JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601

JPET #105601

Anti-allodynic and anti-hyperalgesic effects of selective competitive GLU_{K5} (GluR5) ionotropic glutamate receptor antagonists in the capsaicin and carrageenan models in rats

by

Carrie K. Jones, Andrew Alt, Ann Marie Ogden, David Bleakman, Rosa M. A. Simmons, Smriti Iyengar, Esteban Dominguez, Paul L. Ornstein and Harlan E. Shannon

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 USA (CKJ, AA, AMO, DB, RMAS, SI, PLO, HES)

Centro de Investigación Lilly, S. A. Avda. De la Industria 30, 28108-Alcobendas, Madrid, Spain (ED)

Running title: Reversal of allodynia and hyperalgesia by GLU_{K5} antagonists

Corresponding Author:
Harlan E. Shannon, Ph.D.
Lilly Research Laboratories
Lilly Corporate Center
Indianapolis, IN 46285
Phone: 317-276-4360
Fax: 317-651-6147
E-mail: H.Shannon@Lilly.Com

Text pages: 21
Tables: 2
Figures: 7
References: 39
Words in Abstract: 245
Words in Introduction: 750
Words in Discussion: 1357

Abbreviations:

AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid hydrobromide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; SYM-2081, 2*S*,4*R*)-4-methyl glutamic acid; DRG, dorsal root ganglion; HEK, human embryonic kidney; NMDA, N-methyl-D-aspartate; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline; LY300164, (R)-7-Acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo[4,5-H][2,3]benzodiazepine; NS1209, (R,S)-8-methyl-5-(4-(N,N-dimethylsulfamoyl) phenyl)-6,7,8,9,-tetrahydro-1H-pyrrolo[3,2-h]-isoquinoline-2,3-dione-3-O-(4-hydroxybutyrate-2-yl)oxime; SYM 2081, 2S,4R-4-methylglutamate; NS-102, 6,7,8,9-tetrahydro-5-nitro-1*H*-benz[g]indole-2,3-dione 3-oxime

Abstract

GLU_{K5} kainate receptor subunits are abundant in pain pathways, including dorsal root ganglia and spinothalamic neurons, as well as in 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 AMPA and NMDA receptors in hippocampal pyramidal neurons. The amino acid compounds were poorly efficacious in the pain tests after s.c. or p.o. administration. However, compounds were highly efficacious after central intracisternal administration, and the rank order of potencies correlated with their rank order of affinities at GLU_{K5} receptors determined in vitro, indicating that the lack of activity after systemic administration was due to poor oral bioavailability. In order to increase oral bioavailability, isobutyl or 2-ethylbutyl ester prodrugs of the parent amino acids were prepared. The prodrugs, which produced robust plasma levels of parent amino acids, were highly efficacious in the capsaicin and carrageenan tests. The present studies provide further evidence that selective Glu_{K5} kainate receptor subtype antagonists can reverse allodynia and hyperalgesia, particularly in persistent pain states.

Introduction

Ionotropic glutamate receptors are ion channel-coupled receptors that are classified based on their activation by specific agonists: NMDA, AMPA (McBain and Mayer, 1994) and kainate receptors (e.g., Hollmann and Heinemann, 1994). Moreover, multiple receptor subunit proteins for each class of ionotropic receptors have been identified and cloned (Hollmann and Heinemann, 1994). Of the AMPA and kainate ionotropic glutamate receptor subunit proteins cloned, $GLU_{A1–A4}$ are AMPA-sensitive, while $GLU_{K5–K7}$ and $GLU_{K1–K2}$ are AMPA-insensitive and kainate-preferring (Seeburg, 1993; Hollmann and Heinemann, 1994). Activation of these ionotropic receptors is important for normal CNS functions such as synaptogenesis, synaptic plasticity, and the development of functional neural circuits. However, excessive levels of glutamate may be responsible for pathological CNS processes, including neurodegeneration following stroke and ischemia, and, abnormal 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 primary 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). Further, 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 (see also review by 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, CNQX 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 post-dental 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 Figure 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 efficacy after systemic administration, dose-response curves also were determined for selected compounds after direct intracisternal administration. In order to improve oral bioavailability, ester prodrugs of the parent amino acids were prepared (Dominguez et al., 2005; see also Figure 1) and dose-response curves determined after oral administration.

Methods

Cell culture. All cell and tissue culture reagents were from Invitrogen (Grand Island, New York). Recombinant human glutamate receptors (GLU_{A2} (flip), GLU_{K5}(Q), or GLU_{K6}(Q)) were stably expressed in HEK293 cells. Cells were grown as monolayers under 5% CO_2 at 37°C. Medium used for GLU_{K5}- and GLU_{K6}-expressing cells was Minimum Essential Medium (Invitrogen, catalog #11095-080), with 10% fetal bovine serum and 250 µg/ml geneticin added. Medium for GLU_{A2} cells was Dulbecco's Modified Eagle Medium (Invitrogen, catalog #11965-092), with 5% fetal bovine serum, 250 µg/ml hygromycin, 1,000 units/ml penicillin G sodium and 1 mg/ml streptomycin sulfate added.

Measurement of calcium influx using Fluo-3. Cells were seeded into poly-D-lysine-coated 96-well plates (Becton Dickinson Labware, Bedford, MA) 1 or 2 days prior to experiments at a density of 60,000 cells/well (1 day) or 30,000 cells/well (2 day). Cells were washed 3 times with 100 µl assay buffer composed of Hanks Balanced Salt Solution without phenol red (Invitrogen) with 20 mM HEPES and 3.7 mM $CaCl_2$ added (final [$CaCl_2$] = 5 mM). Plates were then incubated for 2-3 hours in the dark at room temperature in 40 µl assay buffer with 8 µM Fluo3-AM dye (Molecular Probes Inc., Eugene, OR). Following dye incubation, cells were rinsed once and incubated with 100 µl assay buffer containing 250 µg/ml concanavalin A (Sigma, St. Louis, MO) for ½ hour to prevent desensitization of kainate receptors. Finally, 50 µl concanavalin A-containing assay buffer was added to wells and fluorescence measured using a fluorometric imaging plate reader (FLIPR; 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

concanavalin A-containing buffer three minutes 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 GLU_{K5} or GLU_{K6} receptors, and 200 μM when testing compounds at GLU_{A2} (approximate EC_{80} concentrations). Concanavalin A was omitted from experiments using GLU_{A2} receptors.

Electrophysiological Recording Conditions. Whole-cell voltage clamp recordings (Vh = -70mV) 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: 140mM NaCl, 5mM $CaCl_2$, 5mM KCl, 1mM $MgCl_2$, and 10 mM HEPES and 10mM Glucose, pH 7.4 with NaOH (osmolality 315 mosm/kg). Pipette solutions contained: 140mM CsCl, 1mM $MgCl_2$, 14mM diTris creatine phosphate, 50 U/ml creatine phospokinase, 14 mM MgATP, 10mM HEPES, and 15mM BAPTA, pH 7.2 with CsOH (osmolality 295 mosm/kg). Experiments were performed at room temperature (20-22^{o}C) and recorded on an Axopatch 200A amplifier using pClamp 8.0 Software (Axon Instruments, Inc.). Pipette resistance was typically 1.5-2.5 MΩ. Drug application was via a multi-barreled perfusion array. IC_{50} values for compounds were evaluated using 30 μM kainate, 30 μM AMPA or 10 μM NMDA. Data are expressed as mean $\pm$ SEM, n = 3-7.

Kainate currents were measured in acutely prepared isolated DRG from P4-P7 rat neonates as previously described (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 previously described (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 (10μM).

Subjects. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 250 to 290 grams for the capsaicin experiments, 200 – 225 grams for the rotorod test, and 70 to 90 grams for the carrageenan experiments were used. Rats were housed in groups of up to 6 per cage in a large colony room on a 12 hour 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 am and 6:00 pm. All treatment or dose groups consisted of 6 rats. All experiments were conducted in accordance with the NIH regulations of animal care covered in "Principles of Laboratory Animal Care", NIH publication 85-23, and were approved by the Institutional Animal Care and Use Committee.

Capsaicin-Induced Mechanical Allodynia. Groups of 6 rats were injected s.c. with vehicle or a dose of drug 15 minutes before capsaicin (30 μg in 25 μl) was injected into the plantar surface of the right hind paw. 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). Briefly, 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 minutes; 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 hind paw in a

perpendicular fashion at 5, 10, and 15 mm from the primary injection site and depressed slowly (4-5 seconds) 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 intracisternal injections, animals were lightly anesthetized with isoflurane and the back of the rat's head shaved. The head of the animal was placed perpendicular to the body axis as a 25-ga needle, attached to a 25-µl 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 sec and the needle was held in place for an additional 10 sec before being withdrawn. Intracisternal 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 6 rats were injected s.c. with λ-carrageenan (100 µl of a 1.5% solution) into the plantar surface of the right hind paw at time zero followed 90 minutes 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 minutes, respectively. Withdrawal latencies to a nociceptive thermal stimulus were assessed using a modification of the methods of Hargreaves et al. (1988). Each rat was placed in a Plexiglas cubicle with a glass floor through which an infrared photobeam was projected onto the plantar surface of the hind paws 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) was adjusted to produce a mean response latency in untreated rats of

approximately 12 to 15 seconds, and terminated automatically after 27 seconds 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 to the thermal stimulus was reported 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 minus the withdrawal latency of the untreated paw.

Rotorod test. Twenty-four hrs before compound testing, rats were given three training trials to maintain posture on an accelerating rod (Omnitech Electronics Inc., Columbus, OH) 17 rpm in 5 sec and maintaining that speed for 40 sec (Simmons et al., 1998). The following day, rotorod testing was conducted at time points corresponding to the pain testing. Animals that did not fall off the rotorod were given a maximum score of 40 sec. Compounds were evaluated over several doses, varying from a dose that was without effect on the rotorod and increasing in two- or three-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 Figure 1) were dissolved in distilled water or 5% solutol. Morphine sulfate (RBI) and λ-carrageenan (Sigma, St. Louis, MO) were dissolved in double deionized water. Doses refer to the form of the drug listed. All drugs were administered s.c. or PO by gavage in a volume of 1.0 ml/kg, or intracisternally in a volume of 10 µl. Capsaicin (Sigma, St. Louis, MO) was prepared as a 3 mg/2.5 ml

solution, dissolved in olive oil (Sigma, St. Louis, MO) and sonicated for 25 minutes in a 45 °C water bath.

Statistical analysis. Data were expressed as means ± SEM. Affinities of test compounds in transfected cell lines were determined from concentration-response curves for antagonism of glutamate-evoked Ca^{2+} influx. The curves were analyzed using GraphPad Prism 3.02 software (San Diego, CA), with slope factor not fixed, and top and bottom fixed at 100% and 0% inhibition, respectively. The dissociation constant (K_b) was calculated from the IC_{50} value for inhibiting glutamate-induced Ca^{2+} influx according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973): $K_b=IC_{50}/(1+[Glu]/EC_{50\ Glu})$ where [Glu] is the concentration of glutamate (100 μM or 200 μM) and $EC_{50\ Glu}$ is the EC_{50} 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 to appropriate control groups using one-way ANOVA and Dunnett's *t*-test. Statistical analyses were performed using JMP statistical software (SAS Institute Inc., Cary, NC). ED_{50} values and 95% confidence limits were determined using GraphPad Prism. A probability of $p \leq .05$ was taken as the level of statistical significance.

JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Ion flux in HEK293 cells. In order to determine the selectivity of this series of amino acid antagonists for GLU_{K5} versus GLU_{K6} or AMPA receptors, the ability of compounds to inhibit glutamate-evoked calcium influx was measured in HEK293 cells stably expressing either cloned GLU_{K5}, GLU_{K6}, or GLU_{A2}. Table 1 shows calculated K_b values for the antagonists at each cell line.

In the GLU_{K5}–expressing cell line, the rank order of potency was compound **6** (K_b = 3 nM) ≈ **4a** > **5** ≈ **3a** ≈ **2a** > LY377770 ≈ **4b** (prodrug of **4a**) ≈ LY293558 ≥ LY382884 ≈ **2b** (prodrug of **2a**) ≈ **3b** (prodrug of **3a;** K_b = 3 μM). Compound **1** (prodrug of LY382884) had no effect at GLU_{K5} receptors at concentrations up to 100 μM.

In GLU_{A2}–expressing cells, the antagonists displayed potency in the order of LY293558 (K_b = 0.4 μM) ≈ **3a** ≈ **2a** ≈ **4a** >> LY377770 ≈ **2b**. LY382884, **1**, **3b**, **4b, 5** and **6** each produced no significant inhibition in GLU_{A2}–expressing cells at concentrations up to 100 μM.

None of the compounds tested had any significant effect in GLU_{K6}–expressing cells at concentrations up to 100 μM.

Electrophysiological recordings. In order to confirm antagonist potency and selectivity for GLU_{K5} versus AMPA receptors in native tissues, as well as to determine the selectivity for GLU_{K5} versus NMDA receptors, selected compounds were examined for their ability to antagonize kainate-evoked inward currents in rat DRG neurons and AMPA- or NMDA-evoked currents in cultured rat hippocampal neurons (Figure 2). Compound **2a** antagonized kainate-evoked currents in rat DRG with IC_{50} = 0.15 ± 0.06 μM, and AMPA- and NMDA-evoked currents in hippocampal neurons with IC_{50} values

JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601
This article has not been copyedited and formatted. The final version may differ from this version.

of 2.1 ± 0.8 μM and 90 ± 41 μM, respectively. Compound **3a** blocked kainate-, AMPA- and NMDA-evoked currents with IC_{50} values of 0.18 ± 0.11 μM, 1.4 ± 0.9 μM 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_{K5} receptor antagonists. All three compounds produced dose-related reduction in mechanical allodynia in the capsaicin test (Figure 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 SC, or higher, producing statistically significant effects.

LY382884 s.c. and compound 1 PO in capsaicin test. We have previously reported that amino acid compounds such as LY382884 have poor oral bioavailability. Therefore, ester prodrugs were synthesized in order to increase oral bioavailability (Dominguez et al., 2005). The isobutyl ester of LY382884, compound **1**, had virtually no measurable affinity for GLU_{K5} receptors (Table 1), but produced a dose-related anti-allodynic effect in the capsaicin test, with doses of 10 and 30 mg/kg producing statistically significant effects (Figure 4). However, the effects of the prodrug administered PO were relatively modest in magnitude when compared to the complete reversal of capsaicin-induced allodynia produced by the parent compound LY382884 administered SC.

Oral efficacy of amino acids and prodrugs. Several additional amino acid compounds (see Figure 1 and Table 1) were synthesized which had relatively high

affinity and selectivity for GLU_{K5} receptors; however, these compounds were largely inactive after oral administration (data not shown). Ester prodrugs (Figure 1) of these amino acid compounds were therefore prepared. Ester prodrugs of the amino acids were largely devoid of affinity for GLU_{K5} 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** $\geq$ **4b** > **3b** > **1** (Figure 5 and Table 2).

Comparison of amino acid decahydroisoquinolines administered subcutaneously and intracisternally. In addition to being relatively inefficacious after oral administration, a number of amino acid GLU_{K5} antagonists, with a range of affinities for the GLU_{K5} receptor, were also ineffective in the capsaicin test after s.c. administration (Figure 6, left panel). 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 SC, whereas compound **6** had little or no efficacy over the dose-range of 3.0 to 30 mg/kg SC, even though the latter compound has a higher affinity than LY382884 for GLU_{K5} 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 than GLU_{K5} receptors, or due to a lack of blood-brain barrier penetration of the amino acids.

If the lack of efficacy after s.c. administration was due to poor brain penetration, then the amino acid GLU_{K5} receptor antagonists would be expected to be efficacious after central administration, whereas if the efficacy of LY382884 and compound **5** was due to activity via other mechanisms, then compound **6** would not be expected to be efficacious

JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601
This article has not been copyedited and formatted. The final version may differ from this version.

after central administration, and/or the rank order of potencies *in vivo* might be expected to differ from the rank order of potencies determined *in vitro*. We therefore evaluated the efficacy in the capsaicin test of these amino acid antagonists, with a range of affinities for the GLU_{K5} receptor, after intracisternal administration. The GLU_{K5} antagonists evaluated all produced dose-related anti-allodynic effects after intracisternal administration (Figure 6, right panel). All three of the amino acids completely reversed the capsaicin-induced mechanical allodynia (Figure 6, right panel) with a rank order of potencies of compound **6** > **5** > LY382884. Thus, the rank order of potencies *in vivo* after central administration was the same as the rank order of potencies at GLU_{K5} receptors *in vitro*.

Carrageenan-induced thermal hyperalgesia. The efficacy of the prodrug esters was also evaluated after oral administration on carrageenan-induced thermal hyperalgesia. The prodrug esters examined all produced dose-related anti-hyperalgesic effects (Figure 7), and except compound **2b** produced 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** (Figure 7 and 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 GLU_{K5} receptors *in vitro* and for reversing capsaicin-induced mechanical allodynia (see Figure 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 ED_{50} in the capsaicin test. For the prodrugs, the MED values ranged from 3 mg/kg for compound **4b** to 20 mg/kg for compounds **2b** and **3b**,

JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601
This article has not been copyedited and formatted. The final version may differ from this version.

>30 mg/kg for compound **1**. In general, the MED values in the rotorod test were approximately 4 to 20 fold higher than the ED_{50} values in the capsaicin or carrageenan tests (Table 2).

Discussion

The present studies evaluated the anti-mechanical allodynic and anti-thermal hyperalgesic effects of a series of amino acid decahydroisoquinoline GLU_{K5} receptor competitive antagonists in the capsaicin and carrageenan tests in rats. While these antagonists were efficacious when administered intracisternally, the majority of the amino acid GLU_{K5} receptor antagonists were ineffective after either oral or s.c. administration, suggesting that they either had very low oral bioavailability, poor blood-brain barrier penetration, or both. We have previously reported that these amino acid decahydroisoquinoline compounds have very low oral bioavailability (Dominguez et al., 2005). We therefore evaluated ester prodrugs 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 prodrugs tested herein were efficacious in reversing mechanical allodynia in the capsaicin assay after oral administration, which, together with our previous report (Dominguez et al., 2005), demonstrate that ester prodrugs of amino acid decahydroisoquinoline GLU_{K5} receptor antagonists can reverse allodynia after oral administration.

GLU_{K5} receptors are located in multiple sites on pain pathways (see also review by Ruscheweyh and Sandküler, 2002). GLU_{K5} receptors are well documented to occur in dorsal root ganglion cells and small diameter C-fibers (Agrawal and Evans, 1986; Huettner, 1990; Tölle et al., 1993; Furuyama et al., 1993). GLU_{K5} receptors are also located on spinothalamic tract neurons and contribute to high-threshold primary afferent fiber stimulation (Li et al., 1999; Palecek et al., 2004). In addition, GLU_{K5} receptors are abundant in the adult rat in the thalamus and brain stem structures important in pain

modulation (Bettler et al., 1990). Moreover, GLU_{K5} receptors have also been reported to be located in rat skin and peripheral sensory and sensorimotor nerves (e.g., Carlton et al., 1995; Coggeshall and Carlton, 1998).

The GLU_{K5} amino acid antagonists used in the current study were examined for their potency at GLU_{K5} receptors and their selectivity for GLU_{K5} versus GLU_{K6} or GLU_{A2} receptors, using calcium influx measurements at recombinant human glutamate receptors in vitro. Potency at GLU_{K5} receptors ranged from Kb = 3 nM for compound **6** to Kb = 0.02 μM for compound **5**. Ester prodrugs exhibited approximately 40- to 100-fold lower potency than the parent amino acid compounds (or, in the case of compound **1**, showed no measurable potency at GLU_{K5} receptors).

All of the amino acid parent compounds tested were found to be completely selective for GLU_{K5} receptors versus GLU_{K6} receptors, in that none displayed any measurable affinity (up to 100 μM) for GLU_{K6} receptors. Selectivity for GLU_{K5} versus GLU_{A2} receptors varied. LY293558 was found to be approximately equipotent at GLU_{K5} and GLU_{A2} receptors, in agreement with previous reports (Simmons et al., 1998). The remaining compounds all displayed 10-fold or greater selectivity for GLU_{K5} versus GLU_{A2} receptors. Several of the compounds that were efficacious when administered intracisternally in the capsaicin model (compounds **5** and **6**) showed no measurable affinity (up to 100 μM) at GLU_{A2} receptors, strengthening the interpretation that the efficacy observed was due to antagonism of GLU_{K5} receptors, rather than AMPA receptors.

The selectivity profile of selected antagonists was also confirmed electrophysiologically in primary neuronal cultures. Compounds **2a** and **3a** displayed

approximately 8- and >100-fold selectivity, respectively, for blocking kainate-versus AMPA-evoked currents in native rat tissues and were in general agreement with the results obtained using recombinantly expressed human channels. Additionally, the compounds tested displayed selectivity for GLU_{K5} versus NMDA receptors ranging from approximately 11-fold (compound **3a**) to 500-fold or greater (compound **2a**).

Although ester prodrugs of selected amino acid decahydroisoquinoline GLU_{K5} receptor antagonists were efficacious in reversing allodynia and hyperalgesia, not all parent amino acid decahydroisoquinolines were efficacious even after s.c. administration, suggesting that these compounds might vary in their ability to penetrate the blood-brain barrier. We therefore selected a series of compounds that varied over a wide range in their affinity for the GLU_{K5} receptor and administered them by the intracisternal route to circumvent the blood-brain barrier. After intracisternal administration, the compounds reversed allodynia in the capsaicin test, and, the rank order of potencies of the antagonists in the capsaicin test was the same as the rank order of potencies for GLU_{K5} receptors determined in vitro. Thus, the present correlative data provide additional strong support for the interpretation that the reversal of allodynia and hyperalgesia produced by these amino acid decahydroisoquinolines is mediated by antagonism of GLU_{K5} receptors. Moreover, the present results indicate that the efficacy of GLU_{K5} antagonists is mediated, at least in part, at supraspinal levels. Previous studies have demonstrated the importance of kainate receptors on dorsal root ganglion cells, as well as on intrinsic spinal cord dorsal horn neurons (e.g., Palecek et al., 2004), and in skin (Carlton et al., 1995; Coggeshall et al., 1998) in nociception. Thus, the present studies are the first to provide

evidence for a supraspinal site of action of kainate receptors in modulating nociceptive signaling.

The present studies replicate and extend previous findings that GLU_{K5} 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 intrathecal 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). Similarly, Guo et al. (2002) found that the intrathecal 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 which caused motor impairment on the rotarod, 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 GLU_{K5} receptors, or desensitization by selective GLU_{K5} agonists, can effectively reduce allodynia or hyperalgesia produced by direct stimulation of C-fibers or inflammation at doses which do not produce motor impairment.

A growing body of evidence also indicates that selective GLU_{K5} 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 GLU_{K5} 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. Further, 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 (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. Taken together, the data indicate that GLU_{K5} receptors play an important role in a variety of persistent pain states, and that GLU_{K5} 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 GLU_{K5} 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 either oral or s.c. administration, but were efficacious after intracisternal 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 GLU_{K5} 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. Further, taken together with previous reports, the site of action of GLU_{K5} antagonists in producing antinociception appears to be both supraspinal and spinal. The present findings thus provide further evidence that GLU_{K5} receptors are likely 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

Agrawal SG and Evans RH (1986) The primary afferent depolarizing action of kainate in the rat. *Brit J Pharmacol* **87**:345–355.

Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M and Heinemann S (1990) Cloning of a novel glutamate receptor subunit GluR5: Expression in the nervous system during development. *Neuron* **5**:583-595.

Blackburn-Munro G, Bomholt SF and Erichsen HK (2004) Behavioural effects of the novel AMPA/GluR5 selective receptor antagonist NS1209 after systemic administration in animal models of experimental pain. *Neuropharmacology* **47**:351-362.

Bleakman D, Ogden AM, Ornstein PL and Hoo K (1999) Pharmacological characterization of a GluR6 kainate receptor in cultured hippocampal neurons. *Eur J Pharmacol* **378**:331-337.

Bortolotto ZA, Clarke VR, Delany CM, Parry MC, Smolders I, Vignes M, Ho KH, Miu P, Briton BT, Fantaske R, Ogden A, Gates M, Ornstein PL, Lodge D, Bleakman D and Collingridge GL (1999) Kainate receptors are involved in synaptic plasticity. *Nature* **402**:297-301.

Carlton SM, Hargett GL and Coggeshall RE (1995) Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. *Neurosci Lett* **197**:25-28.

Chaplan SR, Bach FW, Pogrel JW, Chung JM and Yaksh TL (1994) Quantitative assessment of allodynia in the rat paw. *J Neurosci Methods* **53**:55-63.

Cheng YC and Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22**:3099-3108.

Coderre TJ (1993) The role of excitatory amino acid receptors and intracellular messengers in persistent nociception after tissue injury in rats. *Mol Neurobiol* **7**:229-246.

Coderre TJ and van Empel I (1994) The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. *Pain* **59**:345-352.

Coggeshall RE and Carlton SM (1998) Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. *J Comp Neurol* **391**:78-86.

Dominguez E, Iyengar S, Shannon HE, Bleakman D, Alt A, Arnold BM, Bell MG, Bleisch TJ, Buckmaster JL, Castano AM, Del Prado M, Escribano A, Filla SA, Ho KH, Hudziak KJ, Jones CK, Martinez-Perez JA, Mateo A, Mathes BM, Mattiuz EL, Ogden AML, Simmons RMA, Stack DR, Stratford RE, Winter MA, Wu Z and Ornstein PL (2005) Two prodrugs of potent and selective GluR5 kainate receptor antagonists actives in three animal models of pain. *J Med Chem* **48**:4200-4203.

Furuyama T, Kiyama H, Sato K, Park HT, Maeno H, Takagi H and Tohyama M (1993) Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. *Mol Brain Res* **18**:141–151.

Fundytus ME (2001) Glutamate receptors and nociception. Implications for the drug treatment of pain. *CNS Drugs* **15**:29-58.

Gilron I, Max MB, Lee G, Booher SL, Sang CN Chappell AS and Dionne RA (2000) Effects of the 2-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid/kainate antagonist LY293558 on spontaneous and evoked postoperative pain. *Clin Pharmacol Ther* **68**:320-327.

Guo W, Zou S, Tal M and Ren K (2002) Activation of spinal kainate receptors after inflammation: behavioral hyperalgesia and subunit gene expression. *Eur J Pharmacol* **452**:309-318.

Hamill OP, Mary A, Neher E, Sakmann B and Sigworth F (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Plügers Arch* **391**:85-100.

Hargreaves K, Dubner R, Brown F, Flores C and Joris J (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32:77-88.

Hollmann M and Heinemann S (1994) Cloned glutamate receptors. *Ann Rev Neurosci* **17**:31-108.

Huettner JE (1990) Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron* **5**:255-266.

Jackson DL, Graff CB, Richardson JD and Hargreaves KM (1995) Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. *Eur J Pharmacol* **284**:321-325.

King AE, Thompson SWN, Urban L and Woolf CJ (1988) An intracellular analysis of amino acid induced excitations of deep dorsal horn neurones in the rat spinal cord slice. *Neurosci Lett* **89**:286-292.

Ko S, Zhao MG, Toyoda H, Qui CS and Zhuo M (2005). Altered behavioral responses to noxious stimuli and fear in glutamate receptor 5 (GluR5) - or GluR6-deficient mice. *J Neurosci* **25**:977-984.

Li P, Wilding TJ, Calejesan AA, Huettner JE and Zhuo M (1999) Kainate-receptor-mediated sensory synaptic transmission in mammalian spinal cord. *Nature* **397**:161-164.

McBain CJ and Mayer ML (1994) *N*-methyl-D-aspartic acid receptor structure and function. *Physiol Rev* **74**:723–760.

Palecek J, Neugebauer V, Carlton SM, Iyengar S and Willis WD (2004) The effect of a kainate GluR5 receptor antagonist on responses of spinothalamic tract neurons in a model of peripheral neuropathy in primates. *Pain* **111**:151-61.

Pogatzki EM, Niemeier JS, Sorkin LS and Brennan TJ (2003) Spinal glutamate receptor antagonists differentiate primary and secondary mechanical hyperalgesia caused by incision. *Pain* **105**:97-107.

Procter MJ, Houghton AK, Faber ES, Chizh BA, Ornstein PL, Lodge D and Headley PM (1998) Actions of kainate and AMPA selective glutamate receptor ligands on nociceptive processing in the spinal cord. *Neuropharmacology* **37**:1287-97.

Ruscheweyh R and Sandküler J (2002) Role of kainate receptors in nociception. *Brain Res Rev* **40**:215–222.

Sang CN, Hostetter MP, Gracely RH, Chappell AS, Schoepp DD, Lee G, Whitcup S, Caruso R and Max MB (1998) AMPA/kainate antagonist LY293558 reduces capsaicin-evoked hyperalgesia but not pain in normal skin in humans. *Anesthesiology* **89**:1060-1067.

Sang CN, Ramadan NM, Wallihan RG, Chappell AS, Freitag FG, Smith TR, Silberstein SD, Johnson KW, Phebus LA, Bleakman D, Ornstein PL, Arnold B, Tepper SJ and Vandenhende F (2004) LY293558, a novel AMPA/GluR5 antagonist, is efficacious and well-tolerated in acute migraine. *Cephalalgia* **24**:596-602.

Seeburg PH (1993) The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci* **16**:359–365.

Simmons RMA, Li DL, Hoo KH, Deverill M, Ornstein PL and Iyengar S (1998) Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. *Neuropharmacology* **37**:25-36.

Skilling SR, Smullin DH, Beitz AJ and Larson AA (1988) Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. *J Neurochem* **51**:127-132.

Ta LE, Dionne RA, Fricton JR, Hodges JS and Kajander KC (2000) SYM-2081 a kainate receptor antagonist reduces allodynia and hyperalgesia in a freeze injury model of neuropathic pain. *Brain Res* **858**:106-120.

Tölle TR, Berthele A, Zieglgänsberger W, Seeburg PH and Wisden W (1993) The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. *J Neurosci* **13**:5009–5028.

Turner MS, Hamamoto DT, Hodges JS, Maccecchini ML and Simone DA (2003) SYM 2081, an agonist that desensitizes kainate receptors, attenuates capsaicin and inflammatory hyperalgesia. *Brain Res* 973:252-264.

Urban L, Thompson SWN and Dray A (1994) Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters. *Trends Neurosci* 17:432-438.

Zieglgänsberger W and Herz A (1971) Changes of cutaneous receptive fields of spino-cervical-tract neurones and other dorsal horn neurones by microelectrophoretically administered amino acids. *Exp Brain Res* 13:111-126.

Footnotes

Current Address: Carrie K. Jones, Ph.D., Department of Pharmacology, Vanderbilt University Medical Center, 23rd Avenue South at Pierce, 417C Preston Research Building, Nashville, TN 37232-6600

Legends for Figures

Figure 1. Structures of competitive Glu_{K5} ionotropic glutamate receptor antagonists.

Figure 2. Dose-related antagonism of kainate-induced currents in rat DRG neurons and of AMPA- and NMDA-induced currents in rat hippocampal neurons in vitro by **2a** and **3a**. Each point represents the mean of 3-7 cells. Vertical lines represent ±SEM and are absent when less than the size of the point. Abscissa: concentration of drug in μM; ordinate: percent inhibition.

Figure 3. Dose-related reversal of capsaicin-induced mechanical allodynia by competitive Glu_{K5} ionotropic glutamate receptor antagonists after s.c. administration in rats. Each point represents the mean of 6 rats. Vertical lines represent ±SEM and are absent when less than the size of the point. Points above V represent the effects of vehicle. Abscissa: dose of drug in mg/kg; ordinate: Withdrawal threshold in grams to a mechanical stimulus. * $p < .05$ vs. Vehicle, Dunnett's *t*-test.

Figure 4. Dose-related reversal of capsaicin-induced mechanical allodynia by LY382884 subcutaneous and the ester prodrug **1** after oral administration in rats. Each point represents the mean of 6 rats. Vertical lines represent ±SEM and are absent when less than the size of the point. Points above V represent the effects of vehicle. Points above 10M represent the effects of 10 mg/kg morphine s.c. Abscissa: dose of drug in mg/kg; ordinate: Withdrawal threshold in grams to a mechanical stimulus. * $p < .05$ vs. Vehicle, Dunnett's *t*-test.

Figure 5. Dose-related reversal of capsaicin-induced mechanical allodynia by the ester prodrugs of competitive GluK5 antagonists after oral administration in rats. Each point represents the mean of 6 rats. Vertical lines represent ±SEM and are absent when less

than the size of the point. Points above V represent the effects of vehicle. Points above 10M represent the effects of 10 mg/kg morphine s.c. Abscissa: dose of drug in mg/kg; ordinate: Withdrawal threshold in grams to a mechanical stimulus. * p <.05 vs. Vehicle, Dunnett's *t*-test.

Figure 6. Dose-related reversal of capsaicin-induced mechanical allodynia by competitive Glu_{K5} antagonists after subcutaneous or intracisternal (IC) administration in rats. Each point represents the mean of 6 rats. Vertical lines represent $\pm$SEM and are absent when less than the size of the point. Points above V represent the effects of vehicle. Data for LY382884 administered s.c. are re-produced from Figure 3. Abscissa: dose of drug in mg/kg s.c. or nmoles/rat IC; ordinate: Withdrawal threshold in grams to a mechanical stimulus. * p <.05 vs. Vehicle, Dunnett's *t*-test.

Figure 7. Dose-related reversal of carrageenan-induced thermal hyperalgesia by ester prodrugs of competitive Glu_{K5} antagonists after oral administration in rats. Each point represents the mean of 6 rats. Vertical lines represent $\pm$ SEM and are absent when less than the size of the point. Points above V represent the effects of vehicle. Abscissa: Dose of drug in mg/kg SC. Ordinate: Withdrawal latency difference in seconds to a thermal stimulus. * , p<0.05 vs Veh, Dunnett's *t*-test.

JPET Fast Forward. Published on July 12, 2006 as DOI: 10.1124/jpet.106.105601
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1.

Potency (K_b) 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[a].

Values given represent the mean (and 95% confidence intervals) K_b from 3-4 experiments, each carried out in triplicate. Numbers in brackets are percent inhibition and SEM at 100 μM when the magnitude of the inhibition was statistically significant but less than 50%.

Compound	GLU_{A2}	GLU_{K5}	GLU_{K6}
	μM	*μM*	*μM*
LY293558	0.4 (0.2-0.7)	0.5 (0.2-0.8)	--[b]
LY377770	[35±2]	0.09 (0.04-0.24)	--
LY382884	--	1.1 (0.6-2.1)	--
1 [c]	--	--	--
2a	1.1 (0.6-2.0)	0.04 (0.03-0.05)	--
2b [c]	*[25±8]*	*2 (1-4)*	--
3a	0.4 (0.1-1.1)	0.03 (0.02-0.06)	--
3b [c]	--	*3 (2-4)*	--
4a	2 (1-3)	0.005 (0.002-0.009)	--
4b [c]	--	*0.2 (0.1-0.4)*	--
5	--	0.02 (0.01-0.03)	--
6	--	0.003 (0.001-0.010)	--

[a] Binding affinities and/or functional potency at NMDA receptors and other ionotropic glutamate receptor subtypes were not determined for every compound, but where determined were typically >100 μM.

[b] --: no significant inhibition observed up to 100 μM.

[c] Prodrug of compound listed immediately above.

[d] nt, not tested.

Table 2.

ED50 values of competitive ionotropic glutamate receptor antagonists and prodrugs for in the capsaicin and carrageenan tests, and minimal effective doses (MED) in the rotorod test of motor impairment.

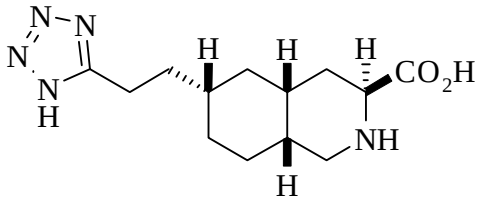
Vehicle or a dose of compound was administered either subcutaneously 30 min (parent compounds) or orally 60 min (prodrug compounds) before testing. Numbers in parentheses are 95% confidence limits.

Compound	Route	Capsaicin Test	Carrageenan Test	Rotorod
		ED_{50}, mg/kg	*ED_{50}, mg/kg*	*MED, mg/kg*
LY293558	SC	4.0 (2.5-6.6)	nt[a]	10
LY377770	SC	6.3 (5.5-7.2)	nt	nt
LY382884	SC	7.6 (5.9-9.8)	nt	>100[b]
1 [c]	*PO*	*>30*	*>30*	*>30*
2a	SC	>10	nt	nt
2b [c]	*PO*	*1.2 (0.9-1.5)*	*2.7 (2.5-3.0)*	*20*
3a	SC	>10	nt	nt
3b [c]	*PO*	*5.1 (3.7-7.0)*	*(2.3 (1.5-3.4)*	*20*
4a	SC	>10	nt	nt
4b [c]	*PO*	*1.6 (1.4-1.8)*	*0.3 (0.2-0.5)*	*3*

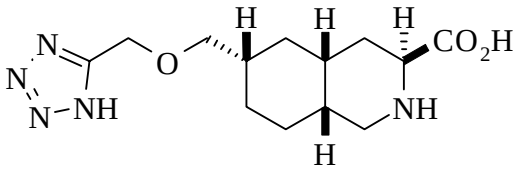
[a] nt, not tested.

[b] From Simmons et al., 1998

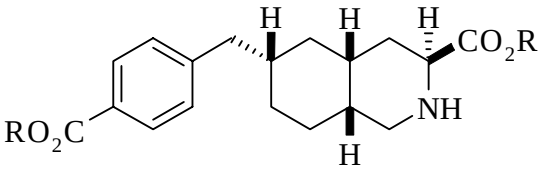
[c] Prodrug of compound listed immediately above.



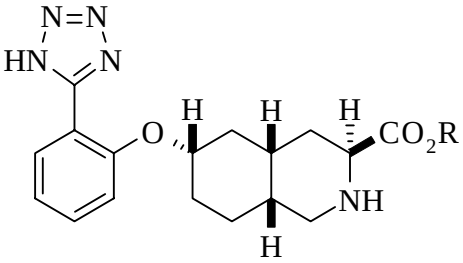
LY293558



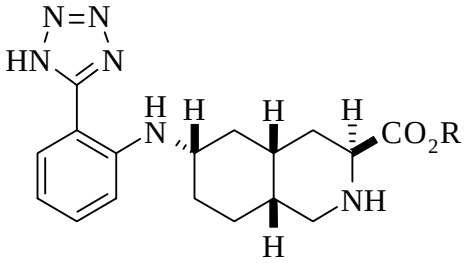
LY377770



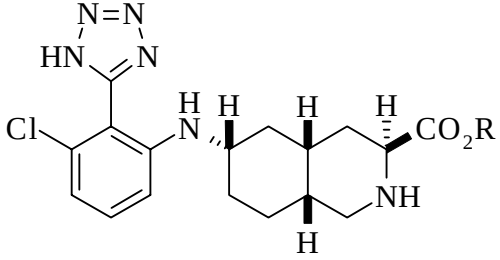
LY382884, R = H
1, R = ethyl



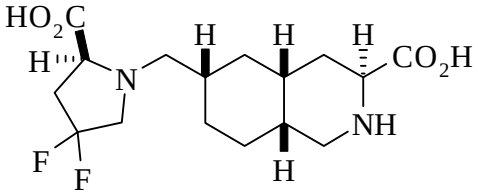
2a, R = H
2b, R = *iso*-butyl



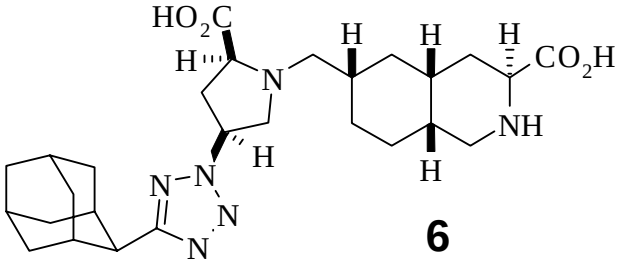
3a, R = H
3b, R = 2-ethylbutyl



4a, R = H
4b, R = 2-ethylbutyl



5



6

Figure 1

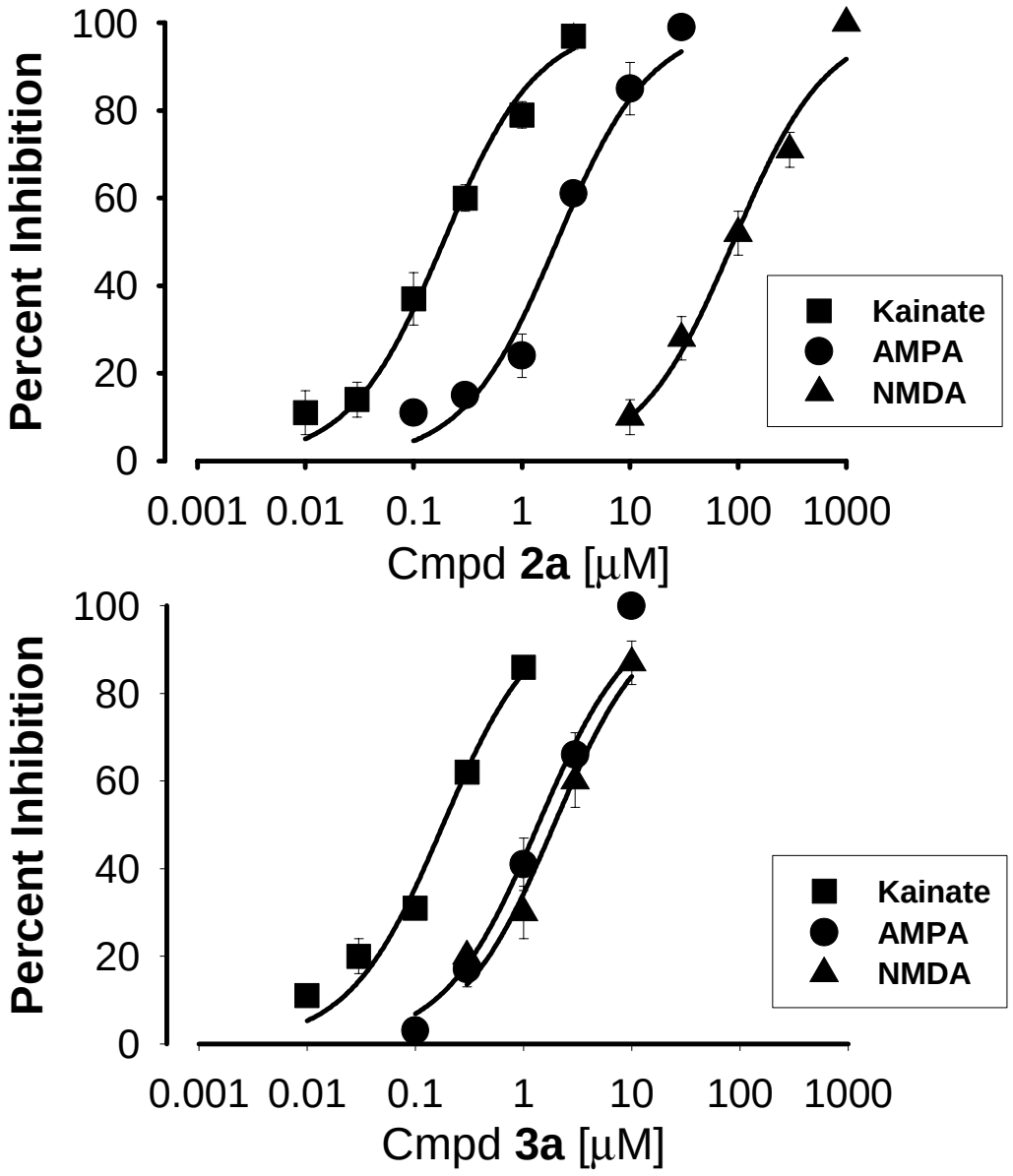


Figure 2

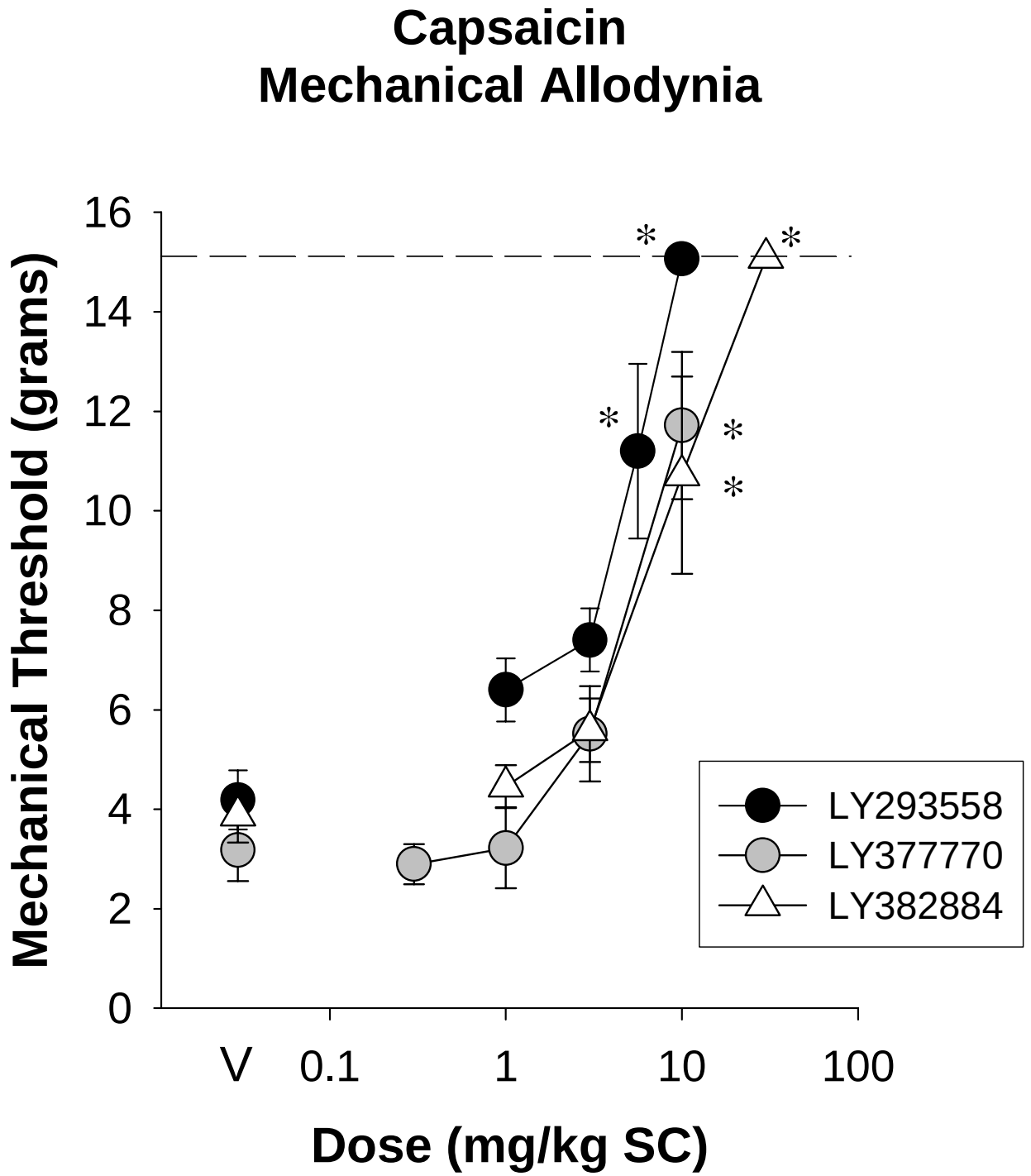


Figure 3

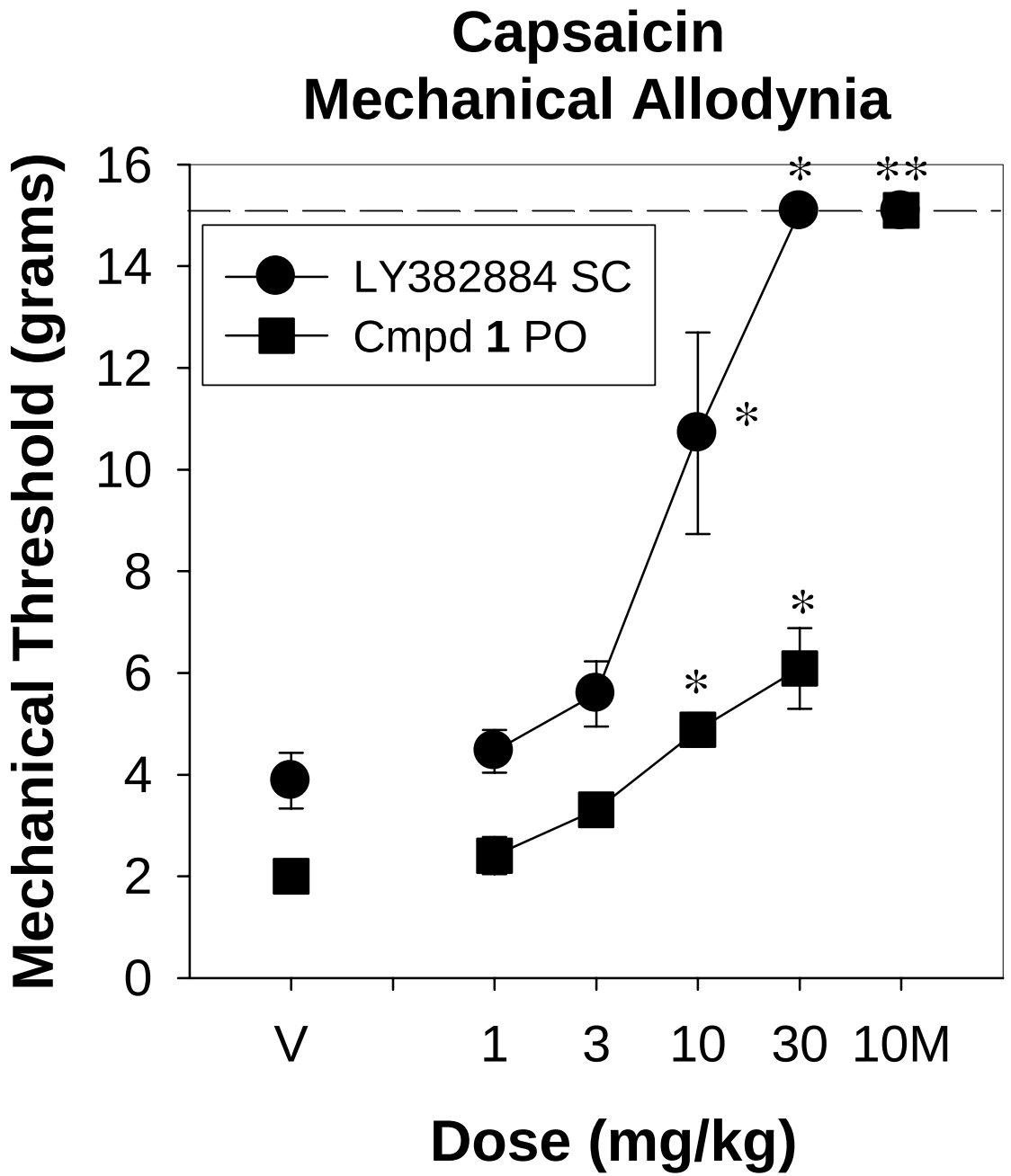


Figure 4

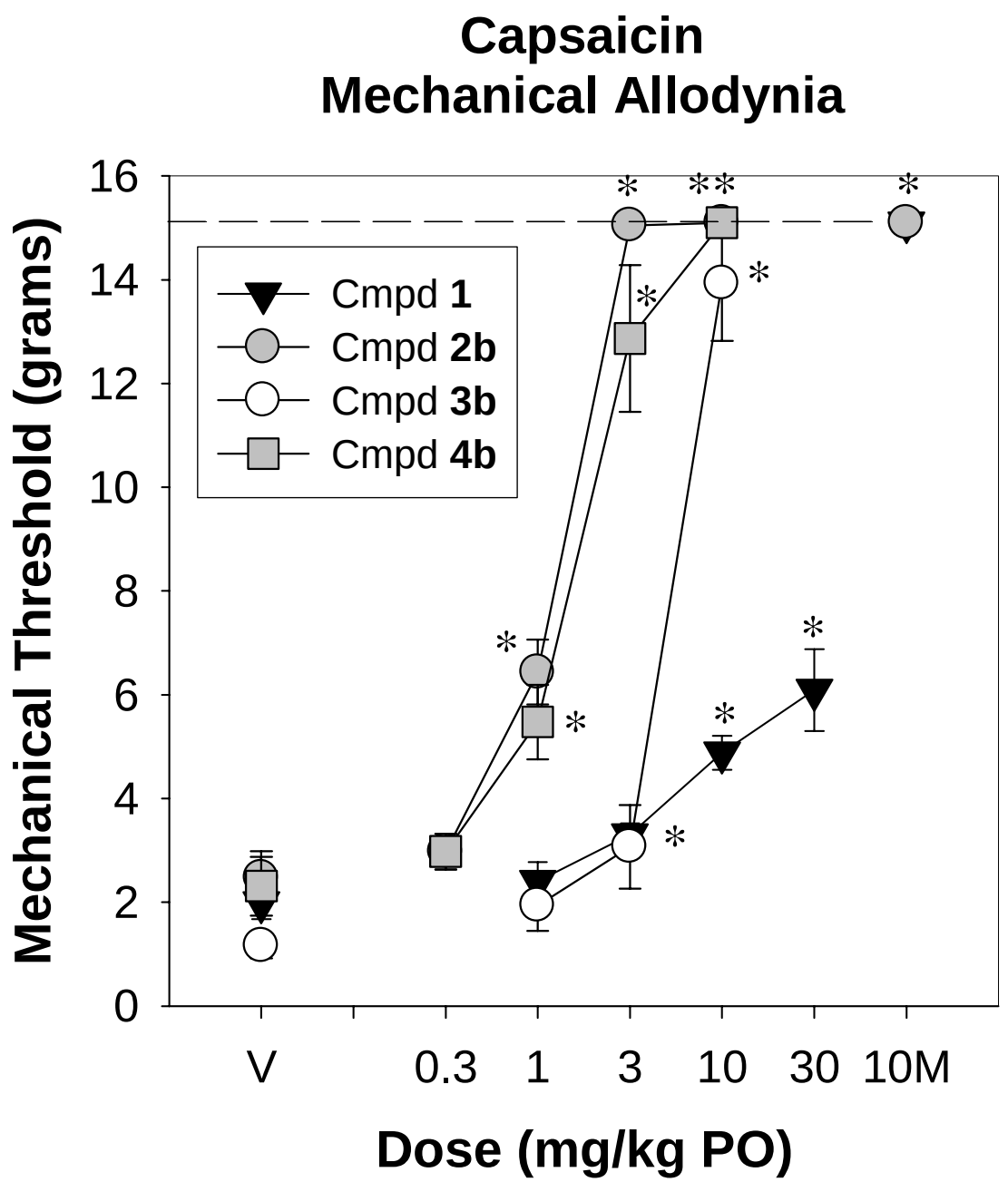


Figure 5

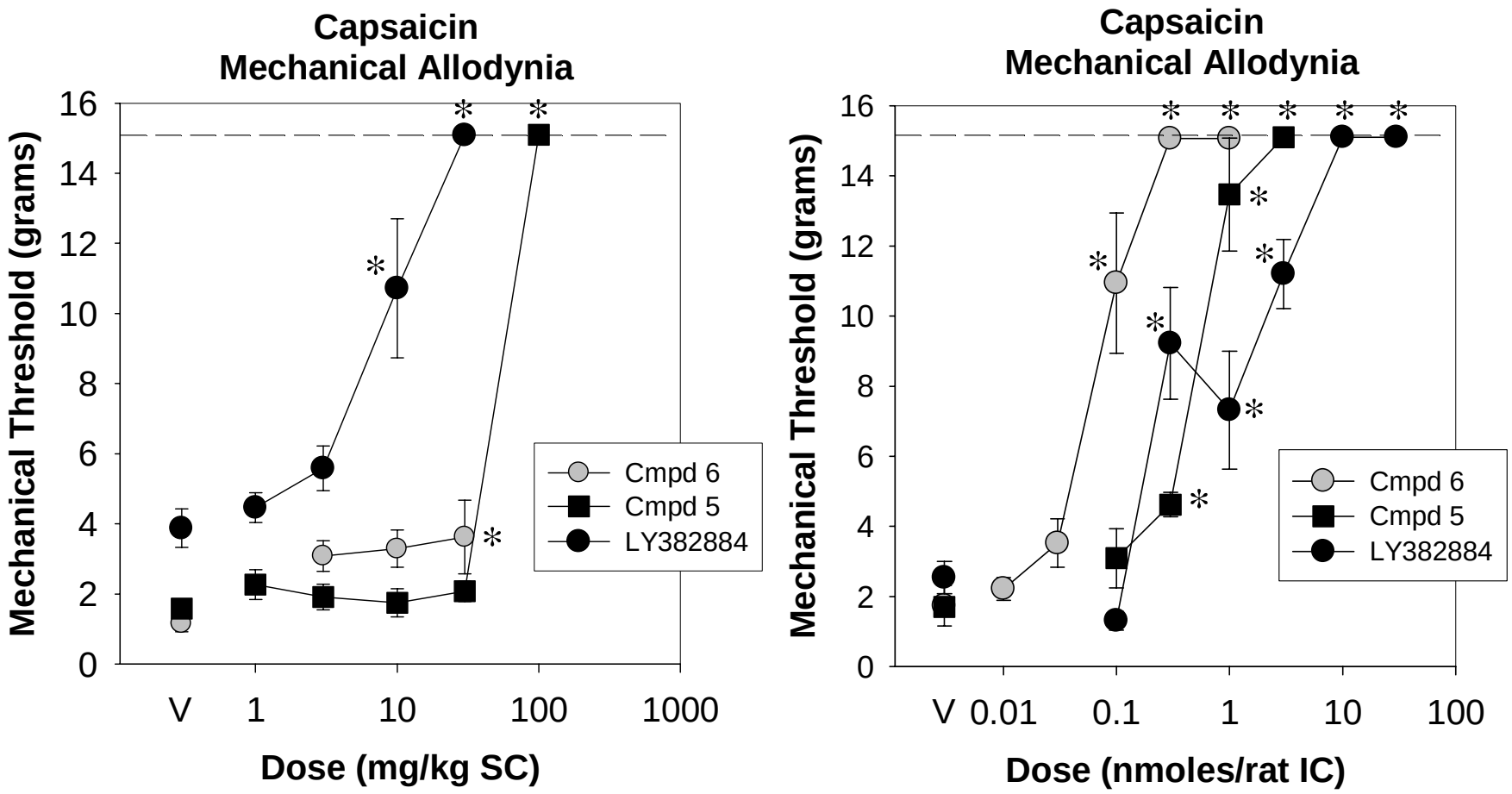


Figure 6

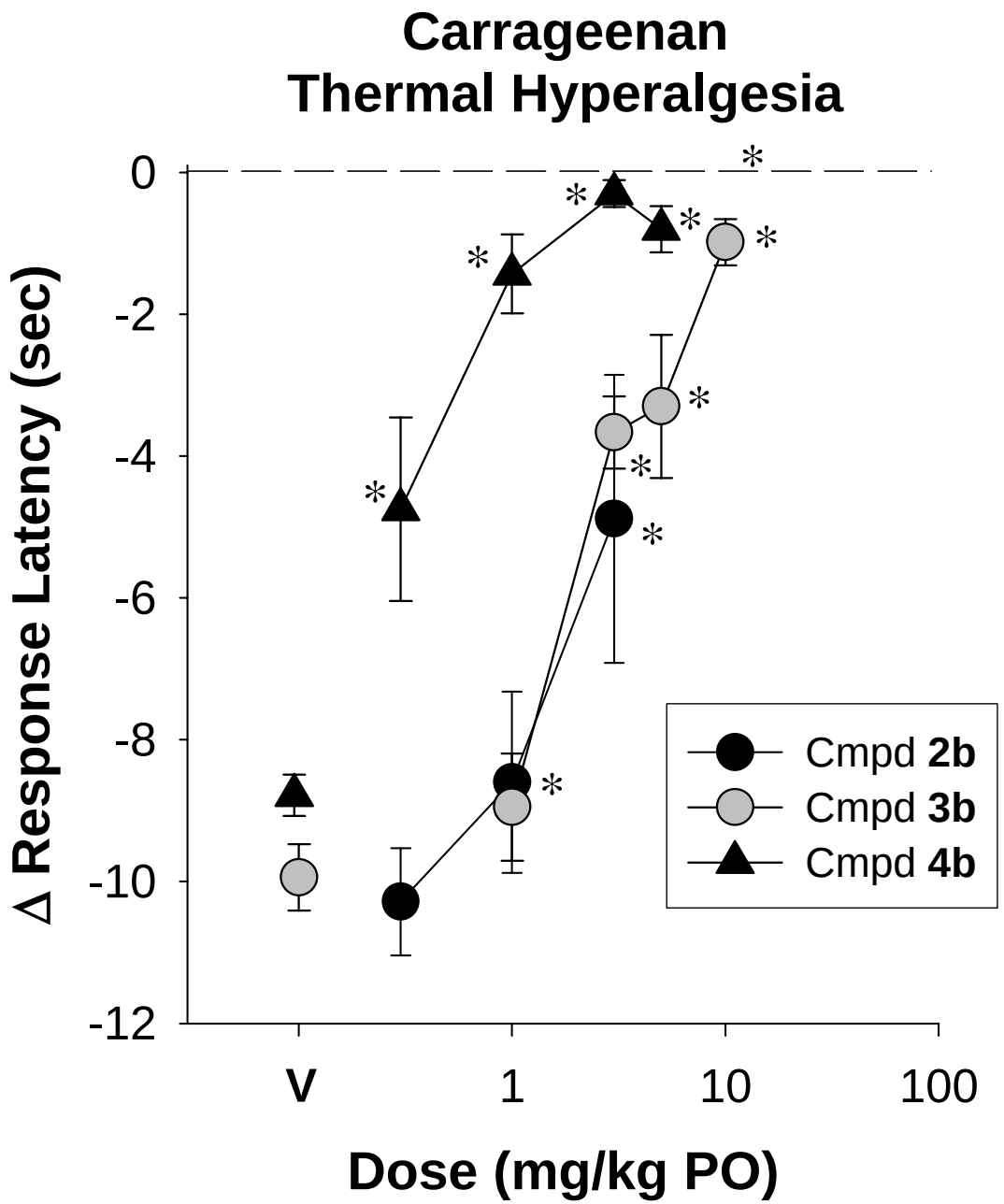


Figure 7