Antiallodynic and Antihyperalgesic Effects of Selective Competitive GLU$_{K5}$ (GluR5) Ionotropic Glutamate Receptor Antagonists in the Capsaicin and Carrageenan Models in Rats

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ABSTRACT

GLU$_{K5}$ kainate receptor subunits are abundant in pain pathways, including dorsal root ganglia and spinthalamic neurons, as well as in the thalamus and brain stem. A growing body of evidence indicates that the GLU$_{K5}$ kainate receptor subtype plays a prominent role in pain transmission, particularly in persistent pain. In the present studies, compounds from a novel series of amino acid GLU$_{K5}$ receptor antagonists were evaluated for their effectiveness in reversing capsaicin-induced mechanical allodynia as well as carrageenan-induced thermal hyperalgesia. In vitro, the amino acid compounds were efficacious in blocking glutamate-evoked calcium flux in cells expressing GLU$_{K5}$ but not GLU$_{K6}$ or GLU$_{A2}$ homomeric receptors. Electrophysiologically, the compounds exhibited selectivity for kainate receptors in dorsal root ganglion cells relative to $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and N-methyl-D-aspartate (NMDA)-insensitive and kainate-preferring (Seeburg, 1993; Hollmann and Heinemann, 1994). Activation of these ionotropic receptors is important for normal central nervous system functions such as synaptogenesis, synaptic plasticity, and the development of functional neural circuits. However, excessive levels of glutamate may be responsible for pathological central nervous system processes, including neurodegeneration following stroke and ischemia and, abnormally processing of pain-related information (McBain and Mayer, 1994). Therefore, the development of ionotropic glutamate receptor antagonists has been viewed as a potentially important therapeutic strategy for the treatment of many neurological disorders.

Glutamate is a major excitatory neurotransmitter in pri-
mary sensory afferent pathways (e.g., Fundytus, 2001). For example, noxious stimulation, such as the administration of formalin into the hindpaw, increases the release of glutamate and aspartate from dorsal horn neurons (Skilling et al., 1988). The persistent release of glutamate in pain pathways can lead to the development of central sensitization, characterized by altered responsiveness of dorsal horn and thalamic neurons, expansion of receptive fields, and plasticity of neuronal connections (e.g., Coderre, 1993; Urban et al., 1994).

Furthermore, repetitive C-fiber stimulation produces a “wind-up” of dorsal horn neuron activity that is mimicked by the application of L-glutamate (Zieglgansberger and Herz, 1971) and NMDA (King et al., 1988). Blockade of the activation of postsynaptic ionotropic receptors has been shown to produce antinociception and decrease central sensitization (e.g., Coderre and van Empel, 1994).

Evidence has begun to accumulate indicating that GLU$_{K5}$ glutamate receptors play an important role in nociception and central sensitization (for review, see Ruscheweyh and Sandküler, 2002). Kainate receptors are present on small diameter C-fibers, and GLU$_{K5}$ receptors have been identified on dorsal root ganglion cells as well as in the spinal cord on spinothalamic tract neurons (e.g., Agrawal and Evans, 1986; Tölle et al., 1993; Furuyama et al., 1993). Nonselective AMPA/kainate receptor antagonists, including NBQX, 6-cyano-7-nitroquinoxaline-2,3-dione, and NS1209, have been shown to produce antinociception in a variety of animal models of acute and persistent pain (e.g., Jackson et al., 1995; Pogatzki et al., 2003; Blackburn-Munro et al., 2004). However, Simmons et al. (1998) demonstrated that the relatively selective GLU$_{K5}$ antagonist LY382884 as well as the nonselective AMPA/kainate receptor antagonists NBQX and LY293558 but not the nonselective AMPA receptor antagonist LY300164, produced antinociception in the formalin test in rats. LY382884 also attenuated the responses of spinothalamic tract neurons to mechanical and thermal stimuli in normal and neuropathic monkeys (Palecek et al., 2004). Recently, Ko et al. (2005) reported that in GLU$_{K5}$-deficient mice, responses to capsaicin and inflammatory pain were substantially reduced. In addition, the mixed AMPA/GLU$_{K5}$ receptor antagonist LY293558 has been demonstrated to produce analgesia in the capsaicin model in humans (Sang et al., 1998) and is efficacious in acute migraine (Sang et al., 2004) and in postdental surgery pain (Gilron et al., 2000). Taken together, these data suggest that GLU$_{K5}$ receptors play an important role in persistent, but not acute, pain. However, most of the studies to date have been conducted using compounds that lack a high degree of selectivity for GLU$_{K5}$ versus AMPA or GLU$_{K6}$ receptors, therefore limiting the strength of this conclusion.

We recently described a new series of decahydroisoquinoline GLU$_{K5}$-selective competitive antagonists (Dominguez et al., 2005) with compounds that have greater potency and selectivity than previous compounds in this pharmacologic class. The improved selectivity of these compounds allows for a more definitive examination of the role of GLU$_{K5}$ signaling in persistent pain states. The purpose of the present experiments was to evaluate the efficacy of these newer antagonists in models of C-fiber activation and inflammatory persistent pain. Concentration-response curves were determined for representative compounds (see Fig. 1) for antagonizing glutamate-induced calcium influx in HEK293 cells stably expressing GLU$_{K5}$, GLU$_{K6}$, or GLU$_{A2}$ homomeric receptors. The selectivity of representative antagonists for blocking kainate-induced currents at the native GLU$_{K5}$ receptors in DRG cells relative to AMPA- or NMDA-induced currents in hippocampal pyramidal cells was determined electrophysiologically. Dose-response curves were also determined after s.c. administration in the capsaicin and carrageenan models in rats. Because the amino acid antagonists exhibited low effi-

![Fig. 1. Structures of competitive Glu$_{K5}$ ionotropic glutamate receptor antagonists.](image-url)
cacy after systemic administration, dose-response curves also were determined for selected compounds after direct intracellular administration. To improve oral bioavailability, ester prodrugs of the parent amino acids were prepared (Dominguez et al., 2005; see also Fig. 1) and dose-response curves determined after oral administration.

Materials and Methods

Cell Culture. All cell and tissue culture reagents were from Invitrogen (Grand Island, New York). Recombinant human glutamate receptors [GLU (flip), GLUT (Q), or GLUT (Q)] were stably expressed in HEK293 cells. Cells were grown as monolayers under 5% CO2 at 37°C. Medium used for GLUT and GLUT-expressing cells was minimum essential medium (catalog no. 11095-080; Invitrogen), with 10% fetal bovine serum and 250 µg/ml genitin added. Medium for GLUTA cells was Dulbecco’s modified Eagle’s medium (catalog no. 11965-092; Invitrogen), with 5% fetal bovine serum, 250 µg/ml hygromycin, 1000 units/ml penicillin G sodium, and 1 mg/ml streptomycin sulfate added.

Measurement of Calcium Influx Using Fluoro-3. Cells were seeded into poly-d-lysine-coated 96-well plates (Becton Dickinson Labware, Bedford, MA) 1 or 2 days before experiments at a density of 60,000 cells/well (1 day) or 30,000 cells/well (2 days). Cells were washed three times with 100 µl of assay buffer composed of Hank’s balanced salt solution without phenol red (Invitrogen) with 20 mM HEPES and 3.7 mM CaCl2 added (final [CaCl2] = 5 mM). Plates were then incubated for 2 to 3 h in the dark at room temperature in 40 mM MgCl2, and 10 mM HEPES, and 10 mM glucose, pH 7.4, with 200 µM kainate receptors. Finally, 50 µl of concanavalin A-containing assay buffer was added to wells and fluorescence measured using a fluorometric imaging plate reader (Molecular Devices, Sunnyvale, CA). A first addition of 50 µl of concanavalin A-containing assay buffer was followed by a second addition of 100 µl of concanavalin A-containing assay buffer 3 min later. Test compounds were added in the absence of agonist during the first addition and in the presence of glutamate during the second addition. Glutamate concentration was 100 µM when testing compounds at GLUTQ or GLUT receptor and 200 µM when testing compounds at GLUTA (approximate EC50 concentrations). Concanavalin A was omitted from experiments using GLUTA receptors.

Electrophysiological Recording Conditions. Whole-cell voltage clamp recordings (Vh = -70 mV) were made from single cells using the tight-seal whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Glass fragments of coverslips with adherent cells were placed in a perfusion chamber and rinsed with buffer of the composition: 140 mM NaCl, 5 mM CaCl2, 5 mM KCl, 1 mM MgCl2, and 10 mM HEPES, and 10 mM glucose, pH 7.4, with NaOH (osmolality, 315 mosm/kg). Pipette solutions contained 140 mM CsCl, 1 mM MgCl2, 14 mM d/Tris creatine phosphate, 50 U/ml creatine phosphokinase, 14 mM MgATP, 10 mM HEPES, and 15 mM BAPTA, pH 7.2, with CsOH (osmolality, 295 mosm/kg). Experiments were performed at room temperature (20–22°C) and recorded on an Axopatch 200A amplifier using pClamp 8.0 Software (Axon Instruments Inc., Union City, CA). Pipette resistance was typically 1.5 to 2.5 MΩ. Drug application was via a multibarreled perfusion array. IC50 values for compounds were evaluated using 30 µM kainate, 30 µM AMPA, or 10 µM NMDA. Data are expressed as mean ± S.E.M. (n = 3–7).

Kainate currents were measured in acutely prepared isolated DRG from P4 to P7 rat neonates as described previously (Bortolotto et al., 1999) in the presence of 250 µg/ml concanavalin A to prevent agonist-induced desensitization. AMPA and NMDA currents were measured in cultured hippocampal pyramidal neurons prepared from E17 rat embryos as described previously (Bleakman et al., 1999; Bortolotto et al., 1999). NMDA currents were activated by application of NMDA in the absence of magnesium in the perfusion buffer with added glycine (10 µM).

Subjects. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 250 to 290 g for the capsaicin experiments, 200 to 225 g for the Rotorod test, and 70 to 90 g for the carrageenan experiments were used. Rats were housed in groups of up to six per cage in a large colony room on a 12-h light/dark cycle (lights on 6:00 A.M.), with food and water provided ad libitum. Each animal was used only once. Test sessions were conducted between 8:00 A.M. and 6:00 P.M. All treatment or dose groups consisted of six to 10 rats. All experiments were conducted in accordance with the National Institutes of Health regulations of animal care covered in Principles of Laboratory Animal Care, National Institutes of Health publication 85-23, and were approved by the Institutional Animal Care and Use Committee.

Capsaicin-Induced Mechanical Allodynia. Groups of six rats were injected s.c. with vehicle or a dose of drug 15 min before capsaicin (30 µg in 25 µl) was injected into the plantar surface of the right hindpaw. Ten minutes after the injection of capsaicin, mechanical hyperalgesia was evaluated with a calibrated series of von Frey filaments using the up-and-down method of Chaplan et al. (1994). In brief, rats were placed in clear plastic cages (17.5 x 15 x 15 cm) fitted with wire mesh flooring and allowed to acclimate for approximately 5 min; the withdrawal thresholds were determined by the up-and-down method (Chaplan et al., 1994) by applying each filament of a graded series of filaments to the midplantar surface of each hindpaw in a perpendicular fashion at 5, 10, and 15 mm from the primary injection site and depressed slowly (4–5 s) until bending occurred, and the maximum force of the fiber was exerted. Any paw withdrawal response in the 5, 10, or 15 mm region outside the primary site of injection was scored as a response to the filament.

For intracranial injections, animals were lightly anesthetized with isoflurane, and the back of the rat’s head was shaved. The head of the animal was placed perpendicular to the body axis as a 25-gauge needle, attached to a 25-glass syringe, was inserted to a depth of 5 mm from the surface of the skin into the cisterna magna. A 10-µl injection was delivered over approximately 10 s, and the needle was held in place for an additional 10 s before being withdrawn. Intracranial injections were administered approximately 15 min before the intraplantar injection of capsaicin, and animals were tested 15 min after capsaicin administration.

Carrageenan-Induced Thermal Hyperalgesia. Groups of six rats were injected s.c. with a-carrageenan (100 µl of a 1.5% solution) into the plantar surface of the right hindpaw at time 0 followed 90 min later by a p.o. or i.p. injection of vehicle or a dose of drug. Withdrawal responses to mechanical and thermal stimuli were determined after approximately an additional 30 and 40 min, respectively. Withdrawal latencies to a noxious thermal stimulus were assessed using a modification of the methods of Hargreaves et al. (1988). Each rat was placed in a Plexiglas cube with a glass floor through which an infrared photobeam was projected onto the plantar surface of the hindpaws, and the latency to withdrawal from the thermal stimulus was determined. The intensity of the infrared photobeam from the plantar reflex device (Plantar Test; Ugo Basile, Comerio, Italy) was adjusted to produce a mean response latency in untreated rats of approximately 12 to 15 s and terminated automatically after 27 s in the absence of a response. The response latency was determined using a timer linked to the photodiode motion sensors in the plantar reflex device. Response latency was defined as the time from the onset of exposure to the infrared photobeam to the cessation of the photobeam when the photodiode motion sensors detected the withdrawal response of the paw of the rat. Response latency was calculated as the difference in withdrawal latency between the treated and untreated paws in seconds and was calculated using the following formula: withdrawal latency of the
carrageenan-treated paw — withdrawal latency of the untreated paw.

Roto rod Test. Twenty-four hours before compound testing, rats were given three training trials to maintain posture on an accelerating rod (OmniTec Electronics Inc., Columbus, OH), 17 rpm for 5 s and maintaining that speed for 40 s (Simmons et al., 1998). The following day, Roto rod testing was conducted at time points corresponding to the pain testing. Animals that did not fall off the Roto rod were given a maximum score of 40 s. Compounds were evaluated over several doses, varying from a dose that was without effect on the Roto rod and increasing in 2- or 3-fold steps until a dose that produced a statistically significant motor impairment, or 100 mg/kg, was reached.

Drugs. LY293558, LY377770, LY382884, and compounds 1, 2a, 2b, 3a, 3b, 4a, 4b, 5, and 6 (Lilly Research Laboratories; see Fig. 1) were dissolved in distilled water or 5% solutol. Morphine sulfate (Sigma/RBI, Natick, MA) and α-carrageenan (Sigma) were dissolved in double-deionized water. Doses refer to the form of the drug listed. All drugs were administered s.c. or p.o. by gavage in a volume of 1.0 ml/kg or intracisternally in a volume of 10 μl. Capsaicin (Sigma) was prepared as a 3 mg/2.5 ml solution, dissolved in olive oil (Sigma), and sonicated for 25 min in a 45°C water bath.

Statistical Analysis. Data were expressed as means ± S.E.M. Affinities of test compounds in transfected cell lines were determined from concentration-response curves for antagonism of glutamate-evoked Ca2+ influx. The curves were analyzed using GraphPad Prism 3.02 software (GraphPad Software Inc., San Diego, CA), with slope factor not fixed and top and bottom fixed at 100 and 0% inhibition, respectively. The dissociation constant (Kb) was calculated from the IC50 values for inhibiting glutamate-induced Ca2+ influx according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973): Kb = IC50/1 + [Glutamate]/EC50 Glu, where [Glutamate] is the concentration of glutamate (100 or 200 μM), and EC50 Glu is the EC50 value of glutamate for evoking calcium influx in the given cell line, determined from glutamate concentration-response curves run in the same plates as the antagonist concentration-response curves. In vivo, treatment groups were compared with appropriate control groups using one-way analysis of variance and Dunnett’s t test. Statistical analyses were performed using JMP statistical software (SAS Institute Inc., Cary, NC). ED50 values and 95% confidence limits were determined using GraphPad Prism. A probability of p ≤ 0.05 was taken as the level of statistical significance.

Results

Ion Flux in HEK293 Cells. To determine the selectivity of this series of amino acid agonists for GLUA5 versus GLUK5 or AMPA receptors, the ability of compounds to inhibit glutamate-evoked calcium influx was measured in HEK293 cells stably expressing cloned GLUK5, GLUK6, or GLUA2. Table 1 shows calculated Kb values for the antagonists at each cell line.

In the GLUK5-expressing cell line, the rank order of potency was compound 6 (Kb = 3 nM) ≈ 4a > 5 > 3a > 2a > LY377770 = 4b (prodrug of 4a) ≈ LY293558 ≈ LY382884 ≈ 2b (prodrug of 2a) > 3b (prodrug of 3a; Kb = 3 μM). Compound 1 (prodrug of LY382884) had no effect at GLUK5 receptors at concentrations up to 100 μM.

In GLUA2-expressing cells, the antagonists displayed potency in the order of LY293558 (Kb = 0.4 μM) ≈ 3a > 2a ≈ 4a ≫ LY377770 ≈ 2b, LY382884, 1, 3b, 4b, 5, and 6 each produced no significant inhibition in GLUA2-expressing cells at concentrations up to 100 μM. None of the compounds tested had any significant effect in GLUK5-expressing cells at concentrations up to 100 μM.

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>GLUA2 IC50 (μM)</th>
<th>GLUK5 IC50 (μM)</th>
<th>GLUA2 IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LY293558</td>
<td>0.4 (0.2–0.7)</td>
<td>0.5 (0.2–0.8)</td>
<td>–</td>
</tr>
<tr>
<td>LY377770</td>
<td>[35 ± 2]</td>
<td>0.09 (0.04–0.24)</td>
<td>–</td>
</tr>
<tr>
<td>LY382884</td>
<td>–</td>
<td>1.1 (0.6–2.1)</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2a</td>
<td>1.1 (0.6–2.0)</td>
<td>0.04 (0.03–0.05)</td>
<td>–</td>
</tr>
<tr>
<td>2b</td>
<td>[25 ± 8]</td>
<td>2 (1–4)</td>
<td>–</td>
</tr>
<tr>
<td>3a</td>
<td>0.4 (0.1–1.1)</td>
<td>0.03 (0.02–0.06)</td>
<td>–</td>
</tr>
<tr>
<td>3b</td>
<td>–</td>
<td>3 (2–4)</td>
<td>–</td>
</tr>
<tr>
<td>4a</td>
<td>2 (1–3)</td>
<td>0.005 (0.002–0.009)</td>
<td>–</td>
</tr>
<tr>
<td>4b</td>
<td>–</td>
<td>0.2 (0.1–0.4)</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>0.02 (0.01–0.03)</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>0.003 (0.001–0.010)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 1**

Potency (Kb) values of competitive ionotropic glutamate receptor antagonists and prodrugs for blocking glutamate-induced calcium influx at cloned human ionotropic receptor AMPA and kainate receptor subtypes

Values given represent the mean (and 95% confidence intervals) Kb from three to four experiments, each carried out in triplicate. Numbers in brackets are percent inhibition and S.E.M. at 100 μM when the magnitude of the inhibition was statistically significant but less than 50%.

Electrophysiological Recordings. To confirm antagonist potency and selectivity for GLUK5 versus AMPA receptors in native tissues, as well as to determine the selectivity for GLUK5 versus NMDA receptors, selected compounds were examined for their ability to antagonize kainate-evoked inward currents in rat DRG neurons and AMPA- and NMDA-evoked currents in cultured rat hippocampal neurons (Fig. 2). Compound 2a antagonized kainate-evoked currents in rat DRG with IC50 = 0.15 ± 0.06 μM and AMPA- and NMDA-evoked currents in hippocampal neurons with IC50 values of 2.1 ± 0.8 and 90 ± 41 μM, respectively. Compound 3a blocked kainate-, AMPA-, and NMDA-evoked currents with...
IC$_{50}$ values of 0.18 ± 0.11, 1.4 ± 0.9, and 1.9 ± 1.6 µM, respectively.

LY293558, LY377770, and LY382884 s.c. in Capsaicin Test. LY293558 is a mixed AMPA/KA receptor antagonist (Table 1), whereas LY377770 and LY382884 are relatively selective GLU$_{K_5}$ receptor antagonists. All three compounds produced dose-related reduction in mechanical allodynia in the capsaicin test (Fig. 3). LY293558 was somewhat more potent than the other two compounds (Table 2), and doses of 5.6 and 10 mg/kg s.c. produced effects that were significantly different from vehicle. LY377770 and LY382884 were approximately equipotent to each other (Table 2) with a dose of 10 mg/kg s.c. or higher, producing statistically significant effects.

LY382884 s.c. and Compound 1 p.o. in Capsaicin Test. We have reported previously that amino acid compounds such as LY382884 have poor oral bioavailability. Therefore, ester prodrugs were synthesized to increase oral bioavailability (Dominguez et al., 2005). The isobutyl ester of LY382884, compound 1, had virtually no measurable affinity for GLU$_{K_5}$ receptors (Table 1) but produced a dose-related antiallodynic effect in the capsaicin test, with doses of 10 and 30 mg/kg producing statistically significant effects (Fig. 4). However, the effects of the prodrug administered p.o. were relatively modest in magnitude when compared with the complete reversal of capsaicin-induced allodynia produced by the parent compound LY382884 administered s.c.

Oral Efficacy of Amino Acids and Prodrugs. Several additional amino acid compounds (see Fig. 1 and Table 1) were synthesized, which had relatively high affinity and selectivity for GLU$_{K_5}$ receptors; however, these compounds were largely inactive after oral administration (data not shown). Ester prodrugs (Fig. 1) of these amino acid compounds were therefore prepared. Ester prodrugs of the amino acids were largely devoid of affinity for GLU$_{K_5}$ receptors (Table 1). However, when administered orally, ester prodrugs produced dose-related reversal of capsaicin-induced allodynia and, unlike 1, produced a complete reversal of capsaicin-induced allodynia. The approximate rank order of potencies of the prodrugs was compound 2b = 4b > 3b > 1 (Fig. 5; Table 2).

Comparison of Amino Acid Decahydroisoquinolines Administered s.c. and Intracisternally. In addition to being relatively inefficacious after oral administration, a number of amino acid GLU$_{K_5}$ antagonists, with a range of affinities for the GLU$_{K_5}$ receptor, were also ineffective in the capsaicin test after s.c. administration (Fig. 6, left). LY382884 produced a dose-related reversal of allodynia over the dose range of 1.0 to 30 mg/kg s.c., and compound 5 was effective at a dose of 100 mg/kg s.c., whereas compound 6 had little or no efficacy over the dose range of 3.0 to 30 mg/kg s.c., even though the latter compound has a higher affinity than LY382884 for GLU$_{K_5}$ receptors (Table 1). The lack of efficacy of these amino acid compounds raised the question of whether the efficacy was due to activity at receptors other

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Capsaicin Test</th>
<th>Carrageenan Test</th>
<th>Rotorod</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$ED_{50}$ mg/kg</td>
<td>$MED$ mg/kg</td>
<td></td>
</tr>
<tr>
<td>LY293558</td>
<td>SC</td>
<td>4.0 (2.5–6.6)</td>
<td>nt$^a$</td>
<td>10</td>
</tr>
<tr>
<td>LY377770</td>
<td>SC</td>
<td>6.3 (5.5–7.2)</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>LY382884</td>
<td>SC</td>
<td>7.6 (5.9–9.8)</td>
<td>&gt;100$^b$</td>
<td></td>
</tr>
<tr>
<td>1$^c$</td>
<td>PO</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>2a</td>
<td>SC</td>
<td>&gt;10</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>2b$^d$</td>
<td>PO</td>
<td>1.2 (0.7–1.5)</td>
<td>2.7 (2.5–3.0)</td>
<td>20</td>
</tr>
<tr>
<td>3a</td>
<td>SC</td>
<td>&gt;10</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>3b$^d$</td>
<td>PO</td>
<td>5.1 (3.7–7.0)</td>
<td>(2.3) (1.5–3.4)</td>
<td>20</td>
</tr>
<tr>
<td>4a</td>
<td>SC</td>
<td>&gt;10</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>4b$^d$</td>
<td>PO</td>
<td>1.6 (1.4–1.8)</td>
<td>0.3 (0.2–0.5)</td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$ nt, not tested.

$^b$ From Simmons et al., 1998

$^c$ Prodrug of compound listed immediately above.

### Figure 3

Dose-related reversal of capsaicin-induced mechanical allodynia by LY382884 s.c. and the ester prodrug 1 after oral administration in rats. Each point represents the mean of six rats. Vertical lines represent ± S.E.M. and are absent when less than the size of the point. Points above V represent the effects of vehicle. Points above 10 M represent the effects of drug in mg/kg; ordinate, withdrawal threshold in grams to a mechanical stimulus.

### Figure 4

Dose-related reversal of capsaicin-induced mechanical allodynia by LY382884 s.c. and the ester prodrug 1 after oral administration in rats. Each point represents the mean of six rats. Vertical lines represent ± S.E.M. and are absent when less than the size of the point. Points above V represent the effects of vehicle. Points above 10 M represent the effects of drug in mg/kg; ordinate, withdrawal threshold in grams to a mechanical stimulus. *, p < 0.05 versus vehicle, Dunnett’s t test.
with a range of affinities for the GLUK5 receptor, after intra-cavity in the capsaicin test of these amino acid antagonists, potencies determined in vitro. We therefore evaluated the effi-vivo might be expected to differ from the rank order of po-
central administration, and/or the rank order of potencies in
compound
compound
administration, whereas if the efficacy of LY382884 and com-
prodrug esters of competitive GluK5 antagonists after oral admin-
ceptivities in vivo after central administration was the same as the
ries in vivo after central administration was the same as the
rank order of potencies at GLUK5 receptors in vitro.

carrageenan-induced Thermal Hyperalgesia. The ef-
cificacy of the prodrg esters was also evaluated after oral
administration on carrageenan-induced thermal hyperalge-
sia. The prodrg esters examined all produced dose-related
antihyperalgesic effects (Fig. 7), except compound 2b pro-
duced a virtually complete reversal of thermal hyperalgesia;
it is possible compound 2b would have produced greater
efficacy had higher doses been tested. The rank order of
potencies in the carrageenan test was compound 4b > 3b 2b (Fig. 7; Table 2). The rank order of potencies of the
prodrugs in reversing thermal hyperalgesia was similar to that
for the binding affinities of the parent compounds to
cloned GLUK5 receptors in vitro and for reversing capsaicin-
induced mechanical allodynia (see Fig. 5).

Rotorod Test. After s.c. administration, the lowest dose of
LY293558 that produced motor impairment was 10 mg/kg (Table 2), a dose approximately 2.5-fold larger in magnitude
than the ED50 in the capsaicin test. For the prodrugs, the
minimal effective dose (MED) values ranged from 3 mg/kg for
compound 4b to 20 mg/kg for compounds 2b and 3b, >30
goingle for compound 1. In general, the MED values in the
Rotorod test were approximately 4- to 20-fold higher than the
ED50 values in the capsaicin or carrageenan tests (Table 2).

Discussion

The present studies evaluated the antimechanical allo-
dynic and antithermal hyperalgesic effects of a series of
amino acid decahydroisoquinoline GLUK5 receptor competi-
tive antagonists in the capsaicin and carrageenan tests in
rats. Although these antagonists were efficacious when ad-
ministered intracisternally, the majority of the amino acid
GLUK5 receptor antagonists were ineffective after either oral
or s.c. administration, suggesting that they had very low oral
bioavailability, poor blood-brain barrier penetration, or both.
We have previously reported that these amino acid decahy-
droisoquinoline compounds have very low oral bioavailable-
Dominguez et al., 2005). We therefore evaluated ester pro-
drugs that are well absorbed after oral administration and
deliver the parent compound in plasma (see also Dominguez
et al., 2005). The diethyl, isobutyl, or 2-ethylbutyl ester pro-
drugs tested herein were efficacious in reversing mechanical
alldynia in the capsaicin assay after oral administration,
The present studies replicate and extend previous findings that GLUK_5 receptor antagonists are efficacious in reversing mechanical allodynia and/or thermal hyperalgesia induced by direct stimulation of C-fiber afferents by capsaicin or by carrageenan-induced inflammation. Turner et al. (2003) reported that the desensitizing kainate receptor agonist SYM 2081 reduced the frequency of hindlimb withdrawal to a normally non-noxious mechanical stimulus and increased the latency to a thermal stimulus. Moreover, SYM 2081 was efficacious after i.t. administration in the capsaicin test (Turner et al., 2003). SYM 2081 also reversed ongoing carrageenan-induced mechanical allodynia and partially reduced ongoing heat hyperalgesia (Turner et al., 2003). Likewise, Guo et al. (2002) found that the i.t. administration of LY382884, as well as NBQX and NS-102, attenuated thermal hyperalgesia induced by complete Freund’s adjuvant. In addition, in the present study, the prodrugs were efficacious at doses 4- to 20-fold lower than the minimal dose that caused motor impairment on the Rotorod, replicating and extending
similar findings in previous studies (Simmons et al., 1998; Blackburn-Munro et al., 2004), indicating that the effects of these antagonists are not simply due to motor impairment. Thus, the preponderance of evidence indicates that selective antagonists of GLUK5 receptors, or desensitization by selective GLUK5 agonists, can effectively reduce allodynia or hyperalgesia produced by direct stimulation of C-fibers or inflammation at doses that do not produce motor impairment.

A growing body of evidence also indicates that selective GLUK5 receptor antagonists, and possibly desensitizing agonists, are also efficacious in other persistent or neuropathic pain states. As mentioned previously, Simmons et al. (1998) demonstrated that the relatively selective GLUK5 antagonist LY382884 as well as the mixed AMPA/kainate receptor antagonists NBQX and LY293558, but not the nonselective AMPA antagonist LY300164, produced antinociception in the formalin test in rats. Furthermore, Procter et al. (1998) demonstrated that LY382884 and LY294486 (the racemate of LY377770), but not the AMPA receptor-selective antagonist GYKI 53655, reduced nociceptive responses recorded electrophysiologically from hemisected spinal cords from neonatal rats in vitro as well as from dorsal horn neurons in adult rats in vivo and in the hot-plate test in conscious mice. Moreover, Ta et al. (2000) found that SYM-2081 reduced mechanical allodynia and thermal hyperalgesia in a freeze nerve injury model of neuropathic pain. In addition, LY382884 attenuated the responses of spinothalamic tract neurons to mechanical and thermal stimuli in normal and neuropathic monkeys (Pacecek et al., 2004). Recently, Ko et al. (2005) reported that in GLUK5-deficient mice, responses to capsaicin and inflammatory pain were substantially reduced. Taken together, the data indicate that GLUK5 receptors play an important role in a variety of persistent pain states and that GLUK5 receptor antagonists may thus be efficacious in the treatment of persistent pain states.

In summary, the present studies compared a series of amino acid selective GLUK5 antagonists and their ester prodrugs in reversing mechanical allodynia and thermal hyperalgesia induced by capsaicin and carrageenan. The amino acid parent compounds, in general, exhibited little efficacy after oral or s.c. administration but were efficacious after intracranial administration. In contrast, the ester prodrugs generally were efficacious in both the capsaicin and carrageenan tests after oral administration. The present findings replicate and extend previous observations that selective GLUK5 receptor antagonists produce antinociceptive effects in persistent pain models including the capsaicin and carrageenan tests, in addition to the formalin test and neuropathic pain models. Furthermore, taken together with previous reports, the site of action of GLUK5 antagonists in producing antinociception appears to be both supraspinal and spinal. The present findings thus provide further evidence that GLUK5 receptors are probably involved in mechanisms of central sensitization such as observed in persistent pain states and suggest they may have therapeutic utility in the clinical treatment of persistent pain states.

References
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