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Interactions Between an NMDA Antagonist and Low-Efficacy Opioid Receptor Agonists in Assays of Schedule-Controlled Responding and Thermal Nociception

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; MOR, μ-opioid receptor; KOR, κ-opioid receptor; LY235959, (-)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid; %MPE, percent maximum possible effect

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ABSTRACT

A growing body of literature has implicated *N*-Methyl-D-Aspartate (NMDA) receptor mechanisms in the acute antinociceptive effects of morphine, however, the nature of this interaction has not been thoroughly quantified. Moreover, it is not clear whether NMDA/morphine interactions extend to less efficacious opioids. Therefore, the present study examined the effects of morphine and various low-efficacy opioid agonists in combination with the NMDA antagonist LY235959 in two different assays: schedule-controlled responding and thermal nociception. Data were examined with dose-addition analysis in order to provide a quantitative assessment of the drug interactions. LY235959 and the opioid agonists morphine, buprenorphine, butorphanol, and nalbuphine all decreased rates of schedule controlled responding. LY235959/morphine and LY235959/buprenorphine mixtures produced additive or subadditive effects in this assay, whereas LY235959/butorphanol and LY235959/nalbuphine mixtures produced additive or supra-additive effects depending on the relative proportions of each drug in mixture. Morphine, buprenorphine, butorphanol, and nalbuphine also produced dose-dependent antinociception in the assay of thermal nociception, whereas LY235959 failed to produce an effect. In this assay, LY235959 potentiated the antinociceptive effects of morphine and each of the low-efficacy opioids tested. These results suggest that LY235959 may selectively increase the antinociceptive effects of morphine and some low-efficacy opioid receptor agonists without increasing their rate altering effects. In addition, these data confirm that the behavioral effects of drug mixtures depend on the relative concentrations of the drugs in the mixture and on the endpoint under study.

INTRODUCTION

A substantial literature has implicated *N*-Methyl-D-Aspartate (NMDA) receptor mechanisms in the acute antinociceptive effects of morphine. For example, behavioral evidence suggests that pretreatment or coadministration of an NMDA receptor antagonist can increase the acute effects of morphine in animal models of thermal nociception (Allen and Dykstra, 2003; Nemmani et al, 2004; Fischer et al., 2005; Grisel et al., 2005). In addition, NMDA antagonists can increase morphine-induced analgesia in humans under certain conditions (Javery et al., 1996; Bossard et al., 2002; Suzuki et al., 2005).

Although NMDA receptor involvement in morphine-induced antinociception has received considerable attention, less is known of NMDA receptor involvement in antinociception induced by low-efficacy opioids. To date, a single study has assessed these interactions, showing that coadministration with the competitive NMDA antagonist LY235959 can increase the antinociceptive activity of the low-efficacy opioids buprenorphine and butorphanol in monkeys responding under a schedule of shock titration, suggesting NMDA receptor involvement in these effects (Allen et al., 2003). This latter finding is particularly intriguing as the enhancement of the antinociceptive effects of low-efficacy opioids may increase their clinical utility.

Taken together, these results suggest that inhibition of the NMDA receptor may increase the antinociceptive effects of morphine and certain low-efficacy agonists. Although these data implicate the NMDA receptor system in opioid antinociception, these interactions have not been quantified precisely in order to determine deviation from simple additivity. The use of dose-addition analysis can provide a more quantitative evaluation of drug interactions and can be used to differentiate effects that are additive from effects that are subadditive or supra-additive (synergistic) (Wessinger, 1986; Tallarida, 2000).

In the present study, dose-addition analysis was used to further evaluate NMDA/opioid interactions. The effects of combinations of the NMDA receptor antagonist LY235959 and various opioid receptor agonists were examined in C57BL/6 mice using two different assays. To assess the extent to which NMDA antagonists selectively increase opioid-induced antinociception, the rate-decreasing effects of LY235959/opioid mixtures were first examined in an assay of schedule-controlled responding maintained by liquid food. Second, the antinociceptive effects of LY235959/opioid mixtures were examined in an assay of thermal nociception. Drug interactions were assessed using a fixed-proportion design, as this has been recommended for the study of drug interactions (Wessinger, 1996; Tallarida, 2000) and has been used to study similar drug mixtures on similar endpoints (Stevenson et al., 2003, 2005).

The opioid receptor agonists examined were morphine and the low-efficacy agonists buprenorphine, butorphanol, and nalbuphine as these are each structurally diverse drugs that differ in pharmacological selectivity (e.g. Chen et al., 1992; Walker et al., 1994, 1998; Emmerson et al., 1996). LY235959, the active isomer of LY274614, was selected because it is a potent NMDA antagonist with high selectivity for the competitive site on the NMDA receptor complex (Schoepp et al., 1991).

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METHODS

Animals. Adult male C57BL/6 mice weighing between 26 and 34 g were purchased from Jackson Labs (Raleigh, NC). Upon arrival, mice were group housed in standard plexiglas cages in a colony room maintained on a 12-hr light/dark cycle (lights on at 7:00 PM). All mice had continuous access to food and water throughout the study and were habituated to the colony room environment for 2 weeks prior to any experimental manipulation. All testing procedures were conducted between 11:00 AM and 3:00 PM. Throughout all testing the "Guide for the Care and Use of Laboratory Animals" (National Research Council, National Academy of Sciences, Washington, D.C., 1996) was adhered to.

Drugs. Morphine sulfate and buprenorphine hydrochloride were provided by NIDA (Bethesda, MD) and LY235959 by Lilly Research Laboratories (Indianapolis, IN). Butorphanol tartate and nalbuphine hydrochloride were purchased from Sigma (St.Louis, MO). All drugs were dissolved in 0.9% phosphate buffered saline. Drugs were injected i.p. at a volume of 0.1 ml/10 g.

Schedule-Controlled Responding. Response rates in the assay of schedule-controlled responding were assessed in an experimental operant chamber (approximately 14 cm×14 cm×14 cm) equipped with a house light, ventilator fan, and two nosepoke holes (1.2 cm diameter) that were located on either side of a liquid dipper. The operant chamber was controlled by a MED-PC interface and an IBM compatible computer programmed with MED Associates software (MED Associates, St. Albans, VT).

Mice were trained under a multiple-cycle procedure during experimental sessions conducted 5 days each week. Each training cycle consisted of a 10-min pretreatment period followed by a 5-min response period. During the pretreatment period, stimulus lights were not illuminated and

responding had no scheduled consequences. During the response period, the right nosepoke was illuminated and mice could obtain up to 10 liquid food reinforcers (8 sec access to 8 µl Ensure®) under a fixed ratio 3 schedule of food presentation. If all 10 reinforcers were earned before 5 min had elapsed, the light was turned off, and responding had no scheduled consequences for the remainder of the response period. The left nosepoke was inactive, and responding at this hole had no scheduled consequences. Training sessions consisted of five consecutive cycles, and testing began once response rates were stable throughout the session.

Test sessions replaced the last training session of each week if responding was stable throughout the preceding training sessions. Test sessions were identical to training sessions except that cumulative doses of drug mixtures were administered i.p. during the first minute of the pretreatment period of each cycle (i.e., 15-min inter injection interval), increasing in one-quarter or one-half log unit increments. Data are expressed as a percentage of control responding using the average rate of responding from the previous day as the control value (average of 5 cycles).

Thermal Nociception. Antinociception was assessed with a tail-flick analgesia meter (Columbus Instruments, Columbus, OH). During this procedure, the stimulus intensity was adjusted to provide baseline latencies between 3-5 sec (stimulus intensity=5). The antinociceptive response was evaluated by recording the latency to flick the tail from the light source. Responses were measured using a stopwatch to the nearest 0.1 sec. A predetermined cutoff time of 10 sec was defined as a maximal response and was employed to prevent tissue damage. Immediately following the termination of a trial, mice were removed from the apparatus and returned to the homecage. The latency to respond to the light source was measured twice at each determination, at least 30 sec apart, at 3 and 5 cm from the tip of the tail. These data were

averaged to yield one value. Following baseline latency measurements, multiple 15 min cycles were run and drugs mixtures were administered cumulatively. During this procedure, cumulative doses of drug mixtures were administered i.p. during the first min of each cycle (i.e., 15-min inter injection interval), increasing in one-quarter or one-half log unit increments and antinociceptive measurements were determined during the last minute of each cycle. Latencies are expressed as a percentage of the maximal possible effect (%MPE) using the following formula: %MPE = [postdrug latency (sec) - baseline latency (sec)] / [cutoff time (20 sec) - baseline latency (sec)].

Data analysis. The dose of each drug mixture required to produce a 50% decrease in responding (ED_{50}) in the assay of schedule-controlled responding was derived using log-linear interpolation by linear regression. The dose of each drug mixture required to produce 50% maximum antinociceptive effect was derived in a similar manner. In each assay, ED_{50} values were determined using the linear portion of the dose-effect curve up to doses that produced a maximal effect.

Interactions between LY235959 and opioid agonists were assessed using both graphical and statistical approaches (Wessinger, 1986; Tallarida, 2000). Graphically, the distinction between subadditive, additive, or synergistic interactions were made with the use of isobolograms. In the current study, isobolograms were constructed by connecting the ED_{50} of LY235959 alone plotted on the abscissa with the ED_{50} of the opioid receptor agonist alone plotted on the ordinate to obtain an additivity line. The additivity line contains the loci of dose pairs that produce an ED_{50} equal to the ED_{50} of LY235959 or an opioid receptor agonist alone. Dose pairs that fall below the additivity line suggest an ED_{50} was reached with lesser quantities of the drugs, suggestive of

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synergism. In contrast, experimental points representing dose pairs that fall above the line are suggestive of subadditivity.

Drug interactions were statistically analyzed by comparing the experimentally determined ED_{50} values for each mixture (Zmix) with predicted additive ED_{50} values (Zadd) as described by Tallarida (2000). Zmix was defined as the total drug dose (i.e., dose LY235959 + dose opioid receptor agonist) that produced a 50% decrease in rates of responding (assay of schedule-controlled responding) or a 50% maximum antinociceptive effect (assay of thermal nociception).

For the assay of schedule-controlled responding, in which all drugs were equieffective, Zadd values were calculated individually for each mouse based on the ED_{50} values of each drug from the following equation: $Zadd = fA + (1 - f)B$, where A is the ED_{50} for LY235959 alone and B is the ED_{50} for the opioid receptor agonist alone. The proportion of LY235959 in each mixture was determined by the equation $fA/[fA + (1 - f)B]$. The present study examined effects produced by mixtures in which $f = 0.25$, 0.5, and 0.75. When $f = 0.25$, the mixture contains a proportion of [A/(A + 3B)] LY235959 and a mixture ratio of [(A/B) ÷ 3] parts LY235959 to 1 part opioid receptor agonist; $f = 0.50$ leads to a proportion of [A/(A + B)] LY235959 in the mixture and a mixture ratio of (A/B) parts LY235959 to 1 part opioid agonist; and $f = 0.75$ leads to a proportion of [A/(A + B/3)] LY235959 in the mixture and a mixture ratio of [(A/B) x 3] parts LY235959 to 1 part opioid receptor agonist.

For the assay of thermal nociception LY235959 alone was ineffective, and the hypothesis of additivity predicts that LY235959 would not contribute to the effects of a mixture of LY235959 in combination with an opioid receptor agonist (Tallarida, 2000). Therefore, Zadd is calculated by dividing the ED_{50} of the opioid receptor agonist by the proportion of opioid receptor agonist

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in the particular mixture. Mean experimentally determined ED_{50} values (Zmix) and predicted additive ED_{50} values (Zadd) for each mixture were compared with a t-test.

Data were further analyzed by calculating opioid ED_{50} values for each opioid receptor agonist alone and in combination with various proportions of LY235959. A dose ratio was defined as the ED_{50} value of the opioid receptor agonist alone divided by the ED_{50} value of the opioid receptor agonist in the mixture. Dose ratios were determined for each LY235959/opioid agonist mixture in both the assay of schedule-controlled responding and the assay of thermal nociception. The dose ratios for each mixture were then compared between assays (Assay Ratio) according to the equation: [Dose Ratio (thermal nociception)] ÷ [Dose Ratio (schedule-controlled responding)].

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RESULTS

Schedule-controlled responding. Fig. 1 (left panel) shows the rate-decreasing effects of LY235959, morphine, buprenorphine, butorphanol, and nalbuphine. Each drug produced dose-dependent decreases in the rate of responding resulting in ED_{50} values (95% CL) of 1.4 (0.97-1.9) for morphine, 0.11 (0.088-0.15) for buprenorphine, 0.15 (0.088-0.24) for butorphanol, and 4.2 (1.6-11) for nalbuphine. Therefore, the relative potencies for the opioid agonists in the assay of schedule-controlled responding was buprenorphine = butorphanol > morphine = nalbuphine. Dose-effect curves for LY235959 were determined separately for each group resulting in ED_{50} values (95% CL) of 5.2 (3.7-7.4), 5.8 (3.9-8.7), 6.1 (3.4-11), and 6.6 (3.5-12) and the relative potencies of these values were used to determine relative proportions of the compounds used in subsequent studies for morphine, buprenorphine, butorphanol, and nalbuphine, respectively (see Data Analysis).

The rate-decreasing effects of each opioid agonist alone and in combination with LY235959 are shown in Fig. 2. Each drug mixture produced dose-dependent decreases in response rates. Addition of LY235959 produced leftward shifts in the dose-effect curves for morphine and buprenorphine, and the magnitude of shift was correlated with the proportion of LY235959 in the mixture. Fig. 2 also shows the graphical analysis of these drug combinations. Mixtures with a lower proportion of LY235959 relative to morphine (i.e., 1.3:1 LY235959/Morphine and 3.9:1 LY235959/Morphine) or to buprenorphine (i.e., 18:1 LY235959/Buprenorphine) produced subadditive effects, as these ED_{50} values fell above the line of additivity. Mixtures with a higher proportion of LY235959 relative to morphine and buprenorphine produced additive effects, as these ED_{50} values fell close to the line of additivity. Statistical comparison of experimentally

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determined ED_{50} values (Zmix) and predicted additive ED_{50} values (Zadd) confirmed these findings (i.e., Zadd > Zmix or Zadd = Zmix) (Table 1).

Addition of LY235959 also produced leftward shifts in the dose-effect curves for butorphanol and nalbuphine, and the magnitude of shift was correlated with the proportion of LY235959 in the mixture. Graphical analysis of these drug combinations indicates that mixtures with a lower proportion of LY235959 relative to butorphanol and nalbuphine produced additive effects, as these ED_{50} values fell close to the line of additivity. Mixtures with a higher proportion of LY235959 relative to butorphanol (i.e., 140:1 LY235959/butorphanol) or to nalbuphine (i.e., 1.4:1 LY235959/nalbuphine and 4.1:1 LY235959/ nalbuphine) produced synergistic effects, as these ED_{50} values fell below the line of additivity. Statistical comparison of experimentally determined ED_{50} values (Zmix) and predicted additive ED_{50} values (Zadd) confirmed these findings (Table 1).

Thermal nociception. Fig. 1 (right panel) shows the antinociceptive effects of LY235959, morphine, nalbuphine, buprenorphine, and butorphanol. LY235959 was without effect in this assay up to a dose of 10 mg/kg, which produced severe motor impairment. Each opioid receptor agonist produced dose-dependent increases in latency to respond to the tail-flick apparatus, resulting in ED_{50} values (95% CL) of 3.5 (2.3-5.1) for morphine, 0.25 (0.21-0.31) for buprenorphine, 0.12 (0.078-0.19) for butorphanol, and 3.4 (2.1-5.5) for nalbuphine. The relative potencies for the opioid agonists in the assay of thermal nociception was similar to the relative potencies determined in the assay of schedule-controlled responding (butorphanol = buprenorphine > nalbuphine = morphine). LY235959 was without effect in this assay, therefore the relative potencies determined in the assay of schedule-controlled responding were used to determine the relative proportions of the compounds in each mixture.

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The antinociceptive effects of the opioid receptor agonists alone and in combination with LY235959 are shown in Fig. 3. Each drug mixture produced dose-dependent increases in antinociception. Addition of LY235959 produced leftward shifts in the dose-effect curves for each of the opioid receptor agonists, and the magnitude of shift was correlated with the proportion of LY235959 in the mixture. Graphical analysis of these drug combinations indicates that each mixture with a relatively lower proportion of LY235959 to morphine (i.e., 1.3:1 LY235959/morphine), buprenorphine (i.e., 18:1 LY235959/buprenorphine), or butorphanol (i.e., 15:1 LY235959/butorphanol) produced synergistic effects, while the effects of the LY235959/nalbuphine mixture was additive. Mixtures with an intermediate or higher proportion of LY235959 relative to all four of the opioid receptor agonists examined also produced synergistic effects, as these ED_{50} values fell to the left of the line of additivity. Statistical comparison determined that the experimentally determined ED_{50} values (Zmix) for these mixtures were less than the predicted additive ED_{50} values (Zadd) (Table 2).

Table 3 shows the dose ratios obtained in the assay of schedule-controlled responding, the dose ratios obtained in the assay of thermal nociception, and the ratios between these assays for each LY235959/opioid agonist mixture. In the assay of schedule-controlled responding, the addition of the lesser proportion LY23959 to morphine, buprenorphine and butorphanol produced rightward shifts in the opioid dose curves relative to the opioid alone. Increasing proportions of LY235959 in the mixture of these drugs produced proportion-dependent leftward shifts in the opioid dose-effect curves. The addition of LY235959 to nalbuphine produced leftward shifts in the nalbuphine dose-effect curve at all proportions in a proportion-dependent manner. In the assay of thermal nociception, the addition of LY235959 to the mixture of each opioid agonist produced leftward shifts in the opioid dose-effect curves. For each drug tested, the

dose-effect curves were shifted in a proportion-dependent manner with the exception of morphine and butorphanol, where the intermediate proportion of morphine was shifted the least relative to the LY235959/morpine mixtures and the intermediate proportion of butorphanol was shifted to the greatest extent relative to the LY235959/butorphanol mixtures.

Assay ratios were determined as a measure of the degree to which addition of LY235959 shifted the opioid dose-effect curve in the assay of thermal nociception relative to the assay of schedule-controlled responding. The addition of LY235959 to morphine, buprenorphine, and butorphanol produced greater shifts in the opioid dose-effect curves in the assay of thermal nociception relative to the assay of schedule-controlled responding across all proportions. Conversely, LY235959 shifted the nalbuphine dose-effect curve to a greater extent in the assay of schedule-controlled responding relative to the assay of thermal nociception.

DISCUSSION

The present study provides a quantitative assessment of the degree to which the NMDA antagonist LY235959 can modulate the antinociceptive and rate-decreasing effects of morphine, buprenorphine, butorphanol, and nalbuphine. Previous research has demonstrated that NMDA antagonists can increase the antinociceptive effects of morphine (Allen and Dykstra, 2003; Nemmani et al, 2004; Fischer et al., 2005; Grisel et al., 2005). Although the interactive effects of morphine and NMDA receptor antagonists have been investigated in various antinociceptive measures, the first purpose of the current study was to assess these interactions using a formally quantitative approach. The quantitative assessment of behavior using dose-addition analysis was used to distinguish interactive effects of morphine and LY235959 that were additive from effects that were subadditive or synergistic.

A second purpose was to determine if NMDA/opioid interactions extend to another behavioral endpoint. Specifically, the effects of NMDA/opioid mixtures unrelated to antinociception can be addressed systematically by, for example, comparing doses required to produce antinociception to doses that eliminate food maintained responding. Therefore, an assay of schedule-controlled responding was used to determine the selectivity of the drug combinations to produce antinociceptive effects vs. nonspecific effects.

A third purpose of the present study was to extend these findings to opioids with lower efficacy. Behavioral evidence has demonstrated differences in the relative efficacies between these opioid receptor agonists (Zimmerman et al., 1987; Adams et al., 1990; Walker et al., 1998). In vitro studies confirm these findings, suggesting that buprenorphine, butorphanol, and nalbuphine are lower in efficacy relative to morphine (Chen et al., 1992; Toll, 1995; Emmerson et al., 1996).

In the current study, three different proportions of LY235959 to each opioid agonist were studied as it has been suggested that deviation from additivity depends on the relative proportions of the drugs under study (Gessner and Cabana, 1970; Tallarida, 2000). In agreement with these findings, the nature of the LY235959/opioid interactions in the assay of schedule-controlled responding was dependent on the proportion of LY235959 in each mixture. For example, the LY235959/morphine mixture with the higher proportion of LY235959 produced additive effects in this assay, whereas LY235959/morphine mixtures with a relatively lower proportion of LY235959 produced subadditive effects. Similar to LY235959/morphine mixtures, the effects of LY235959/buprenorphine mixtures were either additive or subadditive, depending on the proportions of each of the drugs.

In addition to the relative proportions of drugs in a mixture, the effect of an interaction of two drugs may depend on the experimental endpoint under study (Stevenson et al., 2003, 2005). In agreement with these findings, LY235959 produced a synergistic interaction with each of the opioids, regardless of efficacy, in the assay of thermal nociception. These findings agree with data obtained in squirrel monkeys (Allen et al., 2003), suggesting efficacy is not an important determinant of interactions between NMDA and opioid receptors on the endpoint of antincoception.

If the mechanism of action of each of the drugs in a mixture is mediated through different receptors, the detection of synergism suggests an interaction between their receptor mediated signals (Tallarida, 2000). In the current study, LY235959 and each of the low-efficacy opioids produced synergistic effects in the assay of thermal nociception. Previous research has suggested a model of NMDA receptor activation contributing to neural and behavioral plasticity that may underlie the alterations in the antinociceptive effectiveness of morphine. According to this

model, administration of morphine activates NMDA receptors through intracellular mechanisms, increasing intracellular calcium levels, leading to an increase in protein kinase C and subsequent reduction in the sensitivity of μ-opioid receptors (Mao et al., 1995). The current study suggests that similar receptor mediated interactions may occur with opioids of lower efficacy.

The present study demonstrates that studying drug interactions across a range of relative proportions and across experimental endpoints can reveal characteristics of these interactions that may have clinical potential. For example, in the assay of schedule-controlled responding, the LY235959/buprenorphine mixture with the lowest proportion of LY235959 produced sub-additive effects in the assay of schedule-controlled responding while producing synergistic effects in the assay of thermal nociception. This LY235959/buprenorphine mixture resulted in a favorable ED_{50} ratio across experimental endpoints confirming a greater increase in buprenorphine's antinociceptive effects relative to its rate-decreasing effects. This finding was similar to LY235959/morphine mixtures, and suggests that LY235959 may specifically increase the antinociceptive properties of these drugs relative to their nonspecific effects.

Drugs such as buprenorphine, butorphanol and nalbuphine have a lower potential for abuse and exert less side effects relative to morphine (Hoskin and Hanks, 1991; Preston and Jasinski, 1991), however they also are less effective, at least in animal models of antinociception (Dykstra 1990; Walker, et al. 1993; Morgan, et al. 1999). If NMDA antagonists potentiate the antinociceptive effects of low-efficacy opioids without increasing opioid-induced side effects, combination treatment might be useful for the management of various pain states. Therefore, manipulation of pharmacological selectivity and relative concentrations of low-efficacy opioids in a drug cocktail may enhance clinical utility. Further characterization of NMDA antagonist/opioid agonist interactions are necessary to determine their interactive effects on other

behavioral endpoints (such as respiratory depression and self-administration) and to determine if the effects of low-efficacy opioids are modulated by an NMDA antagonist other than LY235959.

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Footnotes

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Legends for Figures

Fig. 1. Effects of LY235959, morphine, buprenorphine, butorphanol, and nalbuphine in the assay of schedule-controlled responding (left) and in the assay of thermal nociception (right). Abscissae, dose of drug in mg/kg. Ordinate, response rate as percentage of control rate of responding (left) or antinociception as percent maximum possible effect (right). Each point shows the mean (± S.E.M.) from 6 mice (schedule-controlled responding) or 8 mice (thermal nociception).

Fig. 2. Effects of cumulative doses of opioid agonists alone and in combination with LY235959 in the assay of schedule-controlled responding. Top, dose-effect curves for morphine, buprenorphine, butorphanol, and nalbuphine alone and in combination with LY235959. Abscissae, dose of opioid in mg/kg. Ordinate, response rate as percentage of control. Bottom, isobolograms for LY235959/opioid mixtures. Abscissae, ED_{50} value for opioid in mg/kg. Ordinate, ED_{50} value for LY235959 in milligrams per kilogram. Each point shows the mean (± S.E.M.) from 5-6 mice. *****Significantly different from additivity.

Fig. 3. Effects of cumulative doses of opioid agonists alone and in combination with LY235959 in the assay of thermal nociception. Top, dose-effect curves for morphine, buprenorphine, butorphanol, and nalbuphine alone and in combination with LY235959. Abscissae, dose of opioid in mg/kg. Ordinate, antinociception as percent maximum possible effect. Bottom, isobolograms for LY235959/opioid mixtures. Abscissae, ED_{50} value for opioid in mg/kg. Ordinate, ED_{50} value for LY235959 in milligrams per kilogram. Each point shows the mean (± S.E.M.) from 7-8 mice. *****Significantly different from additivity.

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Table 1. Predicted additive ED_{50} values (Zadd) and experimentally determined ED_{50} values (Zmix) of mixtures of LY235959 administered in combination with MOR agonists in the assay of schedule-controlled responding

	Z_{add} (95% CL)	Z_{mix} (95% CL)
LY235959 + Morphine		
1.3:1 LY/Morphine	2.2 (1.7-3.0)	4.5 (3.3-6.5)*****
3.9:1 LY/Morphine	3.1 (2.4-4.0)	5.1 (4.2-6.2)*****
12:1 LY/Morphine	3.9 (2.9-5.1)	3.7 (2.7-5.1)
LY235959 + Buprenorphine		
18:1 LY/Buprenorphine	1.5 (0.98-2.3)	2.8 (2.2-3.4)*****
54:1 LY/Buprenorphine	2.9 (1.9-4.4)	3.1 (2.2-4.3)
160:1 LY/Buprenorphine	4.2 (2.7-6.5)	5.5 (4.1-7.2)
LY235959 + Butorphanol		
15:1 LY/Butorphanol	1.9 (1.1-3.3)	2.8 (1.7-4.5)

45:1 LY/Butorphanol	3.5 (2.0-6.1)	2.8 (1.4-5.8)
140:1 LY/Butorphanol	5.1 (2.9-9.0)	1.9 (1.0-3.6)*****
LY235959 + Nalbuphine		
0.46:1 LY/Nalbuphine	4.9 (2.2-11)	1.8 (0.91-3.6)
1.4:1 LY/Nalbuphine	5.7 (3.3-9.8)	1.4 (0.8-2.7)*****
4.1:1 LY/Nalbuphine	5.9 (4.3-8.3)	0.67 (0.099-4.5)*****

CL, confidence limits

***** An experimental ED50 value significantly different from the predicted additive ED50 value ($p < 0.05$)

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Table 2. Predicted additive ED_{50} values (Zadd) and experimentally determined ED_{50} values (Zmix) of mixtures of LY235959 administered in combination with MOR agonists in the assay of thermal nociception

	Z_{add} (95% CL)	Z_{mix} (95% CL)
LY235959 + Morphine		
1.3:1 LY/Morphine	7.7 (5.0-12)	1.4 (0.43-4.5)*****
3.9:1 LY/Morphine	16 (11-25)	5.5 (3.4-8.8)*****
12:1 LY/Morphine	42 (28-64)	5.7 (2.6-13)*****
LY235959 + Buprenorphine		
18:1 LY/Buprenorphine	4.7 (4.0-5.6)	1.5 (1.2-1.9)*****
54:1 LY/Buprenorphine	14 (11-16)	2.9 (1.2-7.0)*****
160:1 LY/Buprenorphine	40 (34-48)	5.5 (3.4-8.9)*****
LY235959 + Butorphanol		

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15:1 LY/Butorphanol	1.9 (0.96-3.8)	0.34 (0.12-1.0)*****
45:1 LY/Butorphanol	5.5 (2.8-11)	0.63 (0.24-1.7)*****
140:1 LY/Butorphanol	16 (8.2-32)	1.3 (0.46-3.5)*****
LY235959 + Nalbuphine		
0.46:1 LY/Nalbuphine	4.8 (2.7-8.4)	4.3 (2.6-6.9)
1.4:1 LY/Nalbuphine	7.8 (4.4-14)	2.8 (1.1-7.1)*****
4.1:1 LY/Nalbuphine	ND	ND

CL, confidence limits; ND, not determined

***** An experimental ED50 value significantly different from the predicted additive ED50 value ($p < 0.05$)

Table 3. Mean opioid ED_{50} Values (95% CL) and Dose Ratios of opioids alone and in mixtures with LY235959 in the assays of schedule-controlled responding and thermal nociception. Assay Ratios were defined for a LY235959/Opioid mixture as [Dose Ratio (thermal nociception)] ÷ [Dose Ratio (schedule-controlled responding)]

	Schedule-Controlled Responding		Thermal Nociception		
	ED50	Dose Ratio	ED50	Dose Ratio	Assay Ratio
Morphine alone	1.4 (0.97-1.9)		3.5 (2.3-5.1)		
1.3:1 LY/Morphine	2.4 (1.8-3.4)	0.58	0.87 (0.51-1.5)*****	4.0	6.9
3.9:1 LY/Morphine	1.1 (0.73-1.7)	1.3	1.2 (0.82-1.7)*****	2.9	2.2
12:1 LY/Morphine	0.31 (0.24-0.42)*****	4.5	0.48 (0.20-1.2)*****	7.3	1.6
Buprenorphine alone	0.11 (0.088-0.15)		0.25 (0.21-0.31)		
18:1 LY/Buprenorphine	0.15 (0.11-0.19)	0.73	0.082 (0.067-0.10)*****	3.0	4.1

54:1 LY/Buprenorphine	0.061 (0.032-0.12)	1.8	0.070 (0.053-0.091)*****	3.6	2.0
160:1 LY/Buprenorphine	0.035 (0.024-0.052)	3.1	0.038 (0.021-0.070)*****	6.6	2.1
Butorphanol alone	0.15 (0.088-0.24)		0.12 (0.078-0.19)		
15:1 LY/Butorphanol	0.16 (0.096-0.28)	0.94	0.034 (0.022-0.053)*****	3.5	3.7
45:1 LY/Butorphanol	0.080 (0.066-0.10)	1.9	0.0086 (0.0047-0.016)*****	14	7.4
140:1 LY/Butorphanol	0.018 (0.013-0.025)*****	8.3	0.012 (0.0057-0.026)*****	10	1.2
Nalbuphine alone	4.2 (1.6-11)		3.4 (2.1-5.5)		
0.46:1 LY/Nalbuphine	2.2 (0.75-6.2)	1.9	2.5 (1.8-3.6)	1.4	0.74
1.4:1 LY/Nalbuphine	0.65 (0.38-1.1)*****	6.5	1.5 (1.1-2.0)*****	2.3	0.35
4.1:1 LY/Nalbuphine	0.14 (0.035-0.59)*****	30	ND	ND	ND

CL, confidence limits; ND, not determined; ***** 95% confidence limits do not overlap with 95% confidence limits after opioid alone.

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Figure 1

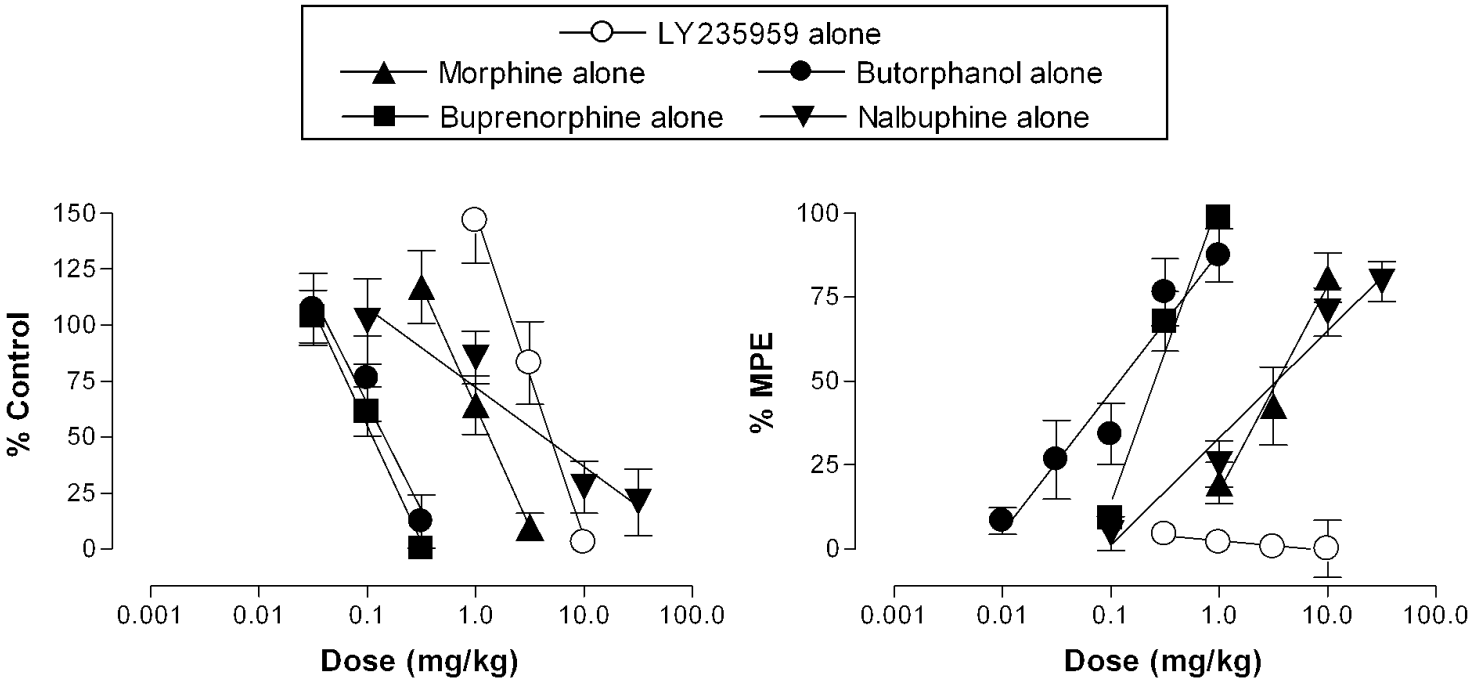

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Figure 2

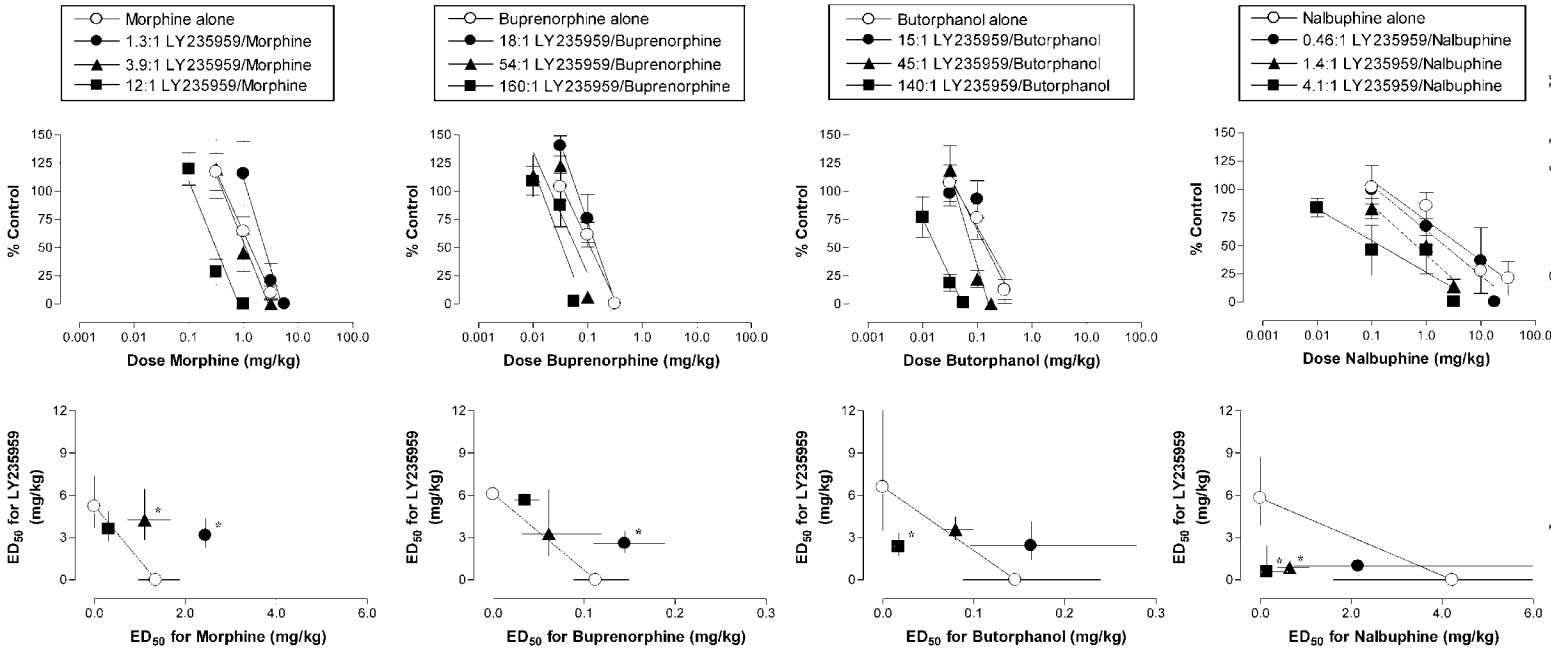

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Figure 3