, 1969). However, the ventilatory effects of 60 and 120 mg of morphine were less in opioid-dependent individuals than the ventilatory effects obtained with much smaller doses of morphine (15 and 30 mg) in nondependent individuals, which suggests that tolerance can develop (Martin et al., 1968). Similar contrasting results are reported in nonhumans, and there is evidence that tolerance can develop differentially to the antinociceptive and ventilatory effects of opioids. For example, greater tolerance developed to the antinociceptive effects of morphine than to the ventilatory effects of morphine after morphine pellet implantation in mice

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ABBREVIATIONS: LAAM, *l*-α-acetylmethadol; DYN, dynorphin A(1–13); V_E, minute volume; FR, fixed ratio; f, frequency; V_T, tidal volume; NMDA, *N*-methyl-D-aspartate.

(McGilliard and Takemori, 1978). In rhesus monkeys treated for 40 weeks with 3.2 mg/kg/12 h morphine, the dose-effect curve for the antinociceptive effects of morphine was shifted 4-fold to the right of the morphine dose-effect curve determined before chronic morphine treatment (Paronis and Woods, 1997b). In contrast, ventilation was depressed throughout the 40 weeks of morphine treatment, and the morphine dose-effect curve for ventilatory depression was not different from the untreated condition. There also was a lack of tolerance to the ventilatory effects of other μ-agonists, such as fentanyl and nalbuphine (Paronis and Woods, 1997b). Collectively, these results suggest that tolerance can develop to some clinically relevant effects of opioids.

The dose and the frequency of treatment (Blasig et al., 1973) can influence the magnitude of tolerance that develops to drugs. For example, greater tolerance typically develops after the administration of drugs that have longer durations of action than with drugs that have shorter durations of action. *l*-α-Acetylmethadol (LAAM) is a μ-opioid agonist that is used clinically as a maintenance therapy for opioid dependence. Importantly, LAAM has a slow onset and a long duration of action, which have been attributed to the formation of two active metabolites, *l*-α-acetylnormethadol (i.e., nor-LAAM) and *l*-α-acetyldinormethadol (i.e., dinor-LAAM; Henderson et al., 1977; Finkle et al., 1982), both of which have equal or greater potency than the parent compound (Holtzman, 1979; Bertalmio et al., 1992; Brandt et al., 1997). The long duration of LAAM not only makes LAAM a useful pharmacotherapy for opioid dependence but also makes it a useful tool for studying opioid tolerance.

Accordingly, the purpose of the current study was to assess the extent to which chronic treatment with LAAM modifies the behavioral effects of other opioids. The ventilatory, rate-decreasing, and antinociceptive effects of μ-opioids were assessed both before and during LAAM treatment. The μ-opioids that were studied vary in efficacy from low (nalbuphine) to intermediate (morphine) to high (alfentanil; Gerak et al., 1994; Walker et al., 1995). Under some conditions, κ-agonists can modify the behavioral effects of μ-opioids. For example, the selective κ-agonist U-50,488 attenuated the respiratory-depressant and antinociceptive effects of morphine (Craft and Dykstra, 1992; Dosaka-Akita et al., 1993), and the κ-opioid peptide dynorphin A(1–13) (DYN) modified tolerance and dependence that developed to some μ-agonists (Tulunay et al., 1981; Takemori et al., 1992). Therefore, the behavioral effects of the selective κ-agonists U-50,488, enadoline, and DYN were also assessed. For comparison with these opioid agonists, the nonopioid *N*-methyl-D-aspartate (NMDA) antagonist ketamine was also tested.

Materials and Methods

Subjects

Two male and two female adult rhesus monkeys (*Macaca mulatta*) were individually housed in stainless steel cages with free access to water. Daily access to food (Teklad Monkey Chow) was restricted, with monkeys maintained at no less than 90% of their free-feeding weights; monkeys also received fresh fruit and peanuts several times each week. A 14:10-h light/dark schedule was in effect (lights on at 6:00 AM). All monkeys were experimentally naive at the beginning of these studies and participated in a companion study (Brandt and France, 1998) that assessed the behavioral effects of dependence and withdrawal midway through the current study. Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, Louisiana State University Health Sciences 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 1996].

Assay of Ventilation

Apparatus and Procedure. Monkeys were seated in metal or Lexan primate chairs within sound-attenuating chambers. A plethysmograph was placed over the head of the monkey and was sealed with alternating plastic and rubber neck dams. Air or 5% CO_2 in O_2 flowed into the plethysmograph at a rate of 10 l/min and was removed with a vacuum pump. Because of procedural changes in this laboratory, redeterminations of the ventilatory effects of drugs during chronic LAAM treatment were assessed in 5% CO_2 in breathing air. Ventilation-induced changes in air pressure were measured with a pressure transducer and recorded by a polygraph trace and by a microprocessor via an analog-to-digital converter.

The ventilatory effects of opioids were assessed in three monkeys before (baseline) and during LAAM treatment. Experimental sessions consisted of multiple, 30-min cycles consisting of a 23-min exposure to breathing air followed by a 7-min exposure to 5% CO_2; studies conducted before LAAM treatment consisted of a 7-min exposure to 5% CO_2 in O_2, whereas studies conducted during LAAM treatment consisted of a 7-min exposure to 5% CO_2 in breathing air. In other monkeys, baseline ventilation and morphine dose-effect curves determined under these two conditions were not different (our unpublished observations). Saline was administered during the first cycle, and increasing doses of morphine, nalbuphine, or naltrexone were administered during the 1st minute of subsequent cycles. Ventilation was monitored continuously, although only the last 3 min of exposure to either air or 5% CO_2 were used for data analyses. Redeterminations of the ventilatory effects of opioids in LAAM-treated monkeys occurred after more than 10 months of twice daily (at 7:00 AM and 7:00 PM) injections of 1.0 mg/kg LAAM (additional details below). The ventilatory effects of drugs were assessed at approximately 11:00 AM. Test sessions ended when ventilation decreased to less than 50% of the saline control value.

Data Analyses. Results of ventilation studies are presented as the average minute volume (V_E), tidal volume (V_T), and frequency (f) $\pm$ 1 S.E.M. of the last 3 min of exposure to either air or 5% CO_2. To determine the ventilatory effects of a wide range of morphine doses before LAAM treatment, two separate tests were combined. Morphine was studied from 0.01 to 1.0 mg/kg during one session and from 0.32 to 10.0 mg/kg during another session; the duplicate data for individual subjects with 0.32 and 1.0 mg/kg morphine were averaged. Comparisons between ventilatory measures under baseline and LAAM-treated conditions were analyzed by paired t tests. Differences between dose-effect curves for the ventilatory effects of drugs under baseline and LAAM-treated conditions were analyzed by two-way ANOVA (general linear model). Significant interactions were analyzed further by Student-Newman-Keuls multiple comparison test. The level of significance was set at $P < .05$.

Assays of Thermal Antinociception and Schedule-Controlled Responding

Apparatus and Procedure. Monkeys were seated in either metal or Lexan primate chairs within ventilated, sound-attenuating chambers. Each chamber contained three response levers; the center lever could be extended into (available) or retracted out of (unavailable) the chamber. Located above each response lever was a green stimulus light. An externally mounted pellet dispenser delivered 300-mg banana-flavored pellets (product F0179; Bio-serve, Frenchtown, NJ) to a food cup located below the center lever. The feet of the monkeys were placed into a pair of shoes located on the front of the

chair. A microprocessor, interface, and commercially available software controlled experiments and recorded data.

Three monkeys were initially trained to respond during daily multiple cycle (two to five) sessions. Each cycle was 15 min in duration and consisted of a timeout period (10 min), a response period (2 min), and a second timeout period (3 min). During the 10-min timeout, the chamber was dark, the center lever was available, and responses had no programmed consequence. During the 2-min response period, the center stimulus light was illuminated green and monkeys could respond on the center lever under a fixed ratio (FR)10 schedule of food presentation (two pellets delivered for each completed ratio). The stimulus light was extinguished after 2 min or when the monkey completed 10 FR10 (i.e., received the maximum 20 food pellets). Responses on either the left or right lever had no scheduled consequence. After this response period was a second timeout period, during which the chamber was dark, the center lever was unavailable, and responses had no programmed consequence. During this 3-min period, the latency for monkeys to remove their tails from warm water was determined and used as a measure of antinociception. The chamber door was opened, and the lower 10 cm of the shaved tail was immersed in a thermos bottle containing 40, 50, or 55°C water. The latency for a monkey to remove its tail from the thermos bottle was measured manually using a hand-held stopwatch. If a monkey did not remove its tail within 20 s, the experimenter removed the tail from the thermos and a latency of 20 s was recorded. After the assessment of tail-withdrawal latencies, the chamber door was closed and no events were scheduled for the remainder of the 3-min period. Testing began when 1) the daily mean response rate for each of 6 consecutive days did not exceed ±15% of the overall mean response rate for those six sessions and 2) monkeys reliably did not remove their tail from 40°C water and reliably removed their tail from 50 and 55°C water within 5 s.

Test sessions were identical with training sessions except that increasing doses of the μ-agonists alfentanil, morphine, or nalbuphine; κ-agonists enadoline or U-50,488; or the NMDA antagonist ketamine were administered. An injection of saline was administered during the 1st minute of the first cycle and cumulative doses of drug, increasing in one-fourth or one-half log unit increments, were administered s.c. during the 1st minute of subsequent cycles. Nalbuphine was tested up to a dose of 56.0 mg/kg, and other drugs were tested up to doses that maximally increased tail-withdrawal latencies (i.e., 20 s) in 50°C water. The κ-selective peptide DYN was administered i.v. 3 min before the start of the first cycle, which was followed by a total of four cycles (i.e., 60-min time course). Previous studies in monkeys have demonstrated that the peak antinociceptive effects of DYN occur between 15 and 30 min after i.v. injection (Butelman et al., 1995). Test sessions were conducted no more than twice per week.

After these studies, a fourth monkey was added to these experiments, and all monkeys were treated for at least 10 months with 1.0 mg/kg/12 h LAAM s.c. (7:00 AM and 7:00 PM) and trained to discriminate between saline and naltrexone (0.01 or 0.0178 mg/kg) s.c. under an FR5 schedule of stimulus shock termination. The behavioral measures of dependence and withdrawal that were assessed during this time are presented elsewhere (Brandt and France, 1998). On completion of the discrimination study, monkeys again responded under an FR10 schedule of food presentation, and drugs were reassessed for their ventilatory, rate-decreasing, and antinociceptive effects. All experimental details and the criteria for testing were identical to the conditions used before LAAM treatment.

A preliminary study was conducted to determine possible variations in the sensitivity of monkeys to thermal stimuli (caused by the onset and offset of LAAM) that could influence potency estimates for the antinociceptive effects of other drugs. Every 2 h for 12 h after the 7:00 AM administration of 1.0 mg/kg LAAM, monkeys were placed into chairs and the tail-withdrawal latencies in 42, 44, 46, 48, 50, 52, and 54°C water were assessed. The antinociceptive effects of the 7:00 AM injection of LAAM appeared to be decreasing at 7:00 PM. Therefore, the antinociceptive and rate effects of drugs were assessed at 7:00 PM to minimize additive effects between LAAM and test drugs.

Data Analyses. Rates of responding are presented as the average number of responses per second (±1 S.E.M.). Antinociception is presented as the average latency in seconds (±1 S.E.M.) for monkeys to remove their tails from 50°C water. To assess shifts in dose-effect curves, response rate data for individual subjects were converted to a percentage of the average rate of the five preceding nontest cycles, and the antinociceptive data for individual subjects were converted to a percentage of the maximum possible effect (MPE) by the following calculation: %MPE = [(test latency − control latency) ÷ (20 − control latency)] × 100%. Individual ED_{50} values were calculated by linear regression when three or more data points were available and by interpolation when two data points (one above and one below 50%) were available. Individual ED_{50} values were converted to their log values for calculation of means and 95% confidence limits and then converted back to linear values for presentation. ED_{50} values (±1 S.E.M.) were averaged across subjects. ED_{50} values were considered to be significantly different from control when the 95% confidence limits did not overlap.

To assess shifts in temperature-effect curves after LAAM administration, the temperature that increased tail-withdrawal latencies to 10 s was calculated by linear regression when three or more data points were available and by interpolation when two data points (one above and one below 10 s) were available. The average temperature (±1 S.E.M.) producing a 10-s increase in tail-withdrawal latencies was averaged across subjects. Differences between dose-effect curves under two treatment conditions were analyzed by two-way ANOVA (general linear model). Significant interactions were analyzed further by Student-Newman-Keuls multiple comparison test. The level of significance was set at $P < .05$.

Drugs

LAAM, DYN, morphine sulfate, and naltrexone hydrochloride were obtained from Research Technology Branch, National Institute on Drug Abuse (Rockville, MD). Enadoline hydrochloride was obtained from Warner-Lambert/Parke-Davis (Ann Arbor, MI). U-50,488 (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzenacetamide methanesulfonate) was obtained from The Upjohn Co. (Kalamazoo, MI). Nalbuphine hydrochloride was obtained from Mallinckrodt Inc. (St. Louis, MO). The commercially available Ketaset solution (Fort Dodge Laboratories, Inc., Fort Dodge, IA) was used for ketamine hydrochloride and was diluted in sterile water. With the exception of LAAM and DYN, all other compounds were dissolved in sterile water and injected s.c. in the back in a volume of 0.01 to 0.4 ml/kg. LAAM was dissolved in 85% H_2O, 10% Emulphor, and 5% ethanol to which a small quantity of 5 M NaOH was added to increase the pH to 6 to 7. DYN was dissolved in sterile water and injected i.v. in the saphenous vein in a volume of 0.2 to 0.5 ml. Doses are expressed in milligrams per kilogram of body weight in terms of the forms described above.

Results

Figure 1 shows ventilation before and during twice-daily LAAM treatment. Under baseline conditions (absence of LAAM), V_E, V_T, and f in air were 1300 ± 108 ml, 34 ± 5 ml, and 39 ± 10 breaths/min, respectively (open bars above "baseline"). After more than 10 months of LAAM treatment, V_E and f were significantly decreased by 44 and 34%, respectively, whereas V_T was not modified (open bars above "LAAM" treatment). In 5% CO_2, V_E, V_T, and f increased to 1814 ± 150 ml, 35 ± 5 ml, and 48 ± 13 breaths/min, respectively (shaded bars above "baseline"). Similar to the effects in air, LAAM treatment significantly decreased V_E (37%) and f (32%) in 5% CO_2 without modifying V_T (shaded bars above

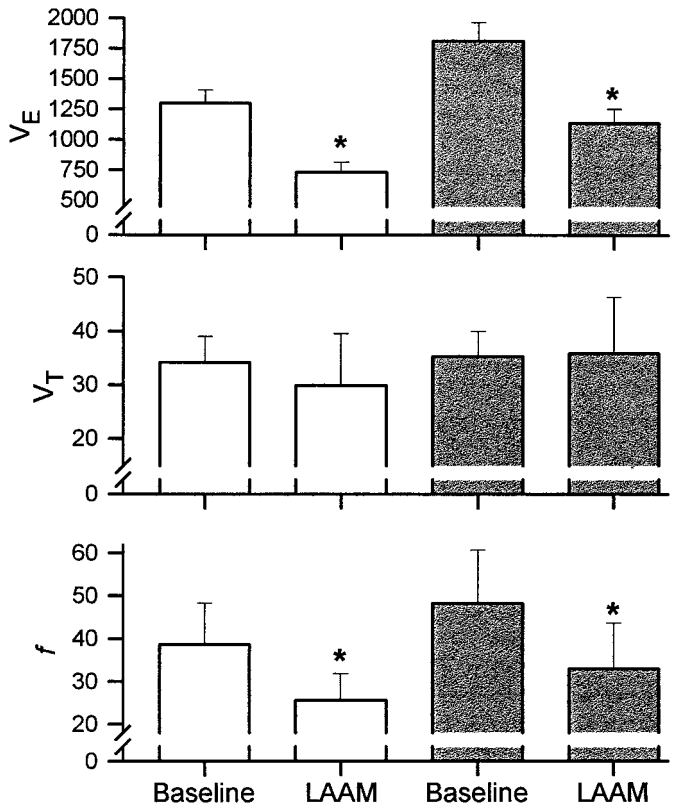

Fig. 1. Ventilation in monkeys breathing air (□) or 5% CO_2 (■) before and during LAAM treatment. V_E (milliliters per minute), V_T (milliliters per inhalation), and f (inhalations per minute) are expressed as absolute values. Abscissa represents before (baseline) and during daily LAAM treatment (LAAM). *, significantly ($P < .05$) different from the baseline condition. All bars show the mean (+1 S.E.M.) from three determinations in three monkeys.

"LAAM" treatment). Sensitivity to the ventilatory-stimulant effects of 5% CO_2 was not substantially modified by LAAM treatment. Under baseline conditions, 5% CO_2 increased V_E by 40%, and during LAAM treatment, 5% CO_2 increased V_E by 55%.

Naltrexone had differential effects on ventilation depending on whether monkeys were receiving LAAM. Under baseline conditions, naltrexone did not modify ventilation in air (Fig. 2, left) or produce any observable changes in behavior up to a dose of 0.032 mg/kg. In contrast, naltrexone dose dependently increased ventilation in LAAM-treated monkeys. A cumulative dose of 0.0032 mg/kg naltrexone increased V_E and f to values similar to those obtained under control conditions (Fig. 2, top and bottom). Doses of naltrexone larger than 0.0032 mg/kg tended to increase f and decrease V_T (Fig. 2, bottom and middle), and a dose of 0.032 mg/kg naltrexone increased f by 220%. Cumulative doses of naltrexone larger than 0.001 mg/kg increased the frequency of grimacing, holding abdomen, and wet-dog shakes (data not shown), and for one monkey, the intensity of behavioral signs precluded the administration of 0.032 mg/kg naltrexone.

Under baseline conditions, naltrexone did not modify ventilation in 5% CO_2 (Fig. 2, right). In LAAM-treated monkeys, a cumulative dose of 0.0032 mg/kg naltrexone increased V_E and f to approximately baseline values. Increases in V_E (Fig. 2, top) were observed primarily because of increases in f (Fig. 2, bottom) rather than changes in V_T (Fig. 2, middle).

Figure 3 shows that morphine dose dependently decreased ventilation in air (left) to similar values under baseline and LAAM-treated conditions. Under baseline conditions, a cumulative dose of 10.0 mg/kg morphine decreased V_E to 645 ± 55 ml. Although ventilation was lower in LAAM-treated monkeys (points above "S"), a similar decrease was observed

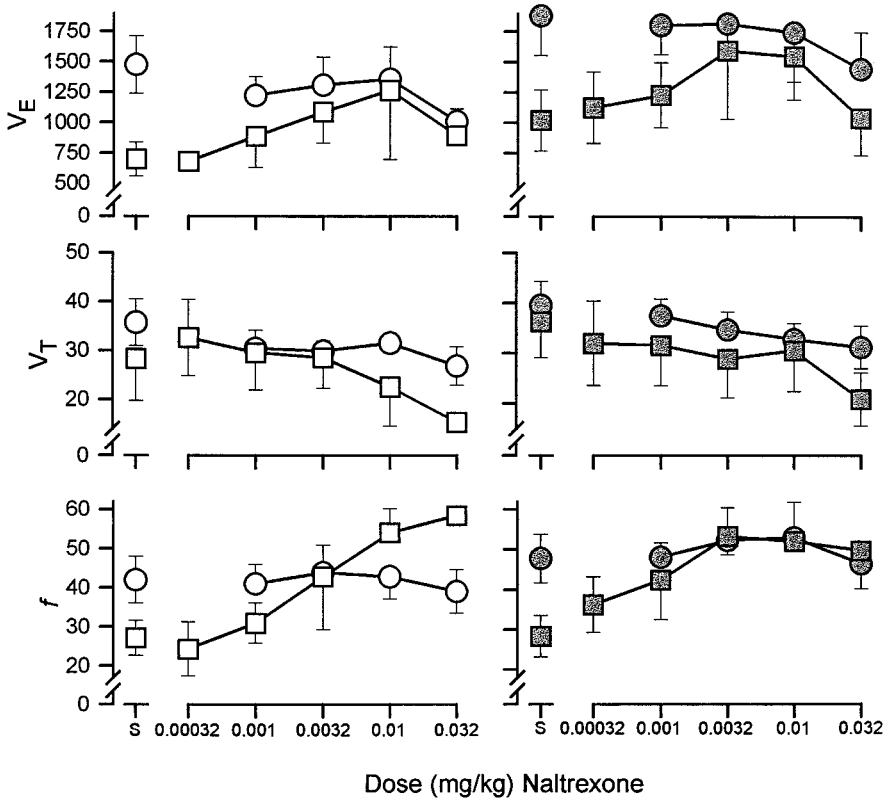

Fig. 2. Ventilatory effects of naltrexone on V_E, V_T, and f in monkeys breathing air (left; ○, baseline; □, LAAM treatment) or 5% CO_2 (right; ●, baseline; ■, LAAM treatment) under baseline and LAAM-treated conditions. Abscissa represents dose (milligrams per kilogram of body weight). All points show the mean (±1 S.E.M.) for three monkeys except for 0.032 mg/kg naltrexone under the LAAM-treated condition ($n = 2$). See the legend for Fig. 1 for other details.

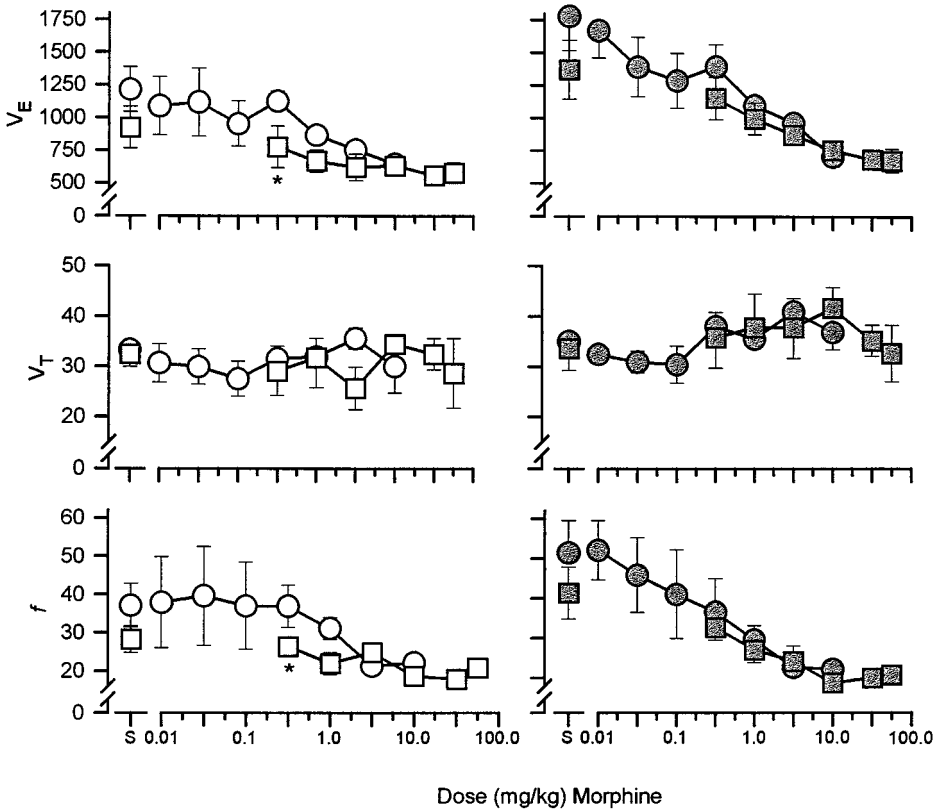

Fig. 3. Ventilatory effects of morphine in monkeys breathing air (left; ○, baseline; □, LAAM treatment) or 5% CO_2 (right; ●, baseline; ■, LAAM treatment) under baseline and LAAM-treated conditions. All points show the mean (±1 S.E.M.) for three monkeys except for 56.0 mg/kg morphine under the LAAM-treated condition (n = 2). See the legends for Figs. 1 and 2 for other details.

at a cumulative dose of 10.0 mg/kg morphine (V_E = 631 ± 52 ml). Decreases in V_E under baseline and LAAM-treated conditions after morphine were due to decreases in f (Fig. 3, bottom) rather than V_T (Fig. 3, middle). V_E and f appeared to plateau with doses of morphine larger than 3.2 mg/kg in monkeys receiving LAAM, although there were considerable differences among monkeys. For example, in one monkey, a dose of 32.0 mg/kg morphine decreased V_E to 44% of the saline control, and therefore, the highest dose (56.0 mg/kg) was not tested. In a second monkey, 56.0 mg/kg morphine decreased V_E to 52% of the saline control, whereas in a third monkey, this dose of morphine did not modify V_E (104% of control).

In monkeys breathing CO_2, similar decreases in ventilation after morphine were observed before and during LAAM treatment (Fig. 3, right). Under baseline conditions, a cumulative dose of 10.0 mg/kg morphine decreased V_E to 707 ± 76 ml. Likewise, during LAAM treatment, a cumulative dose of 10.0 mg/kg morphine decreased V_E to 755 ± 42 ml (Fig. 3, top). Under both conditions, decreases in V_E after morphine were primarily the result of decreases in f (Fig. 3, bottom) rather than changes in V_T (Fig. 3, middle). Although morphine decreased V_E and f to similar values before and during LAAM treatment, V_E and f appeared to plateau at doses of morphine larger that 3.2 mg/kg in LAAM-treated monkeys.

Nalbuphine had little effect on ventilation in LAAM-treated monkeys. Up to a dose of 56.0 mg/kg, nalbuphine did not decrease V_E, V_T, or f in LAAM-treated monkeys breathing air (Fig. 4, left). Similarly, nalbuphine did not modify V_E, V_T, or f in LAAM-treated monkeys breathing 5% CO_2 (Fig. 4, right).

Temperature-response curves were determined every 2 h for 12 h after the 7:00 AM injection of LAAM to determine whether daily injections of LAAM modified tail-withdrawal latencies. The average temperature that produced a 10-s tail-withdrawal latency is presented in Table 1. Both 2 and 12 h after LAAM administration, an average temperature of 48.8°C produced a 10-s latency. The average temperature to produce a 10-s tail-withdrawal latency was maximally increased (51.3°C) 6 and 8 h after LAAM injection, although these changes in temperature were not significantly different from other times.

Figure 5 shows the rate-decreasing and antinociceptive effects of μ-opioids before and during LAAM treatment. After saline (leftmost data points in each panel; circle above "S"), the average rate of food-maintained responding was 1.19 ± 0.06 responses/s in untreated monkeys. Nalbuphine (Fig. 5, left) dose dependently decreased rates of responding with a dose of 10.0 mg/kg decreasing rates to less than 0.1 response/s. LAAM treatment did not substantially modify the average rate of responding (1.30 + 0.04 responses/s; square above "S") compared with the untreated condition. Nalbuphine dose dependently decreased response rates in LAAM-treated monkeys with an ED_{50} value (Table 2) that was significantly (7-fold) greater than the ED_{50} obtained under baseline conditions. A dose of 56.0 mg/kg nalbuphine was required to decrease response rates to less than 0.1 response/s. Tail-withdrawal latencies in 50°C water were less than 2 s before and during LAAM treatment (Fig. 5, bottom, points above "S"). Before LAAM treatment, nalbuphine had limited antinociceptive effects in 50°C water, and the maximum tail-withdrawal latency was 9.1 s at a dose of 32.0 mg/kg. During LAAM treatment, nalbuphine failed to increase tail-withdrawal latencies above 2 s up to a dose of 56.0 mg/kg, resulting in a downward shift in the nalbuphine dose-effect curve.

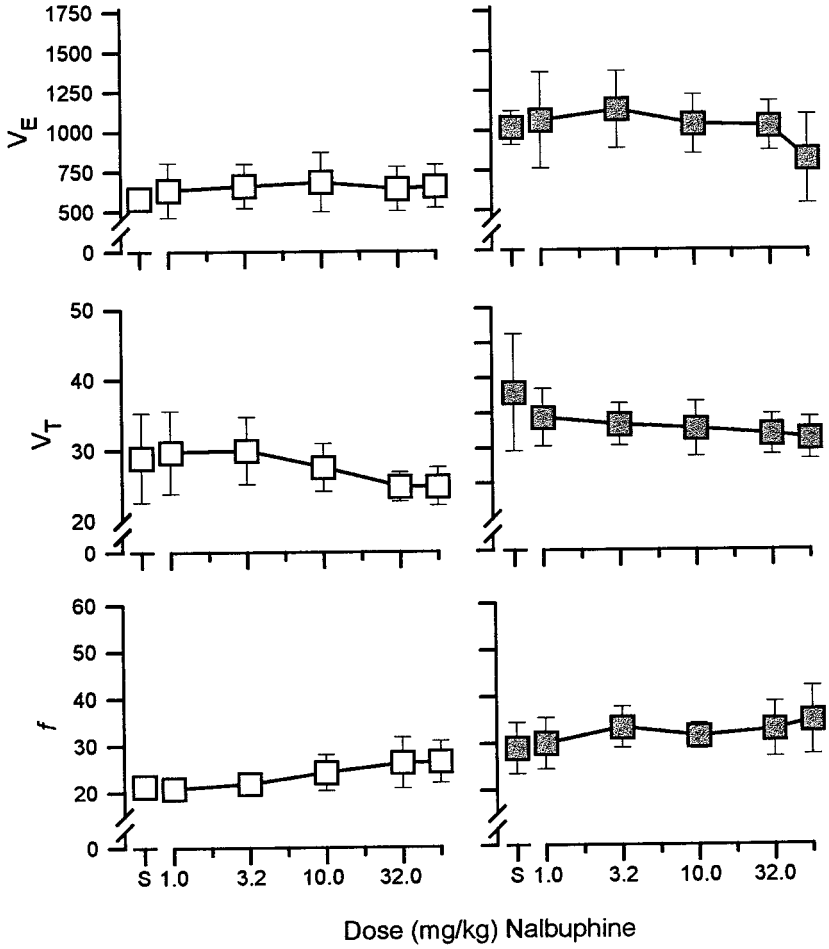

Fig. 4. Ventilatory effects of nalbuphine in monkeys breathing air (left; □) or 5% CO_2 (right; ■) under the LAAM-treated condition. All points show the mean (±1 S.E.M.) for three monkeys. See the legends for Figs. 1 and 2 for other details.

TABLE 1
Average temperature that produced a 10-s tail-withdrawal latency after 1.0 mg/kg LAAM

Time after LAAM	Mean Temperature
h	*°C ± 1 S.E.M.*
2	48.8 ± 0.71
4	49.3 ± 0.74
6	51.3 ± 1.04
8	51.3 ± 0.53
10	50.7 ± 0.71
12	48.8 ± 0.86

Morphine (Fig. 5, middle) dose dependently decreased rates of lever pressing in monkeys before LAAM treatment with a dose of 17.8 mg/kg decreasing rates to less than 0.1 response/s. LAAM treatment shifted the morphine dose-effect curve significantly to the right and produced a 7-fold increase in the ED_{50} value (Table 2). Up to a dose of 56.0 mg/kg morphine, response rates were greater than 0.1 response/s in three of four LAAM-treated monkeys. Under untreated conditions, tail-withdrawal latencies were maximally increased after a dose of 17.8 mg/kg morphine. LAAM treatment shifted the dose-effect curve for the antinociceptive effects of morphine significantly to the right and produced a 5-fold increase in the ED_{50} value. Tail-withdrawal latencies increased to only 15 s after the largest dose of morphine (56.0 mg/kg).

Alfentanil dose dependently decreased rates of responding and increased tail-withdrawal latencies (Fig. 5, right). LAAM treatment produced a significant shift to the right in the alfentanil dose-effect curve and a 2-fold increase in the ED_{50} value (Table 2). A dose of 0.1 mg/kg alfentanil decreased responding to less than 0.1 response/s in all monkeys before LAAM treatment and in three of four monkeys during LAAM treatment. Similarly, LAAM treatment produced a significant shift in the dose-effect curve for the antinociceptive effects of alfentanil and increased the ED_{50} by 3-fold.

The NMDA antagonist ketamine dose dependently decreased response rates and increased tail-withdrawal latencies (Fig. 6, left). LAAM treatment did not modify the ED_{50} values for ketamine (Table 2); under both untreated and LAAM-treated conditions, similar doses of ketamine were required to decrease rates of responding (3.2 mg/kg) and to increase tail-withdrawal latencies (10.0 mg/kg). Similarly, LAAM treatment did not modify the behavioral effects of the κ-agonist U-50,488 (Fig. 6, middle). ED_{50} values (Table 2) and doses of U-50,488 that either eliminated responding or produced maximum tail-withdrawal latencies were similar before and during LAAM treatment. In contrast, LAAM treatment modified the behavioral effect of the κ-agonist enadoline (Fig. 6, right). LAAM-treated monkeys were slightly more sensitive to the rate-decreasing effects of enadoline than control monkeys. For example, a dose of 0.00032 mg/kg enadoline did not modify rates of responding under baseline conditions, whereas this dose significantly decreased rates of responding in LAAM-treated monkeys. Al-

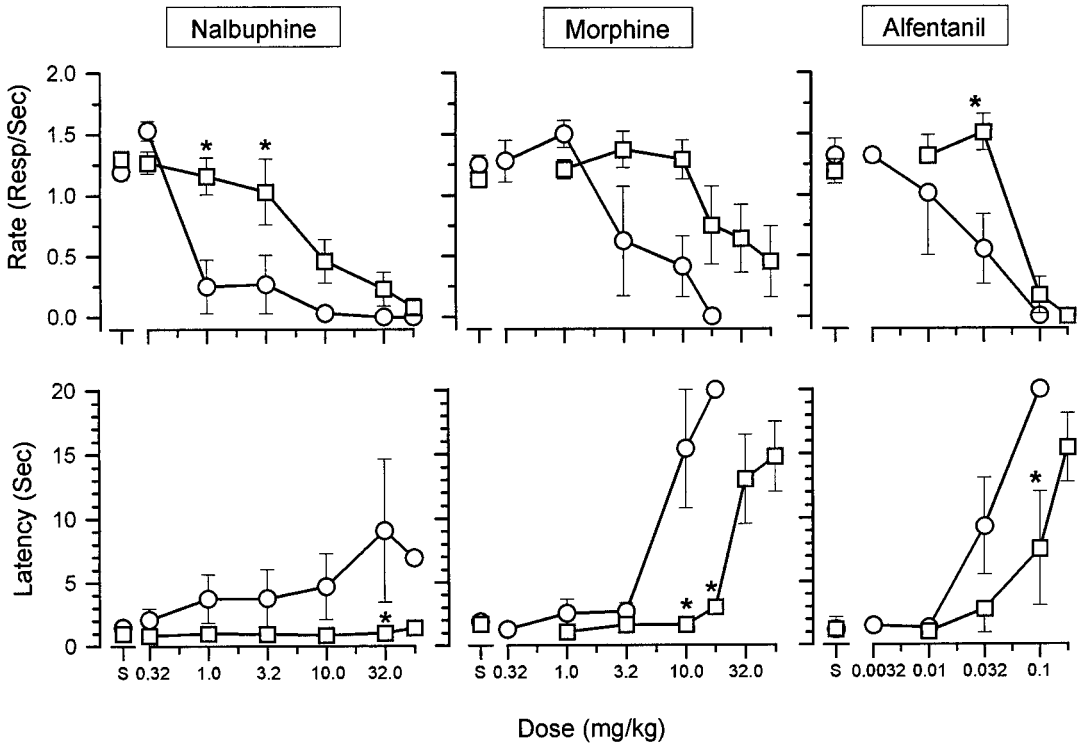

Fig. 5. Rate-decreasing and antinociceptive effects of nalbuphine (left), morphine (middle), and alfentanil (right) under baseline (○; $n = 3$) and LAAM-treated (□; $n = 4$) conditions. Ordinates represent (top) average responses (±1 S.E.M.) per second and (bottom) average tail-withdrawal latency (±1 S.E.M.) in 50°C water. Abscissae represent dose (milligrams per killigram of body weight). Saline was administered on the first cycle, and these data are shown above "S". *, significantly ($P < .05$) different from the baseline condition.

TABLE 2

ED_{50} values to decrease rates of responding under an FR30 schedule of food presentation and to increase tail-withdrawal latencies from 50°C water

Test Drug	Response Rates		Antinociception	
Nalbuphine				
Baseline	1.59	(0.38–3.41)		—[a]
LAAM treatment	9.62	(3.63–15.75)[b]		—
Morphine				
Baseline	5.5	(1.92–10.67)	6.8	(4.54–9.47)
LAAM treatment	37.8	(16.33–62.25)[b]	36.7	(22.12–52.61)[b]
Alfentanil				
Baseline	0.029	(0.006–0.073)	0.038	(0.024–0.055)
LAAM treatment	0.072	(0.060–0.084)	0.117	(0.047–0.204)
Ketamine				
Baseline	0.81	(0.41–1.35)	4.93	(3.88–6.10)
LAAM treatment	0.27	(0.11–0.43)	4.65	(3.10–6.37)
U-50,488				
Baseline	0.19	(0.18–0.20)	0.90	(0.38–1.60)
LAAM treatment	0.14	(0.06–0.26)	1.47	(0.76–2.33)
Enadoline				
Baseline	0.0007	(0.0005–0.0009)	0.0028	(0.0010–0.0054)
LAAM treatment	0.0012	(0.0004–0.0027)	0.0093	(0.0052–0.0139)

[a] Could not be determined.
[b] 95% confidence limits do not overlap with baseline values.

though LAAM-treated monkeys were more sensitive to the rate-decreasing effects of enadoline, they were less sensitive to the antinociceptive effects of enadoline. The dose-effect curve for the antinociceptive effects of enadoline was shifted to the right and the ED_{50} value was increased 5-fold in LAAM-treated monkeys (Table 2).

Like enadoline, acute administration of DYN had greater rate-decreasing effects in LAAM-treated monkeys than in untreated monkeys (Fig. 7). At 15 min after the i.v. administration of 1.0 mg/kg DYN (left), rates of responding were decreased to less than 0.1 response/s in one of three monkeys under baseline conditions. Rates of responding returned to control values by 30 min. In comparison, this dose of DYN decreased rates of responding to less than 0.1 response/s in all LAAM-treated monkeys, and rates did not return to control values until 45 min after DYN. Moreover, under baseline conditions, a dose of 3.2 mg/kg DYN (right) decreased rates of responding for 15 min, with responding returning to control values after 30 min. In LAAM-treated monkeys, this dose of DYN suppressed responding for 30 min, with rates of responding not returning to control values until 45 min after injection. One LAAM-treated monkey did not respond throughout the 60-min session. DYN did not significantly increase tail-withdrawal latencies in either untreated or LAAM-treated monkeys.

Discussion

Tolerance to the behavioral and physiologic effects of opioids is an important concern when these drugs are administered chronically. Under some conditions, tolerance is considered an adverse effect (e.g., pain treatment), whereas under other conditions, tolerance is considered a beneficial effect. For example, the moderate success of pharmacotherapies for treating opioid abuse (e.g., methadone) is attributed in part to the development of cross-tolerance to the subjective effects of other opioids (e.g., heroin), which can decrease illicit drug use (Levine et al., 1973; Kreek, 1992). To further assess the importance of tolerance, in the current study, we systematically assessed the development of cross-tolerance to the behavioral and physiologic effects of opioids in opioid-dependent monkeys.

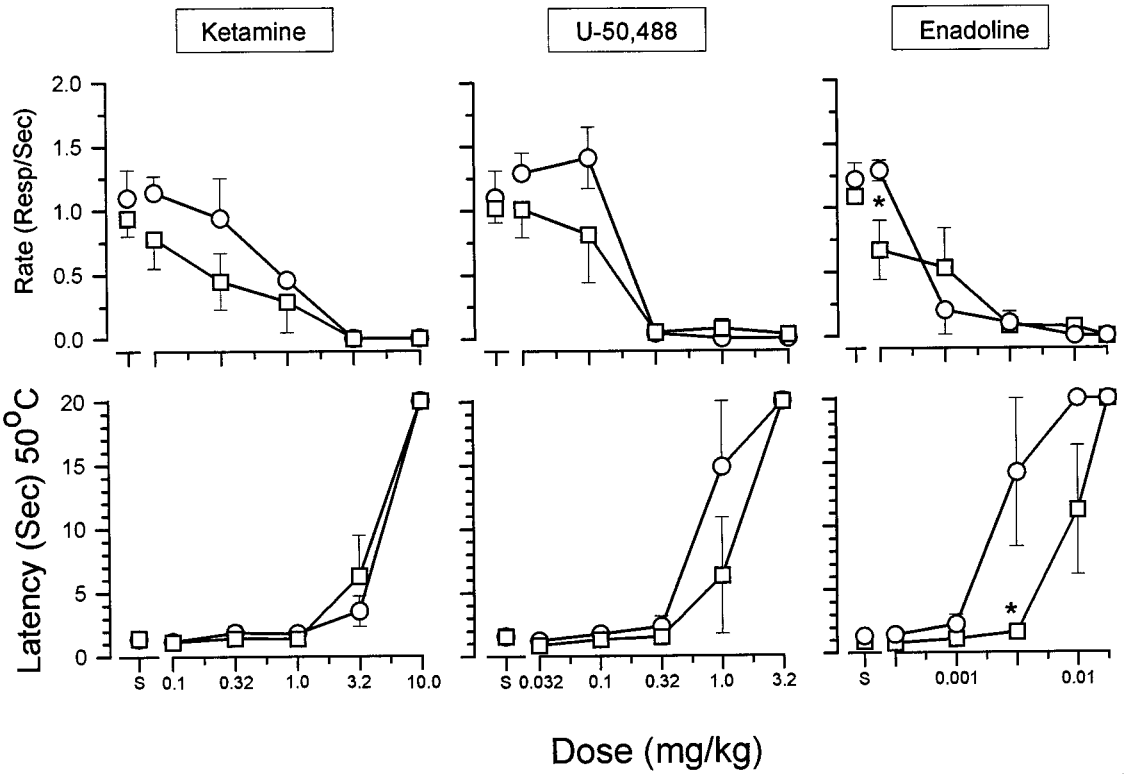

Fig. 6. Rate and antinociceptive effects of ketamine (left), U-50,488 (middle), and enadoline (right) under baseline (○; $n = 3$) and LAAM-treated (□; $n = 4$) conditions. *, significantly ($P < .05$) different from the baseline condition. See the legend for Fig. 5 for other details.

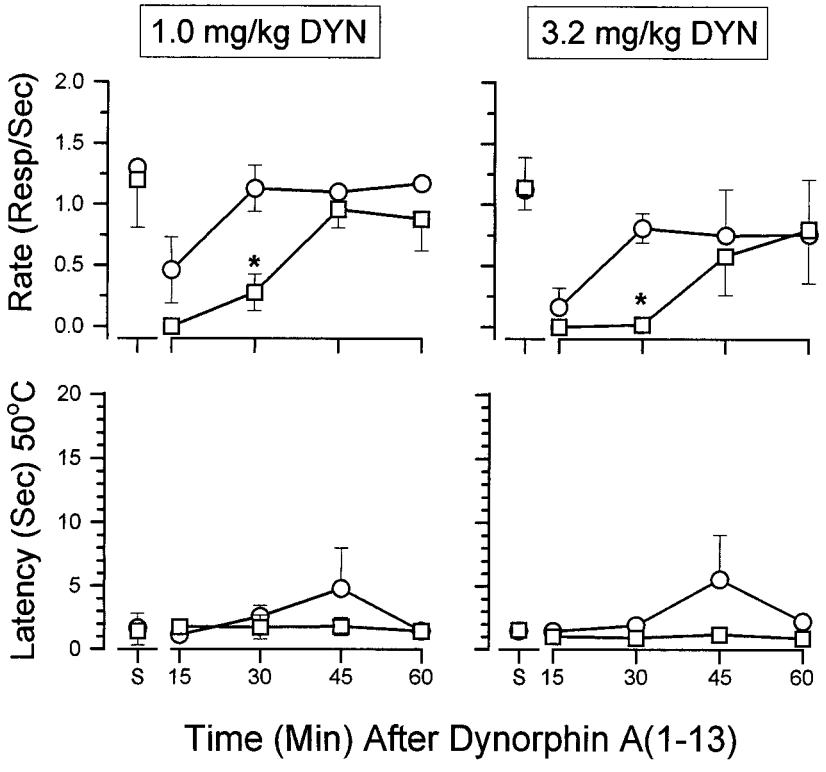

Fig. 7. Time course for the rate and antinociceptive effects of 1.0 mg/kg (left) and 3.2 mg/kg (right) of DYN (i.v.) under baseline (○; $n = 3$) and LAAM-treated (□; $n = 3$) conditions. Abscissa represents time (minutes) after the administration of DYN. *, significantly ($P < .05$) different from the baseline condition. See the legend for Fig. 5 for other details.

Chronic treatment with μ-agonists often produces dependence, which can be quantified by behavioral and physiologic changes that occur either after the termination of drug treatment or after the administration of a pharmacologic antagonist. In the current study, naltrexone did not have behavioral or ventilatory effects in untreated monkeys, whereas naltrexone produced behavioral signs of withdrawal and increased ventilation in monkeys receiving LAAM. Other studies in monkeys (Goldberg, 1976; Paronis and Woods, 1997a) and humans (Martin and Jasinski, 1969) have reported similar

results with the termination of drug treatment or the administration of a pharmacologic antagonist. Moreover, a previous study in these monkeys demonstrated that naltrexone decreases rates of scheduled-controlled behavior and produces behavioral signs and discriminative stimuli related to opioid withdrawal (Brandt and France, 1998). Although monkeys appeared to be dependent in the current study, there was no apparent change in sensitivity to the ventilatory stimulant effects of 5% CO_2; such an effect has been reported for opioid-treated humans (Martin et al., 1968; Marks and Goldring, 1973) but not for opioid-treated monkeys (Paronis and Woods, 1997a).

One purpose of the current study was to determine whether tolerance develops to the ventilatory effects of μ-opioid agonists. During chronic LAAM treatment, V_E was decreased by 44% after more than 10 months of treatment. In humans, a dose of 60 mg of morphine was administered four times daily for 34 weeks with no apparent recovery in ventilatory-depressant effects (Martin and Jasinski, 1969). Thus, tolerance might not develop to the ventilatory effects of μ-opioid agonists. If tolerance does not develop to the ventilatory effects of μ-agonists, then the effects of other μ-agonists (e.g., morphine) should have added to the ventilatory-depressant effects of LAAM in LAAM-treated monkeys, thereby shifting the dose-effect curve for agonists leftward. Such an additivity was not observed in this study, suggesting that cross-tolerance might be masked by the sustained decrease in ventilation produced by twice-daily injections of LAAM. In support of this view, the ventilatory effects of morphine appeared to plateau at doses larger than 3.2 mg/kg in LAAM-treated monkeys. Although the ventilatory effects of large doses of morphine were not assessed in untreated monkeys, studies in this laboratory (our unpublished observations) and others (Paronis and Woods, 1997b) have noted apnea with doses of 10.0 or 32.0 mg/kg morphine (necessitating the administration of an opioid antagonist). Consistent with these results in monkeys, the respiratory effects of 60 and 120 mg of morphine were less in morphine-dependent humans than the effects of 15 and 30 mg of morphine in nondependent humans (Martin and Jasinski, 1969). Taken together, these results suggest that some cross-tolerance can develop to the ventilatory effects of opioids; however, the magnitude of this tolerance might be less than that for other effects (see later).

Unlike the increases in ventilation obtained with naltrexone and the decreases in ventilation obtained with morphine, nalbuphine did not modify V_E, V_T, or f. Other studies, using identical procedures, showed that doses of nalbuphine larger than 1.0 mg/kg decrease V_E by at least 60% and f by at least 75% in untreated monkeys (Gerak et al., 1994). Results of the current study suggest that monkeys receiving 1.0 mg/kg/12 h LAAM were tolerant to the ventilatory depressant effects of nalbuphine. In contrast, tolerance did not develop to the ventilatory effects of nalbuphine in monkeys receiving 3.2 mg/kg/12 h morphine (Paronis and Woods, 1997b). Although the morphine and LAAM dosing conditions used in these studies were adequate to produce opioid dependence (Paronis and Woods, 1997a; Brandt and France, 1998), the longer duration of LAAM, compared with morphine (e.g., Brandt et al., 1997), might have conferred greater tolerance and cross-tolerance.

Different magnitudes of cross-tolerance developed to the rate-decreasing and antinociceptive effects of μ-agonists. For example, LAAM treatment increased the ED_{50} values for the rate-decreasing effects of nalbuphine and morphine by 7-fold, whereas the ED_{50} value for alfentanil was increased by only 2-fold. Similarly, LAAM treatment eliminated the antinociceptive effects of nalbuphine and increased the ED_{50} values for morphine and alfentanil by 5- and 3-fold, respectively. The magnitude of tolerance that develops in response to μ-opioids depends in part on the efficacy of the agonist (Young et al., 1991; Paronis and Holtzman, 1992). Nalbuphine (low), morphine (intermediate), and alfentanil (high) have different efficacies at μ-receptors (Gerak et al., 1994; Emmerson et al., 1996). In morphine-treated rats, greater shifts in agonist dose-effect curves were observed for low-efficacy agonists than for high-efficacy agonists (Young et al., 1991). Behavioral expression of these differences is observed as progressive rightward shifts and an eventual flattening of the agonist dose-effect curve as the magnitude of tolerance increases. Together, these data emphasize that agonist efficacy is an important determinant of tolerance and that the magnitude of tolerance to one drug does not necessarily predict cross-tolerance to a second drug.

LAAM treatment increased the sensitivity of monkeys to the rate-decreasing effects of some κ-agonists. The lowest dose of enadoline decreased rates of responding in LAAM-treated monkeys and not in untreated monkeys, and DYN suppressed responding for a longer period of time in LAAM-treated monkeys. These disruptions were likely not caused by additive rate-decreasing effects with LAAM, because similar results were not obtained with ketamine. These changes might be related to the interoceptive effects of κ-opioids. In humans, μ-opioid withdrawal and the administration of κ-agonists produce similar reports of dysphoria and anxiety (Jasinski et al., 1985; Kanof et al., 1992). Thus, interoceptive stimuli of κ-agonists might overlap with stimuli during μ-opioid withdrawal in monkeys. In support of this view, some κ-agonists substitute for naltrexone in morphine-treated monkeys discriminating between naltrexone and saline (France et al., 1994). It is unclear why LAAM-treated monkeys were not more sensitive to the rate-decreasing effects of U-50,488, although other results from this study suggest that there might be qualitative differences between U-50,488 and enadoline.

Enadoline and U-50,488 are selective κ-agonists (Von-Voigtlander et al., 1983; Hunter et al., 1990), and cross-tolerance typically does not develop between κ- and μ-opioids (Gmerek et al., 1987; Craft et al., 1989; Paronis and Woods, 1997b). In LAAM-treated monkeys, cross-tolerance appeared to develop to the antinociceptive effects of enadoline and not U-50,488. It is possible that LAAM (or one of its metabolites) has activity at κ-opioid receptors. Binding and antinociception studies in monkeys have differentiated among κ-opioids according to selective antagonism by the κ-selective antagonist nor-binaltorphimine (Butelman et al., 1993, 1998). Thus, differential cross-tolerance to U-50,488 and enadoline in LAAM-treated monkeys might be related to differences in selectivity for κ-receptor subtypes between these drugs. Alternatively, efficacy can influence the magnitude of cross-tolerance that develops (e.g., with μ-agonists); however, antinociception studies in monkeys have not provided data that would substantiate differences in efficacy between these κ-agonists (France et al., 1994; Pitts and Dykstra, 1994). The small number of observations in this study preclude any firm

conclusion regarding differential cross-tolerance between LAAM and κ-agonists; however, the data are consistent with the view that the behavioral effects of κ-agonists are not identical and, therefore, that interactions between μ- and κ-agonists might vary markedly.

It is not clear why DYN failed to have antinociceptive effects in this study. Previous studies in rhesus monkeys have shown antinociceptive effects for 1.0 and 3.2 mg/kg DYN in 50°C water (Butelman et al., 1995). In the current study, antinociceptive effects of DYN were assessed immediately after operant responding, whereas in previous studies, antinociceptive effects of DYN were assessed in monkeys not responding under operant procedures. Thus, procedural details might have contributed to this apparent difference in the antinociceptive effects of DYN. Support for this view is provided by data showing that tail-withdrawal latencies in 50°C water were greater than 10 s when monkeys were not responding under an operant procedure (see Table 2), whereas latencies were less than 3 s when monkeys were responding under an operant procedure (see Figs. 5 and 6, squares above "S"). Collectively, these results suggest that environmental factors (e.g., the activity level of monkeys) might influence the antinociceptive effects of drugs.

In the current study, marked tolerance did not appear to develop to the ventilatory effects of LAAM, suggesting that chronic LAAM treatment may be contraindicated in patients with compromised respiration. Some cross-tolerance appeared to develop to the ventilatory effects of μ-opioids because doses of morphine that would have produced toxic effects in untreated monkeys were safely administered to LAAM-treated monkeys. The significant variability that was evident among monkeys might also be observed in humans. The data predict that in humans, the therapeutic ratio of opioids might decrease during chronic opioid treatment (i.e., tolerance to the analgesic effects of opioids might develop to a greater extent than tolerance to the respiratory depressant effects of opioids). Moreover, the magnitude of cross-tolerance that developed to the antinociceptive and rate-decreasing effects of nalbuphine, morphine, and alfentanil demonstrates that agonist efficacy is an important determinant of tolerance and that the magnitude of tolerance to one drug does not necessarily predict equal cross-tolerance to other drugs. This finding has important implications for the use of long-acting opioids (i.e., methadone and LAAM) as substitution therapies for heroin abuse. Because the potency of high-efficacy agonists (i.e., alfentanil) is changed little during LAAM treatment, the abuse liability of high-efficacy agonists (e.g., heroin) might not be significantly changed by LAAM treatment. These results might be relevant to the high relapse rates that have been observed in either methadone- or LAAM-maintained individuals.

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Send reprint requests to: Charles P. France, Ph.D., Department of Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900.