Morphine in Combination with Metabotropic Glutamate Receptor Antagonists on Schedule-Controlled Responding and Thermal Nociception


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ABSTRACT

The present study examined the interactive effects of morphine in combination with metabotropic glutamate (mGlu) receptor antagonists on schedule-controlled responding and thermal nociception. Drug interaction data were examined with isobolographic and dose-addition analysis. Morphine, the mGlu1 receptor antagonist JNJ16259685 \([3,4\text{-dihydro}-2H\text{-pyrano}[2,3-b]quinolin-7-yl]-(\text{cis}-4\text{-methoxycyclohexyl})\text{-methanone}\), the mGlu5 receptor antagonist MPEP \(2\text{-methyl}-6\text{-(phenylethynyl)pyridine hydrochloride}\), and the mGlu2/3 receptor antagonist LY341495 \((2\text{S})\text{-2-amino-2\text{-(1S,2S-2-carboxycycloprop-1-yl})-3-(xanth-9-yl) propanoic acid}\) all decreased rates of schedule-controlled responding. JNJ16259685/morphine, MPEP/morphine, and LY341495/morphine mixtures produced additive effects on this endpoint. Morphine also produced dose-dependent antinociception in the assay of thermal nociception, whereas JNJ16259685, MPEP, and LY341495 failed to produce an effect. In this assay, JNJ16259685 and LY341495 potentiated the antinociceptive effects of morphine, whereas MPEP/morphine mixtures produced additive effects. These results suggest that an mGlu1 and an mGlu2/3 receptor antagonist, but not an mGlu5 receptor antagonist, selectively enhance the antinociceptive effects of morphine. In addition, these data confirm that the behavioral effects of drug mixtures depend on the endpoint under study.

Glutamatergic neurotransmission works by activating both ionotropic glutamate (iGlu) and G-protein-coupled metabotropic glutamate (mGlu) receptor subtypes and is thought to play a modulatory role in the behavioral effects of morphine. To date, the most thoroughly investigated system of the glutamate receptor subtypes is the ionotropic N-methyl-D-aspartate (NMDA) receptor. Several lines of evidence suggest that pharmacological antagonism of the NMDA receptor blocks morphine-induced conditioned place preference (Papp et al., 2002), sensitization (Jezierski et al., 1994), and physical dependence (Trujillo and Akil, 1991). NMDA receptor antagonists also attenuate the development of morphine tolerance (Trujillo and Akil, 1991; Kozela et al., 2003) and increase the acute antinociceptive effects of morphine (Nemmani et al., 2004; Fischer et al., 2005). Preclinical data demonstrating that NMDA receptor antagonists increase the acute antinociceptive effects of morphine have led to the development of NMDA antagonist/morphine combinations for clinical use, albeit with mixed results (Bossard et al., 2002; Galer et al., 2005). The rationale for combination therapy is that NMDA antagonist/morphine mixtures may decrease morphine-induced side effects since increased analgesia would be produced at a lower morphine dose. However, one disadvantage of this approach is the undesirable side effects associated with some NMDA antagonists (e.g., nausea, fatigue and dizziness, psychomimetic effects), most probably due to the ubiquitous involvement of NMDA receptors in excitatory neurotransmission throughout the central nervous system.

Interestingly, activation of postsynaptic mGlu receptors results in the potentiation of NMDA-mediated responses in dorsal horn neurons (Cerne and Randic, 1992; Kelso et al., 1992; Skeberdis et al., 2001), a region implicated in acute nociceptive neurotransmission. This potentiation may modulate NMDA-mediated increases in morphine antinociception. Therefore, drugs that have antagonist action at postsynaptic mGlu receptors may provide an alternative to NMDA receptor antagonists in clinically therapeutic drug mixtures for the treatment of pain.

ABBREVIATIONS: iGlu, ionotropic glutamate; mGlu, metabotropic glutamate; NMDA, N-methyl-D-aspartate; JNJ16259685, \((3,4\text{-dihydro}-2H\text{-pyrano}[2,3-b]\text{quinolin-7-yl})\text{-}(\text{cis}-4\text{-methoxycyclohexyl})\text{-methanone}\); MPEP, \(2\text{-methyl}-6\text{-(phenylethynyl)pyridine hydrochloride}\); LY341495, \((2\text{S})\text{-2-amino-2\text{-(1S,2S-2-carboxycycloprop-1-yl})-3-(xanth-9-yl) propanoic acid}\).
Recently, selective and bioavailable mGlu receptor antagonists have been synthesized. The present study was designed to assess the acute interactive effects of mGlu receptor antagonists and morphine on two behavioral endpoints. To assess the extent to which mGlu receptor antagonists selectively enhance morphine-induced antinociception, the rate-decreasing effects of mGlu antagonist/morphine mixtures were first examined in an assay of schedule-controlled responding maintained by liquid food. Second, the antinociceptive effects of mGlu antagonist/morphine mixtures were examined in an assay of thermal nociception. The interactive effects of mGlu antagonist/morphine mixtures were assessed using both graphical (isobolographic analysis) (Loewe, 1953) and statistical (dose-addition analysis) (Tallarida, 2000) approaches to distinguish effects that are additive from effects that are infra-additive or supra-additive.

To date, eight mGlu receptor subtypes have been identified and have been divided into three groups: group I (mGlu1 and mGlu5), group II (mGlu2 and mGlu3), and group III (mGlu4, mGlu6, mGlu7, and mGlu8). The present study investigated the interactive effects of morphine in combination with antagonists selective for group I and group II mGlu receptors because these receptor subtypes have been previously implicated in the behavioral effects of morphine on other endpoints (Popik and Wrobel, 2002; Kozela et al., 2003; Smith et al., 2004).

Materials and Methods

Animals. Adult male C57BL/6 mice weighing between 22 and 30 g were purchased from The Jackson Laboratory (Raleigh, NC). Upon arrival, mice were group housed in standard Plexiglas cages in a colony room maintained on a 12-h 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 for 2 weeks before any experimental manipulation. All testing procedures were conducted between 11:00 AM and 3:00 PM. Animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill, and all testing adhered to the Institute of Laboratory Animal Resources (1996).

Drugs. The mGlu receptor antagonists JNJ16259685, MPEP, and LY341495 display selectivity for group I (mGlu1 and mGlu5) and group II (mGlu2/3) receptors, respectively (Kingston et al., 1998; Gasparini et al., 1999; Lavreysen et al., 2004). JNJ16259685, MPEP, and LY341495 were purchased from Tocris (Ellisville, MO). Morphine sulfate was provided by the National Institute on Drug Abuse (Bethesda, MD). Morphine, MPEP, and LY341495 were dissolved in 0.9% phosphate-buffered saline, and the pH of LY341495 solutions was adjusted to 7.0 with NaOH. JNJ16259685 was dissolved in 45% (w/v) 2-hydroxypropyl-β-cyclodextrin. All drugs were injected i.p. at a volume of 0.1 ml/10 g.

Schedule-Controlled Responding. Schedule-controlled responding was assessed in an experimental operant chamber (approximately 14 × 14 × 14 cm) equipped with a house light, ventilator fan, and two nose-poke 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 conducted 5 days each week. Each training cycle consisted of a 25-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 nose poke was illuminated, and mice could obtain up to 10 liquid food reinforcers (8-s access to 8 µl of 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 nose poke 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. For dose-effect curve determinations, test sessions were identical to training sessions except that cumulative doses of drug mixtures were administered i.p. at the start of each cycle (i.e., 30-min interinjection interval), increasing in one-quarter or one-half log unit increments. For time course determinations, a single dose of a drug was administered i.p. at the start of the session, and 5-min response periods identical to those described above began at 10, 20, 40, 80, 160, and 240 min after the injection. 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 five cycles).

Thermal Nociception. Antinociception was assessed using a tail-flick analgesia meter (Columbus Instruments, Columbus, OH). For this procedure, the stimulus intensity was adjusted to provide baseline latencies between 3 and 5 s. 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 s. A predetermined cutoff time of 10 s 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 home cage. The latency to respond to the light source was measured twice at each determination, at least 30 s apart, with the light source focused 3 and 5 cm from the tip of the tail. These data were averaged to yield one value. Following baseline latency measurements, multiple 30-min cycles were conducted, and drug mixtures were administered cumulatively. During dose-effect determinations, cumulative doses of drug mixtures were administered i.p. at the start of each cycle (i.e., 30-min interinjection interval), increasing in one-quarter or one-half log unit increments, and antinociceptive measurements were determined during the last minute of each cycle. For time course determinations, a single injection of a drug or drug mixture was administered i.p., and antinociceptive measurements were assessed at 15, 30, 45, and 60 min. Latencies are expressed as a percentage of the maximal possible effect (%MPE) using the following formula: %MPE = [(postdrug latency (seconds) – baseline latency (seconds))/cutoff time (10 s) – baseline latency (seconds)] × 100.

Dose-Effect Analysis. The dose of each drug mixture required to produce a 50% decrease in schedule-controlled responding or 50% maximal antinociceptive effect in the assay of thermal nociception was derived using log-linear interpolation by linear regression. Individual ED50 values were converted to their log values for calculation of means and 95% confidence limits and then converted back to linear values for presentation.

Isobolographic Analysis. The effects of mGlu antagonist/morphine mixtures were assessed graphically with the use of isobolograms. In the present study, isobolograms were constructed by connecting the ED50 of morphine alone plotted on the abscissa with the ED50 of the mGlu antagonist alone plotted on the ordinate to obtain an additivity line. The additivity line contains the loci of dose pairs that would produce an ED50 equal to the ED50 of morphine or an mGlu receptor antagonist alone if the combination is additive. Dose pairs that fall below the additivity line suggest an ED50 was reached with lesser quantities of the drugs, suggestive of supra-additivity. In contrast, experimental points representing dose pairs that fall above the line are suggestive of infra-additivity.

Isobolograms of dose pairs (a, b) were constructed based on the relative efficacy of morphine and an mGlu receptor antagonist. Morphine was fully efficacious in both assays and was used as the...
reference drug. Therefore, the shape of the isobologram is dependent on the efficacy of the mGlu receptor antagonist under study from the equation:

$$ B = b + \frac{B}{\left(\frac{100}{A_{\text{max}}} \left(1 + \frac{A_{\text{max}}}{a}\right) - 1\right)} $$  \hspace{1cm} (1)

where $B$ is the ED$_{50}$ for morphine alone, $A_{\text{max}}$ is related to the efficacy of the mGlu antagonist, and $A$ is the dose of mGlu antagonist that attains half of $A_{\text{max}}$ (Grabovsky and Tallarida, 2004). For the assay of schedule-controlled responding, in which all drugs were fully efficacious, the equation describing the isobologram is reduced to:

$$ B = b + \frac{B}{(A/a)} \text{ or } A = b \Rightarrow A = b $$  \hspace{1cm} (2)

For the assay of thermal nociception, the mGlu antagonists JNJ16259685, MPEP, and LY341495 were ineffective when administered alone. Therefore, as the value of $A_{\text{max}}$ approaches 0, the equation describing this isobologram reduces to:

$$ B = \lim_{A_{\text{max}} \to 0} \left( b + \frac{B}{\left(\frac{100}{A_{\text{max}}} \left(1 + \frac{A_{\text{max}}}{a}\right) - 1\right)} \right) $$  \hspace{1cm} (3)

**Dose-Addition Analysis.** Drug interactions were statistically analyzed by comparing the experimentally determined ED$_{50}$ values for each mixture ($Z_{\text{mixture}}$) with predicted additive ED$_{50}$ values ($Z_{\text{add}}$) as described by Tallarida (2000). $Z_{\text{mixture}}$ was defined as the total drug dose (i.e., dose morphine + dose mGlu receptor antagonist) that produced a 50% decrease in rates of responding (schedule-controlled responding) or a 50% maximal antinociceptive effect (thermal nociception).

For the assay of schedule-controlled responding, in which all drugs were equieffective, $Z_{\text{add}}$ values were calculated individually for each mouse based on the ED$_{50}$ values of each drug from the equation:

$$ Z_{\text{add}} = fA + (1-f)B $$  \hspace{1cm} (4)

where $A$ is the ED$_{50}$ for the mGlu receptor antagonist alone, $B$ is the ED$_{50}$ for morphine alone, and $f$ is related to the proportion of the mGlu receptor antagonist in the mixture. The proportion of mGlu receptor antagonist ($p_A$) in each mixture is determined by the equation:

$$ p_A = \frac{fA}{fA + (1-f)B} $$  \hspace{1cm} (5)

and the proportion of morphine ($p_B$) in each mixture is determined by the equation:

$$ p_B = \frac{(1-f)B}{fA + (1-f)B} $$  \hspace{1cm} (6)

For the assay of thermal nociception, the mGlu antagonists JNJ16259685, MPEP, and LY341495 were ineffective when administered alone, and the hypothesis of additivity predicts that these drugs would not contribute to morphine’s effects when administered in combination (Tallarida, 2000). Therefore, $Z_{\text{add}}$ is calculated based on the proportion of morphine in each particular mixture from the equation:

$$ Z_{\text{add}} = \frac{B}{p_B} $$  \hspace{1cm} (7)

Mean experimentally determined ED$_{50}$ values ($Z_{\text{mixture}}$) and predicted additive ED$_{50}$ values ($Z_{\text{add}}$) for each mixture were compared with a Student’s $t$ test.

## Results

### Schedule-Controlled Responding

**Morphine and mGlu Antagonists Alone.** Figure 1 (top) shows that morphine, JNJ16259685, MPEP, and LY341495 produced dose-dependent decreases in the rate of responding. A statistical test for parallelism revealed that the morphine dose-effect curve was parallel to the dose-effect curves for JNJ16259685, MPEP, and LY341495 ($p < 0.05$). The mean ED$_{50}$ values for each drug and the relative potencies for each mGlu antagonist in comparison to morphine are shown in Table 1. These relative potency values were used to determine relative proportions of the compounds used in subsequent studies assessing mGlu antagonist/morphine mixtures.

To confirm a similar duration of action, the rate-decreasing effects of morphine, JNJ16259685, MPEP, and LY341495 were assessed over time. As shown in Fig. 2, each drug produced similar decreases in response rates (>$50\%$ control) for 40 min. Rates of responding gradually returned to near control rates at 160 min for morphine, JNJ16259685, and MPEP (91, 88, and 86% control, respectively) and 240 min for LY341495 (81% control). These data suggest that morphine, JNJ16259685, MPEP, and LY341495 produce similar peak effects and duration of action.

**Morphine and mGlu Antagonist Mixtures.** The rate-decreasing effects of morphine alone and in combination with
TABLE 1

<table>
<thead>
<tr>
<th>Assay/Drug</th>
<th>ED$_{50}$ Value (95% CL)</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schedule-controlled responding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>3.0 (1.9–5.0)</td>
<td></td>
</tr>
<tr>
<td>JNJ16259685</td>
<td>2.6 (1.6–4.2)</td>
<td>0.87</td>
</tr>
<tr>
<td>MPEP</td>
<td>17 (10–28)</td>
<td>5.7</td>
</tr>
<tr>
<td>LY341495</td>
<td>2.8 (1.6–4.8)</td>
<td>0.93</td>
</tr>
<tr>
<td>Thermal nociception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>5.6 (4.8–7.4)</td>
<td>N.D.</td>
</tr>
<tr>
<td>JNJ16259685</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>MPEP</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>LY341495</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

CL, confidence limit; N.D., not determined.

Morphine and mGlu Antagonists Alone. Figure 1 (bottom) shows the antinociceptive effects of morphine, JNJ16259685, MPEP, and LY341495. Morphine produced dose-dependent increases in latency to respond on the tail-flick apparatus, and the resulting ED$_{50}$ value is shown in Table 1. JNJ16259685, MPEP, and LY341495 were 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.

Morphine and mGlu Antagonist Mixtures. The antinociceptive effects of morphine alone and in combination with JNJ16259685, MPEP, and LY341495 are shown in Fig. 4. Each drug mixture produced dose-dependent increases in antinociception, and addition of JNJ16259685 or LY341495 produced leftward shifts in the morphine dose-effect curve. Graphical analysis of the JNJ16259685/morphine mixtures indicates that each mixture produced supra-additive effects.
because these ED_{50} values fell to the left of the line of additivity. Graphical analysis of the LY341495/morphine mixture indicates that the mixture with a lower proportion of LY341495 (i.e., 0.3:1 LY341495/morphine) produced additive effects because these ED_{50} values fell close to line of additivity. In contrast, mixtures with a higher proportion of LY341495 (i.e., 0.93:1 and 2.8:1 LY341495/morphine) produced supra-additive effects because these ED_{50} values fell to the left of the line of additivity. Statistical comparison determined that the experimentally determined ED_{50} values (Z_{mix}) for these mixtures were significantly less than the predicted additive ED_{50} values (Z_{add}) (Table 3).

Addition of MPEP did not significantly shift the morphine dose-effect curve because the ED_{50} values for MPEP/morphine mixtures were similar to the ED_{50} value of morphine alone. Graphical analysis of these drug combinations indicates that mixtures of MPEP and morphine produced additive effects across a range of proportions because these ED_{50} values fell close to the line of additivity. Statistical comparison of experimentally determined ED_{50} values (Z_{mix}) and predicted additive ED_{50} values (Z_{add}) confirmed these findings (i.e., Z_{add} = Z_{mix}) (Table 3).

The antinociceptive effects of morphine alone and in combination with JNJ16259685, MPEP, and LY341495 assessed over time are shown in Fig. 5 (top). Morphine alone produced a minimal antinociceptive effect, with a peak effect reached at 30 min postinjection (13% maximal possible effect). Addition of JNJ16259685 and LY341495 produced a significant increase (p < 0.05) in morphine-induced antinociception. The JNJ16259685/morphine mixture produced a peak effect at 45 min postinjection (47% maximal possible effect), whereas the peak effect of the LY341495/morphine mixture was reached at 30 min postinjection (69% maximal possible effect). Coadministration of morphine and MPEP did not produce an antinociceptive effect that was significantly different from morphine alone at any time point.

Discussion

The purpose of the present study was to assess the interactive effects of morphine and antagonists selective for group I and group II mGlu receptors on schedule-controlled responding and thermal nociception. The main finding from these experiments is that an mGlu1 receptor antagonist and an mGlu2/3 receptor antagonist potentiate the antinociceptive effects of morphine, whereas mGlu5 receptor antagonist/morphine mixtures produce an additive effect. Each mGlu antagonist/morphine mixture, however, produced an additive effect on schedule-controlled responding. Taken together, these data suggest that the interactive effects of mGlu antagonist/morphine mixtures depend on the mGlu receptor antagonist under study and the experimental endpoint.

Morphine and mGlu Receptor Antagonists Administered Alone. In the assay of schedule-controlled responding, the μ-opioid receptor agonist morphine, the mGlu1 receptor antagonist JNJ16259685, the mGlu5 receptor antagonist MPEP, and the mGlu2/3 receptor antagonist LY341495 dose-dependently decreased rates of responding. In addition, the decreases in operant responding are time-dependent, with peak effects occurring at 10 to 40 min. Previous research suggests that MPEP also reduces operant responding at doses similar to those used in the present experiment (Varty et al., 2005), whereas the response rate-altering effects of JNJ16259685 and LY341495 have not been assessed.

Previous research suggests that mGlu receptor antagonists produce antinociceptive effects in animal models of chronic pain (see Neugebauer, 2002). Reports on the acute antinociceptive effects of mGlu antagonists in assays of thermal nociception are limited, although at least one report suggests that they do not modulate acute nociceptive processing (Walker et al., 2001). The present study further suggests that mGlu antagonists selective for group I and group II receptor subtypes do not modulate acute nociceptive processing after systemic administration.

Morphine and Group I mGlu Receptor Antagonist Interactions. The findings from the present study suggest that the mGlu1 receptor antagonist JNJ16259685 potentiates the antinociceptive effects of morphine, while producing additive effects on schedule-controlled responding. Although the present study is the first to demonstrate that an mGlu1 receptor antagonist potentiates morphine-induced antinociception, these effects have been evaluated in rats following antisense knockdown of mGlu1 receptors. In this study, knockdown of mGlu1 receptors increased the effectiveness of morphine in an animal model of neuropathic pain (Fundytus et al., 2001). The finding from the present study that JNJ16259685 increased morphine’s antinociceptive effects corroborates this report. Taken together, these data suggest that mGlu1 receptors mediate the antinociceptive effects of morphine.

In contrast to the supra-additive effect of JNJ16259685/morphine mixtures on thermal nociception, mixtures containing the mGlu5 antagonist MPEP and morphine produced an additive effect on this endpoint. This agrees with and extends a study assessing single-dose combinations of MPEP and morphine (Kozela et al., 2003). Taken together, these data suggest that the antinociceptive effects of morphine may not be modulated by mGlu5 antagonists.

Both mGlu1 and mGlu5 receptors are expressed postsynaptically on dorsal horn neurons in the spinal cord (Vidnyanszky et al., 1994; Jia et al., 1999; Alvarez et al., 2000). Interestingly, these mGlu receptors are physically linked to NMDA receptors in this region (Naisbitt et al., 1999; Tu et al., 1999). Activation of mGlu1 and mGlu5 receptors results in the potentiation of NMDA-mediated responses, through an activation of protein kinase C and a reduction of the voltage-dependent block of NMDA receptors by Mg^{2+} (Cerne and Randic, 1992; Kelso et al., 1992; Skeberdis et al., 2001). Increases in morphine-induced

<table>
<thead>
<tr>
<th>Drug Mixture</th>
<th>Z_{mix} (95% CL)</th>
<th>Z_{add} (95% CL)</th>
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</thead>
<tbody>
<tr>
<td>JNJ16259685 + morphine</td>
<td>1.7 (1.3–2.3)</td>
<td>3.1 (1.8–5.3)</td>
</tr>
<tr>
<td>0.29:1 JNJ16259685/morphine</td>
<td>3.5 (2.3–5.3)</td>
<td>3.1 (2.0–4.9)</td>
</tr>
<tr>
<td>0.57:1 JNJ16259685/morphine</td>
<td>1.1 (0.5–1.9)</td>
<td>3.1 (2.0–4.9)</td>
</tr>
<tr>
<td>2.6:1 JNJ16259685/morphine</td>
<td>3.1 (2.7–3.6)</td>
<td>3.0 (2.0–4.5)</td>
</tr>
<tr>
<td>MPEP + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1 MPEP/morphine</td>
<td>4.7 (2.1–11)</td>
<td>6.1 (3.9–9.4)</td>
</tr>
<tr>
<td>5.7:1 MPEP/morphine</td>
<td>6.9 (3.2–15)</td>
<td>9.5 (6.1–15)</td>
</tr>
<tr>
<td>17:1 MPEP/morphine</td>
<td>14.5 (5.5–35)</td>
<td>13 (8.2–21)</td>
</tr>
<tr>
<td>LY341495 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.31:1 LY341495/morphine</td>
<td>3.4 (2.0–4.9)</td>
<td>3.3 (2.2–4.9)</td>
</tr>
<tr>
<td>0.93:1 LY341495/morphine</td>
<td>2.9 (1.7–5.1)</td>
<td>3.3 (2.2–5.0)</td>
</tr>
<tr>
<td>2.8:1 LY341495/morphine</td>
<td>2.7 (1.3–3.6)</td>
<td>3.1 (2.0–4.9)</td>
</tr>
</tbody>
</table>
antinociception after NMDA antagonist administration have been demonstrated in assays of acute thermal nociception (Nemmani et al., 2004; Fischer et al., 2005). Therefore, the results from the present study suggest that inactivation of mGlu1 receptors, but not mGlu5 receptors, increases morphine-induced antinociception, probably via an NMDA-dependent mechanism.

In view of the functional similarities of mGlu1 and mGlu5 receptors, the additive effects of MPEP/morphine mixtures on thermal nociception are surprising. Indeed, a growing body of experimental evidence indicates that MPEP modu-

TABLE 3
Predicted additive ED_{50} values (Z_{add}) and experimentally determined ED_{50} values (Z_{mix}) of morphine + mGlu receptor antagonist mixtures in the assay of thermal nociception

<table>
<thead>
<tr>
<th>Drug Mixture</th>
<th>Z_{add} (95% CL)</th>
<th>Z_{mix} (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ16259685 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.28:1 JNJ16259685/morphine</td>
<td>8.7 (6.2–12)</td>
<td>3.7 (2.5–5.4)*</td>
</tr>
<tr>
<td>0.87:1 JNJ16259685/morphine</td>
<td>13 (8.9–18)</td>
<td>2.6 (2.1–3.2)*</td>
</tr>
<tr>
<td>2.6:1 JNJ16259685/morphine</td>
<td>24 (17–34)</td>
<td>6.5 (4.1–10)*</td>
</tr>
<tr>
<td>MPEP + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9:1 MPEP/morphine</td>
<td>17 (12–26)</td>
<td>13 (10–16)</td>
</tr>
<tr>
<td>5.7:1 MPEP/morphine</td>
<td>40 (27–59)</td>
<td>34 (25–46)</td>
</tr>
<tr>
<td>17:1 MPEP/morphine</td>
<td>110 (71–160)</td>
<td>&gt;94 (84–100)*</td>
</tr>
<tr>
<td>LY341495 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.31:1 LY341495/morphine</td>
<td>7.0 (4.7–10)</td>
<td>5.0 (3.2–8.0)</td>
</tr>
<tr>
<td>0.93:1 LY341495/morphine</td>
<td>10 (7.0–15)</td>
<td>3.6 (2.1–6.3)*</td>
</tr>
<tr>
<td>2.8:1 LY341495/morphine</td>
<td>20 (14–30)</td>
<td>5.4 (3.5–8.3)*</td>
</tr>
</tbody>
</table>

CL, confidence limit.
* Z_{mix} significantly different from Z_{add} (p < 0.05).
* The highest dose tested (5.6 mg/kg morphine + 95.2 mg/kg MPEP) did not increase the latency to respond to the tail-flick apparatus to >50% in three mice. For analysis, a Z_{mix} of 100.8 was assigned to these mice.

antinociception after NMDA antagonist administration have been demonstrated in assays of acute thermal nociception (Nemmani et al., 2004; Fischer et al., 2005). Therefore, the results from the present study suggest that inactivation of mGlu1 receptors, but not mGlu5 receptors, increases morphine-induced antinociception, probably via an NMDA-dependent mechanism.

In view of the functional similarities of mGlu1 and mGlu5 receptors, the additive effects of MPEP/morphine mixtures on thermal nociception are surprising. Indeed, a growing body of experimental evidence indicates that MPEP modu-

Fig. 4. Morphine alone and in combination with JNJ16259685, MPEP, or LY341495 in the assay of thermal nociception. Top, dose-effect curves for morphine alone and in combination with JNJ16259685, MPEP, or LY341495. Abscissae, cumulative dose of morphine in milligrams per kilogram. Ordinate, antinociception as percent maximal possible effect. Bottom, isobolograms for mGlu antagonist/morphine mixtures. Abscissae, ED_{50} value for morphine in milligrams per kilogram. Each point shows the mean (±S.E.M.) from eight mice.

Table 3

<table>
<thead>
<tr>
<th>Drug Mixture</th>
<th>Z_{add} (95% CL)</th>
<th>Z_{mix} (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ16259685 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.28:1 JNJ16259685/morphine</td>
<td>8.7 (6.2–12)</td>
<td>3.7 (2.5–5.4)*</td>
</tr>
<tr>
<td>0.87:1 JNJ16259685/morphine</td>
<td>13 (8.9–18)</td>
<td>2.6 (2.1–3.2)*</td>
</tr>
<tr>
<td>2.6:1 JNJ16259685/morphine</td>
<td>24 (17–34)</td>
<td>6.5 (4.1–10)*</td>
</tr>
<tr>
<td>MPEP + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9:1 MPEP/morphine</td>
<td>17 (12–26)</td>
<td>13 (10–16)</td>
</tr>
<tr>
<td>5.7:1 MPEP/morphine</td>
<td>40 (27–59)</td>
<td>34 (25–46)</td>
</tr>
<tr>
<td>17:1 MPEP/morphine</td>
<td>110 (71–160)</td>
<td>&gt;94 (84–100)*</td>
</tr>
<tr>
<td>LY341495 + morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.31:1 LY341495/morphine</td>
<td>7.0 (4.7–10)</td>
<td>5.0 (3.2–8.0)</td>
</tr>
<tr>
<td>0.93:1 LY341495/morphine</td>
<td>10 (7.0–15)</td>
<td>3.6 (2.1–6.3)*</td>
</tr>
<tr>
<td>2.8:1 LY341495/morphine</td>
<td>20 (14–30)</td>
<td>5.4 (3.5–8.3)*</td>
</tr>
</tbody>
</table>

CL, confidence limit.
* Z_{mix} significantly different from Z_{add} (p < 0.05).
* The highest dose tested (5.6 mg/kg morphine + 95.2 mg/kg MPEP) did not increase the latency to respond to the tail-flick apparatus to >50% in three mice. For analysis, a Z_{mix} of 100.8 was assigned to these mice.

antinociception after NMDA antagonist administration have been demonstrated in assays of acute thermal nociception (Nemmani et al., 2004; Fischer et al., 2005). Therefore, the results from the present study suggest that inactivation of mGlu1 receptors, but not mGlu5 receptors, increases morphine-induced antinociception, probably via an NMDA-dependent mechanism.

In view of the functional similarities of mGlu1 and mGlu5 receptors, the additive effects of MPEP/morphine mixtures on thermal nociception are surprising. Indeed, a growing body of experimental evidence indicates that MPEP modu-

Fig. 5. Morphine alone and in combination with JNJ16259685, MPEP, or LY341495 in the assay of thermal nociception as assessed over time. Abscissae, time after drug administration. Ordinate, antinociception as a percentage of maximal possible effect. Each point shows the mean (±S.E.M.) from eight mice. *, significantly different from morphine alone.
lates the effects of morphine on other endpoints (Popik and Wrobel, 2002; Kozela et al., 2003; Smith et al., 2004). Nota-
bly, as demonstrated in the current study, the behavioral
effects of two drugs may depend on the experimental end-
point under study (Stevenson et al., 2005; Fischer and Dyk-
stra, 2006). In addition, these interactive effects may depend
on factors including the relative proportion of the drugs in
the mixture and the type of nociceptive assay used (Tallarida,
2000; Nemmani et al., 2004; Craft and Lee, 2005). Therefore,
studies with MPEP/morphine mixtures in other proportions
or in other assays of thermal nociception could yield results
that are different from those reported here. Nevertheless,
these data suggest that despite the functional similarities of
mGlu1 and mGlu5 receptors, the manner in which they in-
teract with morphine may be fundamentally different.

**Morphine and Group II mGlu Receptor Antagonist Interactions.** The results from this study suggest that the
mGlu2/3 receptor antagonist LY341495 potentiates the an-
tinociceptive effects of morphine in an acute model of thermal
nociception after systemic administration. This finding
agrees with a recent preliminary report by Yoon et al. (2006),
which demonstrated that an LY341495/morphine mixture
produces a supra-additive effect in the rat formalin test after
i.t. administration.

The underlying mechanisms mediating the interactive ef-
fects of LY341495 and morphine in the assay of thermal
nociception are not clear. Numerous reports have demons-
trated that mGlu2/3 receptors are located presynaptically in
numerous brain regions and that drugs that act as agonists
at these receptors decrease glutamate release and subse-
quently postsynaptic binding to both iGlu and mGlu receptor
subtypes (Pin and Duvoisin, 1995). Indeed, some behavioral
reports are consistent with this finding, suggesting that the
mGlu2/3 agonist LY354740 attenuates behavioral signs of
morphine tolerance and withdrawal in a similar manner as
agonists with affinity at the NMDA receptor or group I
mGlu receptors (Klodzinska et al., 1999; Popik et al., 2000).

The specific function of mGlu2/3 receptors in the dorsal
horn of the spinal cord, a region thought to mediate the
behavioral responses in the assay of thermal nociception,
is less clear. Both pre- and postsynaptic mGlu2/3 receptors
have been identified in the dorsal horn (Carlton et al., 2001),
and conflicting results are often reported from both in
vitro and in vivo preparations. For example, mGlu2/3 agonists
produce both facilitation and inhibition of neuronal activity
(Bond and Lodge, 1995; Cao et al., 1995; King and Liu, 1996).
In addition, both agonism and antagonism of mGlu2/3 recep-
tors decrease pain related behaviors in animal models of
inflammation (Jones et al., 2005; Yoon et al., 2006). These
results may reflect the complex interaction of pre-
and postsynaptic mGlu2/3 receptors in the dorsal horn and make
the interpretation of mGlu2/3 antagonist/morphine interac-
tions difficult. Therefore, further research is necessary to
elucidate the mechanisms mediating the interactive effects of
mGlu2/3 antagonist/morphine mixtures on nociceptive
behavior.

**Drug Interactions across Behavioral Endpoints.** Pre-
vious research suggests that interactions between two drugs
may depend on the experimental endpoint under study
(Stevenson et al., 2005; Fischer and Dykstra, 2006). For
example, we have previously demonstrated that mixtures
containing the NMDA antagonist LY235959 and morphine
produced supra-additive effects on thermal nociception and
additive or infra-additive effects on schedule-controlled re-
spinding (Fischer and Dykstra, 2006). Similarly, in the
present study, mixtures of morphine and the mGlu1 antag-
onist JNJ16259685 and mixtures of morphine and the
mGlu2/3 antagonist LY341495 produced supra-additive ef-
fects in the assay of thermal nociception, whereas the same
drug mixtures produced additive effects in the assay of sched-
ule-controlled responding.

The behavioral selectivity of these interactions suggests that
there is corresponding selectivity across brain regions
mediating each behavior, rather than a general enhancement
of all behavioral effects. The data from the present study
suggest that it is possible to develop mGlu antagonist/mor-
phine mixtures that interact in a supra-additive manner
specifically at brain regions that mediate the targeted behav-
ior of antinociception. Further research is necessary to ex-
pand the current findings across different measures of an-
tinociception and on behavior maintained by other schedules
of reinforcement. In addition, research assessing mGlu an-
tagons/morphine mixtures on other behavioral endpoints
(e.g., respiratory depression and/or drug self-administration)
is necessary to predict further the clinical utility of these
drug mixtures.

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