Pharmacology, Biochemistry and Behavior 96 (2010) 108–113

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Potentiation of morphine antiallodynic efficacy by ACPT-III, a Group III metabotropic glutamate receptor agonist, in rat spinal nerve ligation-induced neuropathic pain

Hyung Gon Lee [a], Sung Keun Park [a], Myung Ha Yoon [a,b,*]

[a] Department of Anesthesiology and Pain Medicine, Chonnam National University, Medical School, 8 Hakdong, Donggu, Gwangju 501-757, Republic of Korea
[b] Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, 8 Hakdong, Donggu, Gwangju 501-757, Republic of Korea

ARTICLE INFO

Article history:
Received 31 December 2009
Received in revised form 2 April 2010
Accepted 18 April 2010
Available online 29 April 2010

Keywords:
Antinociception
Group III mGluRs
Morphine
Intrathecal
Neuropathic pain

ABSTRACT

Despite the importance of spinal metabotropic glutamate receptors (mGluRs) and opioid receptors in nociceptive processing, the roles of these receptors in the modulation of neuropathic pain at the spinal level have not been thoroughly investigated. The purpose of this study was to investigate the effects of spinal mGluR agents and opioids (morphine) on neuropathic pain. Male Sprague–Dawley rats underwent L5 and L6 spinal nerve ligation to induce neuropathic pain and intrathecal catheterization for drug administration. A paw-withdrawal threshold to mechanical stimulus was measured using the "up and down" method. When administered intrathecally, neither Group I mGluR antagonists nor Group II or III agonists modified the withdrawal threshold after spinal nerve ligation. Intrathecal administration of morphine dose-dependently increased the withdrawal threshold. Whereas ACPT-III, a Group III mGluR agonist, enhanced the antiallodynic action of morphine, other mGluR agents did not. Collectively, mGluRs may not directly modulate the processing of spinal nerve ligation-induced neuropathic pain at the spinal level. However, Group III mGluR agonists in the spinal cord may indirectly contribute to the potentiation of morphine antiallodynia, indicating that these agonists might be used as adjuvants for spinal morphine.

1. Introduction

When injured or diseased, the peripheral or central nervous system (CNS) can enter a pathologic pain state referred to as neuropathic pain (Finnerup et al., 2007). Neuropathic pain is associated with abnormal sensory change phenomena such as increased responsiveness to painful stimuli (hyperalgesia) and the perception of pain in response to normally innocuous stimuli (allodynia). Unfortunately, chronic neuropathic pain is often refractory to commonly used analgesics (Dray, 2008). Therefore, the effective and safe management of neuropathic pain poses an important and difficult therapeutic challenge.

Although the mechanisms underlying neuropathic pain are poorly understood, they involve several releasable substances such as glutamate, cytokines, and neurotrophic factors have been identified to play an active role in the evolution of spinal cord sensitization (Ledeboer et al., 2005; Willis, 2001). In particular, the excitatory amino acid glutamate plays a major role in modulation of nociceptive processing (Dickenson et al., 1997). Glutamate exerts its action in the spinal cord by activating two distinct types of receptors, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). The iGluRs are ligand-gated ion channels responsible for fast synaptic transmission (Madden, 2002). The mGluRs are members of the large G-protein-coupled receptor family and have been implicated in synaptic plasticity in the CNS. Accumulating evidence suggests a pivotal role for mGluRs in nociceptive processing (Conn and Pin, 1997; Neugebauer, 2002).

To date, eight mGluRs have been identified; based on their sequence similarities, signal transduction mechanisms, and drug selectivity, these mGluRs have traditionally been divided into three groups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) (Conn and Pin, 1997). In some studies, intrathecal administration of Group I mGluR antagonists or Group II or III mGluR agonists was reported to attenuate mechanical hypersensitivity in neuropathic pain models (Chen and Pan, 2005; Fisher et al., 1998; Fisher et al., 2002; Goudet et al., 2008; Yashpal et al., 1995). On the other hand, in other studies, intrathecal injection of mGluR antagonists did not alleviate mechanical allodynia in neuropathic pain (Chaplan et al., 1997; Hama and Urban, 2004; Nguyen et al., 2009).

The opioid morphine acts via a number of CNS sites, including the spinal cord, at which opioid receptors modulate nociceptive transmission. Whether intrathecal morphine is effective against neuropathic pain induced by peripheral nerve injury has not been clearly established, with different studies reporting contradictory results (Lee et al., 1995; Zhang et al., 2005).

* Corresponding author. Department of Anesthesiology and Pain Medicine, Chonnam National University, Medical School, 8 Hakdong, Donggu, Gwangju 501-757, Republic of Korea. Tel.: +82 62 220 6893; fax: +82 62 232 6294.
E-mail address: mhyoon@chonnam.ac.kr (M.H. Yoon).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.04.014

Although mGluRs and opioid receptors may be involved in nociception modulation in neuropathic pain, their exact roles are not yet clearly understood. Moreover, little is known about their patterns of interaction. Therefore, a better understanding of the functional roles of these receptors in altered spinal nociception after nerve injury may facilitate the discovery of novel targets for neuropathic pain therapy. In the present study, we assessed the effects of spinal mGluR agonists and antagonists and opioids (morphine) on neuropathic pain induced by spinal nerve ligation. In addition, we characterized the interactions between intrathecal mGluR agents and morphine.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats weighing 100–200 g were used in all experiments. The animals were acclimated to the laboratory environment for 5–7 days before use. While in their home cage environment, they were allowed free access to a standard rat diet and tap water. The room was maintained at 20–23 °C with a 12 h/12 h light/dark cycle. The study proposal was reviewed and approved by the Institutional Animal Care Committee, Research Institute of Medical Science of Chonnam National University (Gwangju, Korea).

2.2. Neuropathic pain model

Neuropathic pain was evoked in the experimental rats ($n=8$) by spinal nerve ligation as previously described (Kim and Chung, 1992). Briefly, the left L5 and L6 spinal nerves were isolated adjacent to the vertebral column during sevoflurane anesthesia and tightly ligated with a 6-0 silk suture distal to the dorsal root ganglia. Care was taken to avoid injury of the L4 spinal nerve. Animals were considered to be in neuropathic pain when they exhibited mechanical allodynia, i.e., a paw-flinch behavioral response to the application of a bending force of less than 4 g (Calcutt et al., 1996). Animals were allowed to recover from surgery for at least 1 week. Sham rats underwent identical anesthetic and surgical procedures without spinal nerve ligation. After surgery, the development of neuropathic pain was evaluated daily between 13:00 h and 15:00 h for 21 days by measuring the mechanical sensitivity of the injured paw.

2.3. Implantation of intrathecal catheter

Seven days after spinal nerve ligation, a polyethylene-10 tube was inserted into the subarachnoid space of the rats through a slit that had been made in the atlantooccipital membrane under sevoflurane anesthesia (Yaksh and Rudy, 1976). Any rats exhibiting neurological deficit after catheterization were excluded and euthanized immediately with an overdose of volatile anesthetics. Another 7 days of recovery were allowed after catheterization before commencing the behavioral study.

2.4. Drugs

The following drugs were used in this study: (S)-(+)-α-amino-4-carboxy-2-methylbenzeneaceric acid (LY 367385; Tocris Cookson Ltd., Bristol), 2-methyl-6-(phenylethynyl)-pyridine (MPEP; Tocris), (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC; Tocris), (3RS,4RS)-1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-III; Tocris), and morphine sulfate (Sigma-Aldrich Co., St. Louis, MO). LY 367385 and MPEP were dissolved in 20% dimethylsulfoxide (DMSO), and APDC, ACPT-III, and morphine were dissolved in normal saline. These agents were administered intrathecally using a hand-driven, gear-operated syringe pump. All drugs were delivered in a volume of 10 µl, and an additional 10 µl of normal saline was then used to flush the catheter.

2.5. Assessment of mechanical allodynia

To determine withdrawal thresholds, rats were placed individually in plastic cages with a plastic mesh floor and tested after accommodation to the environment (typically 20–30 min after being placed in the cage). The paw-withdrawal threshold in response to mechanical stimulation was measured using the "up and down" method (Chaplan et al., 1994) by applying calibrated von Frey filaments (Stoelting, Wood Dale, IL) from underneath the cage through openings in the mesh floor to the hindpaw. A series of eight von Frey filaments (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, and 15 g) was applied vertically to the plantar surface of the hindpaw for 6–7 s while the hair was bent. Brisk withdrawal or paw flinching was considered a positive response. If a response was absent even at 15 g pressure, this value was assigned as the cutoff value. Tests were performed in duplicate, with an approximately 3-min rest period between tests, and the average value was used. Only rats exhibiting marked allodynia (a withdrawal threshold less than 4 g) after spinal nerve ligation were studied.

2.6. Experimental paradigm

On the day of experiments, the rats were allocated into experimental and control groups for the tested drugs. Control studies were performed using intrathecal saline ($n=5$) or DMSO ($n=5$) according to the solvent used for the tested drug. All experiments were carried out by an observer blinded to the drug treatment.

2.6.1. Effects of intrathecal LY 367385, MPEP, APDC, ACPT-III and morphine

The effects of Group I mGluR antagonists against mGlu1a (LY 367385, 200 µg, $n=6$) and mGlu5 (MPEP, 300 µg, $n=5$), a Group II mGluR agonist (APDC, 100 µg, $n=6$), a Group III mGluR agonist (ACPT-III, 10 µg, $n=6$), and morphine (0.03, 0.1, 0.3, and 1 µg, $n=26$) were investigated in the neuropathic pain state. Measurement of the mechanical threshold was carried out before spinal nerve ligation (pre-ligated, baseline threshold). The withdrawal threshold was determined at 15, 30, 60, 90, 120, 150, and 180 min after intrathecal administration of experimental drugs. The withdrawal threshold measured immediately before intrathecal delivery of drugs was regarded as post-ligated baseline threshold (control). The agents LY 367385, MPEP, and APDC were not soluble at higher doses than those used in this study, and the agent ACPT-III caused motor dysfunction at doses greater than 30 µg. Hence, the highest doses of the mGluR agents administered in the present study were regarded as maximum possible doses.

2.6.2. Drug interaction

After intrathecal administration of mGluR agents, no antinociceptive effect was seen in our neuropathic pain model. Therefore, the mGluR agents were co-administered at fixed doses with various doses of morphine to assess the possible modulatory effects of the agents on the antinociception activity of morphine ($n=103$) based on previously described experiments (Nishiyama, 2000). The doses of the mGluR agents were fixed at the maximum ineffective doses used in this study.

2.7. General behavior

To evaluate the behavioral changes induced by the administration of mGluR agents and/or morphine, additional rats ($n=25$) were examined 5, 10, 20, 30, 40, 50, and 60 min after intrathecal administration of the highest doses of agents used in this study. Motor functions were assessed by examining the righting and placing/

stepping reflexes. The former was evaluated 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. The latter was evoked by drawing the dorsum of either hind paw across the edge of the table. Normally rats try to put their paws forward into a position for walking. Changes in motor functions were scored as 0 (normal), 1 (slight deficit), 2 (moderate deficit), or 3 (severe deficit). Pinnal and corneal reflexes were also evaluated and scored as present or absent. Other abnormal behaviors, such as serpentine movement or tremor, were also noted if observed.

2.8. Statistical analysis

The data are expressed as mean ± SEM. The time–response data are presented as the withdrawal threshold in grams. The dose–response data are presented as the percentage of maximum possible effect (%MPE). To calculate the ED_{50} value (effective dose producing a 50% reduction of the control response) of each drug, the withdrawal threshold data from von Frey filament testing were converted to % MPE according to the formula %MPE = [(postdrug threshold − post-ligated baseline threshold)/(cutoff threshold − post-ligated baseline threshold)] × 100. Dose–response data were analyzed by one-way analysis of variance (ANOVA) with Bonferroni correction for post hoc analysis. ED_{50} values and their 95% confidence intervals (CIs) were calculated using linear regression according to the method described by Tallarida (2000). Differences between the withdrawal thresholds of the ligated and sham groups were analyzed by unpaired *t*-test, and comparisons of ED_{50} values obtained with morphine and those obtained with morphine combined with mGluR agents were performed with modified *t*-test (Tallarida, 2000). Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Time course for development of mechanical allodynia in spinal nerve-ligated rats

Before spinal nerve ligation, paw-withdrawal thresholds ranged from 12 g to 14 g. After ligation, the withdrawal threshold of the nerve-ligated group was significantly reduced in comparison with that of the sham-ligated group, and this reduced threshold persisted for 21 days (Fig. 1). The withdrawal threshold of the sham group did not change during the observation period. In control groups, the post-ligated baseline thresholds evoked by the application of von Frey filaments to the ligated paws did not differ from each other ($p>0.05$; Figs. 2–4A).

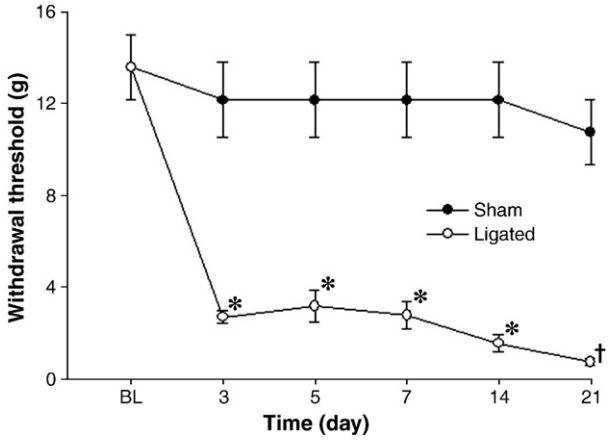

Fig. 1. Time course of the hindpaw-withdrawal response to von Frey filament stimulation after spinal nerve ligation. Data are presented as the withdrawal threshold (g). Each line represents mean ± SEM of 8 rats. BL = baseline withdrawal threshold measured before spinal nerve ligation. Significant differences between the spinal nerve-ligated group and the sham-ligated group are indicated. *$p<0.01$, †$p<0.001$, vs. sham.

3.2. Effects of intrathecal LY 367385, MPEP, APDC, ACPT-III and morphine

Several intrathecal mGluR agents were tested for their effects on the withdrawal threshold in the paws of nerve-ligated rats. Neither LY 367385 (200 μg; a Group I mGlu1a antagonist), MPEP (300 μg; a Group I mGlu5 antagonist), APDC (100 μg; a Group II mGluR agonist), nor ACPT-III (10 μg; a Group III mGluR agonist), had any significant effect on the withdrawal threshold ($p>0.05$; Figs. 2 and 3). On the other hand, intrathecal morphine [$F(4,26)=17.093$, $p<0.001$] dose-dependently increased the withdrawal threshold in the paws of nerve-ligated rats (Fig. 4B). The ED_{50} value of morphine was 0.19 μg (95% CIs: 0.13–0.3 μg).

3.3. Drug interactions

The intrathecal co-administration of ACPT-III (10 μg) with morphine increased the antinociceptive effect of morphine (Fig. 5). The ED_{50} value of morphine in the morphine/ACPT-III mixture was 0.03 μg (95% CIs: 0.02–0.07 μg) (Table 1), which was significantly less than that of morphine alone ($p<0.001$), as determined by the modified *t*-test described by Tallarida (2000); thus, the effect was synergistic. None of the other mGluR agents tested (LY 367385, MPEP, or APDC) increased the antiallodynic effect of morphine ($p>0.05$; Fig. 6).

3.4. General behavioral effects

After intrathecal delivery of mGluR agents and/or morphine, the rats retained their pinnal and corneal reflexes and displayed normal motor functions. Additionally, no overt behavioral changes were observed after the administration of any of the drugs. All rats remained alert during the experiment.

4. Discussion

Neuropathic pain occurs as a result of various conditions that cause functional abnormalities or direct injury in the peripheral or central nervous system (Finnerup et al., 2007). In this study, we tested the effectiveness of intrathecally administered mGluR agents against spinal nerve ligation-induced neuropathic pain in rats. Neither Group

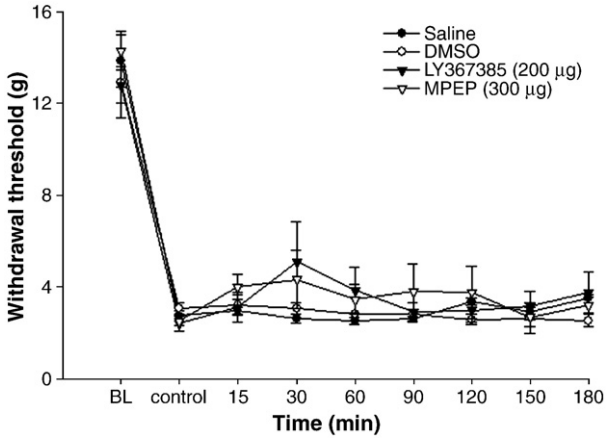

Fig. 2. Effects of intrathecal Group I mGluR antagonists on the hindpaw-withdrawal response to von Frey filament stimulation after spinal nerve ligation. Data are presented as the withdrawal threshold (g). Each line represents mean ± SEM of 5 or 6 rats. BL = baseline withdrawal threshold measured before spinal nerve ligation. Control data were measured immediately before intrathecal delivery of drugs. Neither the Group I mGlu1a antagonist LY 367385 (200 μg) nor the Group I mGlu5 antagonist MPEP (300 μg) affected the withdrawal threshold in hindpaws of nerve-ligated rats.

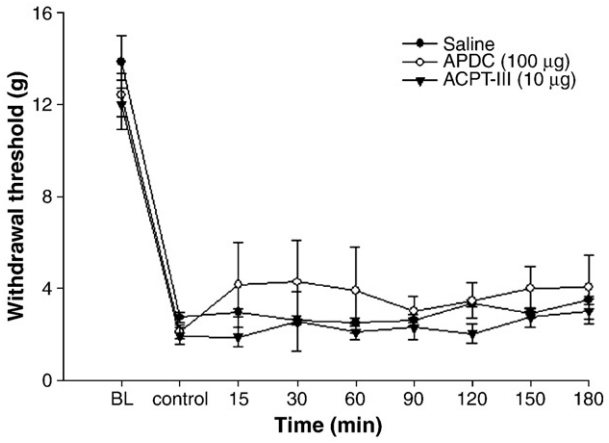

Fig. 3. Effects of intrathecal Group II and III mGluR agonists on the hindpaw-withdrawal response to von Frey filament stimulation after spinal nerve ligation. Data are presented as the withdrawal threshold. Each line represents mean ± SEM of 5 or 6 rats. BL = baseline withdrawal threshold measured before spinal nerve ligation. Control data were measured immediately before intrathecal delivery of drugs. Neither the Group II mGluR agonist APDC (100 μg) nor the Group III mGluR agonist ACPT-III (10 μg) affected the withdrawal threshold in hindpaws of nerve-ligated rats.

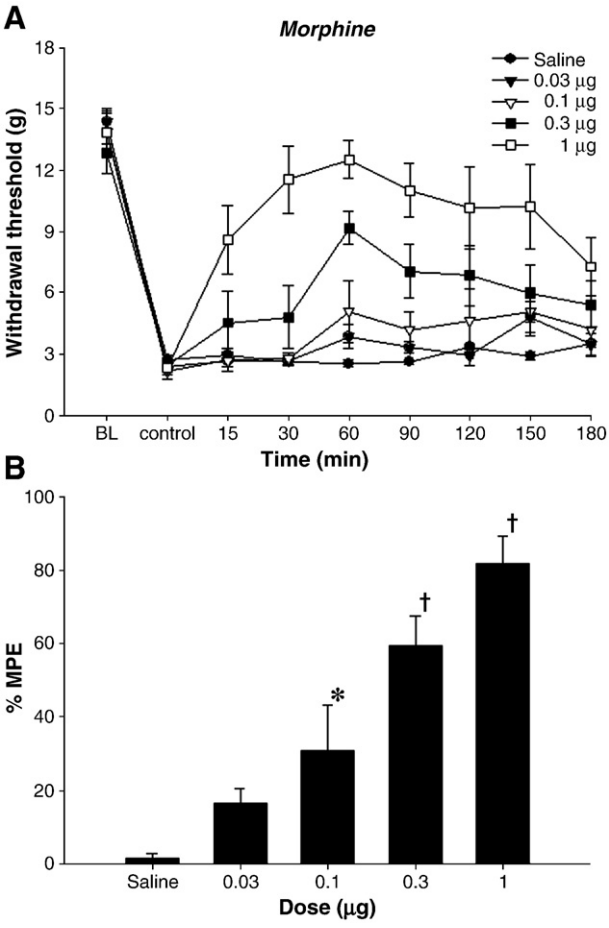

Fig. 4. Effects of intrathecal morphine on the hindpaw-withdrawal response to von Frey filament stimulation after spinal nerve ligation. Data are presented as the withdrawal threshold (A) or the percentage of maximal possible effect (%MPE, B). Each line or bar represents mean ± SEM of 5–7 rats. BL = baseline withdrawal threshold measured before spinal nerve ligation. Control data were measured immediately before intrathecal delivery of drugs. Morphine produced a dose-dependent increase of the withdrawal threshold. *$p<0.01$, †$p<0.001$ vs. control.

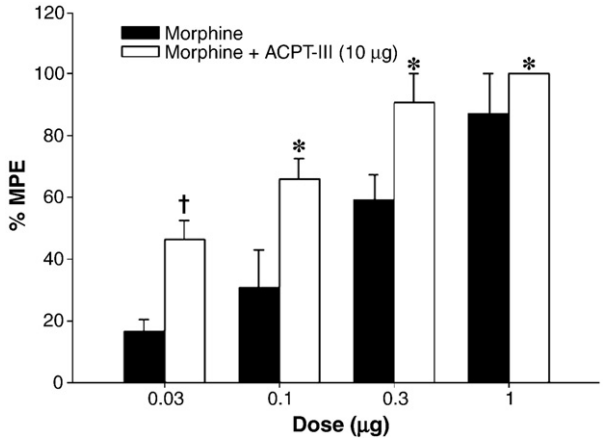

Fig. 5. Effect of co-administration of ACPT-III (10 μg) on the antinociception activity of morphine. ACPT-III was intrathecally co-administered with various doses of morphine. Data are presented %MPE. Each bar represents mean ± SEM of 5–7 rats. ACPT-III enhanced the antiallodynic action of morphine (*$p<0.05$, †$p<0.01$ vs. morphine alone).

I mGluR antagonists nor Group II or III mGluR agonists exhibited any activity against neuropathic pain, suggesting that spinal mGluRs are not directly involved in the development of neuropathic pain after spinal nerve ligation.

After injury, peripheral nerves exhibit spontaneous and persistent afferent discharges, leading to a sensitization of the spinal cord that drives the development of neuropathic pain. The mechanisms underlying the sensitization within the spinal cord involve changes in cell-surface receptor and ion-channel expression, the activation of intracellular signaling cascades, and glial proliferation (Coderre, 1992). As the predominant neurotransmitter released from primary afferents, the excitatory amino acid glutamate also likely plays an important role in central sensitization via iGluRs and mGluRs in the spinal cord after nerve injury (Dickenson et al., 1997). Consistent with this hypothesis, spinal cord levels of glutamate and mGluR5 are elevated during the development of neuropathic pain after nerve injury (al-Ghoul et al., 1993; Hudson et al., 2002).

Recent research has emphasized the possible role of mGluRs in the development of neuropathic pain after nerve injury. Several studies have addressed the expression of the three classical mGluR subtypes (Conn and Pin, 1997) in the spinal cord. Group I and II mGluR mRNAs are expressed in the spinal cord (Valerio et al., 1997), and immunoreactivity to the corresponding proteins is detected pre- and postsynaptically in the superficial dorsal horn, an area intimately associated with nociceptive processing (Jia et al., 1999; Tang and Sim, 1999; Vidnyanszky et al., 1994). Group III mGluR mRNAs are expressed, and immunoreactivity is detected, in the dorsal horn of the spinal cord (Azkue et al., 2001; Valerio et al., 1997).

Group I mGluRs primarily stimulate phosphoinositide hydrolysis, which increases the intracellular Ca^{2+} concentration, promotes the production of protein kinase C (Conn and Pin, 1997), and has been

Table 1
ED_{50} (μg) with 95% confidence intervals (CI) of intrathecal drugs.

	ED_{50} (95% CI)
Morphine ($n=26$)	0.19 (0.13–0.3)
Morphine[a] + LY367385 ($n=26$)	0.15 (0.11–0.22)
Morphine[a] + MPEP ($n=26$)	0.16 (0.09–0.29)
Morphine[a] + APDC ($n=27$)	0.11 (0.06–0.18)
Morphine[a] + ACPT-III ($n=24$)	0.03 (0.02–0.07)*

ED_{50}: effective dose producing a 50% reduction of control response.
[a] Means the ED_{50} of morphine in the mixture of other drugs.
* $p<0.001$ vs. morphine.

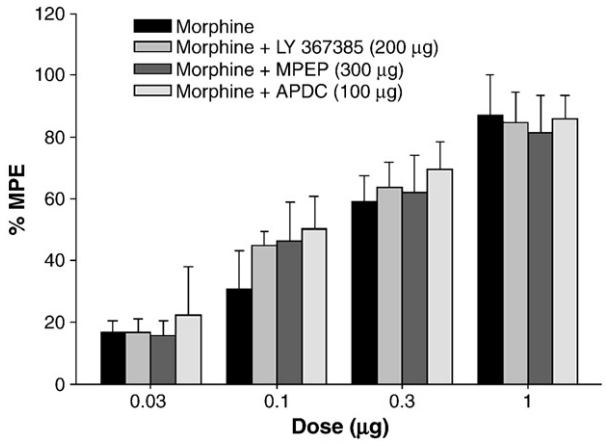

Fig. 6. Effects of LY 367385 (200 μg), MPEP (300 μg), and APDC (100 μg) on the antinociception activity of morphine. Each agent was intrathecally co-administered with various doses of morphine. Data are presented as %MPE. Each bar represents mean ± SEM of 6–8 rats. None of these agents affected the antiallodynic action of morphine.

shown to contribute significantly to the development of pain (Yashpal et al., 1995). Additionally, Group I mGluRs facilitate the release of glutamate in the CNS (Reid et al., 1999). On the other hand, Group II and III mGluRs are negatively coupled to adenylate cyclase and act to decrease the release of glutamate in the CNS (Battaglia et al., 1997). Thus, one might expect that antagonists of the Group I mGluRs or agonists of the Group II or III mGluRs would suppress the nociceptive state, thereby producing an antinociceptive effect.

The results of several studies support a role for mGluRs in the processing of neuropathic pain in the spinal cord. Intrathecal pretreatment with Group I mGluR antagonists significantly reduced mechanical hypersensitivity in a rat model of neuropathic pain (Fisher et al., 2002), and a spinally microinjected mGluR5 antagonist reduced the spontaneous and noxious evoked activity and significantly decreased the suppression of the afterdischarge duration in a mononeuropathic pain rat model (Sotgiu et al., 2003). Elevation of extracellular *N*-acetylaspartylglutamate (NAAG) levels induced by the inhibition of NAAG peptidase activated Group II mGluRs and attenuated the level of mechanical allodynia induced by sciatic nerve ligation at the spinal level (Yamamoto et al., 2004), and intrathecal administration of Group II and III mGluR agonists reduced mechanical hypersensitivity in nerve-injured models (Chen and Pan, 2005; Fisher et al., 2002; Goudet et al., 2008). Furthermore, activation of Group III mGluRs inhibited spinal synaptic transmission in a neuropathic pain model (Zhang et al., 2009).

The above findings suggest that spinal mGluRs might play an important role in the modulation of neuropathic pain induced by spinal nerve ligation. However, our findings differ unexpectedly from those described above. In our experiments, none of the intrathecal mGluR agents had any effect on spinal nerve ligation-induced neuropathic pain when administered alone; this discrepancy might result from differences in drug selection, methods of drug administration, or models of neuropathic pain. In particular, the time point of drug injection may affect the outcome. In one report, intrathecal Group I mGluR antagonists administered before nerve injury or in the early stage of nerve injury reduced mechanical allodynia, whereas late treatment did not alter the nociceptive behavior (Fisher et al., 1998). In other studies, intrathecal Group I mGluR antagonists given after nerve injury failed to reduce mechanical allodynia (Chaplan et al., 1997; Hama and Urban, 2004; Nguyen et al., 2009). These findings suggest that Group I mGluRs might not be directly associated with neuropathic pain at the spinal level. On the other hand, intrathecal Group II and III mGluR agonists administered before or after nerve injury were reported to be antiallodynic (Chen and Pan, 2005; Fisher et al., 2002; Goudet et al., 2008), suggesting that Group II and III mGluRs might play a role in neuropathic pain.

Another possible source of the apparent inconsistency between our results and some others is the possible difference in the contributions of iGluRs and mGluRs to nerve injury-associated neuropathic pain. As described above, mGluR activation is important for the initiation of central sensitization in the dorsal horn of the spinal cord (Neugebauer, 2002). Behavioral studies also have indicated that spinal mGluRs can be important for the development of pain behaviors (Fisher et al., 2002; Goudet et al., 2008). Intrathecal *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists have been shown to attenuate mechanical allodynia in spinal nerve ligation-induced neuropathic pain models (Chaplan et al., 1997; Chen et al., 2000). However, in the current study, no intrathecal mGluR agents affected the mechanical allodynia, suggesting that mGluRs might be less important than iGluRs in the development and maintenance of neuropathic pain at the spinal level.

Intrathecal administration of morphine, on the other hand, dose-dependently increased the withdrawal threshold of the injured paw in our study, consistent with previous studies (Zhang et al., 2005). This finding provides further support for the effectiveness of spinal opioids in the management of neuropathic pain.

In our study, the co-administration of intrathecal ACPT-III, which is ineffective against neuropathic pain by itself, with intrathecal morphine increased the antiallodynic action of morphine. Thus, the combination of the Group III mGluR agonist and morphine augmented the antiallodynic effect of morphine in a neuropathic pain state evoked by spinal nerve ligation. In contrast, Group I mGluR antagonists and a Group II mGluR agonist did not increase the antiallodynic effect of morphine, suggesting that Group III mGluRs, but not Group I or II mGluRs, may play a critical role in the potentiation of the effect of morphine. Although pharmacological interactions between two kinds of drugs are often very complicated, there are several possible explanations for this potentiation effect. First, the drugs might interact by altering each other's kinetics. One agent might alter the actions of the other agent at a receptor or channel. A synergistic effect would arise if the activation of opioid receptors in turn activates Group III mGluRs. Second, drug interactions might occur if the drugs affect different critical points along a common pathway (Berenbaum, 1989). Group III mGluRs and opioid receptors act on G-protein-coupled receptors. Hence, the actions of ACPT-III and morphine might independently alter intracellular second-messenger systems linked to G-protein activation, resulting in potentiation (Malmberg and Yaksh, 1993).

This report is the first to describe an increase in the antiallodynic efficacy of intrathecal morphine caused by a co-injection of a Group III mGluR agonist (ACPT-III) in a spinal nerve ligation-induced neuropathic pain model. This synergism was linked only to Group III mGluRs, and not to Group I and II mGluRs, for reasons that were not determined in the current study. Thus, closer investigation of these differences is needed.

In clinical medicine, central sensitization after nerve injury leads to hyperalgesia or allodynia. The management of neuropathic patients is complex, and patient responses to established treatments have not been consistent. Because the well-established neuropathic medications are inadequate for treatment of neuropathic pain and have undesirable side effects, new treatment modalities are needed. Unfortunately, spinal Group III mGluR agonists are not yet available for clinical use. However, selective Group III mGluR agonists might be used in combination with morphine in the treatment of neuropathic pain in the future.

In summary, all of intrathecal group I mGluRs antagonist, group II and III mGluRs agonists themselves are not active to neuropathic pain evoked by spinal nerve ligation, but the addition of group III mGluRs agonist (ACPT-III) to morphine increases the antiallodynic effect of morphine alone in the spinal cord.

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.

Azkue JJ, Murga M, Fernandez-Capetillo O, Matoes JM, Elezgarai I, Benitez R, et al. Immunoreactivity for the group III metabotropic glutamate receptor subtype mGluR4a in the superficial laminae of the rat spinal dorsal horn. J Comp Neurol 2001;430:448–57.

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.

Berenbaum MC. What is synergy? Pharmacol Rev 1989;41:93-141.

Calcutt NA, Jorge MC, Yaksh TL, Chaplan SR. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. Pain 1996;68:293–9.

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

Chaplan SR, Malmberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J Pharmacol Exp Ther 1997;280:829–38.

Chen SR, Pan HL. Distinct roles of group III metabotropic glutamate receptors in control of nociception and dorsal horn neurons in normal and nerve-injured rats. J Pharmacol Exp Ther 2005;312:120–6.

Chen SR, Eisenach JC, McCaslin PP, Pan HL. Synergistic effect between intrathecal non-NMDA antagonist and gabapentin on allodynia induced by spinal nerve ligation in rats. Anesthesiology 2000;92:500–6.

Coderre TJ. Contribution of protein kinase C to central sensitization and persistent pain following tissue injury. Neurosci Lett 1992;140:181–4.

Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Ann Rev Pharmacol Toxicol 1997;37:205–37.

Dickenson AH, Chapman V, Green GM. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen Pharmacol 1997;28:633–8.

Dray A. Neuropathic pain: emerging treatments. Br J Anaesth 2008;101:48–58.

Finnerup NB, Sindrup SH, Jensen TS. Chronic neuropathic pain: mechanisms, drug targets and measurement. Fundam Clin Pharmacol 2007;21:129–36.

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.

Fisher K, Lefebvre C, Coderre TJ. Antinociceptive effects following intrathecal pretreatment with selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain. Pharmacol Biochem Behav 2002;73:411–8.

Goudet C, Chapuy E, Alloui A, Acher F, Pin JP, Eschalier A. Group III metabotropic glutamate receptors inhibit hyperalgesia in animal models of inflammation and neuropathic pain. Pain 2008;137:112–24.

Hama AT, Urban MO. Antihyperalgesic effect of the cannabinoid agonist WIN 55, 212-2 is mediated through an interaction with spinal metabotropic glutamate-5 receptors in rats. Neurosci Lett 2004;358:21–4.

Hudson LJ, Bevan S, McNair K, Gentry C, Fox A, Kuhn R, et al. Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced cold hyperalgesia. J Neurosci 2002;22:2660–8.

Jia H, Rustioni A, Valtschanoff JG. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. J Comp Neurol 1999;410:627–42.

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–63.

Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain 2005;115:71–83.

Lee YW, Chaplan SR, Yaksh TL. Systemic and supraspinal, but not spinal, opiates suppress allodynia in a rat neuropathic pain model. Neurosci Lett 1995;199:111–4.

Madden DR. The structure and function of glutamate receptor ion channels. Nat Rev Neurosci 2002;3:91-101.

Malmberg AB, Yaksh TL. Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. Anesthesiology 1993;79:270–81.

Neugebauer V. Metabotropic glutamate receptors—important modulators of nociception and pain behavior. Pain 2002;98:1–8.

Nguyen D, Deng P, Matthews EA, Kim DS, Feng G, Dickenson AH, et al. Enhanced presynaptic glutamate release in deep-dorsal horn contributes to calcium channel alpha-2-delta-1 protein-mediated spinal sensitization and behavioral hypersensitivity. Mol Pain 2009;5:6.

Nishiyama T. Interaction between intrathecal morphine and glutamate receptor antagonists in formalin test. Eur J Pharmacol 2000;395:203–10.

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.

Sotgiu ML, Bellomi P, Biella GE. The mGluR5 selective antagonist 6-methyl-2-(phenylethynyl)-pyridine reduces the spinal neuron pain-related activity in mononeuropathic rats. Neurosci Lett 2003;342:85–8.

Tallarida RJ. Drug synergism and dose–effect data analysis. 1st. ed. New York: Chapman & Hall/CRC; 2000. 21–71.

Tang FR, Sim MK. Pre- and/or post-synaptic localisation of metabotropic glutamate receptor 1alpha (mGluR1alpha) and 2/3 (mGluR2/3) in the rat spinal cord. Neurosci Res 1999;34:73–8.

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

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

Willis WD. Role of neurotransmitters in sensitization of pain responses. Ann NY Acad Sci 2001;933:142–56.

Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yamamoto T, Hirasawa S, Wroblewska B, Grajkowska E, Zhou J, Kozikowski A, et al. Antinociceptive effects of N-acetylaspartylglutamate (NAAG) peptidase inhibitors ZJ-11, ZJ-17 and ZJ-43 in the rat formalin test and in the rat neuropathic pain model. Eur J Neurosci 2004;20:483–94.

Yashpal K, Pitcher GM, Parent A, Quirion R, Coderre TJ. Noxious thermal and chemical stimulation induce increases in 3H-phorbol 12, 13-dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C. J Neurosci 1995;15:3263–72.

Zhang Y, Conklin DR, Li X, Eisenach JC. Intrathecal morphine reduces allodynia after peripheral nerve injury in rats via activation of a spinal A1 adenosine receptor. Anesthesiology 2005;102:416–20.

Zhang HM, Chen SR, Pan HL. Effects of activation of group III metabotropic glutamate receptors on spinal synaptic transmission in a rat model of neuropathic pain. Neuroscience 2009;158:875–84.