The Competitive α-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionate Receptor Antagonist LY293558 Attenuates and Reverses Analgesic Tolerance to Morphine But Not to Delta or Kappa Opioids

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ABSTRACT
Antagonists of the NMDA type of excitatory amino acid (EAA) receptor attenuate or reverse the development of tolerance to the analgesic effects of the mu opioid agonist morphine, the delta-1 opioid agonist DPDPPE but not the kappa-1 agonist U50,488H or the kappa-3 agonist naloxone benzoylehydrazone. The role of the AMPA subtype of EAA receptor in analgesic tolerance was examined using LY293558, a selective competitive antagonist that is active after systemic administration. Administration of morphine, DPDPPE, or U50,488H three times daily for 3 days according to an escalating dosing schedule resulted in analgesic tolerance as indicated by an increase in analgesic ED₅₀ values using the tail-flick test in mice. Analgesic tolerance was attenuated when mice received a continuous subcutaneous infusion of LY293558 at doses of 30, 45 or 60 mg/kg/24 hr via an osmotic pump concurrent with the morphine treatment. Continuous subcutaneous infusion of LY293558 (45 mg/kg/24 hr) also reversed established morphine tolerance. In contrast, continuous subcutaneous infusion of the highest dose of LY293558 (60 mg/kg/24 hr) was ineffective in preventing the development of analgesic tolerance to DPDPPE or U50,488H. Continuous subcutaneous infusion of LY293558 (60 mg/kg/24 hr) for 3 days protected mice from generalized convulsions produced by the selective AMPA agonist ATPA, indicating that the dosage of LY293558 that attenuated morphine tolerance was effective as an antagonist at AMPA receptors. These results demonstrate that AMPA receptors may play a role in the development and maintenance of morphine, but not DPDPPE or U50,488H, analgesic tolerance.

Excitatory amino acids including glutamate and aspartate are neurotransmitters in the vertebrate central nervous system (Bettler and Mulle, 1995; Watkins, 1994). The NMDA, AMPA and kainate receptors are named on the basis of the selective agonists used for their initial characterization. These three types are directly coupled to cation channels (ionotropic receptors). AMPA receptors are involved in the generation of the fast component of synaptic transmission, and NMDA receptors contribute to a slow component of repetitive synaptic activity generated primarily by AMPA and other non-NMDA EAA receptor coupled channels (Watkins, 1994). Pharmacological, electrophysiological and molecular cloning studies have demonstrated that these EAA receptors are functionally and constitutively distinct (Bettler and Mulle, 1995; Watkins, 1994). Through the use of selective competitive and noncompetitive antagonists that are active after systemic administration, it is well established that NMDA receptor blockade can attenuate or reverse the development of morphine tolerance in the mouse or rat (see reviews by Elliott et al., 1995; Pasternak and Inturrisi, 1995). The potential clinical utility of NMDA receptor antagonists is enhanced by the observation that these antagonists act directly on the processes underlying the development of morphine tolerance and do not potentiate morphine analgesia (Elliott et al., 1995; Pasternak and Inturrisi, 1995).

The recent cloning of three separate genes encoding opioid receptors supports pharmacological studies indicating that opioids exert their effects at mu, delta and kappa receptor types (Knapp et al., 1995; Mansour et al., 1995). Using cloning studies have demonstrated that these EAA receptors are functionally and constitutively distinct (Bettler and Mulle, 1995; Watkins, 1994). Through the use of selective competitive and noncompetitive antagonists that are active after systemic administration, it is well established that NMDA receptor blockade can attenuate or reverse the development of morphine tolerance in the mouse or rat (see reviews by Elliott et al., 1995; Pasternak and Inturrisi, 1995). The potential clinical utility of NMDA receptor antagonists is enhanced by the observation that these antagonists act directly on the processes underlying the development of morphine tolerance and do not potentiate morphine analgesia (Elliott et al., 1995; Pasternak and Inturrisi, 1995).

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gands with relative selectivity, it has been shown that analgesia can be mediated by each of the three opioid receptors (Pasternak, 1993; Porreca and Burks, 1993; Suh and Tseng, 1990). Although morphine exerts its analgesic effects primarily at the mu receptor type (Matthes et al., 1996), U50,488H is the prototypic kappa-1 receptor agonist (VonVoigtlander et al., 1983). The opioid effects of the conformationally restricted enkephalin analog DPDPE and [D-Ala³]deltorphin II are differentially affected by several selective delta receptor antagonists and therefore characterized as delta-1 and delta-2 opioid receptor agonists, respectively (Jiang et al., 1990; Suh and Tseng, 1990). Tolerance to the analgesic effects of opioids after repeated administration occurs independently of changes in the potency of opioids acting at other receptor types. For example, tolerance to the analgesic effects of morphine after chronic administration does not produce a concomitant loss in the analgesic potency of U50,488H, nor does U50,488H tolerance produce cross-tolerance to morphine analgesia (VonVoigtlander et al., 1983). Reciprocal cross-tolerance is also not observed among DPDPE, [D-Ala³]deltorphin II and the mu receptor selective opioid DAMGO (Mattia et al., 1991). Thus, the mechanisms of opioid analgesic tolerance for each opioid receptor type appear to be selective. This differential organization is thought to underlie the selective ability of NMDA receptor antagonists to prevent analgesic tolerance. In our laboratory, the competitive and noncompetitive NMDA receptor antagonists LY274614 and MK-801, respectively, as well as the nitric oxide synthase inhibitor Nω-nitro-l-arginine were able to attenuate and reverse analgesic tolerance to morphine but not to U50,488H or to the kappa-3 agonist naloxone benzhydrazone (Elliott et al., 1994, 1995; Tiseo et al., 1994; Tiseo and Inturrisi, 1993). LY235959 (the active isomer of LY274614) and MK-801 attenuated tolerance to morphine but not to the delta-2 agonist [D-Ala²,Glul⁴]deltorphin (Bilsky et al., 1996), whereas blockade of the allosteric glycine site of the NMDA receptor by ACPC prevents tolerance to the analgesic effects of morphine and DPDPE but not to U50,488H (Kolesnikov et al., 1994). NPC17742, a competitive NMDA receptor antagonist that differs structurally from the LY274614 and MK801 type compounds, prevents morphine- and U50,488H-induced analgesic tolerance (Kolesnikov et al., 1993).

In contrast to the extensive studies with NMDA receptor antagonists, the role of AMPA receptors in opioid tolerance is unknown. This may reflect the investigational constraints of the available AMPA receptor antagonists, which either have limited solubility or are not active after systemic administration. Recently, Ornstein et al. (1993) reported the synthesis of LY215490, a structurally novel and systemically active competitive AMPA receptor antagonist. Resolution of LY215490 yields LY293558, the levorotatory isomer that is the active AMPA receptor antagonist. In ligand binding assays, LY293558 selectively displaces AMPA receptor ligands. In these assays, the affinity of LY293558 at AMPA receptors is 9-fold greater than that at NMDA receptors and 21-fold greater than at kainate receptors (Schoepf et al., 1995). In the in vitro functional assays (rat cortical wedge preparation and EEA agonist-evoked release of ³H-norepinephrine), LY293558 and/or LY215490 demonstrates selective AMPA receptor antagonist activity (Schoepf et al., 1995). Both LY293558 and LY215490 were anticonvulsant against maximal electroshock seizures in mice (Ornstein et al., 1993) and protect against the convulsions produced by ATPA, a selective AMPA receptor agonist.¹

The aim of the present study was to examine the role of AMPA receptors in the development and maintenance of opioid tolerance in mice using LY293558. Because opioid agonists differ in their sensitivity to the modulation of tolerance by NMDA receptor antagonists (Bilsky et al., 1996; Elliott et al., 1995, 1994; Kolesnikov et al., 1994; Pasternak and Inturrisi, 1995), the effects of LY293558 on analgesic tolerance to morphine, U50,488H, DPDPE and [D-Ala³]deltorphin II were assessed. We also determined whether LY293558, administered by osmotic pump, had any effects on motor performance or was able to protect mice against convulsions produced by ATPA.

Materials and Methods

Subjects. Male adult CD-1 mice (25–35 g, Charles River, Kingston, NY) were housed five to a cage and maintained on a 12:12 hr light/dark cycle in a temperature-controlled environment with unrestricted food and water. Each treatment condition was replicated once, and each treatment group consisted of 10 mice unless otherwise indicated.

Drugs. LY293558 and ATPA were generously provided by Dr. Paul L. Ornstein (The Lilly Research Laboratories, Indianapolis, IN). Morphine sulfate and DPDPE were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Resources program of the National Institute on Drug Abuse (Rockville, MD). [D-Ala³]Deltorphin II was purchased from Molecula, (Durham, NC), and NMDA was from Sigma Chemical (St. Louis, MO). U50,488H was a gift of Dr. P. Von Voigtlander (The Upjohn Co., Kalamazoo, MI). The compounds were dissolved in saline, which served as vehicle control, and the pH adjusted to 7.0. The dosage of morphine sulfate is expressed as the free base. Morphine, U50,488H, NMDA and ATPA were administered subcutaneously in an injection volume of 10 ml/kg. LY293558 was administered by continuous s.c. infusion via an osmotic pump (model 2001, Alza Co., Palo Alto, CA) implanted under halothane anesthesia subcutaneously on the dorsal surface of mice (Elliott et al., 1994). Control animals underwent identical surgical procedures but did not receive pumps (sham group). In a previous study (Elliott et al., 1994) we compared sham surgery with the implantation of saline pumps and found no difference in the base-line TF response or in the development of morphine tolerance. DPDPE and [D-Ala³]Deltorphin II were administered via i.c.v. injection after the method of Haley and McCormick (1957). Under halothane anesthesia, an incision was made in the scalp, and bregma was located. The drug was then injected directly through the skull at a point 2 mm caudal and lateral to bregma at a depth of 3 mm using a Hamilton (Hamilton Co., Reno, NV) microliter syringe with a 27-gauge needle. All i.c.v. injections were made in a volume of 5 μl. After each injection, the incision was closed by a stainless steel wound clip.

Tail-flick test. TF latencies were assessed using a standardized TF apparatus (EMDIE, Richmond, VA) with a radiant heat source connected to an automatic timer. Latencies were measured from the onset of the heat stimulus applied to the distal 2 cm of the tail and was terminated on the flick of the tail. The intensity of the stimulus was adjusted to yield baseline latencies between 2.5 and 3.5 sec. A maximum latency of 10 sec (i.e., cutoff) was used to minimize damage to the tail. Base-line latency was the mean of two determinations and was assessed before ED₅₀ determinations.

Dose-response studies. Tolerance was operationally defined as an increase in ED₅₀ value of the opioid agonist. To reduce the number of animals required to assess tolerance, ED₅₀ values were derived from cumulative dose-response curves constructed from mice injected with increasing doses of opioid until each animal became an
analgesic responder. The increment of each increase in dose was 0.25 log unit (Elliott et al., 1994). A responder was operationally defined as a mouse whose TF latency on two consecutive determinations at a particular dose was equal to or greater than double that animal's mean base line. Mice were tested at 30 min after each dose of morphine and U50,488H or at 20 and 10 min after each dose of DPDPE and [d-Ala²]deltorphin II, respectively. Each analgesic responder was not subjected to further TF assessments but was injected with the subsequent dose of opioid agonist until every animal was a responder, so each animal received the same cumulative dose. The ED⁵₀ values presented in the tables are the values derived by combining the observations from two separate studies.

**Tolerance paradigms.** Tolerance was produced by injection of an opioid three times daily (at 9:00 a.m., 1:00 p.m. and 5:00 p.m.) for 3 days according to an escalating dose schedule. Mice rendered tolerant to morphine and U50,488H received 10, 20 and 40 mg/kg s.c. on days 1, 2 and 3, respectively, whereas mice in the DPDPE and [d-Ala²]deltorphin II groups received 10, 20 and 40 μg/mouse i.c.v. on days 1, 2 and 3, respectively. LY293558 was delivered s.c. at a rate of 1 μl/hr via an osmotic pump implanted 16 hr before the first opioid injection on day 1 at concentrations of 15, 30, 45 and 60 mg/kg/24 hr. The ED⁵₀ value for each opioid agonist was assessed before (day 1) and after (day 4) tolerance induction and compared. The ability of LY293558 to restore the analgesic potency of morphine in tolerant mice (i.e., reversal of tolerance) was also assessed. In this paradigm, the morphine ED⁵₀ values of two groups of mice were assessed on day 1 and then they were made tolerant as described above. On day 4, after assessment of morphine ED⁵₀ values to confirm tolerance, one group was implanted with a pump and began receiving continuous s.c. infusion of LY293558 (45 mg/kg/24 hr) for 3 days, whereas the control morphine tolerant group underwent sham surgery. Morphine ED⁵₀ values were reassessed in both groups on day 7.

**Motor performance.** The effects of LY293558 on motor performance were assessed after 16 hr (the morning of day 1) and 88 hr (the morning of day 4) of continuous s.c. infusion of 60 mg/kg/24 hr. Reflexes for righting and placing/stepping were evaluated. In the former, the latency required for a mouse to regain normal position after being placed on its back horizontally was measured. In control mice, this response occurs immediately. Thus, mice with latencies of >1.5 sec were considered impaired. To assess the placing/stepping reflex, the dorsum of either hind paw was drawn over the edge of a table top. Criteria for impairment was the absence of “stepping” with the drawn paw onto the table top. Also, the ability of mice to navigate or remain balanced for 30 sec on a wire grid inclined 90° was assessed.

**ATPA- and NMDA-induced convulsions.** The AMPA receptor agonist ATPA and the NMDA receptor agonist NMDA given s.c. produced dose-dependent generalized convulsions (tonic-clonic convulsions). The percentage of animals convulsing was determined using a cumulative dosing procedure (Comer et al., 1993). Cumulative doses of ATPA from 60 to 800 mg/kg and of NMDA from 60 to 510 mg/kg were administered s.c. to mice (n = 15) using a 15-min inter-injection interval. Immediately after each injection, the mice were placed in a clear plastic cylinder (11 × 25 cm). If a convolution occurred, then higher doses were not tested in that animal. The ability of LY293558 to shift the dose response curve for ATPA- or NMDA-induced convulsions to the right was assessed on the morning of day 4 in mice receiving LY293558 at 60 mg/kg/24 hr by s.c. infusion. Mice that received sham surgery were also challenged with ATPA or NMDA. In contrast to ATPA, NMDA-induced convulsions were invariably followed by death.

**Data analysis.** Base-line TF latencies for each group were subjected to analysis of variance. Motor performance tests were assessed using the Fisher exact test. The quantal dose-response data for the TF response and ATPA- or NMDA-induced convulsions were analyzed using the BLISS-21 computer program. This program maximizes the log-likelihood function to fit a parallel set of gaussian normal sigmoid curves to the dose-response data and provides ED⁵₀ values, 95% confidence intervals and estimates of relative potency (Umans and Inturrisi, 1981). The criterion for significance in each test was P < .05.

**Results**

When tested at 30 min after a single dose of 50 mg/kg s.c. of LY293558, the righting reflex was not affected, whereas 100% of the mice failed the placing/stepping test and 50% failed the inclined plane test. In contrast, LY293558 given s.c. by osmotic pump at 60 mg/kg/24 hr did not affect motor performance as assessed by these three tests. None of the responses of the LY293558 treated mice were significantly different from sham surgery controls on treatment days 1 and 4 (data not shown). Both the behavioral and pharmacokinetic advantages of the administration of excitatory amino acid receptor antagonists by an osmotic pump have been discussed previously (Tiseo et al., 1994; Tiseo and Inturrisi, 1993).

The dose-response curve for ATPA-induced convulsions was shifted to the right when mice implanted with a pump that delivers LY293558 at 60 mg/kg/24 hr were tested on treatment Day 4 (fig. 1). The ED⁵₀ values for the LY293558 group was increased 2.5 fold (P < .05) from an ED⁵₀ value 128 mg/kg (104–154, 95% CI) in sham animals to 319 mg/kg (273–370, 95% CI). In contrast, no significant change was observed in the ED⁵₀ values for NMDA, which were 256 mg/kg (194–331, 95% to CI) in sham and 311 (229–409) in an LY293558-treated group (data not shown).

Table 1 shows that the continuous s.c. infusion of LY293558 at 15, 30, 45 or 60 mg/kg/24 hr via an osmotic pump did not alter the morphine ED⁵₀ values relative to sham-operated controls (sham + morphine or sham + saline groups) on day 1. Note that table 1 presents three separate sham + morphine groups that were evaluated concurrently with the LY293558-15 and -30 treatments, the LY293558-45 treatment and the LY293558-60 treatment. In sham-operated mice, 3 days of morphine, given three times per day, were invariably followed by death.

**Fig. 1.** The AMPA receptor antagonist LY293558 protects mice against convulsions. On day 1, mice were implanted with an osmotic pump delivering LY293558 (60 mg/kg/24 hr) or received sham surgery. On day 4 (after 88 hr), mice were subjected to cumulative doses of ATPA from 60 to 800 mg/kg s.c. given every 15 min until a generalized convolution was produced (see text for the ED⁵₀ values for ATPA in sham and LY293558-treated mice).
increased the day 4 ED50 values for morphine ~2.5-fold relative to day 1 values, indicating the development of tolerance. In contrast, the day 4 ED50 values for morphine obtained from mice coadministered LY293558 (LY293558 + morphine group) at doses ranging from 30 to 60 mg/kg/24 hr were significantly lower than the day 4 sham and morphine group, indicating the attenuation of the development of morphine tolerance by LY293558. Neither sham surgery nor LY293558 at 60 mg/kg/24 hr altered the day 4 ED50 values for morphine compared with the day 1 sham + saline group values. Figure 2 shows the shift to the right of the dose-response curve on day 4 in morphine-treated mice and the prevention of this shift by the coadministration of LY293558 at 45 mg/kg/24 hr. The lowest dose of LY293558 tested (15 mg/kg/24 hr) was not effective in reducing morphine tolerance.

Table 2 shows that after 3 days of escalating morphine treatment, the ED50 values for morphine for groups 1 and 2 were significantly increased on day 4, indicating that morphine tolerance of an equal magnitude had developed in each group. Sham surgery had no effect on the morphine ED50 value, and this group (group 1) remained tolerant when retested on day 7. In contrast, the morphine ED50 value of tolerant mice that received a continuous s.c. infusion of LY293558 (45 mg/kg/24 hr) on days 4, 5 and 6 (group 2) was significantly decreased, indicating a more rapid reversal in the magnitude of tolerance compared with group 1. Furthermore, the reversal of morphine tolerance was complete because the analgesic potency of morphine on day 7 in this group (group 2) was not significantly different from the day 1 morphine ED50 value for this group (table 2). During the 3 days after morphine administration was discontinued, neither group showed measurable signs of spontaneous opioid withdrawal as assessed by behavior and loss of weight (data not shown).

As indicated in tables 3 and 4, the s.c. infusion of LY293558 (60 mg/kg/24 hr) did not affect the respective DPDPE or U50,488H, ED50 values relative to the values on day 1. Three days of DPDPE administration resulted in a 3-fold shift in the ED50 value for DPDPE in both sham and LY293558-treated mice, indicative of the development of tolerance in both groups (table 3). Administration of i.c.v. saline for 3 days does not affect the day 4 ED50 value for DPDPE (Kest et al., 1994). Likewise, the ED50 values for U50,488H were also increased nearly 8-fold in both sham and LY293558-treated mice on day 4 (table 4).

The administration of [D-Ala2]deltorphin II according to the tolerance protocol described above in mice receiving a continuous infusion of LY293558 (30 or 60 mg/kg/24 hr) produced malaise, labor breathing and hypoactivity in 70% of animals on day 1 and a 30% lethality rate by day 3. Surviving mice appeared normal 2 days after the cessation of [D-Ala2]deltorphin II injections and the infusion of LY293558.

Fig. 2. The AMPA receptor antagonist LY293558 prevents morphine tolerance. Tolerance, as assessed by a rightward shift in the cumulative dose-response curve in mice, was produced by the s.c. administration of morphine, three times per day, at 10 mg/kg on day 1, 20 mg/kg on day 2 and 40 mg/kg on day 3. Theordinate indicates the percentage of animals that achieved an analgesic response using the TF test. One group received sham surgery plus morphine as described above. The other group received LY293558 at 45 mg/kg/24 hr via osmotic pump plus morphine as above. On day 4, cumulative morphine dose-response curves were established. On day 4, the sham plus morphine curve had shifted 2.5-fold, whereas the curves for the day 1 sham, day 1 LY293558 and day 4 LY293558 plus morphine groups were not significantly different (see table 1 for ED50 values).

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Morphine ED50 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 4</td>
</tr>
<tr>
<td>Sham + morphine (15 + 30)</td>
<td>2.6 (1.8–3.5)</td>
</tr>
<tr>
<td>LY293558-15 + morphine</td>
<td>2.3 (1.6–3.2)</td>
</tr>
<tr>
<td>LY293558-30 + morphine</td>
<td>1.9 (1.3–2.7)</td>
</tr>
<tr>
<td>Sham + morphine (45)</td>
<td>4.3 (3.4–5.1)</td>
</tr>
<tr>
<td>LY293558-45 + morphine</td>
<td>4.0 (3.3–4.9)</td>
</tr>
<tr>
<td>Sham + morphine (60)</td>
<td>4.1 (3.4–5.0)</td>
</tr>
<tr>
<td>Sham + saline</td>
<td>3.9 (2.9–5.3)</td>
</tr>
<tr>
<td>LY293558-60 + morphine</td>
<td>2.8 (2.1–3.6)</td>
</tr>
<tr>
<td>LY293558-60 + saline</td>
<td>2.8 (2.1–3.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from corresponding day 1 group.  
<sup>b</sup> Significantly different from corresponding day 4 sham + morphine group.
TABLE 3
Tolerance to the delta-1 opioid agonist DPDPE is not affected by coadministration of LY293558

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental day</th>
<th>DPDPE ED₅₀ (μg/mouse)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + DPDPE</td>
<td>1</td>
<td>3.2</td>
<td>2.3–4.3</td>
</tr>
<tr>
<td>LY 293558 + DPDPE</td>
<td>1</td>
<td>4.5</td>
<td>3.4–5.8</td>
</tr>
<tr>
<td>Sham + DPDPE</td>
<td>4</td>
<td>10.1*</td>
<td>8.0–12.6</td>
</tr>
<tr>
<td>LY 293558 + DPDPE</td>
<td>4</td>
<td>12.4*</td>
<td>9.8–15.4</td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from corresponding day 1 value.

TABLE 4
Tolerance to the kappa-1 opioid agonist U50,488H is not affected by coadministration of LY293558

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental day</th>
<th>U50,488H ED₅₀ (mg/mouse)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + U50</td>
<td>1</td>
<td>6.8</td>
<td>4.5–9.5</td>
</tr>
<tr>
<td>Sham + saline</td>
<td>1</td>
<td>8.4</td>
<td>6.0–11.9</td>
</tr>
<tr>
<td>LY 293558 + U50</td>
<td>1</td>
<td>7.2</td>
<td>4.9–10.0</td>
</tr>
<tr>
<td>Sham + U50</td>
<td>4</td>
<td>54.0*</td>
<td>40.4–71.9</td>
</tr>
<tr>
<td>Sham + saline</td>
<td>4</td>
<td>8.9</td>
<td>8.7–12.6</td>
</tr>
<tr>
<td>LY 293558 + U50</td>
<td>4</td>
<td>43.7*</td>
<td>32.0–57.9</td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from corresponding day 1 value.

Discussion

In this study, the effects of the competitive AMPA receptor antagonist LY293558 on opioid tolerance was assessed. Concurrent administration of LY293558 by continuous s.c. infusion produced a dose-dependent attenuation of morphine tolerance (table 1). When animals made tolerant to morphine were given LY293558 at 45 mg/kg/hr for 3 days, the morphine ED₅₀ was reduced and the potency of morphine returned toward day 1 control values (table 2). Because this change in the potency of morphine was not seen in sham-treated tolerant mice, it can be assumed that LY293558 reversed morphine tolerance. The effects of LY293558 on morphine tolerance cannot be attributed to effects on basal nociceptive thresholds because TF latencies were not altered by LY293558 treated mice relative to controls in morphine naïve or tolerant mice (data not shown). The highest dose of LY293558 (60 mg/kg/hr) that was effective in inhibiting and reversing morphine tolerance also did not produce motor impairment (see Results). Finally, LY293558 (60 mg/kg/hr) attenuated the convulsions produced by the AMPA-selective agonist ATTPA but not the convulsions produced by NMDA, indicating that AMPA but not NMDA receptor blockade was present after more than 3 days (88 hr) of continuous treatment with LY293558 by the same mode of administration (osmotic pump) that was used in studies demonstrating the attenuation and/or reversal of morphine tolerance (tables 1 and 2, figs. 1 and 2). In this regard, Ornstein et al. (1993) have demonstrated that doses of LY293558 that attenuate ATTPA induced convulsions do not affect NMDA-induced lethality. In both in vivo and in vitro systems, LY293558 over a range of doses produces AMPA receptor antagonism at doses that are several-fold lower than those required for NMDA receptor antagonist activity (Ornstein et al., 1993; Schoepf et al., 1995). Thus, the present study indicates that activation of AMPA receptors are involved in both the development and maintenance of morphine tolerance and that both processes may be inhibited by AMPA receptor blockade at doses that are not accompanied by motor impairment.

The consequences of the antagonism of AMPA receptors by LY293558 that result in the attenuation of morphine tolerance can only be speculated on given our incomplete understanding regarding the complex mechanisms underlying morphine tolerance. It is, however, known that stimulation of EAA receptors provides for the rapid cellular influx of monovalent and divalent cations, including Na⁺ and Ca²⁺ ions (Bettler and Mule, 1995; Bliss and Collingridge, 1993). Increased influx of Ca²⁺ (Diaz et al., 1995) and high levels of basal free intracellular Ca²⁺ (Welch and Olson, 1991) have been observed in mu opioid-tolerant rats and mice, respectively. NO levels may also be increased by intracellular Ca²⁺ activation of nitric oxide synthase. NO mediates the formation of cGMP after stimulation of EAA receptors (Vincent, 1994). Changes in cGMP hydrolysis have been reported in morphine-tolerant rats (Burton et al., 1990), and interruption of the NO/cGMP cascade by NO synthase inhibitors such as NG-nitro-L-arginine or methylene blue attenuates the development of morphine tolerance in mice (see Pasternak and Inturrisi, 1995). It is thought that EAA antagonists may inhibit morphine tolerance by decreasing the initial intracellular influx of Ca²⁺ through the NMDA-gated ion channel, thereby inhibiting the accumulation of this ion and the subsequent physiological events described above (Diaz et al., 1995; Elliott et al., 1994; Tiseo et al., 1994; Tiseo and Inturrisi, 1993). Although NMDA receptor-gated ion channels are typically regarded as the most important EAA receptor subtype regulating the effects of glutamate on the flux and levels of intracellular Ca²⁺, several recent reports indicate that native and recombinant AMPA receptor channels display relatively high Ca²⁺ permeability. Activation of AMPA receptors can produce a marked increase in cytoplasmic free Ca²⁺ (Cebers and Lijfhequeit, 1995; de Erausquin et al., 1994; Ozawa and Iino, 1993). Additionally, there is in vitro evidence to suggest that NO synthesis and cGMP production can be suppressed by AMPA receptor blockade (Marin et al., 1993). These data suggest that AMPA receptor antagonism may block morphine tolerance through mechanisms similar to those suggested for NMDA receptor antagonists (i.e., blockade of the Ca²⁺/NO/cGMP cascade). Furthermore, AMPA receptors have a highly overlapping anatomical distribution with NMDA receptors and are thought to assist in their activation by providing the postsynaptic depolarization necessary to remove the voltage-sensitive Mg²⁺ blockade of NMDA receptors (Bliss and Collingridge, 1993; Cotman et al., 1987; Patel and McCulloch, 1995). Thus, it is possible that blockade of AMPA receptors by LY293558 might reduce the Ca²⁺/NO/cGMP cascade, and morphine tolerance, indirectly via an AMPA receptor-mediated reduction in NMDA receptor activation.

Our findings with LY293558 parallel previous observa-
tions of the selective protection against the development and reversal of morphine, but not U-50,488H, analgesic tolerance by competitive and noncompetitive NMDA receptor antagonists in mice (Elliott et al., 1995; Pasternak and Inturrisi, 1995). While it is true that a greater degree of analgesic tolerance was observed for U-50,488H relative to morphine (table 4), it is not likely that the degree of U50,488H tolerance was the limiting factor since the magnitude of a reduction in the analgesic potency of DPDPE was similar to that of morphine but without similar protection against the development of tolerance to LY293558 (table 3). The differential ability of LY293558 to inhibit opioid tolerance may, however, reflect the distinct mechanisms that are presumed to mediate tolerance at mu, delta-1 and kappa-1 opioid receptors and that contribute to the lack of reciprocal cross-tolerance between selective opioid ligands (Mat\-tia et al., 1991; Von Voigtlander et al., 1983). Regardless of mechanism, our data demonstrate that AMPA receptor activation is part of the substratum underlying morphine (mu) but not DPDPE (delta-1) or U-50,488H (kappa-1) analgesic tolerance.

Previous studies (Tiseo et al., 1994; Tiseo and Inturrisi, 1993) have shown that for NMDA receptor antagonists an acceptable balance of safety and efficacy is achieved by use of the continuous s.c. infusion. Furthermore, this mode of drug administration minimizes the effects in differences in pharmacokinetics between the opioid and the EAA receptor antagonist (Tiseo and Inturrisi, 1993). It is also clear that continuous s.c. infusion of LY293558 provides systemic drug availability that is sufficient to block AMPA receptors (fig. 1). Marek et al. (1991) reported that delaying injections of the NMDA receptor antagonist MK-801 for 2 hr after morphine administration was still effective in blocking the development of morphine tolerance. Given the role of NMDA receptors in neuronal plasticity, the authors suggested that morphine tolerance results from enduring or delayed changes caused by the activity of EAA receptors. AMPA receptors have also been reported to mediate long-term central nervous system changes (Bettler and Mulle, 1995; Shahi and Baudry, 1993). Perhaps the enduring or delayed changes underlying the development of morphine tolerance are subserved by long-lasting physiological events that are particularly sensitive to AMPA receptor blockade. In such a case, the continued presence of LY293558, released continuously via a pump, would be effective in inhibiting morphine tolerance.

The present data suggest that activation of AMPA receptors during chronic morphine treatment may contribute to analgesic tolerance. Because NMDA receptor antagonists are also effective in attenuating the development of morphine tolerance (Elliott et al., 1995; Pasternak and Inturrisi, 1995), the present data indicate that activation of more than one EAA receptor system mediates the development of morphine tolerance. Most NMDA receptor antagonists and the AMPA receptor antagonist LY293558 cannot attenuate kappa-1 opioid agonist tolerance (Elliott et al., 1994, 1995). The AMPA receptor antagonist LY293558 also is not effective in attenuating DPDPE (delta-1 opioid receptor) induced analgesic tolerance. Collectively, these findings suggest that AMPA and NMDA receptors make similar but not identical contributions to the development of opioid tolerance. The elucidation of the relative contribution of EAA receptor sys-tem to opioid tolerance could provide a new understanding of the interactions of EAAs and opioid receptor systems that result in the neuronal adaptations that are manifest behaviorally as tolerance to opioid anal-

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