The Competitive \(N\)-Methyl-\(d\)-aspartate Receptor Antagonist (\(-\)\()-6\)-Phosphonomethyl-deca-hydroisoquinoline-3-carboxylic Acid (LY235959) Potentiates the Antinociceptive Effects of Opioids That Vary in Efficacy at the \(\mu\)-Opioid Receptor

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ABSTRACT

(\(-\)\()-6\)-Phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959) is a competitive \(N\)-methyl-\(d\)-aspartate receptor antagonist shown to prevent the development of tolerance to the antinociceptive effects of morphine in rodents. Although administration of LY235959 alone generally does not produce antinociception, LY235959 potentiates the antinociceptive effects of morphine in squirrel monkeys. The present study was designed to determine whether LY235959 would potentiate the acute antinociceptive effects of morphine as well those of the opioid receptor agonists \(l\)-methadone, levorphanol, butorphanol, and buprenorphine. A squirrel monkey titration procedure was used in which shock (delivered to the tail) increased in intensity every 15 s (0.01–2.0 mA) in 30 increments. Five lever presses during any given 15-s shock period (fixed ratio 5) 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.), \(l\)-methadone (0.1–0.56 mg/kg i.m.), levorphanol (0.1–1.0 mg/kg i.m.), butorphanol (1.0–10 mg/kg i.m.), and buprenorphine (0.01–0.03 mg/kg i.m.), but not LY235959 (0.1–1.0 mg/kg i.m.), dose and time dependently increased the intensity below which monkeys maintained shock 50% of the time (median shock level, MSL). LY235959 dose dependently potentiated the effect of each opioid agonist on MSL when concurrently administered to monkeys. Although LY235959 potentiated the antinociceptive effect of each opioid examined in a statistically significant manner, LY235959 seemed more potent and effective when combined with higher efficacy opioids. The present data suggest that the \(N\)-methyl-\(d\)-aspartate antagonist, LY235959, can potentiate the antinociceptive effects of a range of opioid receptor agonists independently of nonspecific motor effects.

An important goal of research in opioid pharmacology is to develop medications with the therapeutic effects of \(\mu\)-opioid receptor (MOR) agonists (e.g., analgesia) without untoward effects, such as respiratory suppression and abuse potential. To this end, low-efficacy and/or mixed action opioids have been studied extensively in humans and in various animal models that assess the analgesic and abuse-related effects of opioid agonists. Several low-efficacy and/or mixed action opioids, such as butorphanol, seem to have a lower potential for abuse than drugs such as morphine (for review, see Preston and Jasinski, 1991). However, some have argued that there exists a limited effective dose range for these compounds relative to morphine (for review, see Hoskins and Hanks, 1991). In preclinical evaluations, these opioids tend to be less effective than morphine in rodent and primate models of antinociception (Dykstra, 1990; Walker et al., 1993; Morgan et al., 1999).

In addition to having lower antinociceptive efficacy, preclinical data show that chronic administration of drugs with lower efficacy at the MOR tend to produce more tolerance than drugs with higher efficacy at the MOR. For example, rats administered equieffective doses of morphine or fentanyl, an opioid with higher efficacy than morphine (Magnan et al., 1982; Adams et al., 1990), showed greater tolerance to morphine than to fentanyl in antinociception assays (Stevens and Yaksh, 1989; Paronis and Holtzman, 1992). Similarly, chronic administration of buprenorphine, an opioid agonist with lower efficacy than morphine (Traynor and Nahorski, 1995; Pitts et al., 1998), produced more antinociceptive tolerance than did chronic administration of equieffective doses of morphine in rats tested with a warm-water tail with-

ABBREVIATIONS: MOR, \(\mu\)-opioid receptor; NMDA, \(N\)-methyl-\(d\)-aspartate; SNC80, (+)\(-\)(\(\alpha\)R)-\(\alpha\)\((-\)(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl\)-3-methoxy-benzyl]-\(N\)-diethyl-benzamid; FR, fixed ratio; MSL, median shock level; RR, response rate; ANOVA, analysis of variance; (+)HA-966, (+)-(1-hydroxy-3-aminopyrrolidine-2-one).
drawal procedure (Walker and Young, 2001). Such an effect in humans could further limit the utility of low-efficacy and/or mixed action opioids for the treatment of severe and persistent pain.

One recent approach in the development of effective pain medications is the combination of an opioid agonist with an NMDA receptor antagonist. MorphiDex, a combination of morphine and the noncompetitive NMDA receptor antagonist dextromethorphan, shows good analgesic effectiveness in humans (Caruso, 2000), and the repeated administration of the combination over 2 weeks does not result in the dose escalation that occurs with the repeated administration of morphine alone (Katz, 2000). This latter finding is concordant with animal research conducted over the past 12 years showing that the coadministration of NMDA receptor antagonists with morphine prevents or attenuates the development of tolerance that occurs when morphine alone is administered repeatedly (Trujillo and Akil, 1991; Tiseo and Inturrisi, 1993; Allen and Dykstra, 2000a,b).

The finding that dextromethorphan potentiates the analgesic effectiveness of morphine in humans is also supported by some animal research that shows the acute administration of an NMDA receptor antagonist potentiates the antinociceptive effect of an acute injection of morphine. For example, recently published data using a squirrel monkey titration procedure show that the antinociceptive effects of morphine are potentiated by the competitive NMDA receptor antagonist LY235959, the noncompetitive NMDA receptor antagonists dizocilpine and dextromethorphan, and the glycine-site partial agonist (+)-HA-966 (Allen and Dykstra, 2001; Allen et al., 2002). Dextromethorphan also potentiated the antinociceptive effects of the δ-opioid agonist SNC80 in this procedure (Allen et al., 2002).

To date, however, less is known about acute interactions between NMDA receptor antagonists and drugs with varying efficacy at the MOR. If the analgesic effects of low-efficacy and/or mixed action opioids were potentiated by NMDA receptor antagonists independently of abuse-related effects, then such a combination would have considerable value for the treatment of pain. The purpose of the present study, then, was to determine first whether the potentiating effects of NMDA receptor antagonists on the antinociceptive effects of morphine extend to opioids that vary in efficacy at the MOR. To this end, LY235959 was administered in combination with buprenorphine, butorphanol, morphine, levorphanol, and l-methadone to monkeys responding under a schedule of shock titration.

Materials and Methods

Subjects

Eight adult male squirrel monkeys (Saimiri sciureus) with maximum weight values between 730 and 1140 g were housed in pairs in a colony room set on 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 previously received various opioid compounds.

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, Graphics 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 house light 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 MED Associates (St. Albans, VT) software and hardware through a microcomputer located in the adjacent room.

Behavioral Procedure

A shock titration procedure nearly identical to that described by Dykstra (1985) was used. In each session, periods of blackout alternated with periods during which a fixed ratio 5 (FR5) schedule of shock titration was in effect. Each FR5 titration period began with the illumination of the house light and presentation of 0.01-mA shock. Shock intensity increased from 0.01 to 2.0 mA in 30 increments. Completion of the FR5 requirement at a given shock intensity initiated a time-out during which shock was off and the house light remained illuminated. After the 15-s time-out, the shock resumed at the next lower intensity. If a monkey failed to complete the FR5 during 15 s at a given shock intensity, the intensity increased by one increment and the response requirement was reset to 5. The FR5 titration periods usually lasted 15 min. An FR5 period terminated automatically, however, if the shock intensity rose to the peak intensity of 2.0 mA and the FR5 requirement was not completed during any of five consecutive 15-s periods. During the blackouts that separated the FR5 titration periods, the chamber was dark, no shock was delivered, and lever presses had no programmed consequences. Blackouts lasted 20 min. Each session began with an FR5 titration period and ended after completion of five FR5 periods. The intensity below which monkeys maintain shock 50% of the time, the median shock level (MSL, milliamperes) is the measure of antinociceptive effect.

Pharmacological Procedure

Time-effect curves for morphine, l-methadone, levorphanol, buprenorphine, butorphanol, LY235959, and all drug combinations were obtained by administering a dose or dose combination to monkeys 20 min before the first FR5 period. On completion of the first and all subsequent FR5 periods (at the onset of each blackout), vehicle (sterile water) was injected. The effects of LY235959 in combination with an opioid agonist were determined by administering both LY235959 and the opioid agonist together 20 min before the start of the first FR5 component.

The behavior of monkeys was assessed daily, Monday through Friday. Drugs were generally administered on Tuesdays and Fridays. The behavior of monkeys after vehicle injections was determined periodically throughout the course of the study.

The National Institute on Drug Abuse generously provided morphine sulfate, buprenorphine, and l-methadone. Eli Lilly & Co. (Indianapolis, IN) generously provided (−)-6-phosphonomethyl-deca-hydroisooquinoline-3-carboxylic acid (LY235959). Butorphanol tartrate was purchased from Sigma-Aldrich (St. Louis, MO), and levorphanol tartrate was purchased from Sigma/RBI (Natick, MA). All drugs were dissolved in sterile water and injected intramuscularly into the calf in a volume of 0.5 ml/kg body weight.

Data Analysis

Vehicle and Opioid Alone Control Data. For each monkey, data from the 10 most recent vehicle control sessions were averaged, and the average for a monkey was used in the statistical analysis.
For opioid/LY235959 combinations, the effects of the opioid alone were assessed before and after each series of dose combinations. Thus, there were two opioid alone curves for each monkey in each of these experiments. Because there was little variability in the effectiveness of each opioid within a given monkey, these opioid dose-effect curves were averaged, and the average for a monkey represented that monkey in the statistical analysis.

**Statistical Analysis.** Two dependent variables were analyzed extensively in the present study: MSL (milliamperes) and response rate during shock (RR, responses per second). Because the same monkeys received all drug doses within an experiment, and because the drug effects were measured over time in the same monkeys, a repeated measures analysis of variance (ANOVA) was used to analyze all of the data in the present study. Opioid dose, LY235959 dose, and time were within-subjects variables. All data were analyzed using SAS for Windows version 6.1 (SAS Institute, Inc., Cary, NC).

### Results

**Effect of LY235959 Alone on MSL and RR.** The MSL values for eight monkeys tested with LY235959 (0.1–1.0 mg/kg) are presented in Table 1. When administered alone, LY235959 did not increase MSL in a statistically significant manner. Thus, the repeated measures ANOVA in which LY235959 dose (0, 0.1, 0.3, and 1.0 mg/kg) and time (components 1, 2, 3, 4, and 5) served as within-subjects variables did not reveal a main effect of LY235959 dose ($F_{3,21} = 1.45$, $P = 0.2579$) or a dose by time interaction ($F_{12,84} = 0.92$, $P = 0.5349$). The statistical analysis did reveal a main effect of time ($F_{4,98} = 5.00$, $P = 0.0036$). That is, regardless of LY235959 dose (0, 0.1, 0.3, and 1.0 mg/kg), MSL values increased across the five components of the experimental session. This main effect of time is sometimes revealed when sterile water control injection data alone are analyzed with repeated measures ANOVA and has been reported previously with this procedure (Allen and Dykstra, 2001). It is important to note that 0.19 ($\pm 0.08$) mA was the highest MSL measured when LY235959 was administered alone. Although this represents a slight increase over sterile water control injection values, the increase is small relative to the total increase in MSL possible in this procedure (0.09 mA above control values with a possible increase of 1.70 mA). There was a statistically significant main effect of LY235959 dose when RR was the dependent measure ($F_{3,21} = 5.55$, $P = 0.0058$), but no main effect of time and no dose by time interaction. Mean RRs were as low as 0.26 responses/s after administration of 1.0 mg/kg LY235959, compared with mean RRs as low as 0.31 responses/s after administration of sterile water control injections. However, this small decrease in RR is not a meaningful change in rate in this procedure.

**Effect of Opioid Agonists Alone on MSL and RR.** The effect of $l$-methadone, morphine, levorphanol, buprenorphine, and butorphanol alone on MSL and RR were determined before any combination tests with LY235959. Data for each agonist were analyzed statistically using repeated measures ANOVA with dose (including sterile water control or 0.0 mg/kg) and time as within-subjects variables. Only data from the first two components were entered when morphine was the dependent variable because the session was terminated early for more than one-half of the monkeys tested with 3.0 mg/kg morphine. Only data for the lowest two doses of levorphanol were entered into the statistical analysis when levorphanol was the dependent variable for similar reasons.

The MSL values for each opioid agonist are presented in Table 1. All of the opioid agonists tested in the present study increased MSL in a dose-dependent manner. These dose-dependent increases were statistically significant for each agonist, as revealed by main effects of opioid dose for morphine ($F_{3,21} = 28.22$, $P < 0.0001$), levorphanol ($F_{2,18} = 12.62$, $P = 0.0007$), $l$-methadone ($F_{3,21} = 10.59$, $P = 0.0002$), buprenorphine ($F_{3,21} = 10.17$, $P = 0.0002$), and butorphanol ($F_{3,21} = 5.68$, $P = 0.0052$). The time course of effect for each agonist differed, and these effects were also revealed as sig-

### Table 1

Effects of water, LY235959, $l$-methadone, morphine, levorphanol, buprenorphine, and butorphanol on MSL over the five components of an experimental session

<table>
<thead>
<tr>
<th></th>
<th>25–35 min</th>
<th>60–70 min</th>
<th>95–105 min</th>
<th>130–140 min</th>
<th>165–175 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td>0.07 (0.03)</td>
<td>0.08 (0.04)</td>
<td>0.09 (0.05)</td>
<td>0.09 (0.05)</td>
<td>0.10 (0.05)</td>
</tr>
<tr>
<td>LY235959</td>
<td>0.08 (0.03)</td>
<td>0.10 (0.03)</td>
<td>0.12 (0.04)</td>
<td>0.12 (0.05)</td>
<td>0.13 (0.03)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>0.09 (0.02)</td>
<td>0.11 (0.04)</td>
<td>0.13 (0.04)</td>
<td>0.13 (0.03)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>0.11 (0.05)</td>
<td>0.15 (0.08)</td>
<td>0.17 (0.08)</td>
<td>0.19 (0.08)</td>
<td>0.19 (0.08)</td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td>0.12 (0.07)</td>
<td>0.13 (0.08)</td>
<td>0.07 (0.03)</td>
<td>0.08 (0.03)</td>
<td>0.08 (0.03)</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>0.19 (0.09)</td>
<td>0.20 (0.10)</td>
<td>0.14 (0.04)</td>
<td>0.15 (0.07)</td>
<td>0.14 (0.07)</td>
</tr>
<tr>
<td>0.56 mg/kg</td>
<td>0.79 (0.21)</td>
<td>0.89 (0.24)</td>
<td>0.67 (0.23)</td>
<td>0.41 (0.15)</td>
<td>0.33 (0.15)</td>
</tr>
<tr>
<td><strong>Levorphanol</strong></td>
<td>0.10 (0.06)</td>
<td>0.15 (0.09)</td>
<td>0.13 (0.08)</td>
<td>0.14 (0.08)</td>
<td>0.15 (0.09)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>0.15 (0.08)</td>
<td>0.22 (0.13)</td>
<td>0.27 (0.15)</td>
<td>0.31 (0.17)</td>
<td>0.31 (0.19)</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>0.96 (0.26)</td>
<td>1.55 (0.20)</td>
<td>1.48 (0.25)</td>
<td>1.25 (0.28)</td>
<td>1.30 (0.00)</td>
</tr>
<tr>
<td><strong>Buprenorphine</strong></td>
<td>0.12 (0.07)</td>
<td>0.18 (0.13)</td>
<td>0.24 (0.19)</td>
<td>0.12 (0.08)</td>
<td>0.20 (0.16)</td>
</tr>
<tr>
<td>0.01 mg/kg</td>
<td>0.73 (0.24)</td>
<td>0.93 (0.26)</td>
<td>0.82 (0.22)</td>
<td>0.62 (0.19)</td>
<td>0.44 (0.17)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>1.73 (0.06)</td>
<td>1.80 (0.00)</td>
<td>1.80 (0.00)</td>
<td>1.80 (0.00)</td>
<td>1.80 (0.00)</td>
</tr>
<tr>
<td><strong>Butorphanol</strong></td>
<td>0.01 mg/kg</td>
<td>0.23 (0.11)</td>
<td>0.19 (0.08)</td>
<td>0.17 (0.07)</td>
<td>0.15 (0.06)</td>
</tr>
<tr>
<td>0.03 mg/kg</td>
<td>0.21 (0.09)</td>
<td>0.49 (0.22)</td>
<td>0.38 (0.16)</td>
<td>0.28 (0.10)</td>
<td>0.25 (0.09)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>0.81 (0.25)</td>
<td>1.01 (0.27)</td>
<td>0.86 (0.24)</td>
<td>0.73 (0.23)</td>
<td>0.60 (0.22)</td>
</tr>
<tr>
<td><strong>Butorphanol</strong></td>
<td>1.0 mg/kg</td>
<td>0.28 (0.09)</td>
<td>0.29 (0.09)</td>
<td>0.36 (0.13)</td>
<td>0.37 (0.13)</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>0.52 (0.18)</td>
<td>0.50 (0.18)</td>
<td>0.57 (0.20)</td>
<td>0.43 (0.18)</td>
<td>0.39 (0.16)</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>1.53 (0.27)</td>
<td>0.56 (0.20)</td>
<td>0.34 (0.09)</td>
<td>0.26 (0.07)</td>
<td>0.28 (0.10)</td>
</tr>
</tbody>
</table>
significant in the repeated measures ANOVA. For example, the greatest increase in MSL produced by butorphanol (1.08 ± 0.27 mA) occurred in the first component after administration of 10 mg/kg, and quickly decreased thereafter. Lower doses of butorphanol did not increase MSL as substantially in the first component. This was revealed in the statistical analysis as a butorphanol dose by time interaction \((F_{12,84} = 5.38, P < 0.0001)\). Significant dose by time interactions were also observed for morphine \((F_{3,21} = 7.53, P = 0.0013)\) and l-methadone \((F_{12,84} = 3.59, P = 0.0002)\). Although both levorphanol and buprenorphine produced statistically significant, dose-dependent increases in MSL (main effect of dose; see above), these increases were apparent in the first component and consistent throughout the time points entered into the statistical analysis. Thus, the statistical analyses for levorphanol and buprenorphine did not reveal main effects of time or dose by time interactions.

All of the opioids tested increased MSL at doses that did not significantly suppress RRs. However, the highest dose of morphine (3.0 mg/kg) suppressed RR to values as low as 0.09 responses/s. The highest dose of levorphanol suppressed RR to 0.0 responses/s as early as component 2 \((n = 5)\). Although this dose was not included in the repeated measures ANOVA, still there was a statistically significant decrease in RR produced by levorphanol (main effect of dose: \(F_{2,14} = 4.39, P = 0.0322\)); the lowest mean RR was measured after 0.3 mg/kg levorphanol (0.21 mA). Similarly, there was a statistically significant decrease in RR produced by buprenorphine (main effect of dose: \(F_{3,21} = 5.95, P = 0.0042\)). The lowest mean RR when buprenorphine was administered followed administration of a dose of 0.1 mg/kg of buprenorphine (0.20 mA). Neither decrease, although statistically significant, represents a meaningful change in rate in this procedure (data not shown).

**LY235959 Potentiates the Antinociceptive Effects of Buprenorphine and Butorphanol.** Figure 1 shows the effect of LY235959 (0.1, 0.3, and 1.0 mg/kg) when administered in combination with buprenorphine (0.01 and 0.03 mg/kg) and butorphanol (1.0 and 3.0 mg/kg) on MSL (top) and RR (bottom). These particular doses of buprenorphine and butorphanol were chosen because they represented doses of the opioids that produced modest or no antinociceptive effects on their own. Data were analyzed for each agonist using repeated measures ANOVA with LY235959 dose, opioid dose, and time included as within-subjects variables.

LY235959 potentiated the antinociceptive effect of buprenorphine in a dose-dependent manner. The greatest increases in MSL values were observed after administration of 1.0 mg/kg LY235959. The highest MSL value, 0.83 mA \((\pm 0.25)\) was measured in the third component (95–105 min) after coadministration of 0.03 mg/kg buprenorphine and 1.0 mg/kg LY235959. The statistical analysis revealed that this effect was statistically significant (main effect of LY235959 dose: \(F_{2,12} = 4.79, P = 0.0296\)). There was no main effect of buprenorphine dose, no buprenorphine dose by LY235959 interaction, and no three-way interaction between the two drugs and time. Thus, although LY235959 increased the antinociceptive effects of 0.01 and 0.03 mg/kg buprenorphine in a dose-dependent and statistically significant manner, it did not increase the antinociceptive effects of one dose of buprenorphine to a greater or lesser degree that the other. Although the effects of LY235959 did not differ by buprenorphine dose, the effects of LY235959 did depend on time (LY235959 dose by time interaction: \(F_{8,48} = 2.26, P = 0.0389\)).

The effect of LY235959 in combination with butorphanol (Fig. 1) was nearly identical to the effect measured when LY235959 was combined with buprenorphine. LY235959 potentiated the antinociceptive effect of butorphanol in a dose-dependent manner. The greatest antinociceptive effect was measured in component 3 (95–105 min) after coadministration of 3.0 mg/kg butorphanol and 1.0 mg/kg LY235959 \((0.69 \pm 0.18\) mA). As with buprenorphine, the potentiation of the effects of butorphanol by LY235959 were revealed in the statistical

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**Fig. 1.** Effects of buprenorphine (0.01 and 0.03 mg/kg) and butorphanol (1.0 and 3.0 mg/kg) alone and in combination with LY235959 (0.1, 0.3, and 1.0 mg/kg) on MSL (top) and response rate (bottom) in monkeys \((n = 8)\). Components 1, 2, 3, 4, and 5 represent data collected 25 to 30 min, 60 to 70 min, 95 to 105 min, 130 to 140 min, and 165 to 175 min after drug administration, respectively.
analysis as a main effect of LY235959 dose ($F_{3,21} = 9.03$, $P = 0.0005$). There was also an interaction between LY235959 dose and time ($F_{12,84} = 2.72$, $P = 0.0037$), but no main effect of butorphanol dose and no interaction effects that included butorphanol dose. Thus, although LY235959 increased MSL values when administered in combination with butorphanol in a dose- and time-dependent manner, LY235959 did not increase the effect of one dose of butorphanol to a greater or lesser degree than the other.

Statistically significant differences between dosing conditions were revealed when RR was the dependent measure in the repeated measures ANOVA for both butorphanol and butorphanol. RR never fell below a mean 0.22 response/s under any condition, however, indicating that monkeys were consistently capable of responding in the procedure (data not shown).

**LY235959 Potentiates the Antinociceptive Effects of Morphine and Levorphanol.** Although LY235959 potenti- ated the antinociceptive effects of both buprenorphine and butorphanol in a dose-dependent manner, the magnitude of overall effect was small relative to that observed when mor- phine was administered in combination with LY235959 and other NMDA receptor antagonists as previously reported with this procedure (Allen and Dykstra, 2001; Allen et al., 2002). To more directly compare the effects of LY235959 when administered in combination with low-efficacy opioids and high-efficacy opioids, LY235959 was administered to- gether with morphine and levorphanol in the present study using the same monkeys that received buprenorphine/ LY235959 and butorphanol/LY235959 combinations.

Figure 2 shows the effect of LY235959 (0.1, 0.3, and 1.0 mg/kg) when administered in combination with morphine (0.3 and 1.0 mg/kg) and levorphanol (0.1 and 0.3 mg/kg) on MSL (top) and RR (bottom). These particular doses of mor- phine and levorphanol were chosen because they represented doses of the opioids that produced modest or no antinocicep- tive effects on their own. Data were analyzed for each agonist using repeated measures ANOVA with LY235959 dose, opioid dose, and time included as within-subjects variables.

LY235959 potenti- ated the antinociceptive effect of morphine in a dose- and time-dependent manner. The results of the repeated measures ANOVA in which morphine dose, LY235959 dose, and time were included as within-subjects variables revealed a significant three-way interaction between LY235959 dose, morphine dose, and time ($F_{12,84} = 5.85$, $P < 0.0001$). The highest MSL, 1.14 mA ($\pm 0.20$), was measured in the fifth component when 1.0 mg/kg morphine was administered in combination with 1.0 mg/kg LY235959.

LY235959 also potentiated the antinociceptive effect of levorphanol in a manner that was a function of LY235959 dose, levorphanol dose, and time as revealed by a three-way interaction ($F_{6,24} = 4.55$, $P = 0.0033$). Because only five of the eight monkeys were tested with the highest levorphanol/ LY235959 dose combination (three showed maximal effects with the 0.3 levorphanol/0.3 LY235959 combination), only three doses of LY235959 (0.0, 0.1, and 0.3) were included in the repeated measures ANOVA. Although not included in the repeated measures ANOVA, the greatest MSL (1.50 $\pm 0.30$ mA) was measured in the third component after administration of 0.3 mg/kg levorphanol and 1.0 mg/kg LY235959 ($n = 5$).

As with butorphanol and buprenorphine, there were statisti- cally significant differences between dosing conditions revealed when RR was the dependent measure in the repeated measures ANOVA for both morphine and levorphanol. However, RR never fell below a mean 0.16 response/s under any condition, indicating that monkeys were consistently capable of respond- ing in the procedure (data not shown).

**LY235959 Potentiates the Antinociceptive Effects of l-Methadone.** The highest dose of l-methadone that could be tested in combination with LY235959 safely in all monkeys

![Fig. 2. Effects of morphine (0.3 and 1.0 mg/kg) and levorphanol (0.1 and 0.3 mg/kg) alone and in combination with LY235959 (0.1, 0.3, and 1.0 mg/kg) on MSL (top) and response rate (bottom) in monkeys ($n = 8$). Components 1, 2, 3, 4, and 5 represent data collected 25 to 30 min, 60 to 70 min, 95 to 105 min, 130 to 140 min, and 165 to 175 min after drug administration, respectively.](https://example.com/figure2.png)
was 0.3 mg/kg, due to the steep dose-response curve for \textit{l}-methadone in this procedure. LY235959 potentiated the antinociceptive effects of 0.3 mg/kg \textit{l}-methadone, revealed as a significant dose by time interaction in the repeated measures ANOVA ($F_{12,72} = 2.27, P = 0.0167$). The magnitude of this effect was small, however, because MSL values did not rise above 0.39 ($\pm 0.15$) mA (0.3 mg/kg \textit{l}-methadone + 1.0 LY235959, component 5; data not shown). A subset of monkeys ($n = 3$) that showed smaller effects when administered 0.56 mg/kg \textit{l}-methadone alone were subsequently tested with combinations of 0.56 mg/kg \textit{l}-methadone and LY235959 (0.0, 0.1, 0.3, and 1.0 mg/kg). The results of these combinations are presented in Fig. 3. LY235959 markedly increased MSL in these monkeys, with maximal effects produced with the combination of 0.56 mg/kg \textit{l}-methadone and 0.3 mg/kg LY235959. The highest dose of LY235959 (1.0 mg/kg) was tested in two of the three monkeys only. Although the highest dose combination suppressed responding in these animals, maximal and near maximal antinociceptive effects were produced by dose combinations that did not completely suppress responding.

**Discussion**

In the present study, butorphanol, buprenorphine, levorphanol, morphine, and \textit{l}-methadone all increased MSL in a dose-dependent and statistically significant manner. Although the highest doses of morphine and levorphanol alone suppressed RR to zero or near zero, MSL was increased in the absence of rate suppression by some dose of all drugs tested (including morphine and levorphanol), indicating that the antinociceptive effects of these drugs were not due to nonspecific motor impairment or sedation. This ability to separate an antinociceptive effect from sedation or some other motor-impairing effect of a drug is a unique strength of the titration procedure, and especially valuable when evaluating the antinociceptive effect of drugs with known effect on motor performance, such as NMDA receptor antagonists (e.g., Rupniak et al., 1993; Carter, 1994).

It is important to note that the competitive NMDA receptor antagonist LY235959, when administered alone, did not increase MSL at any dose tested (0.1, 0.3, and 1.0 mg/kg) nor did LY235959 suppress responding at any of the doses tested. This is a reliable finding with LY235959 when tested in the squirrel monkey titration procedure and has been reported previously by this laboratory (Allen and Dykstra, 2001). As noted previously, 3.0 mg/kg LY235959 was administered to two monkeys and that dose produced profound sedation and motor impairment, the sedation lasting approximately 36 h. It was therefore not possible to test doses higher than those used in the present study. Although we are confident that LY235959 does not produce antinociceptive effects when administered alone across the dose range tested in this procedure, it is unknown whether higher doses would produce such effects.

The finding that LY235959, when administered alone, does not produce antinociceptive effects in squirrel monkeys is also consistent with our own published data from Sprague-Dawley rats (Allen and Dykstra, 2000a) as well as data published by others (Bilsky et al., 1996). One report, however, shows an antinociceptive effect of LY235959 in rats tested with a thermal tail-flick procedure (Bargava and Thorat, 1997). Although statistically significant, the antinociceptive effect of LY235959 in rats using this procedure seems small. In our own experiments with rats, a dose of LY235959 as high as 30 mg/kg did not significantly increase tail withdrawal latencies from warm water, but did produce profound ataxia (Allen and Dykstra, 2000a). It is not clear what factors may account for this reported difference in antinociceptive effects with LY235959.

Previous research from this laboratory has demonstrated that LY235959, when administered together with morphine, increases the antinociceptive effectiveness of low doses of morphine (Allen and Dykstra, 2001). The purpose of the present study was to assess the effects of LY235959 in combination with MOR agonists other than morphine that vary along the dimension of efficacy. There are several published experimental results that demonstrate differences in relative efficacy among the MOR agonists chosen for the present

**Fig. 3.** Effects of \textit{l}-methadone (0.56 mg/kg) alone and in combination with LY235959 (0.1, 0.3, and 1.0 mg/kg) on MSL (top) and response rate (bottom) in monkeys ($n = 3$). Components 1, 2, 3, 4, and 5 represent data collected 25 to 30 min, 60 to 70 min, 95 to 105 min, 130 to 140 min, and 165 to 175 min after drug administration, respectively.
with the interpretations that relative to morphine, squirrel monkey shock titration procedure are also consistent with morphine. Studies conducted previously in this laboratory with the squirrel monkey shock titration procedure are also consistent with the interpretations that relative to morphine, l-methadone is a higher efficacy compound and buprenorphine is a lower efficacy compound. For example, the repeated administration of morphine to squirrel monkeys resulted in tolerance to the antinociceptive effects of morphine and cross-tolerance was conferred to l-methadone; however, the magnitude of morphine tolerance was greater than the magnitude of cross-tolerance conferred to l-methadone (Craft and Dykstra, 1990). The insurmountable MOR antagonist clonixin was more potent and the duration of antagonism longer against buprenorphine than against morphine in squirrel monkeys responding in the same shock titration procedure (Pitts et al., 1998), suggesting a greater receptor reserve for morphine than for buprenorphine.

The findings from the present study show that regardless of efficacy, cotreatment with LY235959 potentiated the antinociceptive effects of low doses of butorphanol, buprenorphine, morphine, levorphanol, and l-methadone in a dose-dependent and statistically significant manner. Our ability to quantify the magnitude with which LY235959 increased the antinociceptive effects of each opioid agonist with the aim of comparing differences in magnitude across opioids was compromised by several factors related to the study design. For example, baseline opioid alone values differed across the opioid agonists tested. Also, measurement of the effects of levorphanol/LY235959 combinations were terminated earlier than for other opioid/LY235959 combinations, and the effects of l-methadone/LY235959 combinations were measured in a smaller group of monkeys (l-methadone; n = 3) than for other opioid/LY235959 combinations, both to ensure the safety of the experimental animals.

Despite these limitations, three observations suggest that efficacy may be a factor in the magnitude of the potentiation. First, the greatest increases in MSL were observed when LY235959 was combined with l-methadone. In this case, both 0.3 and 1.0 mg/kg LY235959 produced maximal increases in MSL at some time point. Second, increases in MLS were less pronounced when 0.3 or 1.0 mg/kg LY235959 was combined with morphine or levorphanol compared with l-methadone. Third, increases in MSL after combinations of LY235959 and buprenorphine or butorphanol were very modest. A formal approach, such as an isobolographic analysis, would help to identify the nature of these interactions.

The combinations of LY235959 with each agonist produced antinociceptive effects at least as great as those of higher doses of the agonists alone. Butorphanol was a notable exception: the highest dose of butorphanol alone (10 mg/kg) produced the greatest increase in MSL in the first component (mean MSL = 1.53 mA), and this effect was dramatically reduced by component 2 (mean MSL = 0.56 mA). In contrast, when LY235959 was administered in combination with 3.0 mg/kg butorphanol, the greatest MSL (mean = 0.69 mA) was measured in component 3, and there was no peak response during the first component.

Bespalov et al. (1998) have argued that NMDA receptor antagonists function to prolong, rather than potentiate, the analgesic effects of morphine. In their study, the competitive NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene) increased the duration of morphine analgesia observed in Sprague-Dawley rats (tail-pinch and tail-flick assays), but only after 60 min. Our findings are partly consistent with this interpretation. Whereas higher doses of all opioids alone produced increases in MSL in the first component of the experimental session (25–35 min postinjection), there was no significant potentiation of opioid effects by LY235959 in this same component. Combination MSL values in later components of the experimental sessions were similar to those produced by higher doses of the opioids alone. Others, however, have published data that show potentiation of the antinociceptive effects of morphine and methadone by the noncompetitive NMDA receptor antagonist dextromethorphan that have a time course similar to the effects of higher doses of the opioid alone (Bulka et al., 2002). In the present study, the effect of the combination of LY235959 with each opioid was greater than the effect of the same dose of the opioid alone at any time point in the experimental session, suggesting that LY235959 also potentiated, rather than simply prolonged, the antinociceptive effect.

Some recently published data show that noncompetitive NMDA receptor antagonists potentiate the antinociceptive effects of morphine and other high efficacy MOR agonists (methadone, fentanyl, and sufentanil) when administered to rats (Baker et al., 2002; Bulka et al., 2002). In the present study with squirrel monkeys, the competitive NMDA receptor antagonist LY235959 produced marked increases in the antinociceptive effects of l-methadone and levorphanol. The present data extend previous findings with morphine alone and suggest that it is the agonist action of low doses of morphine at the MOR that is the basis for potentiation of morphine’s antinociceptive effects by NMDA receptor antagonists. To our knowledge, these are the first published data that demonstrate potentiation of the antinociceptive effects of low-efficacy opioids by an NMDA receptor antagonist. Given the structural diversity of the compounds tested in the present study and others, it is unlikely that this potentiation is the result of alterations in metabolic or dispositional factors.

Recently published data also show that the noncompetitive NMDA receptor antagonist dextromethorphan potentiates the analgesic effects of morphine in humans (Caruso, 2000). We have previously shown that dextromethorphan potentiates the antinociceptive effects of morphine using this procedure (Allen et al., 2002). If NMDA antagonists potentiate the antinociceptive effects of MOR agonists in a manner independent of abuse potential, such drug combinations will be especially attractive for the treatment of pain. The findings presented in the present study showing potentiation of the antinociceptive effects of buprenorphine and butorphanol, opioids thought to have lower abuse potential that morphine, are especially exciting in this respect.
References


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