ABSTRACT

Data from rodent antinociception models indicate that N-methyl-D-aspartate (NMDA) receptor antagonists do not produce antinociception alone or potentiate morphine antinociception, but do attenuate the development of morphine tolerance. This study examined the antinociceptive effects of the noncompetitive NMDA receptor antagonist dizocilpine, the competitive NMDA receptor antagonist (+)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid (LY235959), and the glycine-site antagonist (+)-(1-hydroxy-3-aminopyrrolidine-2-one) ( (+)-HA-966), alone and in combination with morphine in a squirrel monkey titration procedure. In this procedure, shock (delivered to the tail) increased in intensity every 15 s from 0.01 to 2.0 mA in 30 increments. Five lever presses during any given 15-s shock period produced a 15-s shock-free period after which shock resumed at the next lower intensity. Morphine (0.3–3.0 mg/kg i.m.) dose-dependently increased the intensity below which monkeys maintained shock 50% of the time (median shock level; MSL). In contrast, dizocilpine (0.003–0.1 mg/kg i.m.) produced only modest increases in MSL in some monkeys (three of five) at the highest dose tested. Neither LY235959 (0.1–1.0 mg/kg i.m.) or (+)-HA-966 (10–56 mg/kg i.m.) increased MSL in any monkey tested. Dizocilpine, LY235959, and (+)-HA-966, when administered in combination with doses of morphine (1.0 mg/kg, 1.7 mg/kg) that either produced no antinociception or produced very little antinociception, were all found to dose-dependently potentiate the antinociceptive effect of morphine. Importantly, although these NMDA antagonists in combination with morphine produced marked increases in MSL, these combinations did not alter response rate, demonstrating that the potentiation was not due to nonspecific motor effects.

Empirical research demonstrates that N-methyl-D-aspartate (NMDA) receptor antagonists attenuate the development of tolerance to the antinociceptive effects of opiates (Trujillo and Akil, 1991; Tiseo and Inturrisi, 1993; Allen and Dykstra, 1999, 2000). Additionally, NMDA receptor antagonists attenuate the development of physical dependence under some conditions (Trujillo and Akil, 1991; Manning et al., 1996; Popik and Skolnick, 1996; Medvedev et al., 1998). Taken together with data showing that NMDA receptor antagonists can reverse pre-existing morphine tolerance (Tiseo and Inturrisi, 1993; Elliott et al., 1994), the implication of this research for substance abuse treatment and chronic pain management is profound. It is clear that NMDA receptor blockade can prevent and possibly reverse the development of tolerance to the antinociceptive effects of morphine. However, acute antinociceptive effects of NMDA receptor antagonists or potentiation of the acute antinociceptive effects of morphine by NMDA receptor antagonists in nontolerant animals has not been demonstrated consistently. For example, studies have demonstrated that the prevention of morphine tolerance by NMDA receptor antagonists occurs despite the lack of antinociceptive effect of the NMDA receptor antagonist alone or the potentiation of morphine’s acute antinociceptive effects by the NMDA receptor antagonist (Trujillo and Akil, 1991; Tiseo and Inturrisi, 1993; Allen and Dykstra, 2000). In contrast, several reports demonstrate marked antinociceptive effects of NMDA receptor antagonists when administered alone (France et al., 1989, 1990; Nelson et al., 1997; Plesan et al., 1998). Similarly, other reports indicate that NMDA receptor antagonists can potentiate the antinociceptive effects of low doses of morphine (Bernardi et al., 1996; Mao et al., 1996; Plesan et al., 1998; Lutfy et al., 1999).

A confounding variable in the assessment of the acute antinociceptive effects of NMDA receptor antagonists alone or in combination with opioids is the effect of NMDA receptor antagonists on motor function. NMDA receptor antagonists often produce marked effects on motor function, character-
ized by ataxia, bradykinesia, and hyperlocomotion (Koek and Colpaert, 1990; Carter, 1994; Geter-Douglass and Witkin, 1999). Because the assessment of pain relief in an animal model is generally a measure of the animal's capacity to remove a portion of its body from exposure to a painful stimulus (e.g., tail withdrawal from hot water, tail-flick from radiant heat, jumping or paw-licking from a hot-plate surface), compromised motor performance may confound interpretation of changes in the dependent measure.

The squirrel monkey shock titration procedure is an animal model that simultaneously provides a measure of response rate and antinociception (Dykstra, 1985; Dykstra and Massie, 1988). During 15-min components, shock (delivered to the tail) increases in intensity (0.01–2.0 mA) every 15 s in 30 increments. Five lever presses during any given 15-s shock period produce a 15-s shock free period after which shock resumes at the next lower intensity. The level below which monkeys maintain the shock intensity 50% of the time, the median shock level (MSL), is the antinociceptive measure. In contrast to procedures in which responding (e.g., tail-flick or paw lick) is measured over short time periods (from 8–60 s, depending on the procedure), data are collected from monkeys over multiple 15-min components. Most important, because termination of shock requires an operant response, responding on the lever is recorded along with the antinociceptive measure (MSL). We have previously demonstrated antinociceptive effects of opioids at doses that do not abolish responding using this procedure (Dykstra, 1979; Craft and Dykstra, 1992; Dykstra et al., 1993; Pitts et al., 1998).

Thus, the present study was designed to assess the antinociceptive activity of several NMDA receptor antagonists alone and in combination with morphine in the squirrel monkey shock titration procedure. To this end, the noncompetitive NMDA receptor antagonist dizocilpine (MK-801; Wong et al., 1986), the competitive NMDA receptor antagonist LY235959 (Schoepf et al., 1991), and the glycine-site antagonist (+)-HA-966 (Foster and Kemp, 1989; Pullan et al., 1990) were administered alone and in combination with various doses of morphine to squirrel monkeys responding in the shock titration procedure. We chose to investigate a range of doses of morphine/NMDA receptor antagonist combinations, rather than multiple classes of NMDA receptor antagonists rather than multiple examples of a single class to provide convergent evidence for a role of the NMDA receptor in any observed effect. Dizocilpine, (+)-6-phosphonomethyl-deca-hydrosoquinoline-3-carboxylic acid (LY235959), and (+)-1-hydroxy-3-aminopyrrolidine-2-one ([(+)-HA-966] were selected in particular because these compounds have been shown to suggest the development of tolerance to the antinociceptive effects of morphine in rodent antinociception procedures (Trujillo and Akil, 1991; Allen and Dykstra, 2000; Christensen et al., 2000).

Materials and Methods

Animals. Five adult male squirrel monkeys (Saimiri sciureus) weighing between 0.70 and 0.95 kg were housed individually or in pairs in a colony room with a 12-h light/dark cycle. All monkeys had continuous access to water and were maintained on a high-protein monkey diet and given fresh fruit and nuts daily. All of the monkeys had previous experience with the shock titration procedure and had received various opioid compounds, but had not received drugs for at least 30 days before the start of the present experiment.

Apparatus. During experimental sessions, each monkey sat in a Plexiglas chair and was held in place by a waist support with its tail secured by a small stock (Dykstra, 1985). The tail was coated with a noncorrosive electrode paste (EKG Sol; Graphic Controls Corporation, Buffalo, NY) to provide a low resistance electrical contact. Electric shock (110 V AC, 60 Hz) was delivered through two hinged brass plates that rested on a shaved portion of the tail. Each chair was enclosed within a ventilated sound-attenuating chamber and was illuminated by a 10-W white houselight during experimental sessions. A lever was mounted on the right side of the front panel, 8.5 cm above the waist plate and 4.0 cm from the right side wall. During experimental sessions, presses on the lever with a downward force of 0.15 N produced an audible click and were recorded as responses. White noise was presented continuously both inside the chamber and throughout the experimental room. Experimental events, including control of shock intensity, were controlled using software and hardware from Med Associates (St. Albans, VT) through a microcomputer located in the adjacent room.

Behavioral Procedure. A shock titration procedure nearly identical to that described by Dykstra and Massie (1998) was used. In each session, periods during which an FR 5 schedule of shock titration was in effect alternated with periods of blackout. Each FR 5 titration period began with the illumination of the houselight and presentation of 0.01 mA shock. Shock intensity increased from 0.01 to 2.0 mA in 30 increments. Completion of the FR 5 requirement at a given shock intensity initiated a timeout during which shock was off and the houselight remained illuminated. After the 15-s timeout the shock resumed at the next lower intensity. If a monkey failed to complete the FR 5 during 15 s at a given shock intensity, the intensity increased by one increment and the response requirement was reset to 5. The FR 5 titration periods usually lasted 15 min. An FR 5 period terminated automatically, however, if the shock intensity rose to the peak intensity of 2.0 mA and the FR 5 requirement was not completed during any of five consecutive 15-s periods. During the blackouts that separated the FR 5 titration periods, the chamber was dark, no shock was delivered, and lever presses had no consequences. Blackouts lasted 20 min. Each session began with an FR 5 titration period and ended after completion of five FR 5 periods.

Pharmacological Procedure. Dose-effect curves for dizocilpine and LY235959 were first obtained using a cumulative dosing procedure. Under this procedure, vehicle (saline) was injected 20 min before the first FR 5 period. On completion of the first FR 5 period (at the onset of the first blackout), the lowest dose was injected and its effects were assessed in the following FR period. At the onset of each subsequent blackout period, an amount of drug that increased the cumulative dose by 0.5 log unit was injected. Injections continued in this manner for three or four dose increments.

Time-effect curves for dizocilpine, LY235959, (+)-HA-966, morphine, and various morphine/NMDA receptor antagonist combinations were obtained by administering the dose or dose combination to monkeys 20 min before the first FR period. On completion of the first and all subsequent FR 5 periods (at the onset of each blackout), vehicle (saline) was injected. Combinations of dizocilpine with morphine used both the cumulative dosing and time course procedures described above. For these dose combinations, saline or a single dose of morphine (0.3, 1.0, or 1.7 mg/kg) was administered 20 min before the start of the first FR 5 period. On completion of the first FR 5 period (at the onset of the first blackout), saline was injected and its effects were assessed in the following FR period. At the onset of each subsequent blackout period (prior to components 3, 4, and 5), dizocilpine (0.003–0.03 mg/kg) was injected using the cumulative dosing procedure described above.

The behavior of monkeys was assessed daily, Monday through Friday. Drugs were generally administered on Tuesdays and Fridays. The behavior of monkeys following saline vehicle injections was determined periodically throughout the course of the study. Morphine sulfate and (+)-HA-966 were generously provided by

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the National Institute on Drug Abuse and LY235959 by Lilly Research Laboratories (Indianapolis, IN). Dizocilpine (MK-801) was purchased from Research Biochemicals, Inc. (Natick, MA). All drugs were dissolved in sterile saline and injected intramuscularly into the calf.

**Data Analysis.** There were two dependent variables extensively analyzed in this study: MSL (mA) and response rate during shock (RR, responses/s). Response rate during timeout, a third dependent variable, was only analyzed for data from monkeys that received vehicle control injections for all five components of an experimental session.

Because the same monkeys received all drug dosages within an experiment, and because the drug effects were measured over time in the same monkeys, a repeated-mea-sures analysis of variance (ANOVA) was used to analyze all of the data in this study. Both dose and time were within-subjects variables. When the data from a time course experiment were analyzed [saline control injections, morphine alone, NMDA antagonists alone, morphine plus LY235959, morphine plus (+)-HA-966], raw data were entered into the statistical analysis. When data from an experiment that used cumulative dosing were analyzed (dizocilpine and LY235959 dose-effect curves, morphine plus dizocilpine), data entered into the analysis were difference-scored. Difference scores were calculated as the difference between drug treatment and saline control injection (for drug combinations, the difference between morphine plus dizocilpine and morphine alone) for the appropriate component. This was done for two reasons. First, there were small increases in MSL over time when monkeys received saline control injections. Although this effect was small (a difference of 0.04 mA, compared with a total possible increase of 1.95 mA), it was statistically significant. Analysis of difference scores for a treatment relative to the effect of saline at that time point accounted for this small effect. Second, the analysis of difference scores simplified the analysis by replacing two within-subject variables (dose and time) with one.

**Saline and Morphine Control Data.** For each monkey, data from the 7 most recent saline control days were averaged and the average for a monkey was used in the statistical analysis. For morphine/LY235959 and morphine/(+)-HA-966 combinations, the effects of morphine alone were assessed before and after each dose combination. Thus there were six morphine-alone curves for each monkey in each of these experiments. Since there was little variability in the effectiveness of morphine for a given monkey, these morphine curves were averaged and the average for a monkey represented that monkey in the statistical analysis. The results of the statistical analysis did not differ, however, when only one morphine-alone curve was used in place of the averaged morphine curve (data not shown).

**Contrasts.** When a post hoc analysis of a factor in the repeated-measures ANOVA was conducted, the analysis made the following comparisons: for time, all time points versus time 1; for multiple doses of a drug tested alone, all doses versus saline control; and for dose combinations, all combinations versus the effect of morphine or LY235959 alone.

All data were analyzed using SAS for Windows version 6.1 (SAS Institute, Cary, NC).

**Results**

**Control Performance.** To examine the effects of saline injections on MSL, a repeated-measures ANOVA was performed using time as the within-subject variable. Small increases in MSL over time were observed when monkeys were injected with saline. Post hoc analysis of the repeated-measures ANOVA main effect of time ($F_{1,4} = 9.68, P = 0.0004$) revealed a trend toward significance, with MSL at component 5 significantly higher than MSL at component 1 (0.09 versus 0.05 mA, $F_{1,4} = 12.25, P = 0.0249$). However, this difference in mean MSL is small relative to the magnitude of increase possible in this procedure.

To compare response rates during shock and during the postshock timeouts, a repeated-measures ANOVA was performed using time and shock condition (shock or postshock timeout) as within-subject variables. The ANOVA revealed a significant main effect of shock condition ($F_{1,4} = 10.17, P = 0.0333$). Response rates during shock were significantly higher than response rates during the postshock timeouts (data not shown). The ANOVA did not reveal a significant main effect of time ($F_{4,16} = 1.39, P = 0.2814$) or a significant shock condition by time interaction ($F_{4,16} = 1.52, P = 0.2439$).

**Effects of Morphine on MSL and RR.** Figure 1 shows the time course of the antinociceptive effects of morphine in five monkeys. Morphine (0.3, 1.0, 1.7, and 3.0 mg/kg) dose and time-dependently increased MSL in all five monkeys tested. Average MSLs are presented in Table 1. Modest increases in MSL occurred following administration of 1.7 mg/kg morphine, whereas 3.0 mg/kg morphine produced maximal increases in all five monkeys tested. For three of the five monkeys that received 3.0 mg/kg morphine, the session was terminated after the second component, and naltrexone was administered to prevent possible adverse effects of this high dose of morphine. The remaining two monkeys were injected with naltrexone following the fourth and fifth com-

![Fig. 1. Effects of morphine alone on MSL in mA (top panel) and RR in responses/s (bottom panel) over the five components of an experimental session. Value on the abscissas represent the time during which MSL was calculated.](image-url)
The effects of each cumulative dose of dizocilpine were consistently observed when monkeys were injected with saline. The effects of saline, morphine, dizocilpine, LY235959, and (+)-HA-966 on MSL and RR over the five components of an experimental session were measured over time, as shown in Table 1. Dizocilpine, morphine, and LY235959 increased MSL relative to saline. The average MSL presented in Fig. 2. Only the highest dose of dizocilpine (0.1 mg/kg) increased MSL relative to saline. The effects of cumulatively administered dizocilpine and LY235959 on MSL and RR are also presented in Table 1. Dizocilpine, morphine, and LY235959 increased MSL relative to saline. In addition, neither dizocilpine nor LY235959 altered RR when administered cumulatively to monkeys in the shock titration procedure. The effects of NMDA Receptor Antagonists Alone on MSL and RR: Time Course Analysis. The effects of various doses of dizocilpine (0.03 mg/kg) and LY235959 (0.1, 0.3, and 1.0 mg/kg) on MSL and RR were measured over time, as were the effects of (+)-HA-966 (10, 30, and 56 mg/kg). Table 2 presents the results of repeated-measures analyses performed for each drug and for each measure (MSL and RR). The effects of each of these three NMDA receptor antagonists on MSL and RR are also presented in Table 1. Dizocilpine, LY235959, and (+)-HA-966 did not significantly increase MSL or alter RR relative to saline control injections.

Effects of Dizocilpine in Combination with Morphine on MSL and RR. Figure 3 shows the antinociceptive effect of morphine alone and in combination with dizocilpine. Doses of morphine from 0.3 to 1.7 mg/kg produced either no antinociceptive effect or a modest increase in antinociception when administered alone. For example, the mean MSL for monkeys treated with 1.7 mg/kg morphine was 0.14, 0.21, 0.30, 0.30, and 0.36 mA in components 1, 2, 3, 4, and 5, respectively. Dizocilpine (0.003–0.03 mg/kg) dose-dependently potentiated the modest antinociceptive effect of morphine.
The mean MSL for monkeys treated with 1.7 mg/kg morphine and 0.03 mg/kg dizocilpine was 1.14 (0.25) mA. A repeated-measures ANOVA was performed on MSL difference scores (morphine plus dizocilpine–morphine alone), and the results of that analysis are presented in Table 3. There was a significant morphine dose–dizocilpine dose interaction (F9,36 = 4.50, P = 0.0005). In contrast to the effects on MSL, no dose of morphine alone or in combination with dizocilpine decreased the mean rate of responding (Fig. 3, Table 3), demonstrating that the potentiation of the effect of morphine on MSL is not due to motor impairment.

Although the mean MSL for 1.7 mg/kg morphine was increased in all monkeys when combined with 0.03 mg/kg dizocilpine, the increase was modest in two monkeys. The difference scores for these two monkeys (0.15 mA for M290 and 0.55 mA for M540) were low compared with the other three monkeys (0.70, 1.10, and 1.40 mA). Furthermore, MSLs following this drug combination in these two monkeys (M290 and M540) were less than 1.0 mA, whereas the three other monkeys had MSLs that were greater than 1.3 mA and approached maximal antinociceptive effectiveness in this procedure. To begin to address whether these individual differences were related to time course variables not accounted for with the cumulative dosing procedure, monkeys M290 and M540 were administered 1.7 mg/kg morphine in combination with 0.03 mg/kg dizocilpine 20 min before the start of the first session. The effect of these drugs on MSL and RR was measured over the five components of the experimental session. Both monkeys showed substantial increases in MSL following administration of 1.7 mg/kg morphine in combination with 0.03 mg/kg dizocilpine 20 min before the start of the first session. The effect of these drugs on MSL and RR was measured over the five components of the experimental session.
creased in a similar manner from 0.25 mA in the first component to 1.20, 1.50, 1.20, and 1.20 mA in components 2, 3, 4, and 5. Although monkey M290 did not respond during the third component, both monkeys responded within the range of control response rates during the second component, and monkey M540 responded normally throughout five components of the session. Following these results, cumulative dosing was abandoned and time course analyses were conducted for all subsequent morphine/NMDA antagonist combinations.

**Effects of LY235959 in Combination with Morphine on MSL and RR.** Figure 4 shows the effects of the competitive NMDA receptor antagonist LY235959 on the antinociceptive effect of 1.0 mg/kg morphine. When administered

### Table 3

Results from the repeated-measure ANOVAs of the effects of morphine in combination with dizocilpine, LY235959, and (+)-HA-966 on MSL and RR

For morphine/dizocilpine combinations, difference scores were analyzed. Otherwise, raw data were used in the analysis.

<table>
<thead>
<tr>
<th>morphine (S, 0.3, 1.0, 1.7 mg/kg) + dizocilpine (S, 0.003, 0.01, 0.03 mg/kg)</th>
<th>Morphine (S, 0.3, 1.0, 1.7 mg/kg) + LY235959 (S, 0.1, 0.3, 1.0 mg/kg)</th>
<th>Morphine (S, 0.3, 1.0, 1.7 mg/kg) + (+)-HA-966 (S, 10, 30, 56 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.v.</td>
<td>d.v.</td>
<td>d.v.</td>
</tr>
<tr>
<td>Morphine Dose</td>
<td>LY235959 Dose</td>
<td>(+)-HA-966 Dose</td>
</tr>
<tr>
<td>MSL</td>
<td>F3,12 = 3.06, P = 0.0695</td>
<td>F3,9 = 10.46, P = 0.0027**</td>
</tr>
<tr>
<td>RR</td>
<td>F3,12 = 0.97, P = 0.4393</td>
<td>F3,9 = 0.57, P = 0.6851</td>
</tr>
<tr>
<td>F9,36 = 10.28, P = 0.0001***</td>
<td>F9,36 = 0.63, P = 0.7678</td>
<td>F9,36 = 0.64, P = 0.7973</td>
</tr>
<tr>
<td>F9,36 = 12.34, P = 0.0007***</td>
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</tr>
<tr>
<td>F9,36 = 3.41, P = 0.0050**</td>
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<tr>
<td>F9,36 = 0.23, P = 0.9942</td>
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S, saline; d.v., dependent variable.

*P < 0.05, **P < 0.01, ***P < 0.001.
alone 20 min before the start of the first component, 1.0 mg/kg morphine produced minimal increases in MSL. For example, the mean MSL for monkeys administered 1.0 mg/kg morphine was 0.06, 0.11, 0.14, 0.16, and 0.19 in components 1, 2, 3, 4, and 5, respectively (Table 4). LY235959, when administered with morphine 20 min before the start of the first component, dose-dependently increased MSL in all five monkeys tested for the duration of the experimental session. For example, the mean MSL values at component 5 (165–175 min postinjection) were 0.19, 0.19, 0.70, and 1.23 for morphine alone, morphine plus 0.1 mg/kg LY235959, morphine plus 0.3 mg/kg LY235959, and morphine plus 1.0 mg/kg LY235959, respectively. The results of a repeated-measures ANOVA of the MSL values are presented in Table 3. There were highly significant main effects of the LY235959 dose (0, 0.1, 0.3, and 1.0), time (components 1, 2, 3, 4, and 5), and a highly significant dose × time interaction. In contrast, response rate was unchanged over the five components of the experimental session following administration of 1.0 mg/kg morphine alone or in combination with LY235959 (Fig. 4, Table 3, and Table 4). These data demonstrate that the potentiation of the antinociceptive effect of morphine is not due to motor impairment.

Figure 5 shows the effects of 0.3 mg/kg LY235959 administered alone and in combination with 0.3, 1.0, and 1.7 mg/kg morphine in a subset of monkeys (n = 3). The results of a repeated-measures ANOVA are presented in Table 3. These data demonstrate that the interaction between morphine and LY235959 is dependent on both the dose of LY235959 (Fig. 4) and the dose of morphine (Fig. 5). Once again, response rate was not altered in monkeys that received 0.3 mg/kg LY235959 alone or in combination with morphine, demon-

**Fig. 4.** Effects of 1.0 mg/kg morphine alone and in combination with LY235959 on MSL in mA (top) and RR in responses/s (bottom). Value on the abscissas represent the time during which MSL was calculated.

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
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<th>5</th>
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<td></td>
<td></td>
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<td>1.0 Morphine alone</td>
<td>0.06 (0.01)</td>
<td>0.11 (0.01)</td>
<td>0.14 (0.02)</td>
<td>0.16 (0.03)</td>
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<td>0.70 (0.36)</td>
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<td>+0.3 mg/kg morphine</td>
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<td>0.07 (0.02)</td>
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<td>0.17 (0.03)</td>
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<td>1.08 (0.51)</td>
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<td>1.27 (0.29)</td>
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<tr>
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<td>0.05 (0.01)</td>
<td>0.09 (0.04)</td>
<td>0.11 (0.05)</td>
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<td>+10 mg/kg (+)-HA-966</td>
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<td>0.05 (0.02)</td>
<td>0.06 (0.02)</td>
<td>0.05 (0.02)</td>
<td>0.09 (0.04)</td>
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<td>+56 mg/kg (+)-HA-966</td>
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<td>0.47 (0.27)</td>
<td>0.80 (0.45)</td>
<td>1.07 (0.47)</td>
<td>1.07 (0.38)</td>
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<td>RR</td>
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<td></td>
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</tr>
<tr>
<td>1.0 Morphine alone</td>
<td>0.354 (0.043)</td>
<td>0.355 (0.054)</td>
<td>0.309 (0.010)</td>
<td>0.304 (0.021)</td>
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<tr>
<td>+0.1 mg/kg LY235959</td>
<td>0.312 (0.034)</td>
<td>0.313 (0.030)</td>
<td>0.342 (0.083)</td>
<td>0.300 (0.031)</td>
<td>0.301 (0.026)</td>
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<tr>
<td>+0.3 mg/kg LY235959</td>
<td>0.335 (0.034)</td>
<td>0.276 (0.022)</td>
<td>0.281 (0.037)</td>
<td>0.270 (0.018)</td>
<td>0.235 (0.042)</td>
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<tr>
<td>+1.0 mg/kg LY235959</td>
<td>0.300 (0.025)</td>
<td>0.237 (0.041)</td>
<td>0.277 (0.024)</td>
<td>0.278 (0.039)</td>
<td>0.277 (0.032)</td>
</tr>
<tr>
<td>0.3 LY235959 alone</td>
<td>0.305 (0.007)</td>
<td>0.263 (0.007)</td>
<td>0.312 (0.021)</td>
<td>0.269 (0.031)</td>
<td>0.278 (0.055)</td>
</tr>
<tr>
<td>+0.3 mg/kg morphine</td>
<td>0.518 (0.222)</td>
<td>0.322 (0.041)</td>
<td>0.358 (0.088)</td>
<td>0.322 (0.049)</td>
<td>0.313 (0.037)</td>
</tr>
<tr>
<td>+1.0 mg/kg morphine</td>
<td>0.373 (0.043)</td>
<td>0.265 (0.030)</td>
<td>0.238 (0.042)</td>
<td>0.254 (0.026)</td>
<td>0.208 (0.071)</td>
</tr>
<tr>
<td>+1.7 mg/kg morphine</td>
<td>0.270 (0.045)</td>
<td>0.232 (0.035)</td>
<td>0.252 (0.027)</td>
<td>0.211 (0.089)</td>
<td>0.289 (0.024)</td>
</tr>
<tr>
<td>1.0 Morphine alone</td>
<td>0.401 (0.044)</td>
<td>0.399 (0.086)</td>
<td>0.401 (0.082)</td>
<td>0.404 (0.087)</td>
<td>0.348 (0.068)</td>
</tr>
<tr>
<td>+10 mg/kg (+)-HA-966</td>
<td>0.410 (0.129)</td>
<td>0.343 (0.050)</td>
<td>0.329 (0.010)</td>
<td>0.324 (0.025)</td>
<td>0.316 (0.043)</td>
</tr>
<tr>
<td>+30 mg/kg (+)-HA-966</td>
<td>0.326 (0.040)</td>
<td>0.338 (0.048)</td>
<td>0.286 (0.018)</td>
<td>0.276 (0.023)</td>
<td>0.248 (0.013)</td>
</tr>
<tr>
<td>+56 mg/kg (+)-HA-966</td>
<td>0.275 (0.024)</td>
<td>0.254 (0.022)</td>
<td>0.230 (0.023)</td>
<td>0.172 (0.074)</td>
<td>0.197 (0.061)</td>
</tr>
</tbody>
</table>
strating that this antinociceptive effect is not due to motor impairment.

Effects of (+)-HA-966 in Combination with Morphine on MSL and RR. A subset of monkeys (n = 3) were administered 1.0 mg/kg morphine alone or in combination with the glycine-site antagonist (+)-HA-966. Figure 6 shows that (+)-HA-966 dose- and time-dependently increased the antinociceptive effect of 1.0 mg/kg morphine. For example, the mean MSL values following administration of 1.0 mg/kg morphine alone were 0.05, 0.09, 0.11, 0.13, and 0.14 mA during components 1, 2, 3, 4, and 5. Following 1.0 mg/kg morphine plus 56 mg/kg (+)-HA-966, mean MSL values were 0.08, 0.47, 0.80, 1.07, and 1.07 during components 1, 2, 3, 4, and 5 (Table 4). The mean MSL values at time 5 following morphine alone, morphine plus 10 mg/kg (+)-HA-966, morphine plus 30 mg/kg (+)-HA-966, and morphine plus 56 mg/kg (+)-HA-966 were 0.14, 0.09, 0.37, and 1.07 mA, respectively. The results of the repeated-measures ANOVA revealed a significant (+)-HA-966 dose × time interaction when MSL was the dependent variable; however, as with the noncompetitive antagonist, dizocilpine, and the competitive antagonist, LY235959, there was no such interaction when response rate was the dependent measure (Table 3).

Discussion

In this study, the acute administration of morphine produced a maximal increase in MSL that was dose- and time-dependent. Dizocilpine, LY235959, and (+)-HA-966 produced little to no increase in MSL across the range of doses tested. However, when these drugs were combined with doses of morphine that alone produced little or no increase in MSL, MSL was greatly increased relative to treatment with morphine alone. Several other laboratories have demonstrated similar effects in both rodent models of antinociception (Mao et al., 1996; Lutfy et al., 1999) and in human experimental and clinical pain (Sethna et al., 1998; Caruso, 2000; Katz, 2000).

The NMDA receptor antagonists used in this study represented several chemically and functionally distinct classes of drugs. We chose to investigate a range of classes of NMDA receptor antagonist rather than multiple examples of a single class to provide convergent evidence for a role of the NMDA receptor in any observed effect. Because an NMDA receptor channel blocker (dizocilpine), a competitive NMDA receptor antagonist (LY235959), and a glycine-site antagonist [(+)-HA-966] all effectively increased MSL relative to morphine alone, this suggests that blockading activity at the NMDA
receptor plays an important role in the potentiation of the antinociceptive effect of morphine in this procedure.

A second major finding from this study is that the increases in MSL observed following morphine/NMDA receptor antagonist combinations occurred without disruptions in RR during shock. That is, following a morphine/NMDA receptor antagonist combination, monkeys actively titrated the shock intensity at significantly higher mA values than following treatment with saline or morphine alone, yet the rate at which they responded on the lever to titrate the shock was no different than following treatment with saline or morphine alone. Thus, the increases in MSL that followed morphine/NMDA receptor antagonist combinations cannot be attributed to the effect of these drugs or drug combinations on motor performance.

One strength of the shock titration procedure is its ability to differentiate the antinociceptive effect of a drug from changes in motor function that can confound the interpretation of latency increases in other antinociception assays. Traditionally, inferences about the contribution of motor effects within antinociception assays have been drawn from analyses of potency differences. For example, the noncompetitive NMDA receptor antagonist dizocilpine was equipotent in increasing squirrel monkey tail-withdrawal latencies from warm water and impairing motor function as assessed with an observer-scored rating scale (Rupniak et al., 1993). In contrast, France et al. (1989) demonstrated maximal increases in tail-withdrawal latencies using a warm water tail-withdrawal procedure when rhesus monkeys were tested following administration of several noncompetitive NMDA receptor antagonists. Also, the doses for antinociception were 3- to 10-fold lower than the doses for anesthesia. Unfortunately, these data do not critically test the hypothesis that motor effects are causally related to antinociceptive efficacy. For example, it is not known to what extent the ataxia produced by dizocilpine in the Rupniak study was related to the increases observed in squirrel monkey tail-withdrawal latencies. Similarly, potency differences in anesthetic and analgesic effects only demonstrate that increases in tail-withdrawal latency are not due to anesthesia, but leave open the possibility that other motor effects may be causally related to the increase in tail-withdrawal latencies. In the shock titration procedure, however, RR is inextricably linked to the antinociceptive measure MSL, and inference regarding the relationship between potentially unrelated motor tasks and the measure of antinociception is not confounded.

It is important to note that motor impairments were sometimes observed in monkeys that received selected morphine/NMDA antagonist combinations. These effects can be described as periods of inactivity and difficulty maneuvering and maintaining balance on a perch. Still, data from the titration procedure clearly indicate that response rate was unaltered under these conditions.

Here, however, we have a separation of the goals of theoretical basic science research and those of clinical practice for the treatment of pain. Although these data clearly demonstrate that NMDA receptor antagonists can potentiate morphine analgesia despite gross motor effects, it is the clinical question of tolerability that will determine the utility of such drug combination for the treatment of chronic pain. Some data already suggest that the combination of morphine with dextromethorphan, a noncompetitive NMDA receptor antag-
jillo and Akil, 1991; Tiseo and Inturrisi, 1993; Allen and Dykstra, 2000). These studies tend to use methods that may not be adequate for demonstrating these effects of NMDA receptor antagonists. For example, LY235959 did not potentiate the antinociceptive effects of morphine when tested with the rat warm-water tail-withdrawal procedure; however, morphine was administered in a cumulative fashion. Thus, the effect of LY235959 on low doses of morphine was only assessed at a single time point (Allen and Dykstra, 2000). A thorough time-dependent analysis of the effects of LY235959 in combination with morphine is necessary to address this difference.

From an empirical perspective, prevention of tolerance in the absence of acute antinociceptive effects of NMDA receptor antagonists is important for demonstrating that NMDA receptor antagonists prevent the development of opioid tolerance rather than simply enhancing the effectiveness of an opioid through an additive interaction with its own acute antinociceptive effects. From a treatment perspective, however, an acute interaction in addition to a role in preventing tolerance is a benefit. For example, research shows that the magnitude of tolerance that develops to the effects of an opioid is related to the amount of the drug administered chronically, with greater tolerance resulting from higher maintenance doses (Fernandes et al., 1982; Schuh et al., 1996; Allen and Dykstra, 2000). A drug combination that increases the effectiveness of lower doses of morphine might be expected to produce less tolerance relative to an equianalgesic dose of morphine alone independently of its potential to block the mechanism of tolerance. Thus, the results from this and other studies suggest that NMDA antagonists/opioid combinations have promise as analgesic agents for the long-term treatment of pain.

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References


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