ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 411–418

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Antinociceptive effects following intrathecal pretreatment with selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain

Kim Fisher[a,b], Celeste Lefebvre[a], Terence J. Coderre[a,b,c,d,*]

[a]*Pain Mechanisms Laboratory, Clinical Research Institute of Montreal, Montreal, Canada*
[b]*Department of Psychology, McGill University, Montreal, Canada*
[c]*Department of Anesthesia, McGill University, Montreal, Canada*
[d]*McGill University Health Centre Research Institute, Montreal, Canada*

Received 14 August 2001; received in revised form 12 January 2002; accepted 18 February 2002

Abstract

In the present study, we examined the effects of intrathecal pretreatment (twice daily injections on postoperative (PO) days 0–3 with the selective Group I (mGluR1a) mGluR antagonist, (*RS*)-1-aminoindan-1,5-dicarboxylic acid ((*RS*)-AIDA), the selective Group I (mGluR5a) antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), the selective Group II mGluR agonist, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC) or the selective Group III mGluR agonist, L-2-amino-4-phosphonobutyrate (L-AP4), on mechanical and cold hypersensitivity associated with chronic constriction injury (CCI) of the sciatic nerve in rats. Mechanical and cold sensitivity was assessed prior to surgery (baseline) and then at 4, 8 and 12 days following CCI. Pretreatment with all of the mGluR agents produced reductions in the development of mechanical hypersensitivity. In addition, all the mGluR agents, except MPEP, were effective in reducing the development of cold hypersensitivity. This study demonstrates that spinal Group I mGluR antagonism, and Group II or III mGluR agonism, can effectively decrease the development of mechanical and cold hypersensitivity associated with CCI in rats. In addition, the results can be interpreted to suggest that activation of spinal Group I mGluRs contributes to spinal plasticity leading to the development of neuropathic pain, and that this effect is offset by activation of groups II and III mGluRs.

Keywords: Mechanical and cold hypersensitivity; Chronic constriction injury; Metabotropic glutamate receptors; mGluR; Neuropathic pain

1. Introduction

Within the last decade, metabotropic glutamate receptors (mGluRs), in particular Group I mGluRs, have been implicated in persistent and chronic nociceptive processing (see Fundytus, 2001 for review). mGluRs are activated by excitatory amino acids (EAA) (glutamate and aspartate), and are coupled to guanine nucleotide regulatory (G) proteins that modulate intracellular messenger systems. The mGluR family consists of eight receptor subtypes that are classified into three groups based on sequence homology, signal transduction mechanisms and receptor pharmacology. These include: Group I (mGluR1/5), Group II (mGluR2/3) and Group III (mGluR4/6–8) (see Conn and Pin, 1997 for review).

Electrophysiological and behavioral studies have shown that Group I mGluRs are involved in persistent nociceptive processing in the rat. For example, application of Group I mGluR antagonists to the rat spinal cord attenuates dorsal horn neuronal activity associated with knee joint inflammation (Neugebauer et al., 1994), capsaicin-induced sensitization (Neugebauer et al., 1999) or by repeated cutaneous application of the selective C-fiber activator mustard oil (Young et al., 1994, 1995). Conversely, intrathecal administration of the selective and potent Group I agonist, (*RS*)-3,5-dihydroxyphenylglycine (DHPG) induces excitation of dorsal horn cells (Young et al., 1997) and thalamic neurons (Salt and Turner, 1998), of which the latter is reduced by the

* Corresponding author. Anesthesia Research Unit, McGill University, Room 1203, McIntyre Medical Sciences Building, 3655 Dummond Street, Montreal, QC, Canada H3G 1Y6. Tel.: +1-514-398-5773; fax: +1-514-398-8241.

E-mail address: terence.coderre@mcgill.ca (T.J. Coderre).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00832-8

selective Group I mGluR1 antagonist LY3673855. We have previously demonstrated that intrathecal application of DHPG facilitates formalin-induced nociception (Fisher and Coderre, 1996a), and produces persistent thermal and mechanical hypersensitivity (Fisher and Coderre, 1998) and spontaneous nociceptive behaviors that have been observed for over 10 h (Fisher and Coderre, 1996b).

The role of Groups II and III mGluRs in persistent nociceptive processing is less well established. In contrast to Group I mGluR agonists, intrathecal application of the Groups II and III agonists do not produce excitation of dorsal horn neurons (Young et al., 1997), and do not have any behavioral nociceptive effects (Fisher and Coderre, 1996a,b). However, Groups II and III mGluR agonists have been shown to produce an inhibition of dorsal horn neuronal responses after carrageenan injection to the rat hindpaw (Stanfa and Dickenson, 1998), and produce antinociceptive effects in the rat formalin test (Fisher and Coderre, 1996a), respectively. It has also been demonstrated that mGluR3 mRNA is enhanced in spinal cord dorsal horn following hind paw inflammation (Boxall et al., 1998), suggesting that Group II mGluRs are upregulated in association with persistent nociception. Recently, we have also demonstrated that intrathecal application of the selective Group II agonist, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*)-APDC, or the selective Group III agoinst, L-AP4, produce dose-dependent reductions in DHPG-induced spontaneous nociceptive behaviors (Lefebvre et al., 2000).

The involvement of Group I mGluRs in persistent nociception is most likely due to their ability to modulate synaptic transmission by facilitating EAA release in the CNS (Lombardi et al., 1994; Moroni et al., 1998; Reid et al., 1999). This increase follows activation of phosphoinositide hydrolysis, which results in increased intracellular Ca^{2+} concentration and the production of protein kinase C (PKC) (Conn and Pin, 1997). Whereas, the Groups II and III mGluR agonists may be effective at alleviating persistent nociception via their established ability to decrease EAA release in the CNS, following inhibition of cyclic adenosine monophosphate (cAMP) (Cozzi et al., 1997; East et al., 1995; Battaglia et al., 1997).

The electrophysiological and behavioral findings indicating that thermal and mechanical hyperalgesia may be partly mediated by Group I mGluRs, prompted us to examine the possible role of these receptors in the development of pathological pain following nerve injury. Indeed, it has already been established that glutamate levels are enhanced in the spinal cord of neuropathic rats (Al-Ghoul et al., 1993; Kawamata and Omote, 1996). We have previously shown that mGluRs contribute to the development of neuropathic pain-related behaviors by demonstrating that intrathecal pretreatment with the mGluR compound, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) attenuates the development of mechanical and cold hypersensitivity in rats with a chronic constriction injury (CCI) of the sciatic nerve (Fisher et al., 1998). However, (*S*)-4CPG is a Group I mGluR antagonist, as well as a Group II mGluR agonist (Hayashi et al., 1994), and it is unknown which of these actions contributed to its antinociceptive effects.

The aim of the present study is to delineate the involvement of each of the three groups of mGluRs (Groups I, II and III) in the development of neuropathic pain. We hypothesiz that by enhancing the release of EAAs, activation of Group I mGluRs may contribute to hyperalgesia in a rat model of neuropathic pain. Conversely, it is expected that intrathecal administration of Groups II and III mGluR agonists may alleviate neuropathic pain by suppressing the spinal release of EAAs. To test the involvement of spinal mGluRs on the development of neuropathic pain, we examined the effects of 4-day intrathecal pretreatment (twice daily injections on postoperative (PO) days 0–3) with the selective Group I mGluR (mGluR1a) antagonist, (*RS*)-1-aminoindan-1,5-dicarboxylic acid ((*RS*)-AIDA) (Hermans et al., 1999), the selective Group I mGluR (mGluR5a) antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999), the selective Group II mGluR agonist ((2*R*,4*R*)-APDC) (Schoepp et al., 1995) and the selective Group III mGluR agonist (L-AP4) (Thomsen et al., 1992) on the development of mechanical and cold hypersensitivity associated with CCI in rats on PO days 4, 8 and 12.

2. Methods

2.1. Animals

Male Long–Evans rats (270–350 g) were obtained from Charles River (Montreal, QC), and were maintained under controlled lighting conditions (12-h light/dark cycle) with food and water available ad libitum. All experiments were approved by the animal care committee at the Clinical Research Institute of Montreal, and to minimize animal suffering, the guidelines of the Canadian Council of Animal Care were strictly followed.

2.2. CCI

All rats were anaesthetized during surgery by halothane (3.0%) gas anesthesia. CCI was produced by exposing the left sciatic nerve at the mid-thigh level, isolating it from surrounding tissue and by placing a 2-mm polyethylene cuff (PE-90) around the nerve of each rat (method of Mosconi and Kruger, 1996).

2.3. Drug administration

While the CCI rats were under brief halothane anesthesia, they were administered either 0 (vehicle), 30, 90 or 270 nmol of (*RS*)-AIDA, MPEP, (2*R*,4*R*)-APDC or L-AP4 intrathecally by lumbar puncture, in a volume of 30 μl,

between the L4 and L5 vertebrae. Injections were given 15 min prior to surgery and then every 12 h for 4 days (from PO days 0 to 3). All compounds were obtained from Tocris Cookson, St. Louis, MO, USA (except (2*R*,4*R*)-APDC, which was graciously donated by Eli Lilly Laboratories, Indianapolis, IN) and dissolved in 0.9% saline. NaOH was added to L-AP4 to establish a pH between 6 and 8, and 2.5% DMSO was added to MPEP to increase solubility. Appropriate vehicles of 0.9% saline, dilute NaOH in 0.9% saline or 2.5% DMSO in 0.9% saline were used as control treatments for each drug that was dissolved by these solvents. At the doses used, none of the drugs produced motor disturbances or sedation as assessed by grasping, righting and placing reflexes and behavioral observations (see Coderre and Van Empel, 1994 for a description of these tests).

2.4. Sensory testing

Mechanical and cold sensitivity testing was performed 1–2 days prior to surgery to obtain baseline values, and then on PO days 4, 8 and 12. For baseline and PO mechanical sensitivity testing, each rat was placed in a testing box ($27 \times 16 \times 21$ cm) with a 2×2 mm wire-mesh grid floor. A series of von Frey hairs (0.41, 0.7, 1.2, 2.0, 3.63, 5.5, 8.5 and 15.1 g) were applied through to the grid floor to the ventral surface of the operated hindpaw of each rat. Each hair was applied for a 7-s period or until the animal withdrew their hindpaw without ambulating. During each testing trial, the series of hairs were presented following an up–down procedure, described and validated by Chaplan et al. (1994), and the 50% response threshold was calculated for each rat. For baseline and PO cold sensitivity testing, each rat was placed in a 1-cm deep, 1 °C water bath with a metal floor for 75 s. During the testing period, a response was counted if the rat licked, shook or showed a prolonged lift of the operated hindpaw when not ambulating.

2.5. Statistical analyses

The effects of 4-day intrathecal pretreatment with either 0 (vehicle), 30, 90 or 270 nmol for each of the mGluR agents ((*RS*)-AIDA, MPEP, (2*R*,4*R*)-APDC, L-AP4) on mechanical sensitivity during baseline and PO test days 4, 8 and 12 was assessed by a two-way ANOVA with repeated measures (separate ANOVA for each drug trial), followed by subsequent post-hoc comparisons (Fisher LSD protected *t*-tests).

The effect of 4-day intrathecal pretreatment with 0 (vehicle), 30, 90 or 270 nmol for each of the mGluR agents ((*RS*)-AIDA, MPEP, (2*R*,4*R*)-APDC, L-AP4) on cold sensitivity was assessed by a Kruskal–Wallis ANOVA by ranks for multiple independent groups (separate Kruskal–Wallis for each drug and each day), followed by subsequent post-hoc comparisons (Dunn's multiple comparisons for ranked data).

3. Results

3.1. (RS)-AIDA

A two-way repeated ANOVA of the 50% von Frey response thresholds of (*RS*)-AIDA-treated CCI rats indicated a significant main effect of both treatment [$F(3,27) = 4.48$, $P < .05$] and day [$F(3,81) = 31.69$, $P < .001$]. Subsequent post-hoc analyses (Fisher LSD protected *t*-tests) revealed significantly higher 50% von Frey response thresholds in animals pretreated with 270 nmol on test day 4 or with 90 nmol of (*RS*)-AIDA on all test days in comparison to vehicle-treated (0 nmol) rats.

Kruskal–Wallis one-way ANOVA of the cold water response frequencies of (*RS*)-AIDA-treated CCI rats indicated a significant main effect of treatment on PO day 12 [$H(3) = 12.39$, $P < .01$], (but not on day 4 [$H(3) = 4.39$, $P > .05$] or day 8 [$H(3) = 6.0$, $P > .05$]). Subsequent post hoc analyses (Dunn's multiple comparisons for nonparametric data) revealed that, on PO day 12, animals pretreated with

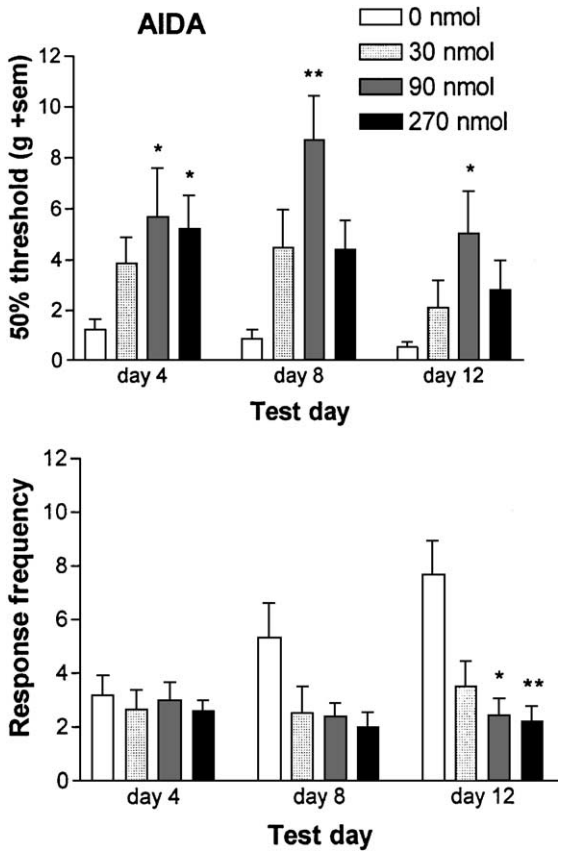

Fig. 1. Mean 50% von Frey response threshold (A) and cold water response frequencies (B) for the operated hindlimb of CCI animals on PO days 4, 8 and 12. Animals were pretreated (twice daily intrathecal injections on PO days 0–3) with vehicle (0 nmol; $n = 10$) or (*RS*)-AIDA (30, 90 or 270 nmol; $n = 6$, 10, 6). (* $P < .05$, ** $P < .01$ significant difference between the experimental and vehicle/control conditions, Fisher LSD multiple comparisons (A) or Dunn's test (B).)

90 or 270 nmol of (*RS*)-AIDA made fewer responses than vehicle-treated animals during the 75-s testing period (Fig. 1B).

3.2. MPEP

A two-way repeated ANOVA of the 50% von Frey response thresholds of MPEP-treated CCI rats indicated a significant main effect of treatment [$F(3,20) = 3.53$, $P < .05$] and a significant main effect of day [$F(3,60) = 141.21$, $P < .001$]. Subsequent post-hoc analyses (Fisher LSD protected *t*-tests) revealed that on PO days 4 and 8 animals pretreated with 90 or 270 nmol of MPEP exhibited significantly higher 50% von Frey response thresholds in comparison to vehicle-treated animals (Fig. 2A).

Kruskal–Wallis one-way ANOVA of the cold water response frequencies of MPEP-treated CCI rats, indicated no significant main effects of treatment on any of the PO days (PO day 4 [$H(3) = 4.47$, $P > .05$], PO day 8 [$H(3) = 6.44$, $P > .05$] or PO day 12 [$H(3) = 4.21$, $P > .05$) (Fig. 2B).

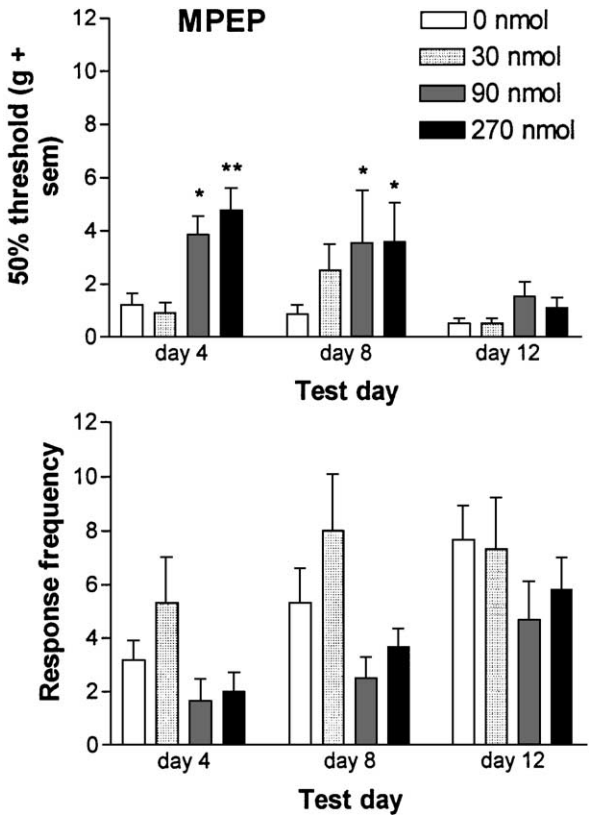

Fig. 2. Mean 50% von Frey response threshold (A) and cold water response frequencies (B) for the operated hindlimb of CCI animals on PO days 4, 8 and 12. Animals were pretreated (twice daily intrathecal injections on PO days 0–3) with either vehicle (0 nmol; $n = 6$) or MPEP (30, 90 or 270 nmol; $n = 6, 6, 6$). ($*p < .05$, $**p < .01$ significant difference between the experimental and vehicle/control conditions, Fisher LSD multiple comparisons (A) or Dunn's test (B).)

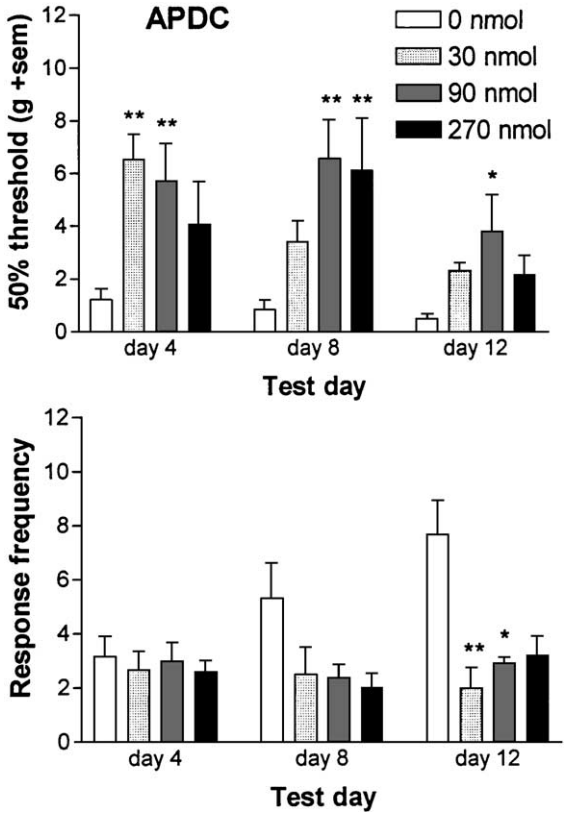

Fig. 3. Mean 50% von Frey response threshold (A) and cold water response frequencies (B) for the operated hindlimb of CCI animals on PO days 4, 8 and 12. Animals were pretreated (twice daily intrathecal injections on PO days 0–3) with vehicle (0 nmol; $n = 10$) or (2*R*,4*R*)-APDC (30, 90 or 270 nmol; $n = 6, 10, 6$). ($*P < .05$, $**P < .01$ significant difference between the experimental and vehicle/control conditions, Fisher LSD multiple comparisons (A) or Dunn's test (B).)

3.3. (2R,4R)-APDC

A two-way repeated ANOVA of the 50% von Frey response thresholds of (2*R*,4*R*)-APDC-treated CCI rats, indicated a significant main effect of day [$F(3,69) = 48.19$, $P < .001$] and a significant Treatment × Day interaction effect [$F(9,69) = 1.99$, $P < .05$]. Subsequent post-hoc analyses (Fisher LSD protected *t*-tests) revealed that animals pretreated with 270 nmol on PO day 8, or 90 nmol on all test days and 30 nmol on PO day 4 of (2*R*,4*R*)-APDC exhibited significantly higher 50% von Frey response thresholds than vehicle-treated animals (Fig. 3A).

Kruskal–Wallis one-way ANOVA for the cold water response frequencies of (2*R*,4*R*)-APDC-treated CCI rats indicated a significant main effect of treatment on PO day 12 [$H(3) = 14.33$, $P < .01$], but not on PO day 4 [$H(3) = 0.41$, $P > .05$] or on PO day 8 [$H(3) = 4.432$, $P > .05$]. Subsequent post hoc analyses (Dunn's multiple comparisons for non-parametric data) revealed that, on PO day 12, animals pretreated with 30 or 90 nmol of (2*R*,4*R*)-APCD, made less responses than vehicle-treated animals during the 75-s testing period (Fig. 3B).

3.4. L-AP4

A two-way repeated ANOVA of the 50% von Frey response thresholds of L-AP4-treated CCI rats indicated a significant main effect of day [$F(3,75) = 72.09$, $P < .001$] and a significant Treatment × Day interaction effect [$F(9,75) = 2.16$, $P < .05$]. Subsequent post-hoc analyses (Fisher LSD protected *t*-tests) revealed that on PO day 4 animals pretreated with 90 or 270 nmol, and on PO days 8 and 12 animals pretreated with 90 nmol of L-AP4 exhibited significantly higher 50% von Frey response thresholds than vehicle-treated animals (Fig. 4A).

Kruskal–Wallis one-way ANOVA for the cold water response frequencies of L-AP4-treated CCI rats indicated a significant main effect of treatment on PO day 12 [$H(3) = 8.81$, $P < .05$], but not on day 4 [$H(3) = 3.71$, $P > .05$] or PO day 8 [$H(3) = 4.34$, $P > .05$]. Subsequent post-hoc analyses (Dunn's multiple comparisons) revealed that, on PO day 12, animals pretreated with 90 or 270 nmol of L-AP4 made fewer responses than vehicle-treated animals during the 75-s testing (Fig. 4B).

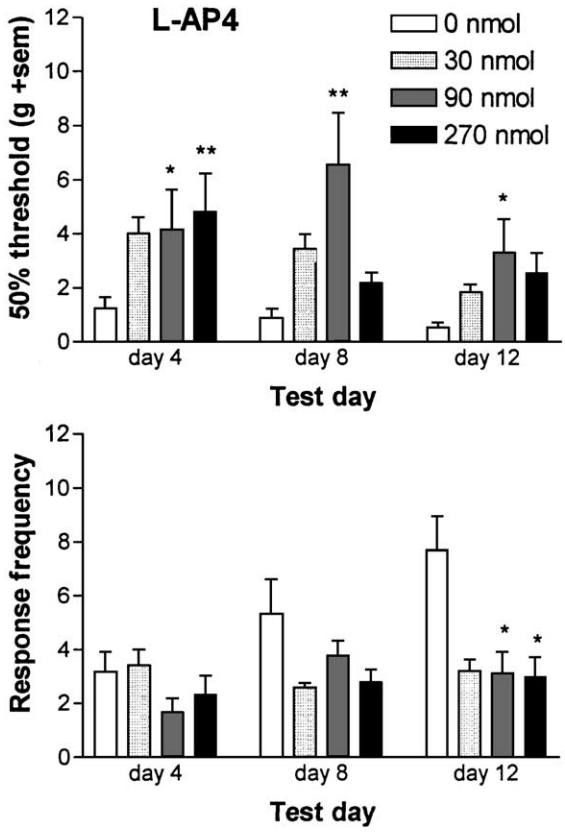

Fig. 4. Mean 50% von Frey response threshold (A) and cold water response frequencies (B) for the operated hindlimb of CCI animals at baseline and on PO days 4, 8 and 12. Animals were pretreated (twice daily intrathecal injections on PO days 0–3) with vehicle (0 nmol; $n = 10$) or L-AP4 (30, 90 or 270 nmol; $n = 6$, 10, 10). (* $P < .05$, ** $P < .01$ significant difference between the experimental and vehicle/control conditions, Fisher LSD multiple comparisons (A) or Dunn's test (B).)

4. Discussion

Results from this study demonstrate the inhibitory effects of spinal Group I mGluR antagonism and Groups II and III mGluR agonism against the development of mechanical and cold hypersensitivity associated with neuropathic pain. Thus, we demonstrated that intrathecal pretreatment with the selective Group I mGluR (mGluR1a) antagonist (*RS*)-AIDA, the selective Group I mGluR (mGluR5a) antagonist MPEP, the selective Group II mGluR agonist (2*R*,4*R*)-APDC, and the selective Group III mGluR agonist, L-AP4, all significantly reduced the development of mechanical hypersensitivity in rats with CCI of the sciatic nerve. In addition, all the mGluR agents, except MPEP, significantly reduced the development of cold hypersensitivity in the CCI operated animals.

The results suggest that the effective antihyperalgesic effects first demonstrated after intrathecal pretreatment with (*S*)-4CPG in the CCI model (Fisher et al., 1998) are likely attributable to the compound's dual action including antagonism of Group I mGluRs and agonism of Group II mGluRs. Since spinal Group I mGluR antagonism was shown to decrease the development of mechanical and cold hypersensitivity in CCI rats, the results can be interpreted to suggest that activation of Group I mGluRs contributes to the spinal cord mechanisms that mediate the development of these neuropathic pain symptoms. In support of this, a large collection of studies, when taken together, provide convincing evidence that Group I mGluRs are involved in the transmission of somatosensory/nociceptive information within the spinal cord, and contribute to the alterations in dorsal horn neuronal function that mediate persistent and chronic nociception. Thus, electrophysiological and behavioral studies show that Group I mGluR agonists can increase the excitability of dorsal horn neurons associated with persistent nociception, and can produce spontaneous nociceptive behaviors and hypersensitivity to thermal and mechanical stimuli. Whereas, Group I mGluR antagonists can reverse these effects (Budai and Larson, 1998; Neugebauer et al., 1994, 1999; Young et al., 1994, 1995, 1997; Salt and Turner, 1998; Fisher and Coderre 1996a,b, 1998).

In addition, the involvement of mGluRs in spinal nociceptive transmission is in accordance with neuroanatomical evidence that has demonstrated that Group I mGluRs (mGluR1a, mGluR5a, mGluR5b, but not mGluR1b mRNA) are localized within the spinal somatosensory/nociceptive pathways (Valerio et al., 1997a). Spinal cord mGluR5a is strongly expressed in the dorsal horn laminae I and II and gradually decreases toward the deeper layers of the rat dorsal horn (Vidnyanszky et al., 1994; Valerio et al., 1997b), and mGluR5a immunoreactive dendrites are often targeted by synaptic boutons of presumed polymodal primary afferent C-fiber terminals (Vidnyanszky et al., 1994).

Taking all the electrophysiological, behavioral and neuroanatomical evidence into account, we propose that the CCI-related discharges of injured primary afferent Aβ-,

Aδ- or C-fibers (Kajander and Bennett, 1992), and the subsequent accumulation of glutamate and/or aspartate in the dorsal horn (Kawamata and Omote, 1996), activate spinal Group I mGluRs in the superficial laminae or deeper layers, and thus acts to sensitize dorsal horn neurons that respond to noxious or innocuous mechanical and cold stimulation.

The recent development of the selective Group I mGluR5a antagonist, MPEP, has allowed us to compare its effects versus that of the Group I mGluR1a antagonist, (*RS*)-AIDA, in an effort to assess a potential receptor subtype specificity. Although the specificity of (*RS*)-AIDA has been questioned (Schoepp et al., 1999), the high mGluR5a selectivity of MPEP allows for comparison. In our study, we found that (*RS*)-AIDA reduced both mechanical and cold hypersensitivity, whereas MPEP only reduced mechanical but not cold hypersensitivity. Thus, while both mGluR1 and mGluR5 may both play a role in neuropathic pain, as we have previously shown with anti-receptor antibodies (Fundytus et al., 1998), there may be some benefit to blocking mGluR1 activity which reduces both mechanical and cold hypersensitivity. Further support for the role of the mGluR1 subtype is demonstrated by studies in which there is a selective knocked-down of mGluR1 in the spinal cord after intrathecal administration of an mGluR1 antisense oligonucleotide. The knockdown of spinal mGluR1 significantly reduces mechanical and cold hypersensitivity in neuropathic rats (Fundytus et al., 2001), and reduces sustained dorsal horn excitatory responses induced by mustard oil or DHPG (Young et al., 1998).

As mentioned earlier, Groups II and III mGluRs have similar physiological roles in the CNS, such that activation of Group II or III mGluRs depresses excitatory transmission by decreasing evoked EAA release. Therefore, activation of Groups II and III mGluRs may play a role in spinal nociceptive processing by inhibiting glutamate release, and can account for the observed antinociceptive effects of the Groups II and III mGluR agonists. Although our use of (2*R*,4*R*)-APDC and L-AP4 provides for a comparison of the differing effects of Groups II and III agonists, it does not allow for comparisons between agonists acting selectively at specific mGluR subtypes within these groups. Given the positive effects of the agents, future investigations of more selective agents (e.g., (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-I) for mGluR4a; (*R*,*S*)-phosphonophenylglycine ((*R*,*S*)-PPG) for mGluR8) may be warranted. Additionally, it would be useful to determine whether the effects of these agonists are blocked by selective agonists (e.g., (2*S*)-α-ethylglutamic acid (Eglu), (α*S*)-α-Amino-α-[(1*S*,2*S*)-2-carboxycyclopropyl]-9*H*-xanthine-9-propanoic acid (LY341495) or (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4)).

Neuroanatomical studies show that Group II mGluRs are localized on neuronal cell bodies in the dorsal horn and lamina X of the rat spinal cord (mGluR3) (Ohishi et al., 1993) and on primary afferent fibers or on interneurons presynaptic to ventral horn neurons (Cao et al., 1995; Jane et al., 1996). However, to our knowledge, there is no evidence that Group II mGluRs are expressed on primary afferent terminals presynaptic to dorsal horn neurons in the rat. Group III mGluRs are intensely expressed in axon terminals of presumed nociceptive primary afferent fibers terminating in laminae I and II of the rat dorsal horn (mGluR7) (Ohishi et al., 1995a; Li et al., 1997) and in the cell bodies of neurons in the dorsal root ganglia (mGluR4 and mGluR7) (Ohishi et al., 1995b; Li et al., 1996).

Although there is no specific physiological evidence for effects of Group II or III mGluRs on the nociceptive responses of dorsal horn neurons, Groups II and III mGluR agonists have been shown to decrease the excitation of other neurons in the CNS. For example, the selective activation of Group II or III mGluRs inhibits hippocampal long-term potentiation in the rat (Holscher et al., 1997; Huang et al., 1997) and attenuates NMDA-induced excitotoxicity in the mouse brain (Bruno et al., 1995; Buisson et al., 1996). As for the effects of brain mGluRs in nociception, their actions may not always be consistent with their spinal effects, and may depend on both the injection site and the nociceptive test. Thus, injection of Group I mGluR agonists directly in the PAG has been shown to induce antinociception in the mouse hot plate test, while injection of Groups II and III mGluRs into this brain area is pro-nociceptive (Maione et al., 1998). Although, in thalamic neurons, Group I mGluR produce excitatory effects (Salt and Eaton, 1995). Conversely, injection of Group I or II agonists into the PAG reduces nociceptive scores, while intra-PAG injection of a Group III agonist enhances nociceptive scores in the late phase of the formalin test (Maione et al., 2000).

Taking together the behavioral and neuroanatomical evidence, we speculate that Group II mGluRs' antinociceptive effects can most likely be attributed to an activation of postsynaptic Group II mGluRs leading to a hyperpolarization of hypersensitive dorsal horn neurons in CCI rats. Alternatively, a Group III mGluR-mediated decrease in EAA release may attenuate CCI-induced alterations in dorsal horn neuronal function (i.e., hypersensitivity of dorsal horn neurons induced by excessive glutamate released) that may underlie mechanical and cold hypersensitivity.

In conclusion, we demonstrated that Group I mGluR antagonism and Groups II and III mGluRs agonism can decrease the development of neuropathic pain-related behaviors in CCI rats. Thus, activation of Group I mGluRs, particularly mGluR1, may play an important role in mediating the initial development of mechanical and cold hypersensitivity associated with neuropathy. Furthermore, the excitatory effects of EAAs at Group I mGluRs may be offset by opposing inhibitory effects at Groups II and III mGluRs. The results suggest that treatments targeting spinal mGluRs may be effective for preventing the development of pain and hyperalgesia in neuropathic pain patients.

Acknowledgments

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and the Astra Research Centre Montreal to TJC. TJC is a CIHR Investigator. The authors wish to thank Eli Lilly & Co. for the generous contribution of (2*R*,4*R*)-APDC.

References

Al-Ghoul WM, Volsi GL, Weinberg RJ, Rustioni A. Glutamate immunocytochemistry in the dorsal horn after injury or stimulation of the sciatic nerve of rats. Brain Res Bull 1993;30:453–9.

Battaglia G, Monn JA, Schoepp DD. In vivo inhibition of veratridine-evoked release of striatal excitatory amino acids by the Group II metabotropic glutamate receptor agonist LY354740 in rats. Neurosci Lett 1997;229:161–4.

Boxall SJ, Berthele A, Laurie DJ, Sommer B, Zieglgansberger W, Urban L, Tolle TR. Enhanced expression of metabotropic glutamate receptor 3 messenger RNA in the rat spinal cord during ultraviolet irradiation induced peripheral inflammation. Neuroscience 1998;82:591–602.

Bruno V, Battaglia G, Copani A, Giffard RG, Raciti G, Raffaele R, Shinozaki H, Nicoletti F. Activation of Class II or Class III metabotropic glutamate receptors prevents cultured cortical neurons against excitotoxicity degeneration. Eur J Pharmacol 1995;7:1906–13.

Budai D, Larson AA. The involvement of metabotropic glutamate receptors in sensory transmission in dorsal horn of the rat spinal cord. Neuroscience 1998;83:571–80.

Buisson A, Yu SP, Choi DW. DCG-IV selectively attenuates rapidly triggered NMDA-induced neurotoxicity in cortical neurons. Eur J Neurosci 1996;8:138–43.

Cao CQ, Evans RH, Headley PM, Udvarhelyi PM. A comparison of the effects of selective metabotropic glutamate receptor agonists on synaptically evoked whole cell currents of rat spinal ventral horn neurones in vivo. Br J Pharmacol 1995;115:1469–74.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.

Coderre TJ, Van Empel I. 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 1994;59:345–52.

Conn PJ, Pin J-P. Pharmacology and function of metabotropic glutamate receptors. Ann Rev Pharmacol Toxicol 1997;37:205–37.

Cozzi A, Attucci S, Peruginelli F, Marinozzi M, Luneia R, Pellicciari R, Moroni F. Type 3 metabotropic glutamate (mGlu) receptors tonically inhibit transmitter release in rat caudate nucleus: in vivo studies with (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-phenylcyclopropyl)-glycine, a new potent and selective antagonist. Eur J Neurosci 1997;9:1350–5.

East SJ, Hill MP, Brotchie JM. Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synpyosomes. Eur J Pharmacol 1995;277:117–21.

Fisher K, Coderre TJ. The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. Pain 1996a;68:255–63.

Fisher K, Coderre TJ. Comparison of nociceptive effects produced by i.t. administration of mGluR agonists. NeuroReport 1996b;7:2743–7.

Fisher K, Coderre TJ. Hyperalgesia and allodynia induced by intrathecal (*RS*)-dihydroxyphenylglycine in rats. NeuroReport 1998;9:1169–72.

Fisher K, Fundytus ME, Cahill CM, Coderre TJ. Intrathecal administration of the mGluR compound, (*S*)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats. Pain 1998; 77:59–66.

Fundytus ME. Glutamate receptors and nociception: implications for the drug treatment of pain. CNS Drugs 2001;15:29–58.

Fundytus ME, Fisher K, Dray A, Henry JL, Coderre TJ. In vivo antinociceptive activity of anti-rat mGluR1 and mGluR5 antibodies in rats. NeuroReport 1998;9:731–5.

Fundytus ME, Yashpal K, Chabot J-G, Osborne MG, Lefebvre CD, Dray A, Henry JL, Coderre TJ. Knockdown of spinal mGluR1 alleviates pain and restores opioid efficacy after nerve injury. Br J Pharmacol 2001; 132:354–67.

Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacaan AI, Santori EM, Velicelebi G, Kuhn R. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 1999; 38:1493–503.

Hayashi Y, Sekiyama N, Nakanishi S, Jane DE, Sunter DC, Birse EF, Udvarhelyi PM, Watkins JC. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J Neurosci 1994;14:3370–7.

Hermans E, Challiss RA, Nahorski SR. Effects of varying the expression level of recombinant human mGlu1 alpha receptors on the pharmacological properties of agonists and antagonists. Br J Pharmacol 1999; 126:873–82.

Holscher C, Anwyl R, Rowan MJ. Activation of Group II metabotropic glutamate receptors blocks induction of long-term potentiation and depotentiation in area CA1 of the rat in vivo. Eur J Pharmacol 1997;322: 155–63.

Huang L, Breakwell NA, Rowan MJ, Anwyl R. PCC-IV inhibits the induction of long-term potentiation in the dentate gyrus in vitro. Eur J Pharmacol 1997;332:161–5.

Jane DE, Thomas NK, Tse HW, Watkins JC. Potent antagonists at the L-AP4- and (1*S*,3*S*)-ACPD-sensitive presynaptic metabotropic glutamate receptors in the neonatal rat spinal cord. Neuropharmacology 1996;35: 1029–35.

Kajander KC, Bennett GJ. Onset of a painful peripheral neuropathy in rat: a partial and differential deafferentation and spontaneous discharge in Aβ and Aδ primary afferent neurons. J Neurophysiol 1992;68:734–44.

Kawamata M, Omote K. Involvement of increased excitatory amino acids and intracellular Ca^{2+} concentrations in the spinal dorsal horn in an animal model of neuropathic pain. Pain 1996;68:85–96.

Lefebvre C, Fisher K, Cahill CM, Coderre TJ. Evidence that DHPG-induced nociception depends on glutamate release from primary afferent C-fibers. NeuroReport 2000;11:1631–5.

Li JL, Ohishi H, Kaneko T, Shigemoto R, Neki A, Nakanishi S, Muzuno N. Immunohistochemical localization of a metabotropic glutamate receptor, mGluR7, in ganglion neurons of the rat; with special reference to the presence of glutamatergic ganglion neurons. Neurosci Lett 1996; 204:9–12.

Li H, Ohishi H, Kinoshita A, Shigemoto R, Nomura S, Mizuno N. Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat. Neurosci Lett 1997;223:153–6.

Lombardi G, Pelligrini-Giampietro DE, Leonard P, Cherici G, Pellicciari R, Moroni F. The depolarization-induced outflow of D-[^{3}H]aspartate from rat brain slices is modulated by metabotropic glutamate receptors. Neurochem Int 1994;24:525–32.

Maione S, Marabese I, Leyva J, Palazzo E, de Novellis V, Rossi F. Characterization of mGluRs which modulate nociception in the PAG of the mouse. Neuropharmacology 1998;37:1475–83.

Maione S, Oliva P, Marabese I, Palazzo E, Rossi F, Berrino L, Filippelli A. Periaqueductal gray matter metabotropic glutamate receptors modulate formalin-induced nociception. Pain 2000;85:183–9.

Moroni F, Cozzi A, Lombardi G, Sourtcheva S, Leonardi P, Carfi M, Pellicciari R. Presynaptic mGlu1 type receptors potentiate transmitter output in the rat cortex. Eur J Pharmacol 1998;347:189–95.

Mosconi T, Kruger L. Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations. Pain 1996;64:37–57.

Neugebauer V, Lucke T, Schaible HG. Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons. Eur J Neurosci 1994;6:1179–86.

Neugebauer V, Chen PS, Willis WD. Role of metabotropic glutamate receptor subtype mGluR1 in brief nociception and central sensitization of primate STT cells. J Neurophysiol 1999;82:272–82.

Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study. J Comp Neurol 1993;335:252–66.

Ohishi H, Akazawa C, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNAs for L-2-amino-4-phosphonobutyrate-sensitive metabotropic glutamate receptors, mGluR4 and mGluR7, in the rat brain. J Neurol 1995;360:555–70.

Ohishi H, Nomura S, Ding YQ, Shigemoto R, Wada E, Kinoshita A, Li JL, Neki A, Nakanishi S, Mizuno N. Presynaptic localization of a metabotropic glutamate receptor, mGluR7, in primary afferent neurons: an immunohistochemical study in the rat. Neurosci Lett 1995;202:85–8.

Reid ME, Toms NJ, Bedingfield JS, Roberts PJ. Group I mGlu receptors potentiate synaptosomal [^{3}H]glutamate release independently of exogenously applied arachidonic acid. Neuropharmacology 1999;38:477–85.

Salt TE, Eaton SA. Modulation of sensory neurone excitatory and inhibitory responses in the ventrobasal thalamus by activation of metabotropic excitatory amino acid receptors. Neuropharmacology 1995;34:1043–51.

Salt TE, Turner JP. Reduction of sensory and metabotropic glutamate receptor responses in the thalamus by the novel metabotropic glutamate receptor-1-selective antagonist *S*-2-methyl-4-carboxy-phenylglycine. Neuroscience 1998;85:655–8.

Schoepp DD, Johnson BG, Salhoff CR, Valli MJ, Desai MA, Burnett JP, Mayne NG, Monn JA. Selective inhibition of forskolin-stimulated cyclic AMP formation in rat hippocampus by a novel mGluR agonist, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate. Neuropharmacology 1995;34:843–50.

Schoepp DD, Jane DE, Monn JA. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 1999;38:1431–76.

Stanfa LC, Dickenson AH. Inflammation alters the effects of mGlu receptor agonists on spinal nociceptive neurones. Eur J Pharmacol 1998;347:165–72.

Thomsen C, Kristensen P, Mulvihill E, Haldeman B, Suzdak PD. L-2-Amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase. Eur J Pharmacol 1992;227:361–2.

Valerio A, Paterlini M, Boifava M, Memo M, Spano P. Metabotropic glutamate receptor mRNA expression in rat spinal cord. NeuroReport 1997a;8:2695–9.

Valerio A, Rizzonelli P, Paterlini M, Moretto G, Knopfel T, Kuhn R, Memo M, Spano P. mGluR5 metabotropic glutamate receptor distribution in rat and human spinal cord: a developmental study. Neurosci Res 1997b;28:49–57.

Vidnyanszky Z, Hamori J, Negyessy L, Ruegg D, Knopfel T, Kuhn R, Gorcs TJ. Cellular and subcellular localization of the mGluR5a metabotropic glutamate receptor in rat spinal cord. NeuroReport 1994;6:209–13.

Young MR, Fleetwood-Walker M, Mitchell R, Munro FE. Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons. Neuropharmacology 1994;33:141–4.

Young MR, Fleetwood-Walker SM, Mitchell R, Dickenson T. The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons. Neuropharmacology 1995;34:1033–41.

Young MR, Fleetwood-Walker SM, Dickinson T, Blackburn-Munro G, Sparrow H, Birch PJ, Bountra C. Behavioral and electrophysiological evidence supporting a role for metabotropic glutamate receptors in the mediation of nociceptive inputs to the rat spinal cord. Brain Res 1997;777:161–9.

Young MR, Blackburn-Munro G, Dickinson T, Johnson MJ, Anderson H, Nakalembe I, Fleetwood-Walker SM. Antisense ablation of type I metabotropic glutamate receptor mGluR1 inhibits spinal nociceptive transmission. J Neurosci 1998;18:10180–8.