Distinct Roles of Group III Metabotropic Glutamate Receptors in Control of Nociception and Dorsal Horn Neurons in Normal and Nerve-Injured Rats

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ABSTRACT
Increased glutamatergic input to spinal dorsal horn neurons constitutes an important mechanism for neuropathic pain. However, the role of group III metabotropic glutamate receptors (mGluRs) in regulation of nociception and dorsal horn neurons in normal and neuropathic pain conditions is not fully known. In this study, we determined the effect of the group III mGluR specific agonist L(+)2-amino-4-phosphonobutyric acid (L-AP4) on nociception and dorsal horn projection neurons in normal rats and a rat model of neuropathic pain. Tactile allodynia was induced by ligation of L5/L6 left spinal nerves in rats. Allodynia was determined by von Frey filaments in nerve-injured rats. The nociceptive threshold was tested using a radiant heat and a Randall-Selitto pressure device in normal rats. Single-unit activity of ascending dorsal horn neurons was recorded from the lumbar spinal cord in anesthetized rats. An intrathecal (5–30 μg) L-AP4 dose-dependently attenuated allodynia in nerve-injured rats but had no antinociceptive effect in normal rats. Topical spinal application of 5 to 50 μM L-AP4 also significantly inhibited the evoked responses of ascending dorsal horn neurons in nerve-ligated but not normal rats. Furthermore, blockade of spinal group III mGluRs significantly decreased the withdrawal threshold and increased the evoked responses of dorsal horn neurons in normal but not nerve-injured rats. These data suggest that group III mGluRs play distinct roles in regulation of nociception and dorsal horn neurons in normal and neuropathic pain states. Activation of spinal group III mGluRs suppresses allodynia and inhibits the hypersensitivity of dorsal horn projection neurons associated with neuropathic pain.

Chronic neuropathic pain remains a significant clinical problem and is often resistant to conventional analgesics (Sindrup and Jensen, 1999; Woolf and Mannion, 1999). Glutamate is the major excitatory neurotransmitter used for synaptic transmission from the primary afferents to dorsal horn neurons. Increased glutamatergic input to the spinal dorsal horn neurons constitutes an important mechanism for central sensitization and the development and maintenance of neuropathic pain syndromes following nerve injury (Dougherty et al., 1992; Kawamata and Omote, 1996). Glutamate acts through two broad classes of glutamate receptors, ion channel-linked (ionotropic) receptors and G protein-coupled metabotropic receptors (mGluRs). Eight mGluRs have been cloned and are classified into three groups. Group I receptors (mGluRs 1 and 5) couple to phospholipase C and increase neuronal firing and synaptic transmission.

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; L-AP4, L(+)2-amino-4-phosphonobutyric acid; MAP4, (S)-2-amino-2-methyl-4-phosphonobutyric acid.
tented by a group II mGlur agonist but slightly attenuated by a group III mGlur agonist administered intrathecally (Fisher and Codere, 1996). However, activation of spinal groups II and III mGlurs both reduces capsaicin-induced sensitization of dorsal horn neurons (Neugebauer et al., 2000).

Two subtypes of group III mGlurs have been located in the rat spinal dorsal horn. In this regard, the mRNA and immunoreactivity for mGlur4 and mGlur7 have been found in the superficial dorsal horn (Ohishi et al., 1995; Li et al., 1997; Azkue et al., 2001). Although intrathecal pretreatment with selective groups II and III mGlur agonists reduces the development of neuropathic pain in rats (Fisher et al., 2002), the action and mechanisms of group III mGlur activation on chronic neuropathic pain remain to be investigated. L(+)-2-amino-4-phosphonobutyric acid (L-AP4) is a potent and specific agonist for the group III mGlurs (Conn and Pin, 1997; Schoepf et al., 1999). In the present study, we determined the spinal effect of L-AP4 on both nociception and functionally identified dorsal horn projection neurons in normal rats and a rat model of neuropathic pain.

Materials and Methods

Induction of Allodynia and Implantation of Intrathecal Catheters. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200 to 220 g were used in this study. Ligation of L5 and L6 spinal nerves in rats was used in this study as an experimental model of neuropathic pain because it produces profound and sustained tactile allodynia, which resembles the condition observed in patients with neuropathic pain (Kim and Chung, 1992). During halothane-induced anesthesia, the left L5 and L6 spinal nerves were isolated and ligated tightly with 4-0 silk suture, according to the method described by Kim and Chung (1992). Intrathecal catheters were inserted in normal and nerve-ligated rats during halothane-induced anesthesia. Intrathecal catheters (PE-10 polyethylene tubing) were advanced 8 cm caudal through an incision in the eusternal membrane and secured to the musculature at the incision site. The rats were allowed to recover for another 7 days before being used for behavioral tests. Only animals with no evidence of neurological deficits after catheter insertion were studied. All the experiments were conducted 4 to 6 weeks after spinal nerve ligation. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and conformed to National Institutes of Health guidelines on the ethical use of animals. All efforts were made to minimize both the suffering and number of animals used.

Behavioral Assessment of Tactile Allodynia. To evaluate the mechanical sensitivity of the injured hind paw, rats were placed in individual plastic boxes on a mesh floor and allowed to acclimate for 30 min. A series of calibrated von Frey filaments (Stoelting Co., Wood Dale, IL) were applied perpendicularly to the plantar surface of the left hind paw with sufficient forces to bend the filaments for 6 s. Brisk withdrawal or paw flinching was considered as a positive response. In the absence of a response, the filament of next greater force was applied. In the presence of a response, the filament of next lower force was applied (Chaplan et al., 1994; Chen and Pan, 2002). The tactile stimulus producing a 50% likelihood of withdrawal response was calculated by using the “up-down” method. Each trial was repeated two to three times at approximately 2-min intervals, and the mean value was used as the force to produce withdrawal responses. Because a large bending force of von Frey filaments can lift the hind paw in normal rats, 25 g was considered as the cutoff value (Chaplan et al., 1994; Chen and Pan, 2002).

Behavioral Assessment of Thermal Nociception. To quantitatively assess the thermal sensitivity of the normal rats, rats were placed on the glass surface of a thermal testing apparatus (IITC Inc., Woodland Hills, CA). The rats were allowed to acclimate for 30 min before testing. The temperature of the glass surface was maintained constant at 30°C. A mobile radiant heat source located under the glass was focused onto the hind paw of each rat. The paw withdrawal latency was recorded by a timer. The apparatus was adjusted at the beginning of the study so that the baseline paw withdrawal latency in normal rats was approximately 10 s. This setting (the light beam intensity) was kept unchanged throughout the study. The cutoff value of 30 s was used to prevent potential tissue damage (Chen and Pan, 2002).

Behavioral Testing of Mechanical Nociception. Because von Frey filaments do not elicit withdrawal responses in normal rats, nociceptive mechanical thresholds, expressed in grams, were measured in normal rats with the Randall-Selitto test using an analgesimeter (Ugo Basile, Comerio, Italy). The test was performed by applying a noxious pressure to the hind paw. By pressing a pedal that activated a motor, the force increased at a constant rate on the linear scale. When the animal displayed pain by withdrawal of the paw or vocalization, the pedal was immediately released and the nociceptive threshold read on a scale. The cut-off of 400 g was used to avoid potential tissue injury (Chen and Pan, 2002). Both hind paws were tested in each rat, and the mean value was used as the withdrawal threshold in response to the noxious pressure.

Motor function was also evaluated in the above behavioral experiments by the placing/stepping reflex and the righting reflex (Chen and Pan, 2003). The former was evoked by drawing the dorsum of either hind paw across the edge of the table. The latter was assessed by placing the rat horizontally with its back on the table, which normally gives rise to an immediate coordinated twisting of the body to an upright position. Changes in motor function were scored as follows: 0, normal; 1, slight deficit; 2, moderate deficit; and 3, severe deficit. Drugs for intrathecal injection were dissolved in normal saline and administered in a volume of 5 μl, followed by a 10-μl flush with normal saline. Repeat intrathecal injections in the same animals were separated by 4 to 5 days. L-AP4 and the specific group III mGlur antagonist, (S)-2-amino-2-methyl-4-phosphonobutyric acid (MAP4), were obtained from Sigma-Aldrich (St. Louis, MO). Both drugs were freshly dissolved in normal saline, and this vehicle has no effect on the withdrawal threshold and dorsal horn neurons in rats (Chen and Pan, 2002, 2003, 2004).

Single-Unit Recording of Ascending Dorsal Horn Neurons. Alloodynic conditions were first verified in all nerve-ligated rats before the electrophysiological experiments. Anesthesia was initially induced with 2% halothane in 100% oxygen. The right jugular vein and left carotid artery were cannulated for administering drugs and monitoring the blood pressure, respectively. Following cannulation, sodium pentobarbital (50 mg/kg) was given intravenously, and the injection was repeated when necessary. The level of anesthesia was maintained at a sufficient level as judged by the absence of corneal reflexes, withdrawal reflexes to noxious pinch, and spontaneous blood pressure fluctuations. The trachea was cannulated, and the rat was ventilated mechanically with a respirator (CWE, Inc., Ardmore, PA). The respirator was adjusted to keep the end-tidal CO2 concentration at 3 to 4%, monitored by a Capstar-100 CO2 Analyzer (CWE, Inc.). Limited laminectomies were performed to expose the spinal cord at the C1–2 and L2–3 levels. Around the exposed lumbar spinal cord, a small pool (−0.2 ml) was formed by the surrounding muscle and soft tissues to serve as a reservoir for topical application of drugs to simulate the intrathecal administration of drugs used in behavioral studies (Chen and Pan, 2004). After the dura was removed at both sites, the spinal cord was covered with artificial cerebrospinal fluid solution. A bipolar, concentric metal stimulating electrode was inserted into the right ventrolateral quadrant of the first cervical segment (Chen and Pan, 2004). Dorsal horn neurons in the left side of the lumbar enlargement were recorded with a glass electrode filled with 5% KCl solution (resistance, 4–6 MΩ). A motorized manipulator (David-Kopf Instruments, Tujunga, CA) was used to gradually
descend the recording electrode until an individual dorsal horn neuron was identified up to 1 mm in depth from the dorsal surface of the spinal cord.

Individual ascending dorsal horn neurons in the lumbar enlargement were antidromically identified and characterized. The search stimulus was 0.5 to 1.0 mA, 0.2 ms, and 0.8 to 1 Hz (S48 stimulator; Grass Instruments, Quincy, MA). The dorsal horn neurons were considered to be antidromically activated if the following criteria were met (Chen and Pan, 2002, 2004): the antidromically evoked spikes were collided at a constant latency, the antidromically evoked spikes followed a high-frequency (400 Hz) stimulation, and the antidromic action potential collided with the orthodromic spike within the critical interval. Single-unit activity of the dorsal horn neuron was isolated using a software window discriminator (DataWave Technology, Longmont, CO). The action potential of the neuron was amplified, filtered with a band-pass filter (DAM 80; World Precision Instruments, Inc., Sarasota, FL), processed through an audioamplifier (Grass Instruments), and monitored on a storage oscilloscope (Tektronix, Beaverton, OR). The neuronal impulse activity also was recorded into a computer through an A/D interface board for subsequent off-line quantitative analysis. Discharge frequency was quantified by using a data acquisition and analysis software (Experimental Workbench; DataWave Technology). After the cutaneous receptive field was located, responses of dorsal horn neurons to touch, pressure, and pinch applied to the receptive field were then determined, as we described previously (Chen and Pan, 2002). The touch stimulus was applied with a cotton tip for two to three back-and-forth cycles. The wooden tip of a cotton-tipped applicator was used to apply the pressure stimulus. The tip was applied perpendicular to the skin for 5 to 6 s to generate an intense pressure (~200 g/mm²), which was perceived by the investigator as mildly painful. The pinch stimulus was applied by means of a small forceps with a strong grip (~560 g/mm²) that produces distinct pain when applied to human skin without causing tissue damage (Chen and Pan, 2002, 2004). In addition, responses to calibrated von Frey filaments of different bending forces (2, 4, 15, and 26 g; Stoelting) applied to the receptive field of dorsal horn neurons were also examined. The filaments were applied in an ascending order, starting with the lowest bending force, each being applied for 5 to 6 s. Only one or two ascending dorsal horn neurons were studied in each rat.

**Inhibitory Effect of L-AP4 on Dorsal Horn Neurons.** The effect of L-AP4 (5, 10, and 50 μM) on identified ascending dorsal horn neurons was studied in 20 L5 and L6 spinal nerve ligated rats. After recording the background activity for 2 to 3 min, responses of dorsal horn neurons to von Frey filaments, touch, pressure, and pinch were examined. L-AP4, starting with the lowest concentration, was applied topically onto the exposed spinal cord. Five to 10 min following L-AP4 application, the response of neurons to graded mechanical stimuli was re-examined. The drug solution was then carefully removed, and the spinal cord was washed with artificial cerebrospinal fluid. The procedure was then repeated to test the other concentrations of L-AP4. To ensure that the effect of L-AP4 on dorsal horn neurons was through group III mGluRs, the inhibitory effect of 50 μM L-AP4 was tested before and after topical application of 100 μM MAP4, a specific antagonist for group III mGluRs (Salt and Eaton, 1995; Conn and Pin, 1997), in another 12 dorsal horn neurons. MAP4 was applied to the recording site 10 min before application of 50 μM L-AP4.

In addition, to determine whether topical application of L-AP4 affects ascending dorsal horn neurons in normal rats, the effect of 50 μM L-AP4 on spontaneous and evoked responses to graded mechanical stimuli was examined in 11 dorsal horn neurons from eight normal rats. Also, the effect of topical spinal application of 100 μM MAP4 alone on these cells was tested 15 to 20 min after washout of L-AP4 solution from the recording site.

**Statistical Analysis.** Data are presented as mean ± S.E.M. Paw withdrawal thresholds in response to mechanical stimulation before and after nerve ligation were compared using a paired Student’s t test. The discharge rate of dorsal horn neurons was averaged during a 2- to 3-min control period, and the evoked responses were quantified as the mean discharge rate during the duration of the stimulus. The effects of L-AP4 and MAP4 on the withdrawal threshold and dorsal horn neurons were compared using repeated measures analysis of variance followed by Tukey’s post hoc test. For calculation of ED₅₀, data were converted to the percentage of the inhibitory effect of L-AP4 based on the following calculation: [(evoked response during control-evoked response during L-AP4)/evoked response during control] × 100%. The ED₅₀ values of L-AP4 and their 95% confidence limits were determined by nonlinear regression analyses of the concentration-response curves using GraphPad Prism (GraphPad Software Inc., San Diego, CA). Differences were considered to be statistically significant when P < 0.05.

**Results**

**Effect of L-AP4 on Tactile Alldynia in Nerve-Injured Rats.** Paw withdrawal threshold in response to application of von Frey filaments before spinal nerve ligation was 22.6 ± 2.4 g. The mechanical threshold decreased significantly (2.3 ± 0.5 g, P < 0.05) within 10 days after nerve ligation and remained stable for at least 8 weeks.

Intrathecal injection of 5 to 30 μg of L-AP4 significantly increased the paw withdrawal threshold in response to application of von Frey filaments in eight nerve-ligated rats in a dose-dependent manner (Fig. 1). The maximal effect of L-AP4 appeared within 45 min and gradually subsided in 120 min following intrathecal administration. To determine the specific effect of L-AP4, 100 μg of MAP4, a specific antagonist for group III mGluRs, was given intrathecally 15 min before 30 μg of L-AP4 was injected in another seven rats. The baseline paw withdrawal threshold was not significantly altered by intrathecal injection of 100 μg of MAP4 (data not shown). In rats pretreated with MAP4, the effect of 30 μg of L-AP4 on the paw withdrawal threshold in response to von Frey filaments was abolished (Fig. 1). The estimated ED₅₀ value (95% confidence limits) of the antiallodynic effect of intrathecal L-AP4 was 8.14 (2.15–27.15) μg.

The motor function, based on the placing/stepping reflex and the righting reflex, appeared normal in MAP4-treated rats. Intrathecal administration of different doses of L-AP4 was not associated with any evident motor dysfunction. All
Effect of L-AP4 and MAP4 on Thermal and Mechanical Nociception in Normal Rats. The withdrawal latency of the hind paw in response to the radiant heat stimulation in normal rats was $9.7 \pm 0.8$ s ($n = 7$). Intrathecal injection of 30 μg of L-AP4 did not significantly alter the paw withdrawal latency in these normal rats (Fig. 2A). However, intrathecal 100 μg of MAP4 alone significantly decreased the withdrawal latency in another eight normal rats (Fig. 2A).

Similarly, the same dose of L-AP4 (30 μg) did not significantly change the paw withdrawal threshold in response to a noxious pressure stimulus, tested with the Randall-Selitto device, in seven normal rats tested (Fig. 2B). Intrathecal 100 μg of MAP4 alone significantly reduced the withdrawal threshold in response to the pressure stimulus in a separate group of normal rats ($n = 8$, Fig. 2B).

Effect of L-AP4 on Ascending Dorsal Horn Neurons in Nerve-Injured Rats. A total of 32 ascending dorsal horn neurons from 22 nerve-injured rats was studied. Also, 11 ascending dorsal horn neurons were recorded from eight normal rats. All the dorsal horn projection neurons included in this study were wide-dynamic range neurons (Chen and Pan, 2002, 2004). The ascending dorsal horn neurons recorded in the lumbar spinal cord in normal and nerve-injured rats had a mean depth of $525 \pm 19$ and $504 \pm 11$ μm ($P > 0.05$), respectively.

In 20 ascending dorsal horn neurons examined in the spinal nerve-ligated rats, the discharge activity increased in a graded manner in response to touch, pressure, pinch, and von Frey filaments (Fig. 3). Topical application of 5 to 50 μM L-AP4 to the spinal cord significantly inhibited the evoked response of neurons to touch, pressure, pinch, and von Frey filaments in a concentration-dependent fashion (Fig. 3, A–C). The effect of L-AP4 appeared in less than 5 min following drug application and was washed out within 5 to 10 min after removing the L-AP4 solution from the recording site. L-AP4 had a significant inhibitory effect on evoked responses of dorsal horn neurons starting at 5 μM, and the maximal inhibition was obtained at 50 μM. L-AP4 at 1 μM had no significant effect on evoked responses of dorsal horn neurons. There was no significant difference between the inhibition effect of L-AP4 on spinal dorsal horn neurons measured at 5

Fig. 2. Effect of intrathecal 30 μg of L-AP4 ($n = 7$) or 100 μg of MAP4 ($n = 8$) on the nociceptive withdrawal threshold in normal rats. The nociceptive threshold was determined by the withdrawal response of the hind paw to noxious radiant heat (A) and a pressure stimulus (B) applied to the hind paw. Data presented as mean ± S.E.M. *, $P < 0.05$ compared with the respective control (time 0).

Fig. 3. Inhibitory effect of L-AP4 on spinal dorsal horn neurons. A, original neurograms showing the inhibitory effect of topical application of 5 to 50 μM L-AP4 on the response of an ascending dorsal horn neuron to application of a 15-g von Frey filament to the receptive field. Arrows indicate the time point of application of the stimulus to the receptive field. B and C, concentration-dependent inhibitory effect of 5 to 50 μM L-AP4 on the discharge activity of 20 ascending dorsal horn neurons in response to von Frey filaments (B) and graded mechanical stimuli (C) in nerve-ligated rats. Data presented as mean ± S.E.M. *, $P < 0.05$ compared with the respective control.
or 10 min following drug application (data not shown). L-AP4 inhibited the evoked responses of dorsal horn neurons to brush, press, and pinch with an ED$_{50}$ value (95% confidence limits) of 7.28 (2.03–32.15), 6.94 (1.66–28.33), and 4.22 (0.77–21.52) µM, respectively.

To examine whether the inhibitory effect of L-AP4 was mediated by group III mGluRs, the effect of 50 µM L-AP4 on evoked responses of another 12 ascending dorsal horn neurons in nerve-ligated rats was tested before and 10 min after topical application of 100 µM MAP4. MAP4 alone had no significant effect on the baseline activity and the evoked response of dorsal horn neurons. In the presence of 100 µM MAP4, the inhibitory effect of 50 µM L-AP4 on these 12 dorsal horn neurons was completely blocked (Fig. 4).

**Effect of L-AP4 on Ascending Dorsal Horn Neurons in Normal Rats.** In 11 dorsal horn projection neurons examined in normal rats, topical application of 50 µM L-AP4 had no significant effect on the spontaneous and evoked responses of neurons to graded mechanical stimuli applied to the receptive field (Fig. 5). Although 100 µM MAP4 alone only slightly increased the baseline activity and evoked responses of dorsal horn neurons at the low intensity, it significantly potentiated the response of these 11 cells evoked at the high intensity of stimulation applied to the receptive field (Fig. 5).

**Discussion**

In this combined behavioral and electrophysiological study, we determined the spinal effect of the group III mGluR agonist L-AP4 on nociception and dorsal horn projection neurons in normal rats and a rat model of neuropathic pain. We first assessed the antiallodynic and antinociceptive effect of intrathecal L-AP4 in spinal nerve-ligated and normal rats. To further determine the mechanism underlying the antiallo-

![Fig. 4.](image_url)

Fig. 4. MAP4 blocks the inhibitory effect of L-AP4 on spinal dorsal horn neurons. A, original traces showing the effect of 100 µM MAP4 on the inhibitory effect of 50 µM L-AP4 on responses of an ascending dorsal horn neuron to brush, pressure, and pinch. Arrows indicate the time point of application of the stimulus to the receptive field. B, summary data showing the effect of 100 µM MAP4 on the inhibitory action of 50 µM L-AP4 on evoked responses of 12 ascending dorsal horn neurons to touch, pressure, and pinch in nerve-ligated rats. Data presented as mean ± S.E.M. *P < 0.05 compared with the respective control.

dynic effect of L-AP4, we examined the effect of topical application of L-AP4 on ascending dorsal horn neurons in normal and nerve-injured rats. We found that intrathecal administration of the group III mGluR specific agonist L-AP4 dose-dependently attenuated allodynia in spinal nerve-ligated rats. On the other hand, intrathecal injection of L-AP4 failed to alter the nociceptive threshold in normal rats. Also, topical application of L-AP4 significantly inhibited the ascending dorsal horn neurons in nerve-injured but not normal rats. The inhibitory effects of L-AP4 on both allodynia and dorsal horn neurons in nerve-injured rats were completely abolished by a specific group III mGluR antagonist, MAP4. Therefore, this in vivo study provides new information that group III mGluRs play a distinct role in regulation of nociceptive processing in the spinal dorsal horn. Activation of spinal group III mGluRs may be an important alternative treatment for neuropathic pain.

The spinal dorsal horn is a major site for transmission and modulation of nociception. Previous studies have shown that increased synaptic glutamate release plays an important role in the alteration of the sensory processing and plasticity of dorsal horn neurons after nerve injury (Dougherty et al., 1992; Leem et al., 1996; Tolle et al., 1996). In the spinal cord, the increased glutamate availability contributes to the enhanced excitability of dorsal horn neurons in the chronic pain state through activation of NMDA and non-NMDA receptors (Dougherty et al., 1992; Harris et al., 1996; Leem et al., 1996). The heterogeneous mGluRs are classified into three groups, based on similarities in their amino acid sequences, their linkage to second messenger systems, and their pharmacology. Group I mGluRs couple to G$_{q/11}$ and phospholipase
C and enhance neuronal excitability and synaptic transmission (Conn and Pin, 1997; Miura et al., 2002). On the other hand, activation of groups II and III mGluRs inhibits adenylate cyclase and reduces neuronal excitability and synaptic neurotransmitter release (Macek et al., 1996; Conn and Pin, 1997; Schoepp et al., 1999). It has been shown that activation of group III mGluRs inhibits both excitatory and inhibitory synaptic transmission in the spinal cord in vitro (Gerber et al., 2000). In the present study, we observed that the selective group III mGluR agonist 1-AP4 produced a dose-dependent reduction in allodynia induced by L5 and L6 spinal nerve ligation in rats. Consistent with the behavioral data, 1-AP4 inhibited the evoked responses of dorsal horn projection neurons to mechanical stimulation of the receptive fields in rats subjected to spinal nerve ligation. We found that the specific antagonist for group III mGluRs, MAP4, completely blocked the inhibitory effect of 1-AP4 on allodynia and dorsal horn neurons in nerve-injured rats. Thus, the antiallodynic effect of intrathecal 1-AP4 likely results from inhibition of dorsal horn neurons through activation of group III mGluRs.

Presynaptic mGluRs have been postulated to function as autoreceptors for regulation of glutamate release from the primary afferents (Gerber et al., 2000; Azkue et al., 2001). The reduction of synaptic transmissions by activation of group III mGluRs at presynaptic sites has been shown in several brain areas and the spinal cord dorsal horn in vitro (Baskys and Malenka, 1991; Trombley and Westbrook, 1992; Gereau and Conn, 1995; Salt and Eaton, 1995; Gerber et al., 2000). Peripheral nerve injury results in ectopic discharges from injured afferent nerves, which trigger persistent and increased glutamate release in the spinal cord and lead to hyperexcitability of dorsal horn neurons (Harris et al., 1996; Leem et al., 1996; Chen et al., 2002). Voltage-gated Ca$^{2+}$ channels are important for presynaptic glutamate release in the spinal cord. Activation of group III mGluRs inhibits voltage-gated Ca$^{2+}$ channels and attenuates presynaptic glutamate release (Trombley and Westbrook, 1992; Conn and Pin, 1997; Gerber et al., 2000). Accordingly, 1-AP4 can suppress the ionotropic glutamate receptor-maintained hypersensitivity of dorsal horn neurons induced by nerve injury (Harris et al., 1996; Leem et al., 1996). The inhibitory effect of 1-AP4 on spinal neurotransmission is probably mediated by the group III mGluR4 or mGluR7 in the spinal cord dorsal horn (Ohishi et al., 1995; Li et al., 1997; Azkue et al., 2001). In situ hybridization and immunocytochemical labeling have demonstrated that both mGluR4 and mGluR7 are expressed in the superficial dorsal horn and located primarily on central terminals of primary afferents nerves (Ohishi et al., 1995; Li et al., 1997; Azkue et al., 2001). It is noteworthy that 1-AP4 has a much higher affinity for mGluR4 than mGluR7 (Tanabe et al., 1993; Saugstad et al., 1994). Thus, it is possible that the spinal effect of 1-AP4 is mainly through activation of mGluR4.

The differential effect of 1-AP4 on nociception and dorsal horn neurons in normal and nerve-injured rats is an unexpected but intriguing finding. Intrathecal injection of 1-AP4 failed to produce an antinociceptive effect in normal rats in responses to both thermal and pressure stimuli. Similarly, topical application of 1-AP4 failed to alter significantly the evoked response of dorsal horn neurons in normal rats. It is possible that the dorsal horn projection neurons are tonically controlled by GABAergic inputs and group III mGluRs under the normal condition. This possibility is supported by our data showing that MAP4 alone significantly augmented evoked responses of dorsal horn neurons to a higher intensity of stimulation and decreased the nociceptive withdrawal threshold in normal rats. It should be noted that the pronociceptive effect of MAP4 in normal rats can be explained by its action on group III mGluRs in the spinal cord. Group III mGluRs function as autoreceptors to limit glutamate release from the primary afferents. It has been demonstrated that blocking group III mGluRs with MAP4 potentiates ~20% of glutamatergic EPSCs evoked from the dorsal root in normal spinal cord slices (Cao et al., 1997). Therefore, increased glutamate release from the primary afferents by MAP4 can augment the evoked responses of dorsal horn neurons and produce a pronociceptive action in normal rats. Furthermore, the differential effect of 1-AP4 may be explained by the different mechanisms that regulate the normal nociception and increased sensitivity (i.e., allodynia) associated with chronic neuropathic pain. The excitability of dorsal horn neurons is controlled by both inhibitory and excitatory synaptic inputs (Yoshimura and Nishi, 1995; Pan and Pan, 2004). Group III mGluRs can function as autoreceptors and heteroreceptors to regulate synaptic release of glutamate and GABA, respectively (Salt and Eaton, 1995; Macek et al., 1996; Conn and Pin, 1997; Gerber et al., 2000). Consequently, activation of the presynaptic group III mGluRs with 1-AP4 could reduce both synaptic glutamate (autoreceptors) and GABA (heteroreceptors) release in the spinal cord. As a result of attenuation of both excitatory and inhibitory inputs, there may be a zero net effect of 1-AP4 on the excitability of ascending dorsal horn neurons and nociception under the normal condition. By contrast, the GABAergic inhibitory input is reduced, whereas the glutamatergic excitatory tone is increased in the spinal dorsal horn (Harris et al., 1996; Moore et al., 2002), resulting in hypersensitivity of dorsal horn neurons and allodynia in the neuropathic pain state. Thus, the major spinal effect of 1-AP4 is likely mediated by inhibition of synaptic glutamate release, which leads to attenuation of allodynia and hyperexcitability of dorsal horn neurons caused by nerve injury. The differential spinal effect of 1-AP4 on glutamatergic and GABAergic synaptic transmission in normal and neuropathic pain states probably accounts for the preferential effect of 1-AP4 in spinal nerve-ligated rats.

Contrary to the finding in a previous study (Neugebauer et al., 2000), we observed that 1-AP4 had no significant effect on dorsal horn neurons in normal animals. Although the reasons for this discrepancy are not entirely clear, different methods used to deliver the drug should be emphasized. Although 1-AP4 was topically applied to the surface of the spinal cord to simulate intrathecal administration in our study, this agent was administered through a microdialysis tubing inserted into the spinal dorsal horn adjacent to the recording site in the previous study (Neugebauer et al., 2000). Thus, the tissue damage associated with the surgical insertion of the microdialysis probe probably results in sensitization of dorsal horn neurons and alters the functional state and responses of the cells in that study.

In summary, our behavioral and electrophysiological study demonstrates that spinal administration of the group III mGluR agonist 1-AP4 alleviated allodynia and inhibited the evoked responses of the dorsal horn projection neurons in rats subjected to spinal nerve ligation. However, the doses of
I-AP4 that attenuated alldynia and responses of dorsal horn neurons in nerve-injured rats had no significant effect on nociceptive thresholds and dorsal horn neurons in normal animals. Therefore, activation of the spinal group III mGluRs can reduce alldynia by suppression of the hypersensitivity of dorsal horn neurons caused by nerve injury. These data suggest that the group III mGluRs in the spinal cord represent an important target for the development of new drugs to relieve neuropathic pain in patients.

References


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