Group I Metabotropic Glutamate Receptor Antagonists Block Secondary Thermal Hyperalgesia in Rats with Knee Joint Inflammation

LIPING ZHANG, YING LU, YING CHEN, and KARIN N. WESTLUND

Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas (L.Z., Y.L., K.N.W.); and Eli Lilly & Co. Ltd., Windlesham, Surrey, United Kingdom (Y.C.)

Received March 9, 2001; accepted September 28, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Activation of ionotropic glutamate receptors has been shown previously to be essential for the development of secondary thermal hyperalgesia. The present study assessed involvement of group I metabotropic glutamate receptors (mGlu) in both the induction and maintenance phases of secondary thermal hyperalgesia initiated by knee joint inflammation in rats. The dose dependence of each drug in antagonism of thermal hyperalgesia was demonstrated in pre- and post-treatment paradigms. Knee joint inflammation was induced by injection of kaolin and carrageenan. Four hours later the paw withdrawal latencies were significantly shorter than baseline values. Rats were pretreated by spinal microdialysis infusion of group I mGlu receptor antagonists, LY393053 [(R)-2-amino-2-(3-cis and trans-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid], LY367385 [(S)-(+)α-amino-4-carboxy-2-methylbenzeneacetic acid], or AIDA [(R,S)-1-aminoindan-1,5-dicarboxylic acid/UPF 523] before knee joint injection. The paw withdrawal latencies measured 4 h after the injection were significantly longer in the presence of group I mGlu receptor antagonists than those of the artificial cerebrospinal fluid-treated arthritic control group. Post-treatment with the group I mGlu receptor antagonists LY367385 and AIDA allowed significant recovery of the paw withdrawal latencies after the onset of the knee joint inflammation. The knee joint inflammation itself was not affected by either treatment. The results of the present study indicate that secondary thermal hyperalgesia can be effectively attenuated during both the development and maintenance phases of acute knee joint inflammation by spinal application of specific group I mGlu receptor antagonists.

ABSTRACT

Activation of ionotropic glutamate receptors has been shown previously to be essential for the development of secondary thermal hyperalgesia. The present study assessed involvement of group I metabotropic glutamate receptors (mGlu) in both the induction and maintenance phases of secondary thermal hyperalgesia initiated by knee joint inflammation in rats. The dose dependence of each drug in antagonism of thermal hyperalgesia was demonstrated in pre- and post-treatment paradigms. Knee joint inflammation was induced by injection of kaolin and carrageenan. Four hours later the paw withdrawal latencies were significantly shorter than baseline values. Rats were pretreated by spinal microdialysis infusion of group I mGlu receptor antagonists, LY393053 [(R)-2-amino-2-(3-cis and trans-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid], LY367385 [(S)-(+)α-amino-4-carboxy-2-methylbenzeneacetic acid], or AIDA [(R,S)-1-aminoindan-1,5-dicarboxylic acid/UPF 523] before knee joint injection. The paw withdrawal latencies measured 4 h after the injection were significantly longer in the presence of group I mGlu receptor antagonists than those of the artificial cerebrospinal fluid-treated arthritic control group. Post-treatment with the group I mGlu receptor antagonists LY367385 and AIDA allowed significant recovery of the paw withdrawal latencies after the onset of the knee joint inflammation. The knee joint inflammation itself was not affected by either treatment. The results of the present study indicate that secondary thermal hyperalgesia can be effectively attenuated during both the development and maintenance phases of acute knee joint inflammation by spinal application of specific group I mGlu receptor antagonists.

Acute knee joint inflammation has been shown to reduce nociceptive threshold within the inflamed area (primary hyperalgesia) and also in a large area surrounding the inflamed area extending to the foot (secondary hyperalgesia) (Coggeshall et al., 1983; Schaible and Schmidt, 1985; Sluka and Westlund, 1992; Sluka et al., 1994). Secondary hyperalgesia is believed to result from sensitization of nociceptive neurons in the dorsal horn of the spinal cord (Schaible et al., 1987; Neugebauer and Schaible, 1990; Simone et al., 1991; Sluka et al., 1994), likely as a result of the unmasking of normally ineffective synapses (Wall, 1977). An increase in release of excitatory amino acids in the dorsal horn after inflammation of the knee joint is one of several factors that may account for secondary hyperalgesia (Sluka and Westlund, 1992, 1993; Sorkin et al., 1992).

Excitatory amino acids, such as L-glutamate, play a major role in sensory processing in neurons throughout the central nervous system, acting on their receptors N-methyl-D-aspartate (NMDA), non-NMDA, or metabotropic glutamate receptors. Sluka and Westlund (1993) and Sluka et al. (1994) demonstrated that release of excitatory amino acids and secondary hyperalgesia after knee joint inflammation were both blocked by the non-NMDA receptor antagonist 6-cyano-2,3-dihydroxy-7-nitroquinoxaline, or by the NMDA receptor antagonist 6-cis-2-amino-5-phosphonopentoic acid. Recently, there is evidence suggesting that metabotropic glutamate receptors are also involved in the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons and the development of mechanical hyperalgesia (Neugebauer et al., 1994; Young et al., 1997; Zhang et al., 2000; Walker et al., 2001a,b).

Group I mGlu receptors mGlu1 and mGlu5 are coupled to phosphoinositide hydrolysis/intracellular calcium mobilization via G proteins. We have chosen three group I metabotropic receptor antagonists, (+)-2-amino-2-(3-cis and trans-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid (LY393053), (S)-(+)α-carboxy-2-methylbenzeneacetic acid (UPF 523; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; aCSF, artificial cerebrospinal fluid.
amino-4-carboxy-2-methylbenzeneacetic acid (LY367385), and (R,S)-1-aminoindan-1,5-dicarboxylic acid/UPF 523 (AIDA), as useful pharmacological tools, because they have demonstrated good solubility with different selectivity and potencies. The antagonists AIDA, LY393053, and LY367385 have been used extensively for the functional blockade of group I mGlu receptors for both in vivo and in vitro studies (Bruno et al., 1999; Neugebauer et al., 1999; Chen et al., 2000).

LY393053 is a novel antagonist at both mGlu1 and mGlu5 receptors (Chen et al., 2000). This compound has IC50 values of 1.0 ± 0.4 μM on mGlu1 and 1.6 ± 1.4 μM on mGlu5 receptors, respectively. LY393053 has been shown to act on group I mGlu receptors in in vitro spinal cord (Chen et al., 2000) and hippocampal preparations (Fitzjohn et al., 1999). In pathological in vivo animal models, this compound has also been shown to have anti-hyperalgesic properties (Salt et al., 1999). Caution needs to be taken in evaluation of physiological experiments using AIDA as a group I mGlu receptor antagonist.

The purpose of the present study was to investigate the role of group I mGlu receptors specifically on dorsal horn neurons involved in secondary thermal hyperalgesia induced by knee joint inflammation while avoiding possible effects of these compounds on receptors of other central or peripheral neuronal components. The decrease in the paw withdrawal latency response of the ipsilateral hindpaw after inflammation was used as an indicator of secondary hyperalgesia at this site remote from the primary injury in the knee joint and indicated that sensitization of spinal neurons has occurred. In this study, each of the three antagonists was delivered in separate experiments by microdialysis fiber into the dorsal horn of segments L4 to L6 of the spinal cord. By administering the drugs before and after the development of acute knee joint inflammation, we have determined that group I mGlu receptors are involved in both the development and the maintenance of secondary thermal hyperalgesia.

Materials and Methods

These experiments were approved by the University of Texas Medical Branch, Animal Care and Use Committee and were in strict accordance with the National Research Council as specified in the Guide for the Care and Use of Laboratory Animals.

Induction of Acute Knee Joint Inflammation

The experiments were carried out with adult male Sprague-Dawley rats (Harlan Bioproducts for Science, Indianapolis, IN). Rats were anesthetized briefly with sodium methohexital (Brevital; 60 mg/kg i.p.; Jones Pharma Inc., St. Louis, MO). Acute knee joint inflammation was induced by injection of a mixture of 3% kaolin (Sigma Chemical, St. Louis, MO) and 3% carrageenan (Sigma Chemical) (0.1 ml in saline) into the left knee joint of each animal after baseline behavioral testing. The injected leg was flexed manually for approximately 10 min.

Assessment of Knee Joint Inflammation and Hyperalgesia

Knee joint inflammation was assessed in three ways: 1) measuring the circumference of the affected knee joint (in centimeters) with a flexible tape measure around the center of the joint while the hindlimb was held in extension both before and 4 h after the injection of kaolin and carrageenan mixture; 2) measuring the temperature of the knee joint by measuring the skin temperature over the affected knee joint with a Digital-Thermo thermometer (Quartz; Fisher Scientific Company, Houston, TX) before and 4 h after the injection of the kaolin and carrageenan mixture; and 3) evaluating the pain-related posture: the abnormal posture of each animal with an affected hindlimb was given a single score by using a subjective pain-related behavioral scale (spontaneous pain rating score) (Salt et al., 1994), i.e., 0, normal; 1, curling of the toes; 2, evasion of the paw; 3, partial weight bearing; 4, non-weight bearing and guarding; and 5, avoidance of any contact with the hindlimb.

Assessment of Noxious Response to Thermal Stimuli

Paw withdrawal latency was measured in response to a radiant heat source shone onto the plantar surface of the hindpaw. Rats were placed in separate clear plastic chambers (25 × 10 × 10 cm) on a glass-top table (approximately 2 mm thick) and allowed to acclimate to their new environment for 20 min before testing. A high-intensity light beam was applied to the plantar surface of the hindpaw through the glass until the rat lifted its paw. The light beam projected through a 5 × 10 mm aperture from a movable metal box that contained a high-intensity projector lamp bulb (Quartzline Lamp; General Electric Co., Cleveland, OH) that was attached to an on/off switch and a digital timer. The time appearing on the timer was expressed as the paw withdrawal latency(s). The cutoff time for paw withdrawal reflex was set to 15 s. To get consistent response values, both hindpaws were tested independently with a single tester for five trials per side with 5-min intervals between trials. The testing session requires 0.6 h to complete. A mean of these five readings was used as the paw withdrawal latency values in this study.

Administration of Drugs

Placement of the Microdialysis Fiber. All drugs tested in these experiments were delivered into the spinal cord dorsal horn with a microdialysis fiber as described in a previous article (Sluka and Westlund, 1992). A microdialysis fiber (200 μm inner diameter, 45,000 mol. wt. cutoff, Hospal AN69; Hospal, Meyzieu, France) was coated with epoxy glue except for a 2-mm permeable length embedded in the spinal gray matter. The animal was deeply anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.; Abbott Diagnostics, Chicago, IL). A midline incision was made in the rat's back at the level of the last rib to expose a portion of the T13 to L1 vertebrae and small holes (1 mm in diameter) were drilled in the lateral aspect of the L1 vertebra. A microdialysis fiber was then inserted transversely through the hole into the dorsal horn of spinal cord (within segments L4-L6) and stabilized with dental cement applied to the bone. The permeable 2-mm length of the fiber was placed in the spinal cord dorsal horn gray matter, and each end of the microdialysis fiber was connected to polyethylene 20 tubing (BD Biosciences, San Jose, CA) tunneled under the skin to the nape of the neck. The aCSF (pH 7.2–7.4; adjusted by bubbling with O2 just...
before use) (McAdoo et al., 1999) was pumped through the tubing at a rate of 5 μl/min for 1 h before the polyethylene 20 tubing was sealed. After the placement of the microdialysis fiber, the animals were allowed to recover overnight. The fiber position was checked histologically for each experimental animal at the end of the experiments. Each fiber always passed through the spinal cord at some point within segments L4 to L6 and within laminae III-V.

Preparation and Administration of Drugs. The group I mGlu receptor antagonists LY393053, LY367385 (Eli Lilly & Co. Ltd., Surrey, UK), or AIDA (Tocris Cookson, Ballwin, MO) were initially dissolved in 20 μl of 1 N NaOH and then diluted with distilled water to produce a 10 mM stock solution. The final concentrations (0.1–1 mM) were achieved by dilution with aCSF and the final pH for the compounds was adjusted to 7.4. All chemicals used in these experiments were pumped into the spinal cord through the microdialysis fiber with a flow rate of 5 μl/min. The assessment of the amount of drug that can diffuse across the semipermeable microdialysis membrane under ideal conditions in vitro was described in our previous articles and has been used extensively by others (McAdoo et al., 1999; Zhang et al., 2000). Briefly, a microdialysis fiber was placed in a bath containing aCSF. The drug at the applied concentrations was infused through the fiber at a flow rate of 5 μl/min, for the same duration as that of the drug treatment used in the animals. The drug concentrations diffusing across the fiber membrane were measured from the collected dialysate with a spectrophotometer (Beckman Coulter, Inc., Fullerton, CA). The percentages of the drug concentration diffusing across the microdialysis membrane under ideal conditions were 11.23% for LY393053 (at the concentration of 1 mM, inside the fiber), 17.34% for LY367385 (at the concentration of 1 mM, inside the fiber), and 2.7% for AIDA (at the concentration of 1 mM, inside the fiber). Thus, the calculated tissue dose for 1 mM LY393053 is 173 μM; the calculated tissue dose of 1 mM LY367385 is 112 μM; and the calculated tissue dose of 2 mM AIDA used in the fiber is 58 μM. The calculated tissue doses are used throughout the results and figures. Because of the drug's molecular weight and lipophilicity as well as tissue diffusion barriers, the actual tissue concentrations of the drugs are likely to be less than the calculated concentrations in vitro. Therefore, the actual tissue concentration is unknown, but the calculated dose is likely to be the maximal dose to which the tissue might be exposed. Verification of the microdialysis method by McAdoo et al. (1999) has shown that after 60 min, amino acid concentration in the tissue at a distance of 1 mm from the fiber reached an equilibrium of 2% of the concentration inside the microdialysis fiber.

Experimental Protocol

Experiments were carried out on male Sprague-Dawley rats weighing between 250 and 300 g. A power analysis showed that a level of significance at 0.05 could be determined with 90% power using n = 3 for all comparisons. The animals were housed in a normal light and dark cycle for 4 to 7 days before testing. They were then divided into groups: vehicle control group with knee joint inflammation (aCSF), and pre- and postdrug treatment groups with inflammation (AIDA, LY393053, and LY367385) (Tables 2 and 3, see n numbers). The effective dose was determined by constructing a dose-response curve with the paw withdrawal latency plotted against the maximum calculated concentrations of each drug (Fig. 1, see n numbers). In addition to paw withdrawal latencies, knee joint circumferences and the joint temperatures were measured after inflammation for both the contralateral and ipsilateral hindpaw for all groups.

The drug treatment groups were subdivided into pre- and posttreatment studies. All studies were observer blind as to treatment allocation. After overnight recovery from the insertion of the microdialysis fiber, baseline paw withdrawal latencies were tested. In the drug pretreatment study, paw withdrawal latency was tested again 1 h after the spinal administration of the drugs. All rats were then given a mixture of kaolin and carrageenan injected into the knee joint cavity (at 1.5 h). Final paw withdrawal latency tests were performed 4 h after injection of the kaolin and carrageenan mixture. In the drug post-treatment study, after baseline paw withdrawal latency testing, rats were injected with kaolin and carrageenan. The pharmacological agents were infused into the spinal cord 4 h after the knee joint injection. The final paw withdrawal latency test was given 1 h after drug administration.

Vehicle-treated (aCSF) control rats with knee joint inflammation were included with each experiment. Each dose was tested on different animals. To ensure knee joint inflammation, the kaolin and carrageenan injection site was checked by dissection at the end of each experiment. Spinal vertebrae were collected and aldehyde fixed.

Fig. 1. Dose-dependent effects of LY393053 (a), LY367385 (b), and AIDA (c) in antagonizing the secondary thermal hyperalgesia measured as paw withdrawal latency. All of the paw withdrawal latency values were taken (c) in antagonizing the secondary thermal hyperalgesia measured as paw withdrawal latency. All of the paw withdrawal latency values were taken 4 h after injection of the kaolin and carrageenan mixture and compared with their respective baseline paw withdrawal latencies. Each antagonist was administrated directly to the spinal cord through a microdialysis fiber for 1.5 h before the knee joint was inflamed. Concentrations shown were corrected for in vitro recovery rate of each compound.
for histological verification of spinal microdialysis fiber placement site as described above.

**Statistical Analysis**

For all of the studies, the knee joint circumferences and knee joint temperatures were compared by paired \( t \) tests. For the pretreatment and post-treatment studies, the paw withdrawal latencies were compared among the different drug treatment groups as well as to their own baseline with a multiple comparisons analysis of variance, followed by post hoc Scheffé test. A \( p \) value of less than 0.05 was used to indicate significance for all comparisons.

**Results**

**Knee Joint Inflammation and Secondary Thermal Hyperalgesia Induced by Intra-Articular Injection of a Kaolin and Carrageenan Mixture.** To assess the knee joint inflammation, circumference and temperature of the knee joint were initially measured for each rat at the beginning of each experiment. The mean knee joint circumference and joint temperature was 5.59 ± 0.04 cm and 29.9 ± 0.14°C (\( n = 23 \)), respectively. The pain-related posture score for each animal at baseline was recorded as zero. Four hours after injection of the kaolin and carrageenan mixture, all experimental animals displayed an abnormal pain-related posture: curling of the toes and decreased weight bearing on the affected limb. These changes are reflected in increased spontaneous pain rating scores, with a median of 3.0. The affected limb was swollen, with a mean knee joint circumference increase to 6.63 ± 0.09 cm, and joint temperature increased to 32.14 ± 0.24°C. The increases in joint circumference and temperature were significant (\( p < 0.001, \text{paired } t \) test) compared with their respective baseline values (Table 1).

All animals showed signs of secondary thermal hyperalgesia after knee joint injection with the kaolin and carrageenan mixture, as indicated by the shortened paw withdrawal latency. The average paw withdrawal latency at baseline for rats before the knee joint injection was 10.24 ± 0.1 s (\( n = 23 \)). Four hours after knee joint injections of kaolin and carrageenan, animals showed a significant 2- to 3-s decrease of their paw withdrawal latencies. The paw withdrawal latency for the inflamed hindlimb averaged 7.49 ± 0.1 s (\( n = 23 \)) compared with baseline values of 10.24 ± 0.1 (\( p < 0.001, \text{Scheffé test} \)), showing a sensitized response to thermal stimulation after inflammation. The paw withdrawal latency of the contralateral hindlimb (10.51 ± 0.13) did not change in any animals throughout the experiment (Table 1).

A dose-response curve for each of the drugs was generated by giving one concentration of each antagonist to each group of rats. In the range of antagonist concentrations used in these experiments (Fig. 1), recovery of paw withdrawal latency toward their own baseline levels occurred in a dose-dependent manner (Fig. 1, a for LY393053, b for LY 367385, and c for AIDA, respectively). Increased drug concentrations resulted in decreased signs of secondary heat hyperalgesia measured as recovery of paw withdrawal latencies toward baseline (Fig. 1).

**Effect of Pretreatment with Group I mGlu Receptor Antagonists on Acute Knee Joint Inflammation and Secondary Hyperalgesia.** Before induction of knee joint inflammation, animals were divided into four groups for pretreatment with aCSF: (vehicle control), LY393053, LY367385, or AIDA. No changes in the paw withdrawal latencies from baseline were observed as a result of the 1-h drug infusions (\( p > 0.05, \text{Scheffé test} \)) (data not shown).

Four hours after the kaolin and carrageenan injection, the paw withdrawal latency for the aCSF-pretreated group of arthritic rats decreased significantly to 7.45 ± 0.19 compared with its own baseline (\( p < 0.001, \text{Scheffé test} \)) (Fig. 2; Table 2). In contrast, after the drug pretreatments there were no significant differences from baseline values (\( p > 0.05, \text{Scheffé test} \)). In the pretreated groups of rats, the paw withdrawal latencies were 9.21 ± 0.23 for LY393053 (173 μM, \( n = 5 \)), 9.15 ± 0.11 for LY367385 (112 μM, \( n = 6 \)), and 8.81 ± 0.04 for AIDA (58 μM, \( n = 3 \)). All of the drug pretreatment groups were significantly different from the inflamed aCSF-treated control group (\( p < 0.001, \text{Scheffé test} \)) (Table 2; Fig. 2). Thus, the drug pretreatments prevented the decrease in the paw withdrawal latency observed after inflammation and only the aCSF control arthritic rats developed secondary hyperalgesia.

![Fig. 2.](image)

**Fig. 2.** Pretreatment. A bar chart comparing the maximum antagonistic effects of LY393053 (112 μM), LY367385 (173 μM), and AIDA (58 μM), respectively. The paw withdrawal latency in aCSF-treated group was significantly reduced 4 h after a mixture of kaolin and carrageenan was injected into the knee joint, in comparison with their own baseline values (\( p < 0.001, \text{Scheffé test} \)). In contrast, the reduction in the paw withdrawal latency after injection of inflammation did not occur in animals pretreated with the group I mGlu antagonists (\( p > 0.05, \text{Scheffé test} \)). *** \( p < 0.001 \) for both comparisons.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Paw Withdrawal Latency</th>
<th>Circumference</th>
<th>Temperature</th>
<th>Pain Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.24 ± 0.1</td>
<td>5.59 ± 0.04</td>
<td>29.9 ± 0.14</td>
<td>0</td>
</tr>
<tr>
<td>Arthritis (4 h)</td>
<td>7.49 ± 0.1</td>
<td>6.63 ± 0.09</td>
<td>32.14 ± 0.24</td>
<td>3.0</td>
</tr>
<tr>
<td>Contralateral</td>
<td>10.51 ± 0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
None of the metabotropic glutamate antagonist pretreatments significantly altered the signs of local knee joint inflammation induced by knee joint injection of kaolin and carrageenan, i.e., knee joint swelling and increased joint temperature (data not shown). As the symptoms of peripheral inflammation persisted, the blockade of the reduction of paw withdrawal latency after spinal drug pretreatment confirmed the central antihyperalgesic effects exerted by the group I mGlu antagonists.

**Effect of Post-Treatment with Group I mGlu Receptor Antagonists on Acute Knee Joint Inflammation and Secondary Hyperalgesia.** Four hours after knee joint injection with kaolin and carrageenan all animals in these studies showed signs of secondary hyperalgesia, with paw withdrawal latencies significantly shorter than that of their own baseline values. Rats with inflamed knee joints were then randomly subdivided into four experimental groups for treatment with aCSF (n = 4), LY393053 (n = 4), LY367385 (n = 3), or AIDA (n = 4). Initially, after the induction of inflammation, the paw withdrawal latencies were measured at 7.79 ± 0.46 for aCSF group, 7.35 ± 0.21 for LY393053 group, 7.55 ± 0.12 for LY367385 group, and 7.43 ± 0.21 for AIDA group. Using the highest effective doses found in the pretreatment dose-response experiments (Fig. 1), LY393053 (112 μM), LY367385 (173 μM), AIDA (58 μM), or aCSF were infused into the spinal cord via microdialysis fiber for 1 h. The paw withdrawal latencies were measured again for the four groups of rats. The paw withdrawal latencies for the aCSF and LY393053 groups were 8.08 ± 0.24 and 7.83 ± 0.18, respectively (Fig. 3; Table 3). The paw withdrawal latencies for the LY367385 and AIDA post-treatment groups recovered to 8.37 ± 0.09 and 8.55 ± 0.36, respectively. Statistical analysis showed that the values of paw withdrawal latencies taken from the LY367385 and AIDA post-treatment groups were significantly different before and after drug treatment (p < 0.05, Scheffé test). On the other hand, the paw withdrawal latency values from the aCSF inflamed control and LY393053 post-treatment groups were not significantly different from the paw withdrawal latencies measured 4 h after injection with kaolin and carrageenan (p > 0.05) (i.e., comparisons before and after drug treatment) (Fig. 3; Table 3).

In these post-treatment experiments, the symptoms of knee joint inflammation before and after the drug infusion were also examined. There were no significant changes in the knee joint circumference or temperature or the animal’s spontaneous pain score after any of the drug treatments (p > 0.05, paired t test).

**TABLE 2**

Paw withdrawal latencies (PWL) for each drug in the pretreatment study. Paw withdrawal latency values (s) are shown at baseline and 4 h after the mixture of kaolin and carrageenan was injected into the knee joint.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline PWL</th>
<th>Arthritis (4 h) PWL</th>
<th>Animal Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>10.05 ± 0.08</td>
<td>7.45 ± 0.19 ***</td>
<td>n = 8</td>
</tr>
<tr>
<td>LY393053</td>
<td>9.94 ± 0.08</td>
<td>9.21 ± 0.23</td>
<td>n = 5</td>
</tr>
<tr>
<td>LY367385</td>
<td>9.65 ± 0.12</td>
<td>9.15 ± 0.1</td>
<td>n = 6</td>
</tr>
<tr>
<td>AIDA</td>
<td>9.95 ± 0.09</td>
<td>8.81 ± 0.04</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

*** p < 0.001

**Fig. 3.** Post-treatment. A bar chart comparing the effects of post-treatment with aCSF, LY393053 (112 μM), LY367385 (173 μM), or AIDA (58 μM) on the paw withdrawal latencies after induction of acute knee joint inflammation. Comparisons made before and after drug treatment in each group showed that the aCSF and LY39053 post-treatment did not significantly impact paw withdrawal latency (p > 0.05, Scheffé test). In contrast, the reversal of paw withdrawal latency by LY367385 or AIDA post-treatment was significant (p < 0.05, Scheffé test). * p < 0.05

**TABLE 3**

Paw withdrawal latencies for each drug in the post-treatment study. Actual paw withdrawal latency values (PWL) (s) (baseline, arthritis 4 h, and post-drug treatment 1.5 h) are shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline PWL</th>
<th>Arthritis (4 h) PWL</th>
<th>Post 5.5 h PWL</th>
<th>Animal Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>10.62 ± 0.09</td>
<td>7.79 ± 0.46</td>
<td>8.08 ± 0.24</td>
<td>n = 4</td>
</tr>
<tr>
<td>LY393053</td>
<td>10.23 ± 0.19</td>
<td>7.35 ± 0.21</td>
<td>7.83 ± 0.18</td>
<td>n = 4</td>
</tr>
<tr>
<td>LY367385</td>
<td>10.19 ± 0.06</td>
<td>7.55 ± 0.12</td>
<td>8.37 ± 0.09*</td>
<td>n = 3</td>
</tr>
<tr>
<td>AIDA</td>
<td>9.90 ± 0.12</td>
<td>7.43 ± 0.21</td>
<td>8.55 ± 0.36*</td>
<td>n = 4</td>
</tr>
</tbody>
</table>

* p < 0.05

**Discussion**

After injection of a mixture of kaolin and carrageenan into the knee joint cavity of rats, an inflammation rapidly develops in the affected knee joint. The inflammation is accompanied by significant increases in knee joint circumference and joint temperature. An abnormal posture develops with curling of the toes and decreased weight bearing on the affected limb, resulting in an increased spontaneous pain rating score. The development of inflammation is followed by hypersensitivity of the hindpaw to noxious thermal (>55°C) stimulation, shown by shortened paw withdrawal latency. Spinal administration of group I mGlu receptor antagonists LY393053, LY367385, or AIDA, before injection of kaolin and carrageenan, significantly blocks the reduction of paw withdrawal latency. Post-treatment with LY367385 and AIDA reverses thermal hyperalgesia. The baseline paw withdrawal latency is not affected by any of the treatments in the dose range tested. Inflation in the knee joint remained unaffected by all drug treatments.

These results have shown that group I mGlu receptor antagonists can act centrally in the spinal cord to block secondary thermal hyperalgesia that occurs in the hindpaw, but they do not affect the development or maintenance of knee joint inflammation. Spinal microdialysis administration of the antagonists would not affect metabotropic glutate...
mGlu receptors on primary sensory neuronal endings in the periphery or in other parts of the central nervous system. We have demonstrated that the activation of group I mGlu receptors in the spinal cord in response to inflammation of the knee joint contributes to the sensitization of spinal neurons and hence the secondary thermal hyperalgesia occurring in the hindpaw.

It is known that spinal dorsal horn neurons undergo plastic changes during the development of peripheral joint inflammation (Schaible et al., 1986, 1987; Neugebauer and Schaible, 1988, 1990; Neugebauer et al., 1994). Responses to innocuous and noxious stimuli are enhanced in wide dynamic range neurons after knee joint inflammation. The threshold of nociceptive responsive neurons (or high-threshold neurons) is lowered such that the neuron is activated by innocuous stimuli (Neugebauer and Schaible, 1988, 1990). We have proposed previously that it is the increased release of glutamate into the dorsal horn during the development of knee joint inflammation in this model (Sluka et al., 1994, 1992, 1993; Sorkin et al., 1992) that activates NMDA and non-NMDA glutamate receptors leading to sensitization of dorsal horn neurons (Sluka et al., 1994).

So far, several studies have provided evidence that the activation of group I mGlu receptors is involved in the sensitization processes in the spinal dorsal horn, initiated by peripheral inflammation (Neugebauer et al., 1994; Young et al., 1997; Zhang et al., 2000) or sustained nociceptive stimulation (Young et al., 1995). Potentiation of the responses to AMPA and NMDA by group I mGlu receptor agonists has been demonstrated in spinal neurons both in vitro (Bleakman et al., 1992; Ugolini et al., 1997) and in vivo (Cerone and Randic, 1992; Bond and Lodge, 1995; Jones and Headley, 1995). This is also undoubtedly an important mechanism through which group I mGlu receptors contribute to sensitization. We have shown previously that spinal administration of either NMDA or AMPA glutamate receptor antagonists are also effective in reducing secondary hyperalgesia in this knee joint inflammation model (Sluka et al., 1994). Thus, it is clear that the development and maintenance of secondary thermal hyperalgesia in this model involves multiple factors that include NMDA, non-NMDA, and metabotropic glutamate receptors.

The mechanisms by which group I mGlu receptors contribute to the sensitization of spinal neurons remain unresolved. Upon the activation of group I mGlu receptors elevation of intracellular calcium levels occurs due to the release of calcium from internal stores, resulting in activation of a number of second messenger systems. The elevated intracellular calcium can lead to the activation of various intracellular kinases modulating function of a variety of receptors and channels (Conn and Pin, 1997). Activation of protein kinase C results in the modulation of ion channels, including the inhibition of some K+ conductance (I_{MHP} and I_{M}) (Conn and Pin, 1997). Inhibition of K+ conductance can result in the depolarization of central neurons (Ugolini et al., 1997; Chen et al., 2000).

In the present study using behavioral assessments in awake rats, although spinal administration of the group I mGlu antagonists before or 4 h after knee joint injection of the mixture of kaolin and carrageenan did promote significant recovery of the paw withdrawal latency, the joint inflammation itself was unaffected. The same group I mGlu receptor antagonists, LY395053 and AIDA, block enhanced dorsal root reflex activity that occurs after knee joint inflammation in the anesthetized preparation, suggesting that group I mGlu receptors in the spinal cord contribute to the generation of dorsal root reflexes in the medial articular nerve (Zhang et al., 2000). It has been suggested that dorsal root reflex activity may contribute to knee joint inflammation as well as development of hyperalgesia (Sluka et al., 1994). Using this knee joint inflammation model we have previously shown that non-NMDA and γ-aminobutyric acidA receptor antagonists can attenuate the neurogenic inflammatory response, whereas NMDA and γ-aminobutyric acidB were ineffective in altering inflammatory signs (Sluka et al., 1994). On the basis of these studies, it appears likely that mGlu receptor activation can also contribute to initiation of inflammation and hyperalgesia, but the cellular cascades that contribute to inflammatory events do not require ongoing mGlu receptor activation.

Several studies have shown that blockade of spinal group I mGlu receptors, especially the mGlu1 receptor, inhibits nociceptive responses in some pain models (Young et al., 1995, 1997, 1998; Fisher and Coderre, 1996; Salt and Turner, 1998; Salt et al., 1999; Chen et al., 2000). In these studies, nociceptive responses were selectively reduced by blocking group I mGlu receptors, whereas normal mechanical or other innocuous sensory processing was not affected by group I mGlu receptor antagonists, mGlu1 antisense (Young et al., 1998), or antibody treatment (Fundytus et al., 1998). Thus, mGlu1 receptor antagonists can block the sensitization of dorsal horn neurons, as well as some nociceptive responses.

It is also interesting to note that group I mGlu receptor antagonists do not affect responses of neurons to innocuous stimuli in a nonsensitized state as noted previously by others (Neugebauer et al., 1994; Young et al., 1995, 1997). In the present study, it was confirmed that baseline paw withdrawal latencies were not affected by spinal administration of mGlu1 (LY367385) or mGlu1 and 5 (LY393053 and AIDA) receptor antagonists in the dose range tested. These characteristics place group I mGlu receptor antagonists as useful analgesics by selectively preventing dorsal horn neurons undergoing sensitization processes.

The LY367385 has recently been reported to be a selective mGlu1 antagonist (Clark et al., 1997), whereas LY393053 has equal potency on mGlu1 and 5 receptors (Chen et al., 2000). The potent mGlu1 and 5 receptor antagonist LY393053, however, did not provide as complete a recovery of the paw withdrawal latency as the selective mGlu1 receptor antagonist LY367385. Our results stressed the importance of blocking spinal mGlu1 receptors for the prevention of secondary thermal hyperalgesia, which is considered to be the result of sensitization of dorsal horn neurons (Schaible et al., 1986, 1987; Neugebauer and Schaible, 1988, 1990; Neugebauer et al., 1994). Although AIDA is not as selective for mGlu1 receptors as LY367385, in the dose range used, it offered recovery of the paw withdrawal latency similar to that of LY367385, exhibiting a predominant mGlu1-antagonizing effect in this case.

The two group I mGlu receptors mGlu1 and mGlu5 share similar cellular mechanisms (Conn and Pin, 1997) and both are present in the spinal cord (Shigemoto et al., 1992, 1993). It is not surprising to find that the most recently available mGlu5 selective antagonists, 2-methyl-6-(phenylethynyl) pyridine and 6-methyl-2-(phenylazo)-3-pyridinol, when ap-
plied spinally, can reduce thermal hyperalgesia (Dogrul et al., 2000) or acute nociception (Bordi and Ugolini, 2000). It would be interesting to compare the effects of the mGlur5-selective antagonist 2-methyl-6-(phenylethynyl)pyridine with the mGlur1-selective antagonist LY367385 in the same model to investigate the relative contributions by the two receptors in a future study. Pretreatment with specific mGlur5 receptor antagonists have been shown to reduce hyperalgesic responses to mechanical stimuli in models of inflammation through peripheral rather than central receptors (Walker et al., 2001a,b).

In conclusion, spinal infusion of group I mGlur receptor antagonists LY395053, LY367385, or AIDA, before a knee joint injection of a mixture of kaolin and carrageenan, significantly reduced the development of secondary thermal hyperalgesia indicative of sensitization of spinal neurons. Treatment with the group I mGlur receptor antagonists LY367385 and AIDA after development of acute knee joint inflammation allowed recovery of the paw withdrawal latency and thus reduction of secondary hyperalgesia. Therefore, group I mGlur receptors contribute to secondary thermal hyperalgesia during the initial development stages immediately after injection of kaolin and carrageenan and also during the maintenance phase. The group I mGlur receptor antagonists were not effective in reducing the inflammation itself, i.e., the joint swelling and temperature increase. Thus, the inflammation induced in this model is not dependent on ongoing spinal metabotropic glutamate receptor activation. Although activation of ionotropic glutamate receptors is also essential for the development of thermal hyperalgesia, group I mGlur receptors can contribute significantly to this event perhaps via enhancing the responsiveness of NMDA and AMPA receptors (Bleakman et al., 1992; Cerne and Randic, 1992; Bond and Lodge, 1995; Jones and Headley, 1995; Ugolini et al., 1997). Only when there is a sufficiently increased glutamate release will group I mGlur receptors be activated. Coactivation of group I mGlur receptors brings about potentiation of the NMDA receptor-mediated response, leading to “wind-up” and the development of the hyperalgesic state. Thus, blocking group I mGlur receptors may offer an add-on therapeutic advantage in neurogenic nociceptive response.

Acknowledgments

We thank Drs. David Lodge, Ann Kingston, and Darryle Schoepf for reading the manuscript and useful discussions.

References


Address correspondence to: Dr. Karin N. Westlund High, Director, Cell Biology Graduate Program, and Professor, Department of Anatomy and Neurosciences, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1043. E-mail: kwhigh@utmb.edu