\(l\)-\(\alpha\)-Acetylmethadol, \(l\)-\(\alpha\)-Acetyl-N-normethadol and \(l\)-\(\alpha\)-Acetyl-N,N-normethadol: Comparisons with Morphine and Methadone in Suppression of the Opioid Withdrawal Syndrome in the Dog

D. BRUCE VAUPEL and DONALD R. JASINSKI

Brain Imaging Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, Maryland (D.B.V.), and Center for Chemical Dependence, Johns Hopkins Bayview Medical Center, Baltimore, Maryland (D.R.J.)

Accepted for publication July 11, 1997

ABSTRACT

\(l\)-\(\alpha\)-Acetyl-N-normethadol (nor-LAAM) and \(l\)-\(\alpha\)-acetyl-N,N-di-normethadol (dinor-LAAM) are active metabolites of the opiate \(l\)-\(\alpha\)-acetylmethadol (LAAM), and they contribute to the prolonged actions of the parent compound. Single doses of nor-LAAM, dinor-LAAM, LAAM, methadone and morphine were given intravenously to the chronic spinal dog to determine acute, single-dose effects and their ability to suppress withdrawal in morphine-dependent dogs. These opioids produced dose-dependent antinociception, decreases in body temperature and pupillary constriction. For these measures, dinor-LAAM was 1.5 to 3 times and nor-LAAM 6 to 12 times as potent as LAAM. Five hours after the acute administration of LAAM or either of the metabolites, a 1-mg/kg dose of naltrexone given intravenously produced withdrawal, indicating the presence of acute physical dependence. In dogs physically dependent on a daily dose of 125 mg of morphine, nor-LAAM was 9 times as potent as either LAAM or dinor-LAAM in suppressing spontaneous withdrawal 40 hr after the last dose of morphine. The efficacies of LAAM and its demethylated metabolites in the dog for producing acute opiate effects were comparable with those of morphine and methadone. There was a trend, however, for LAAM to suppress the expression of abstinence more fully than either metabolite. The usefulness of LAAM as a treatment for opiate addiction is likely due in part to the equivalent efficacies and higher potencies of its nor and dinor metabolites.

For more than 20 years, LAAM, a synthetic derivative of \(d\)-methadone (Pohland et al., 1949), has been proposed as an alternate to methadone in maintenance therapy. In July 1993, LAAM was approved in the United States for use in opioid substitution therapy (Federal Register, 1993). Four decades ago, Fraser and Isbell (1952) characterized the pharmacological actions and assessed the abuse liability of LAAM. They found the effects of a single oral dose of LAAM to be morphine-like. Furthermore, 30 to 60 mg of LAAM, given orally, relieved abstinence in morphine-dependent subjects (400 mg/day). A single, 40 to 60-mg dose of LAAM orally substituted for morphine (60 mg q.i.d.) and suppressed the abstinence syndrome for up to 72 hr. Morphine-like effects of LAAM required 4 to 6 hr to emerge after subcutaneous or intravenous injection and 1.5 hr to appear after oral administration, suggesting the formation of active metabolites.

The initial preclinical pharmacological, metabolic and toxicological studies of LAAM and its metabolites included rodents, dogs and monkeys among the species tested (Archer, 1976). Metabolites of LAAM, particularly the N-demethylated compounds, nor-LAAM and dinor-LAAM, had more rapid onsets of action than LAAM, tended to be more potent than the parent compound (i.e., particularly nor-LAAM) and persisted in animals longer than the metabolites of methadone. For these reasons, the formation of nor metabolites was postulated to confer the long duration of action of LAAM (Archer, 1976; Wolven and Archer, 1976). Except for two analgesic assessments of nor-LAAM (Gruber and Babtisti, 1962; Houde et al., 1962), no other pharmacodynamic data exist for nor- and dinor-LAAM in humans; consequently, their activity profiles must be inferred from animal models. The chronic spinal dog is a validated model for assessing the agonistic actions and dependence-producing properties of morphine-like opioids (Martin and Jasinski, 1977). Using this model, studies were designed to compare the actions and relative potencies of nor-LAAM and dinor-LAAM to morphine, methadone and LAAM. Pharmacological profiles based on single-dose effects, suppression studies of morphine withdrawal and precipitated abstinence were used to define the opiate-like nature of nor-LAAM and dinor-LAAM.

ABBREVIATIONS: ANOVA, analysis of variance; AUC, area under time action curve; dinor-LAAM, \(l\)-\(\alpha\)-acetyl-N,N-normethadol; LAAM, \(l\)-\(\alpha\)-acetylmethadol; nor-LAAM, \(l\)-\(\alpha\)-acetyl-N-normethadol.
Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the NIDA Addiction Research Center (Lexington, KY) and were in accordance with the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health.

Animals. The study included 12 beagle-type, T10 chronic spinal dogs ranging in age from 2 to 6 years and weighing between 7 and 12 kg. One group of six animals was used to evaluate acute opiate effects, and the second group of six came from a colony of morphine-dependent animals and were used to assess the suppression of opiate withdrawal. All dogs had been used in previous studies of opiate-related compounds but had different drug histories.

Physiological and behavioral measurements. The following autonomic, reflex and behavioral measures were acquired using methods that have been previously published (Martin et al., 1974, 1976; Vaupel et al., 1986). The dogs, which were trained to the laboratory setting, were positioned on their right sides and loosely restrained at the neck and abdomen. Vertical pupil diameter and lateral nictitating membrane width of the left eye were measured from photographs taken with a Polaroid close-up camera, and rectal body temperature was recorded continuously. Heart rate and respiration were counted by auscultation and visual observation, respectively. For these five autonomic measures, changes for a selected time period (e.g., 300 min) were based on AUCs: change in response = (AUC_treatment − AUC_pretreatment baseline control) minutes × AUC_pretreatment baseline control.

Spinal and supraspinal antinociception was determined using two nociceptive reflexes: the flexor reflex (reflex arc restricted to the spinal cord below the level of transection), and the skin twitch reflex (reflex arc to supraspinal levels), respectively. The left hindlimb flexor reflex was evoked at 1-min intervals by a programmed pneumatic toe squeezer delivering stimuli of three strengths (4.5, 9 and 18 p.s.i.) in a random order to the superior toe of the ipsilateral limb, which was placed in a freely moving, suspended sling connected to a chart recorder. The flexor reflex, evoked by a 3-sec compression of the toe, and any spontaneous movements or drug-induced (i.e., stepping reflex) reflexes were continuously recorded in an isotonic manner. For the flexor reflex, antinociceptive effects of opiates are measured by the extent to which the reflex amplitude is depressed and the maximally depressed reflex exhibits no response to the toe pinch stimulus. Results were expressed as the percentage of maximum antinociception using the percentage of maximum possible effect method. Individual reflex responses were first normalized by converting their amplitude (in mm) to a percentage of the mean of the nine predrug control values obtained for each stimulus pressure and calculating 5-hr AUCs: Maximum (%) antinociception = (AUC_treatment/AUC_maximally depressed reflex) × 100. The skin twitch reflex was evoked from a dermatome above the level of spinal cord transection using thermal stimulation (i.e., a heat lamp) directed at an India ink-blackened area of shaved skin on the left shoulder. The intensity of the heat lamp was adjusted for each dog to provide a control reaction time of 3 to 5 sec, and a 10-sec cutoff was used. Skin twitch reflex antinociceptive responses were calculated as follows: Maximum (%) antinociception = (AUC_treatment − AUC_maximally depressed reflex) × 100.

Naltrexone-precipitated withdrawal in dogs acutely treated with opiates and suppression of withdrawal abstinence in chronically maintained morphine-dependent spinal dogs were quantified using additional physiological symptoms and behaviors and the revised scoring system of Martin et al. (1976). These measures, with their weighting factors shown in parentheses, were as follows: yawning (4), lacrimation (6), rhinorrhea (8), salivation (4), tremor (4), restlessness (5), whining (16), gnawing (4), head tossing (14), barking (40), retching (38), urination (5), panting (3), fragmentary hindlimb stepping movements (2), continuous hindlimb stepping movements (i.e., the stepping reflex) (7), a 0.1°C change in temperature (24), a 1 beat/min change in heart rate (0.9) and a 1-mm change in pupillary diameter (4). Abstinence or suppression was quantified by multiplying the changes in incidence or magnitude of the response, relative to baseline values, by the weighting factor and summing the values over the three observation periods. According to this system, abstinence scores are positive and suppression scores are negative.

Single-dose studies and precipitated abstinence. Subjects were six nondependent chronic spinal dogs. Their past experience included use in opiate-related studies, including dose-ranging experiments for the present study, but their drug histories were not identical. In the present study, the acute, single-dose effects of the three methadols were compared with those of morphine and methadone in two series of crossover experiments in which all dogs received all treatments using the following design. In the first series, the effects of morphine (0.5 and 2.0 mg/kg), LAAM (0.25 and 1.0 mg/kg), nor-LAAM (0.05 and 0.2 mg/kg), dinor-LAAM (0.1 and 0.4 mg/kg), methadone (0.125 and 0.5 mg/kg) and a double-distilled water vehicle control (administered twice) were studied over a 12-week period using a randomized block design. The design incorporated six blocks, and each block was 2 weeks in length. Within each block, dogs received a pair of different drug treatments (one treatment per week). Treatment pairs were randomly assigned to dogs across the blocks to counterbalance for time. When a preliminary analysis indicated that higher doses of the methadols were needed to produce more complete dose-response curves, a second, 6-week series of experiments was added. The six treatments [LAAM (4.0 mg/kg), nor-LAAM (0.8 mg/kg), dinor-LAAM (1.6 mg/kg), morphine (0.125 and 2.0 mg/kg) and double-distilled water] were tested using a 6 × 6 (dogs × doses) crossover design. In retrospect, a higher dose of methadone should have been included in the second series. However, in making a preliminary assessment of the data, we overestimated the efficacy of methadone. This error limited our data interpretation with respect to the efficacy and relative potency of methadone and its comparison with the LAAM compounds. An additional component to the second series of experiments was the use of a precipitated withdrawal paradigm to assess the development of acute physical dependence 5 hr after drug administration.

On test days, dogs were allowed to habituate to the experimental setting for ≥30 min before observations were begun. Experiments consisted of a 30-min control period followed by a 4-min intravenous administration of saline or drug and 5 hr of observations. Except for the flexor reflex, which was continuously evoked at 1-min intervals, observations were made at 30, 20 and 10 min before the administration of drug or vehicle. After the experimental treatment, observations were continued at 15-min intervals for the first hour and at 30-min intervals for the next 4 hr. In experiments assessing the development of acute physical dependence, naltrexone (1.0 mg/kg i.v. given over 4 min) was administered after the fifth hour. Observations and signs of precipitated abstinence were measured at 5, 15 and 30 min after naltrexone. Computation of withdrawal scores, described above, used measurements from the initial 30-min control period for the base-line values.

Suppression studies. Suppression of morphine abstinence was studied in a second group of six chronic spinal dogs who were physically dependent on morphine. An induction phase of 8 to 10 weeks was required to gradually increase daily subcutaneous and oral doses of morphine until animals were dependent on 125 mg/day morphine sulfate. Thereafter, the maintenance schedule consisted of 25 mg s.c. administered at 8:00 a.m. and 100 mg p.o. administered at 4:00 p.m. The dogs had been used in previous suppression and precipitated studies evaluating the agonist, partial agonist and antagonist effects of opiates.

Suppression studies were conducted 40 hr after the last maintenance dose of morphine, at a time when the dogs were maximally abstinent (Martin et al., 1974). Dogs were then rested 22 to 24 hr later to evaluate the duration of the suppression of abstinence. A 6 × 12 (dogs × doses) randomized block crossover design was used to...
evaluate the following treatments: morphine (0.5 and 2.0 mg/kg), methadone (0.125 and 0.5 mg/kg), LAAM (0.75 and 3.0 mg/kg), nor-LAAM (0.05 and 0.2 mg/kg), dinor-LAAM (0.1 and 0.4 mg/kg) and a double-distilled water vehicle (administered on two occasions). At the completion of these studies, additional doses of LAAM (0.1875 mg/kg), nor-LAAM (0.0125 mg/kg), dinor-LAAM (1.6 mg/kg) and morphine (0.125 mg/kg) were tested, although these three conditions were not retested the next day. Experiments were conducted at weekly intervals. One dog died during the experiments, reducing the number of animals to five.

After habituation of the dogs to the laboratory, base-line observations of abstinence were initiated and made at 60, 30 and 0 min before administration of the drug or vehicle intravenously into the cephalic vein (0.5 ml/kg) over 5 min. Signs of abstinence were evaluated at 30, 60 and 90 min after treatment using the rating scale of Martin et al. (1974) to quantify the degree of suppression. For retests, dogs were returned to the laboratory 22 to 24 hr after the suppression treatment, and signs of abstinence again were scored as previously described using the initial presuppression base-line values to provide a common reference point for both sets of suppression scores.

Data analysis. For the single-dose studies, significance of drug effects was tested against the mean of the three vehicle control experiments using Dunnett’s test. Relative potencies and confidence limits were calculated using standard ANOVA methods and Fieller’s theorem for parallel line bioassays, which were modified for crossover data (Finney, 1978). Relative potency estimates and 95% confidence limits are presented only for valid bioassay ANOVAs, which were defined by a significant regression term, a nonsignificant deviations from parallelism (parallelism term) and a nonsignificant preparations term (no difference in the mean treatment effects). For some bioassays with a significant preparations term, potency estimates without confidence limits were presented for qualitative purposes. Geometric mean values were calculated for the purpose of summarizing the overall relative potency a drug but used only estimates from statistically valid bioassays; the geometric means have no associated confidence limits. The presence of acute physical dependence in the single-dose study and the suppression of withdrawal in dogs chronically dependent on morphine were determined by analyzing total abstinence or suppression scores using a two-way ANOVA (dogs × doses) and a Dunnett’s test (one-tailed, P < .05).

Drugs. The drugs used were morphine sulfate and the hydrochloride salts of dl-methadone, LAAM, nor-LAAM and dinor-LAAM and were kindly provided by the National Institute on Drug Abuse (Rockville, MD). All doses are expressed in terms of the salts. Double-distilled water was the vehicle for all compounds.

Results

Single-dose study. The five opiate treatments had pronounced effects on nociception (see figs. 1–3) and temperature (see fig. 4), whereas changes in pupil diameter and nictitating membrane width were not as robust. Respiration and heart rate were not affected, but the P value for heart rate (.0655) approached the criterion for significance. Analyses to determine which measures were significantly affected in the single-dose study are summarized in table 1. A significant “between animals” term, indicating a difference in animal responsiveness, was associated with every measure (i.e., the comparison of mean responses, collapsed over all treatment conditions for each animal, indicated that some animals had relatively high sensitivity to the opiates and others were relatively less sensitive). Segregation of this significant source of variability emphasized the power of using a crossover design.

Morphine produced statistically significant antinociceptive effects, as manifested by dose-related effects on the flexor (figs. 1 and 2) and skin twitch reflexes (fig. 3), hypothermia (fig. 4) and miosis. With respect to pupils, the largest constriction developed with the intermediate, 0.5 mg/kg, dose of morphine (data not presented). Measures not affected over the 5-hr period included heart rate and respiration (table 1).

Similarly, methadone depressed both the nociceptive reflexes (figs. 1–3) and decreased both temperature (fig. 4) and pupil size. Methadone was the only compound that did not produce nearly maximal depression of the flexor reflex. At 5 hr after the administration of 2 mg/kg morphine and 0.5 mg/kg methadone, the comparable percentages of control amplitude for were 15% and 62%, respectively (data not shown, but see fig. 5 for reference to the LAAM compounds). Potency estimates for antinociception demonstrated that methadone was more potent than morphine on the skin twitch reflex and on the flexor reflex evoked by the low-pressure stimulus; for flexor reflex responses to the medium stimulus, methadone and morphine were equipotent (table 2). With regard to temperature, the greater efficacy of morphine in producing hypothermia at the doses tested prevented attainment of a valid bioassay (fig. 4 and table 2). Both opiates also were equally potent in producing miosis (table 2). Overall, methadone was estimated to be 1.75 times more potent than morphine based on the geometric mean of the four valid bioassay results (table 2).

LAAM, nor-LAAM and dinor-LAAM uniformly produced morphine-like effects characterized by a decrease in body temperature (fig. 4), constricted pupils (table 2 and fig. 5) and nearly maximal levels of analgesia as measured by decreased responsiveness of the flexor (figs. 1 and 2) and skin twitch

### Table 1

#### Single-dose treatment effects

<table>
<thead>
<tr>
<th>Measure</th>
<th>Within doses (14 df); residual (70 df)</th>
<th>Between animals (5 df); residual (70 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexor reflex, low stimulus</td>
<td>F = 14.07; P = .0000</td>
<td>F = 4.00; P = .0030</td>
</tr>
<tr>
<td>Flexor reflex, medium stimulus</td>
<td>F = 16.71; P = .0000</td>
<td>F = 3.57; P = .0062</td>
</tr>
<tr>
<td>Flexor reflex, high stimulus</td>
<td>F = 15.86; P = .0000</td>
<td>F = 4.85; P = .0007</td>
</tr>
<tr>
<td>Skin twitch reflex</td>
<td>F = 11.27; P = .0000</td>
<td>F = 5.92; P = .0001</td>
</tr>
<tr>
<td>Heart rate</td>
<td>F = 1.74; NS</td>
<td>F = 4.44; P = .0014</td>
</tr>
<tr>
<td>Pupil diameter</td>
<td>F = 2.38; P = .0089</td>
<td>F = 6.96; P = .0000</td>
</tr>
<tr>
<td>Nictitating membrane</td>
<td>F = 2.67; P = .0035</td>
<td>F = 5.97; P = .0001</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>F = 0.48; NS</td>
<td>F = 4.06; P = .0027</td>
</tr>
<tr>
<td>Temperature</td>
<td>F = 15.35; P = .0000</td>
<td>F = 3.66; P = .0053</td>
</tr>
</tbody>
</table>
reflexes (fig. 3). Interestingly, LAAM and its metabolites were more effective in depressing the skin twitch reflex than morphine even though all four compounds were equally efficacious in inhibiting the flexor reflex. The hypothermic effect for all opiates tested was developed within the first hour but became more pronounced for the high doses of LAAM, nor-LAAM and dinor-LAAM during the next 4 hr of the 5-hr postobservation period (figs. 4 and 5). In contrast, temperature began returning to base-line values by 3 and 4 hr after the administration of 0.5 mg/kg methadone and 2 mg/kg morphine, respectively (data not shown). Both the nor and dinor metabolites were more potent than LAAM in producing hypothermia (table 2). Compared with morphine, LAAM was slightly but significantly less potent, and dinor-LAAM was twice as potent (table 2).

Overall, pupil and nictitating membrane responses were more variable than other physiological measures, and a substantial amount of this variability was associated with administration of LAAM and its metabolites. In contrast to the antinociceptive and hypothermic effects, for which the highest doses produced the largest effects (figs. 1–4), pupillary responses were not as uniform. The intermediate doses of LAAM (1.0 mg/kg) and nor-LAAM (0.2 mg/kg), like morphine, produced the greatest degree of pupillary constriction, whereas the low and intermediate doses of dinor-LAAM essentially produced no miosis. Doses that were on the extremes of these plateaus were deleted from the relative potency determinations, which are shown in table 2. Several of the potency estimates listed in table 2 had especially wide confidence limits that reflect the high variability, particularly the different geometries of the dose-response curves for pupils relative to other measures (data not presented). Fig. 5 illustrates the time course of the high-dose effects of LAAM and its metabolites on pupil diameter.

The significant dose effect determined for the nictitating membrane (table 1) appeared to represent mainly a relaxation of the membrane produced by 4.0 mg/kg LAAM and 1.6 mg/kg dinor-LAAM. This relaxation was not associated with
any pattern of sleeping behavior or state of unresponsiveness. At this dose, only one of six dogs receiving LAAM was scored for a 30-min period of sleep. After the administration of dinor-LAAM, two of six animals exhibited several episodes of sleeping, totaling 1 and 1.5 hr of sleep of the 5-hr observation period. By comparison, no dogs slept after receiving 2 mg/kg morphine; two of six had brief periods of sleep after 0.5 mg/kg methadone and among the three water vehicle controls, there was only a single 30-min episode of sleep recorded.

The nearly significant overall effect on heart rate (see above) reflected a trend for the highest doses of LAAM and its metabolites to produce bradycardia (fig. 5). Considering behaviors associated with high-dose effects, there was only one brief appearance of nystagmus (nor-LAAM 0.8 mg/kg), three occurrences of myoclonic jerks (LAAM 4 mg/kg and nor-LAAM 0.8 mg/kg [dog 2026] and dinor-LAAM 1.6 mg/kg [dog 3505]) and one report of failure to attend to stimuli (nor-LAAM 0.8 mg/kg [dog 1767]). Occasional whining was produced by morphine (2 mg/kg) and methadone (0.5 mg/kg) as well as the high doses of LAAM, nor-LAAM and dinor-LAAM.

All in all, signs atypical of morphine were few in number, but four of five noted above were associated with the N-demethylated metabolites of LAAM. Geometric means were used to summarize the acute, morphine-like actions based on potencies for valid assays (table 2). These actions include antinociceptive effects at the spinal cord (flexor reflex) and supraspinal (skin twitch reflex) levels, hypothermia and miosis. Relative to LAAM, nor-LAAM was found to be 8.93 times as potent and dinor-LAAM 2.30 times as potent.

**Precipitated withdrawal studies.** Five hours after the administration of LAAM, its metabolites and morphine, the injection of naltrexone elicited a statistically significant withdrawal syndrome, indicating the development of acute physical dependence (figs. 5 and 6). Quantitatively, the withdrawal syndromes for 4 mg/kg LAAM, 0.8 mg/kg nor-LAAM and 1.6 mg/kg dinor-LAAM were statistically equivalent to...
The acute physiological actions of LAAM, nor-LAAM and dinor-LAAM were identical to those of morphine and methadone in the dog. Previous studies in the chronic spinal dog model indicated that morphine produced miosis, bradycardia, hypothermia and depression of nociceptive reflexes (Martin et al., 1976; Martin and Eades, 1964). In the present study, the abilities of these five opiates to depress the flexor and skin twitch reflexes and to lower temperature were the most robust signs of withdrawal syndrome, with lacrimation, rhinorrhea and salivation being present but less prominent. A paired t test comparison of the 40-hr abstinent (−37.4 ± 17.1 points) and 64-hr abstinent (−64.0 ± 30.3 points) (retest) suppression scores after saline administration demonstrated no difference between conditions (t (4 df) = .408, P = NS).

**Discussion**

The effects of LAAM, nor-LAAM and dinor-LAAM on the flexor reflex (fig. 5) are presented as “% Control Amplitude” rather than “% Maximal Antinociception” to more clearly demonstrate reversal by naltrexone and emergence of the stepping reflex as a sign of precipitated withdrawal. Heart rate, although not significantly depressed by LAAM and its metabolites (table 1), is presented because rebound tachycardia after naltrexone was a primary contributor to the withdrawal scores. A low level of hyperthermia developed during withdrawal from morphine but not during withdrawal from LAAM, nor-LAAM or dinor-LAAM, although naltrexone almost reversed their hypothermic effects within the 30-min period for scoring withdrawal (fig. 5).

**Suppression studies.** The metabolites nor-LAAM and dinor-LAAM and LAAM, as well as morphine and methadone, effectively suppressed withdrawal from morphine in 40-hr abstinent dogs [two-way ANOVA (dogs × doses); F(14,56) = 1.99; P < .05]. An apparent ceiling effect was reached in suppressing abstinence in dogs dependent on a daily dose of 125 mg of morphine by morphine, LAAM, nor-LAAM and dinor-LAAM (fig. 7). It could not be determined whether a ceiling effect was attained for methadone because a dose of >0.5 mg/kg was not tested. The relative potency relationships of these opioids in suppressing morphine withdrawal are presented in table 3. Nor-LAAM was approximately one order of magnitude more potent than LAAM or dinor-LAAM in suppressing the canine morphine abstinence syndrome.

Twenty-four hours later, the animals were retested with some of the doses to determine whether withdrawal was still suppressed. There was no statistically significant dose effect as determined with a two-way ANOVA (dogs × doses) (F(10,40) = .65, P = NS), indicating that withdrawal was not suppressed 1 day later by LAAM or its metabolites in the dog. At the time of the retest, the abstinence syndrome was still present although slightly reduced in magnitude. Tremors, the continued presence of the stepping reflex, and persistent elevations in pulse rate, respiratory rate and temperature were the most robust signs of withdrawal syndrome, with lacrimation, rhinorrhea and salivation being present but less prominent. A paired t test comparison of the 40-hr abstinent (−37.4 ± 17.1 points) and 64-hr abstinent (−64.0 ± 30.3 points) (retest) suppression scores after saline administration demonstrated no difference between conditions (t (4 df) = .408, P = NS).
though the dose-effect curves are parallel. These values are included as supplemental information.

Autonomic responses (i.e., miosis and hypothermia) that were less sensitive to opiates than in previous studies. In contrast to the autonomic responses, depression of nociceptive reflexes observed in the present study was comparable to that seen in previous studies (Gilbert and Martin, 1976; Martin et al., 1976).

Based on comparisons with previously established single-dose profiles, the actions of LAAM and its nor and dinor metabolites were representative of those produced by opioid agonists, characterized primarily as mu agonists, and typified by miosis and hypothermia, as opposed to kappa-type opioid agonists. The acute pharmacological actions of the kappa agonists l-ketocyclazocine and U50488H have been established in the dog (Vaupel and Cone, 1991). Although kappa agonists share certain actions, such as depression of nociceptive reflexes and pupillary constriction, with mu agonists, in contrast, they elevate body temperature, relax the nictitating membrane, induce sleep and produce an inability to attend to auditory and visual stimuli. Moreover, U50488H, which is a more selective kappa agonist than l-ketocyclazocine (Lahti et al., 1982; Tang and Collins, 1985), produces additional effects at the highest dose tested. Stimulatory signs, consisting of whining, the emergence of the stepping reflex and occasional myoclonic movements, are accompanied by nystagmus. The appearance of nystagmus, another high-dose effect of U50488H, is produced by the dissociative anesthetic phencyclidine and N-allylnormetazocine (Vaupel et al., 1986). Although mu and kappa opioid activity in the dog can be differentiated on the basis of selective antagonism by naltroxone (Vaupel et al., 1986; Vaupel and Cone, 1991), no attempt was made to demonstrate a selective antagonism of LAAM and its metabolites in this study. Nonetheless, the pharmacologies of LAAM, nor-LAAM and dinor-LAAM are more selective mu agonist than kappa agonist in the dog, and there is minimal evidence to suggest the presence of kappa opiate activity.

Initial studies in humans have indicated that LAAM has a delayed onset of action, regardless of whether it was administered intravenously or subcutaneously (Fraser and Isbell, 1952), and this observation subsequently has been attributed

---

**TABLE 2**

Relative potency estimates from single-dose studies

For each set of comparisons, the relative potency of the standard drug is set equal to 1.00 by convention. By definition, relative potency is defined as the mg/kg of the standard drug equal to 1 mg/kg of the test drug. Relative potency estimates and 95% confidence limits shown in parentheses for test drugs are presented only for valid, parallel line bioassay ANOVAs, which are defined by a significant regression term, nonsignificant deviations from parallelism (parallelism term), and a nonsignificant preparations term (no difference between the mean values of the two treatments).

<table>
<thead>
<tr>
<th></th>
<th>Flexor Reflex; low stimulus</th>
<th>Flexor Reflex; medium stimulus</th>
<th>Flexor Reflex; high stimulus</th>
<th>Skin twitch reflex</th>
<th>Temperature</th>
<th>Pupil diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine standard</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>2.16*</td>
<td>1.54</td>
<td>1.08</td>
<td>3.38*</td>
<td>Not parallel</td>
<td>0.84</td>
</tr>
<tr>
<td>(1.25–3.77)</td>
<td></td>
<td>(1.00–2.32)</td>
<td>(1.11–15.31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAAM</td>
<td>0.86</td>
<td>0.67</td>
<td>0.71</td>
<td>Not parallel</td>
<td>0.62*</td>
<td>0.067*</td>
</tr>
<tr>
<td>(0.42–1.97)</td>
<td></td>
<td>(0.39–1.18)</td>
<td>(0.44–1.19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nor-LAAM</td>
<td>Not parallel</td>
<td>Not parallel</td>
<td>9.23</td>
<td>Not parallel</td>
<td>6.77</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAAM standard</td>
<td>1.94</td>
<td>1.80</td>
<td>1.67</td>
<td>1.93</td>
<td>1.92*</td>
<td>0.23*</td>
</tr>
<tr>
<td>(0.98–4.18)</td>
<td></td>
<td>(0.98–3.52)</td>
<td>(0.87–3.36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nor-LAAM</td>
<td>9.87*</td>
<td>12.70</td>
<td>12.64</td>
<td>6.01</td>
<td>12.13*</td>
<td>8.87</td>
</tr>
<tr>
<td>(3.34–42.99)</td>
<td></td>
<td>(0.59–3.52)</td>
<td>(0.79–5.51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dinor-LAAM</td>
<td>2.19</td>
<td>2.65*</td>
<td>2.28*</td>
<td>1.64</td>
<td>3.08*</td>
<td>2.22</td>
</tr>
<tr>
<td>(0.61–7.34)</td>
<td></td>
<td>(1.04–6.88)</td>
<td>(0.73–3.36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>2.08</td>
<td>2.06</td>
<td>1.48</td>
<td>2.54*</td>
<td>3.05*</td>
<td>7.26*</td>
</tr>
<tr>
<td>(0.49–5.70)</td>
<td></td>
<td>(0.88–4.11)</td>
<td>(1.99–4.56)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Confidence limits that exclude 1.00 represent a significant difference between the standard and test drugs.

Prep difference, a relative potency estimate indicates a significant difference in the two treatment mean values and precludes assigning confidence limits, even though the dose-effect curves are parallel. These values are included as supplemental information.

Not parallel, the two dose-effect curves were not parallel; consequently, no potency estimates can be ascribed to the comparison.
Relative potency estimates, with 95% confidence limits shown in parentheses, were based on suppression scores obtained 40 hr after withdrawal from morphine and are presented only for valid, parallel line bioassay ANOVAs as defined and described in Table 2.

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative potency</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>1.00 (standard)</td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>1.1 (0.09–2.0)</td>
<td></td>
</tr>
<tr>
<td>LAAM</td>
<td>0.5 (0.09–2.0)</td>
<td>1.0 (0.5–3.3)</td>
</tr>
<tr>
<td>nor-LAAM</td>
<td>9.3* (2.9–20)</td>
<td></td>
</tr>
<tr>
<td>dinor-LAAM</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CL for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Confidence limits not including 1.00 represent a significant difference between the standard and test drugs. When morphine was used as the standard drug, there was only one valid bioassay and no difference between the two potencies.

Right column, LAAM was used as the standard drug, and valid bioassays were obtained for all comparisons except with morphine. Note that parallel-line bioassays and relative potency estimates were determined from dose-response curves for which the points are connected by a solid line in figure 7 (i.e., the ceiling effect was excluded).

Suppression of Withdrawal from Morphine

![Graph showing suppression studies.](ja.png)

**Fig. 7.** Suppression studies. The ability of each compound to substitute for morphine was determined by supressing the abstinence syndrome in morphine-dependent dogs. Dogs were maintained on a 125 mg/day total dose of morphine administered in two doses. A withdrawal syndrome was produced by withholding the morphine dosing for 40 hr. The 40-hr morphine-abstinent animals were given a substitute opioid to suppress withdrawal, and suppression scores were computed for the following 90 min. Each point or the vehicle control line represents the mean of five animals. The horizontal line represents the mean water vehicle response, and the dashed line indicates the lower 95% confidence limit (CL). The dashed segments of four dose-response curves represent ceiling effects. Therefore, the highest doses for each of these curves were not used to determine relative potency estimates shown in Table 3. Significant (\(P < .05, \*P < .01\)) suppression of withdrawal for each treatment was based on a comparison with the water vehicle (two-way ANOVA, post hoc one-tailed Dunnett’s test).

motes of the potency of nor-LAAM and dinor-LAAM relative to LAAM found the nor metabolite to be 0.5 to 1.5 orders of magnitude more potent than the parent compound, whereas dinor-LAAM appeared to be equally potent to LAAM.

Although we did not initiate primary addiction studies with LAAM and its metabolites in nondependent animals, the administration of naloxone after acute single doses of LAAM, nor-LAAM and dinor-LAAM demonstrated the development of acute physical dependence within 5 hr of administration for all three compounds. Acute physical dependence to morphine also developed, and it tended to be dose dependent. Previous studies in the dog demonstrated signs of acute dependence after a 7-hr intravenous infusion of morphine (2 mg/kg/hr) (Martin and Eades, 1964) when nalorphine (20 mg/kg s.c.) was used to precipitate withdrawal. The precipitated abstinence syndromes in acutely and chronically physically dependent low spinal dogs can be differentiated by the shorter duration of action, less intense spinal cord signs and the absence of a hyperthermic response in the acute precipitated withdrawal syndrome. Some signs, such as mydriasis and tachycardia, are equal in magnitude. Because precipitated withdrawal is a more sensitive indicator of physical dependence than withdrawal abstinence (Martin and Jasinski, 1977), there is little reason to doubt that primary addiction studies with nor- and dinor-LAAM would have produced a prominent withdrawal syndrome.

In single-dose suppression tests conducted in morphine-dependent (morphine sulfate 3 mg/kg s.c. q.i.d.) rhesus monkeys that were abstinent for 14 to 15 hr, LAAM, nor-LAAM and dinor-LAAM completely suppressed withdrawal signs. The potencies of LAAM and morphine were estimated to be equal. However, the onset of LAAM was somewhat slower, and its duration of action was longer relative to morphine (Aceto et al., 1992). nor-LAAM was estimated to be 6 times as potent as morphine or LAAM. At comparable high doses, the duration of action of nor-LAAM was 2.5 hr longer than that of morphine (Aceto et al., 1991). Also, nor-LAAM did not precipitate withdrawal when administered to morphine-dependent monkeys, suggesting the absence of opiate antago-
nistic-like effects. The potency of dinor-LAAM was estimated to be equivalent to those of morphine and LAAM in this species (Aceto et al., 1992; Jacobson, 1991, 1992). Based on ability to alleviate morphine withdrawal, the estimated potencies of LAAM, nor-LAAM and dinor-LAAM in the morphine-dependent rhesus monkey (1:6:1) (Aceto et al., 1992; Jacobson, 1992), respectively, were similar to the relative potencies calculated for the morphine-dependent chronic spinal dog (1:9:1) in the present study. These ratios also are consistent with the corresponding 1:9:2 potency ratio for the acute, morphine-like effects in the dog reported herein.

The importance of N-dealkylation metabolites in the pharmacology of opiates has been addressed by Fraser et al. (1974, 1975a, 1975b, 1978a, 1978b, 1980), who categorized their pharmacologies into the following four groups: (1) morphine-like activity without significant nonmorphine-like activity (e.g., nor-LAAM and dinor-LAAM); (2) limited morphine-like activity and conspicuous non-morphine-like activity (nor-meperidine); (3) little or no activity, like or unlike morphine (nor-methadone) and (4) morphine-like activity plus non-morphine-like activity (nor-morphine). Clearly, morphine-like activity of both nor- and dinor-LAAM was identified in several tests in the dog, and the occurrences of non-morphine-like actions of nor- and dinor-LAAM were judged to be minimal. These data support the inclusion of both metabolites in category 1 listed above.

LAAM appeared to be pharmacologically active in the dog based on the absence of any consistent differences in the onset of action between the nor and dinor metabolites and LAAM after intravenous administration. This assumption is consistent with the ability of LAAM to inhibit contractions of the guinea pig ileum-myenteric plexus, an in vitro bioassay for determining opiate agonist activity (Nickander et al., 1974), but apparently differs in humans in whom there is a delayed onset for intravenous or oral LAAM (Fraser and Isbell, 1952), which is attributed to the enzymatic biotransformation to two active metabolites.

Among the primary active metabolites of LAAM, nor-LAAM is more potent than the dinor metabolite according to consistent data from primates and dogs. Both metabolites produce opiate-like effects when administered acutely, including acute physical dependence. In morphine-abstinent animals that exhibit physical dependence, both metabolites are capable of fully suppressing the opiate withdrawal syndrome. Historically, drugs demonstrated to have morphine-like properties in animals have been found to be morphine-like in humans (Martin and Jasinski, 1977). Thus, it is reasonable to conclude that nor-LAAM and dinor-LAAM will have the properties of narcotic analgesics in humans. In the dog, the nor and dinor metabolites retained full efficacy compared with the parent LAAM using the intravenous route of administration. Active metabolites that possess full morphine-like pharmacological activity represent one factor contributing to the prolonged action of LAAM, distinguishing it from methadone, whose metabolites lack pharmacological activity (Pohland et al., 1971). The reported half-lives in humans for LAAM and nor-LAAM (2.6 and 2.0 days, respectively) are relatively long, whereas that of dinor-LAAM is even longer (4.0 days) (Henderson et al., 1977; Kalko and Intrurrisi, 1975; Marion, 1995), permitting less frequent dosing than required for methadone. Other processes likely contributing to the prolonged action of LAAM and its metabolites are binding to tissue proteins and enterohepatic circulation (Henderson et al., 1977). The newly available LAAM substitution therapy takes advantage of these pharmacokinetic and pharmacodynamic properties and allows patients to visit treatment sites every other day or three times a week compared with the FDA regulations for methadone, which require at least six visits per week during the first 3 months of treatment. Because LAAM and its metabolites are full agonists, one would anticipate additive or synergistic interactions to develop with other opiates. The ability of the opiate antagonist naltrexone to precipitate acute withdrawal suggests that under conditions in which patients are maintained on long-term, chronic LAAM treatment, the administration of an opiate having antagonist or partial agonist properties (mixed agonists/antagonists) could precipitate opiate withdrawal. Indeed, the potential of these effects have been carefully noted in guidelines for LAAM therapy (Marion, 1995).

Acknowledgments

We wish to thank Dr. Paul Gilbert for his contribution in planning these experiments, Dr. Harlan Shannon for his help with the data analysis, Dr. Edythe London for her beneficial discussions of the manuscript and James Thompson and Richard Huppler for their technical experience. We sincerely appreciate the secretarial assistance of Cindy Ambriz.

References


Houde, R. W., Murphy, T. W. and Wallesen, S. L.: Clinical studies of narcotics at Memorial Sloan-Kettering Cancer Center. A: Relative analgesic potencies of (1) noracetylmethadol (dl-a-3-acetoxy-6-methyamino-4-dipropoxyphenyl diphenylheptane) and morphine; (2) destegropropoxyphene and pethidine; (3) RO-4–1778/1 (1-1-p-chlorphenethyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisquinolone) and codeine; (4) oral codeine and morphine. B: Rel-
ative respiratory depressant potencies of piminodine (ethyl 4-phenyl-l-[3-phenylamino-propyl]-4-piperidine carboxylate) and morphine. Proceedings of the Committee on Drug Addiction and Narcotics and the Committee on Problems of Drug Dependence, National Research Council, Division of Medical Sciences, 24th Meeting, Appendix 14, p. 2852, 1962.


Send reprint requests to: Dr. D. Bruce Vaupel, NIDA Intramural Research Program, Brain Imaging Section, 5500 Nathan Shock Drive, Baltimore, MD 21224. E-mail: bvaupel@nida.nih.gov