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☐ Original Article ☐

Potentiation of Morphine's Antinociception by Group II and Group III Metabotropic Glutamate Receptors Agonists on a Rat Incisional Pain

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= **Abstract** =

Background: The aim of this study was to clarify the role of spinal groups II and III metabotropic glutamate receptors (mGluRs) with respect to postoperative pain at the spinal level. In addition, the nature of the pharmacological interaction between groups II and III mGluRs agonists and morphine was determined.

Methods: Catheters were inserted into the intrathecal space of male SD rats. To induce postoperative pain, an incision was made in the plantar surface of the hind paw. A pharmacological characteristic for the interaction between groups II and III mGluRs agonists and morphine was evaluated using a fixed-dose analysis.

Results: None of intrathecal group II and III mGluRs agonists modified the withdrawal threshold of the incisional pain. The administration of intrathecal morphine resulted in an increase of a dose dependent withdrawal threshold. A fixed-dose analysis revealed that the group III mGluRs agonist, ACPT-III, increased the antinociceptive action of morphine, while the group II mGluRs agonist, APDC, had no effect the antinociception of morphine.

Conclusions: These results suggest that group II and III mGluRs may not play a direct modulatory role in the processing of postoperative pain at the spinal level. However, agonizing group III mGluRs may indirectly contributable to the potentiation of morphines antinociception in the spinal cord. Thus, the combination of morphine and a group III mGluRs agonist may be useful in the management of spinal postoperative pain. **(Korean J Pain 2006; 19: 131－136)**

Key Words: drug interaction, mGluRs, morphine, postoperative pain, spinal cord, Sprague-Dawley rat.

INTRODUCTION

A postoperative pain is directly associated with peripheral tissue damage during surgery. It is widely recognized that peripheral tissue damage results in increased activation of peripheral nociceptors and an activity-dependent increase in central neuronal excitability, inducing peripheral and central sensitization.[1] Recent research suggests that transcriptional alterations in key genes and induction of sustained plasticity of neuronal activity underlies longer-lasting pain.[2]

Accumulating evidence suggests a pivotal role for metabotropic glutamate receptors (mGluRs) in nociceptive processing[3] and they have traditionally been divided into three groups: I (mGluR1 and mGluR5), II (mGluR2 and mGluR3), and III (mGluR4, mGluR6－mGluR8).[4] In particular, group II and group III mGluRs inhibit adenylate cyclase, thereby producing an antinociceptive effect.[5] One study has reported that mGluRs are differentially expressed in the sheep spinal cord following abdominal surgery.[6] These data lead to the hypothesis that agonizing on group II and group III mGluRs activity may contribute to modulation of postoperative pain and hyperalgesia. However, the effects of group II and group III mGluRs agonists in response to surgery have not been determined before.

Therefore, the aim of the present study was to increase our understanding of spinal group II and group III mGluRs signaling mechanisms underlying the development and maintenance of postoperative pain in an animal model of incisional pain, that it closely mimics the human postopeartive pain. In addition, we sought to determine the characteristics of the drug interaction between intrathecal group II agonist (APDC)

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agonist or group III mGluRs (ACPT-III) agonist and morphine.

MATERIALS AND METHODS

1. Animal Preparation

This study was approved by The Institutional Animal Care Committee, Research Institute of Medical Science, Chonnam National University. Adult male Sprague-Dawley rats weighing 250−300 g were used in all experiments. Animals were acclimated to the laboratory environment for 5−7 days before entering the study. While in the home cage environment, the animals were allowed free access to standard rat diet and tap water. Room temperature was maintained at 20−23°C with 12/12 h light/dark cycle. For drug administration, intrathecal catheter (0.28 mm inner diameter, 0.61 mm outer diameter) was implanted via the cisterna magna in enflurane-anesthetized rats.[7] The catheter measured 8.5 cm in length and the distal tip was located at the lumbar enlargement of the spinal cord. The skin margins were closed, leaving only 2 cm of catheter above the skull exposed for injections and plugged with a piece of steel wire. If any motor or sensory deficits were present after intrathecal catheter placement, these rats were dropped from the study and killed immediately with overdose of volatile anesthetics. Three days after catheter implantation, 20 μl of 2% lidocaine was given to verify correct position of catheter at the lumbar enlargement, and only rats with bilateral hindpaw paralysis were re-anesthetized for foot incisional surgery 7 days after catheterization.

2. Drugs

The following drugs were used in this study: APDC (Tocris Cookson Ltd., Bristol, UK), ACPT-III (Tocris), morphine sulfate (Research Biochemical Internationals [RBI], Natick, USA). APDC, ACPT-III and morphine were dissolved with normal saline. Intrathecal administration of these agents was performed using a hand- driven, gear-operated syringe pump. All drugs were delivered in a volume of 10 μl solution.

3. Incisional Pain Model

Postoperative pain was induced by the procedure described by Brennan et al.[8] Under enflurane anesthesia, the plantar surface of the left hindpaw prepared in a sterile manner. A 1 cm longitudinal incision was made through skin and fascia of the plantar aspect of the foot starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally leaving the muscle origin and insertion points intact. After hemostasis with gentle pressure, two 4−0 silk sutures were placed in mattress fashion along the wound. Antibiotic ointment was then applied to the incised site and the postoperative rats were allowed to recover in their cages. Wounds were checked for evidence before behavioral testing.

4. Postoperative Pain Testing

For determining withdrawal threshold, rats were placed individually in plastic cages with a plastic mesh floor. Animals were tested after accommodation to the environment, typically 20−30 min after being placed in the cage. Paw withdrawal threshold in response to mechanical stimulation was measured using the up and down method[9] by applying calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) 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, 15 g) were applied vertically to the plantar surface of the hindpaw for 4 s while the hair was bent. Brisk withdrawal or paw flinching was considered as positive responses. In the absence of a response at the pressure of 15 g, animals were assigned to this cut-off value. Tests were performed in duplicate, with an approximate 3-min test-free period between withdrawal responses, and their average was used. Studies were performed on the first day after paw incision surgery. Only rats with marked allodynia (withdrawal threshold < 5 g) after paw incision were studied.

5. Experimental Paradigm

On the day of experiments, the rats were then allocated to receive one of the experimental drugs. The control study was done using intrathecal saline. Animals were tested only once. All experiments were carried out by an observer blind to drug treatments.

6. Effects of Intrathecal APDC, ACPT-III, and Morphine

The effects of group group II mGluRs agonist (APDC, 100 μg), group III mGluRs agonist (ACPT-III, 10 μg), and morphine (0.1, 0.3, 1, 3 μg) were investigated in the incisional pain state. The withdrawal threshold was determined before and 2 hr after incision, then at 30, 60, 120, 240 min after intrathecal administration of experimental drugs. The withdrawal threshold measured at 2 hr after incision was regarded as baseline postincision threshold. The test drugs were delivered intrathecally after testing 2 hr after incisional surgery. Each ED_{50} value (effective dose producing a 50% reduction of

control response) for the agents was calculated. APDC was not soluble at higher dose than that used in this study. ACPT-III caused a motor dysfunction above 30 μg. Hence, the highest doses of APDC and ACPT-III administered for the present study were regarded as the maximum doses.

7. Drug Interaction

After intrathecal administration of APDC and ACPT-III, no antinociceptive effect was seen in an incisional pain model. Therefore, the fixed dose of APDC or ACPT-III was coadministered with ED_{50} of morphine to assess the modulatory effect of mGluRs on the antinociception of morphine alone. The fixed dose of APDC and ACPT-III indicated the ineffective maximum doses used in this study.

8. General Behavior

In order to evaluate the behavioral changes APDC and ACPT-III, and morphine, additional rats received the highest doses of agents used, and here examined 5, 10, 20, 30, 40, 50, 60 min after intrathecal administration. 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. These behaviors were judged as either present or absent.

9. Statistical Analysis

Data are expressed as means ± SEM. The time response data are presented as the withdrawal threshold in g. The dose-response data are presented as the percent of maximum possible effect (%MPE). To calculate the ED_{50} values of each drug, the withdrawal threshold data from von Frey filament testing were converted to %MPE, according to the formula: %MPE = [(postdrug threshold − baseline postincision threshold) / (preincision threshold − baseline postincision threshold)] × 100. Dose-response data were analyzed by one-way analysis of variance (ANOVA) with Scheffe for *post hoc* comparison. Respective ED_{50} and its 95% confidence intervals were calculated using linear regression. The difference of the withdrawal threshold between injured site and non-injured site, and the difference of %MPE between morphine alone and combination of mGluRs agonists with morphine were analyzed by *t*-test. P values of < 0.05 were considered statistically significant.

RESULTS

Motor functions were normal after intrathecal delivery of group II mGluRs agonist (APDC, 100 μg), group III mGluRs agonist (ACPT-III, 10 μg), and morphine.

Incisional surgery decreased the withdrawal threshold immediate after incision and persisted for approximately 4 days in the injured paw (Fig. 1). Incisional surgery did not affect the withdrawal threshold in the contralateral normal hindpaw (Fig. 1). The preincision withdrawal thresholds were 13.6 ± 0.9 in left and 14.1 ± 0.7 g in right, respectively.

In control groups, baseline postincision threshold evoked by application of von Frey filaments on the incised paw did not differ from each other (Fig. 2, 3).

Neither intrathecal APDC (100 μg) nor intrathecal ACPT-III (10 μg) altered the withdrawal threshold in the injured paw (Fig. 2).

Intrathecal morphine dose-dependently increased the withdrawal threshold in the injured paw (Fig. 3). The ED_{50} value (95% confidence intervals) of morphine was 0.4 μg (0.3−0.5 μg).

The addition of intrathecal ACPT-III to the ED_{50} of intrathecal morphine potentiated the antinociceptive effect of morphine alone, but APDC did not increase the morphine-induced antinociception (Fig. 4).

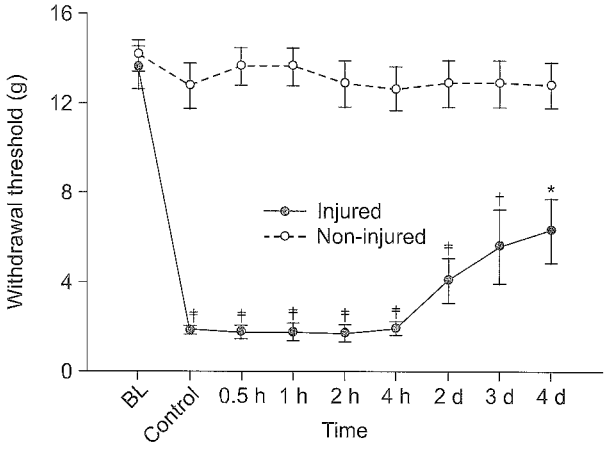

Fig. 1. Time course of hindpaw withdrawal response to von Frey filaments after incisional surgery. Data are presented as the withdrawal threshold. Each line represents the mean ± SEM of 8 rats. BL: baseline withdrawal threshold measured before paw incision. h: hour. d: day. Control data were obtained 2 hr after incision. Significant difference between injured site and non-injured site are indicated, *P $<$ 0.05, †P $<$ 0.01, ‡P $<$ 0.001.

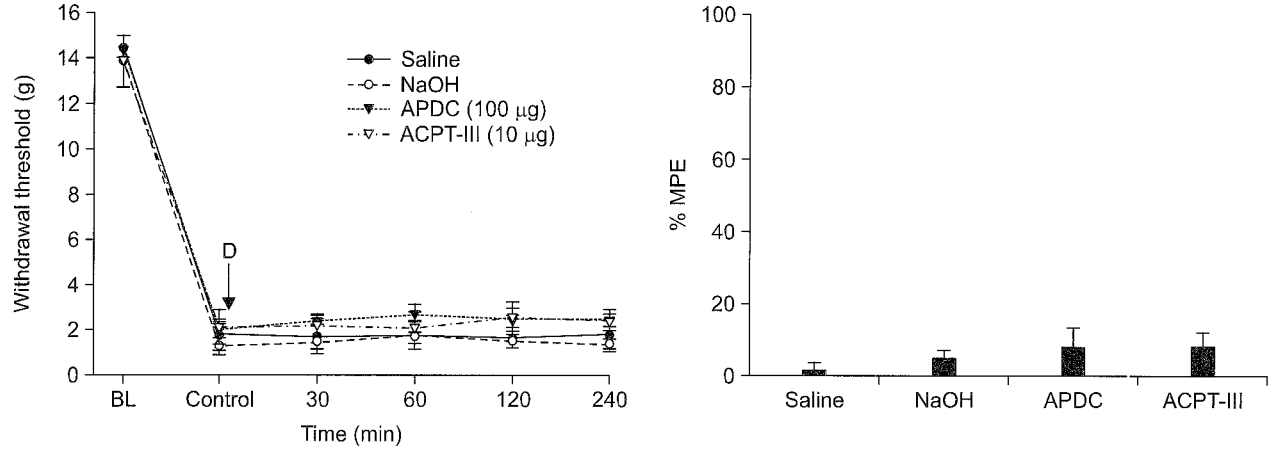

Fig. 2. Effects of intrathecal group II and group III mGluRs agonists for hindpaw withdrawal response to von Frey filaments after incisional surgery. Data are presented as the withdrawal threshold or the percent of maximal possible effect (%MPE). Each point on the graph represents the mean ± SEM of 4−8 rats. BL: baseline withdrawal threshold measured before paw incision. Control data were obtained 2 hr after incision. Neither group II mGluRs agonist APDC (100 μg) nor group III mGluRs agonist ACPT-III (10 μg) affected the withdrawal threshold in injured paw after incision.

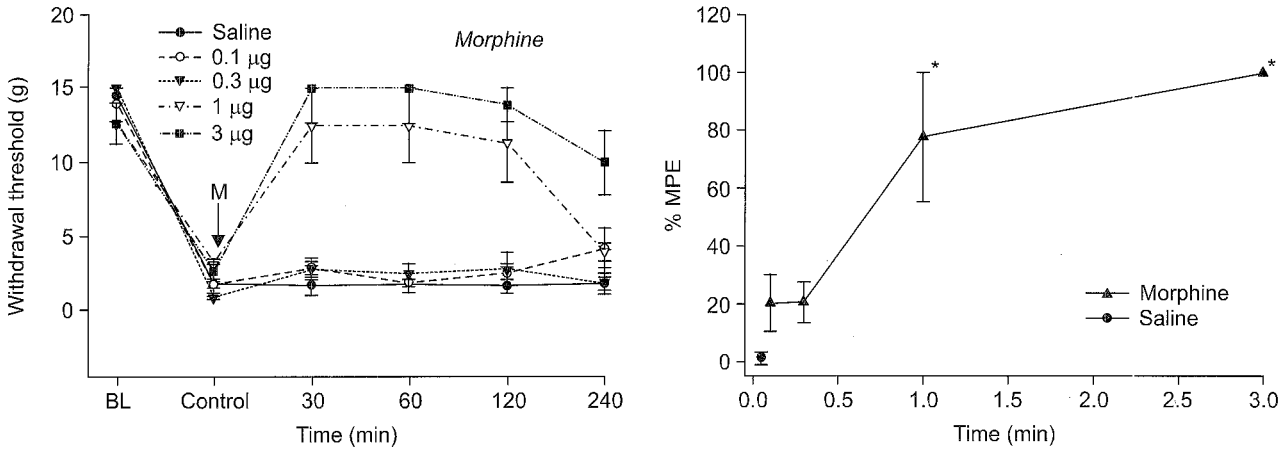

Fig. 3. Effects of intrathecal morphine for hindpaw withdrawal response to von Frey filaments after incisional surgery. Data are presented as the withdrawal threshold or the percent of maximal possible effect (%MPE). Each line represents the mean ± SEM of 5−7 rats. BL: baseline withdrawal threshold measured before paw incision. Control data were obtained 2 hr after incision. Morphine produced a dose-dependent increase of the withdrawal threshold in injured paw after incision. Compared with control (saline), *P < 0.05.

DISCUSSION

Results of the current study showed that intrathecal group II and group III mGluRs agonists did not affect the withdrawal threshold to von Frey filaments after paw incision. These findings suggest that group II and group III mGluRs themselves are not directly involved in the processing of postoperative pain at the spinal level.

Surgery induces two phases of sensory input in the acute postoperative period, first in response to tissue damage from the incision and second, an inflammatory reaction to the tissue damage involving release of proinflammatory mediators at the site.[1]

Excitatory amino acids, such as glutamate and aspartate, are important role in the processing of nociceptive inputs in the dorsal horn of the spinal cord.[10] The actions of excitatory amino acids are mediated either through an interaction with inotropic GluRs and mGluRs.[11] Activation of these receptors facilitate transmission of sensory inputs and contribute the enhanced excitability of nociceptive pathway in the spinal cord, leading to persistent pain states like postoperative pain.[12,13] Less is known about the role of mGluRs compared to the ionotropic GluRs in the development of central sensitization and persistent pain after tissue injury. To date, eight mGluRs have been identified, which can be classified into three subgroups based on their sequence similarities and transduction mechanisms.[4] Expressions of group I and group II mGluRs

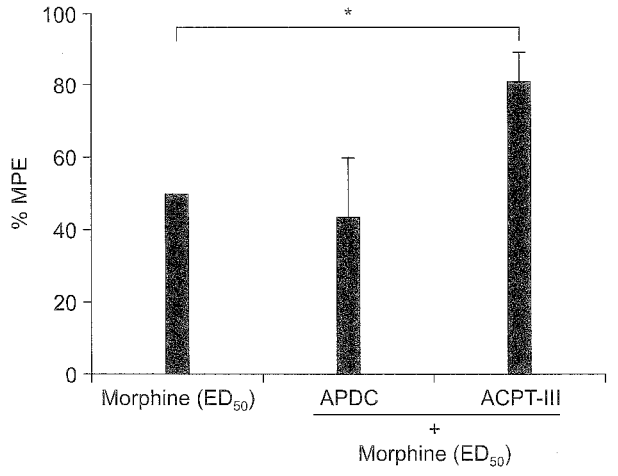

Fig. 4. Effects of APDC (100 μg) and ACPT-III (10 μg) for the antinociception of morphine. APDC and ACPT-III were intrathecally coadministered with the ED_{50} of morphine. Data are presented as the percent of maximal possible effect (%MPE). Each bar represents the mean $\pm$ SEM of 6 rats. Ineffective doses ACPT-III enhanced the antinociceptive action of morphine alone, while APDC did not alter the antinociception of morphine. Compared with morphine alone, *$P < 0.05$.

mRNA have been identified in the spinal cord.[14,15] The group III mGluRs are expressed in the dorsal horn of the spinal cord.[15-17] The metabotropic glutamate5 mRNA was upregulated in the early postoperative period with delayed upregulation of group II mGluRs in the incisional rat spinal cord.[6]

Mechanistically, group I mGluRs are coupled to phospholipase C, which stimulates the production of inositol trisphosphate (IP_3) and diacylglycerol (DAG).[5] DAG, in turn, activates the protein kinase C, which has been shown to contribute significantly to the development of pain.[18,19] Hence, blocking agents for group I mGluRs may suppress the nociceptive state. On the other hand, group II and group III mGluRs couple to inhibition of adenylate cyclase, thereby producing an antinociceptive effect.[5] Pharmacologic research has been shown that group II and group III mGluRs are involved in the modulation of nocicpetive processing in the spinal cord. Activation of group II mGluRs attenuated formalin-induced nociception and neuropathic pain at the spinal level.[20] Intrthecal group II and group III mGluRs agonists reduced mechanical hypersensitivity with after nerve injury.[21] Based on the above mentioned findings, we might assume that spinal group II and group III mGluRs may play an important role in modulation of postoperative pain induced by plantar incision. However, unfortunately, the present study indicated that intrathecal group II and group III mGluRs agonists were without effect to incisional pain. Therefore, group II and group III mGluRs activity is not directly involved in the maintenance of hyperalgesia after incision at the spinal level.

Furthermore, there are another several possibilities why these spinal mGluRs are not linked to postoperative pain. Activation of the mGluRs is important for the initiation of central sensitization in the dorsal horn of the spinal cord.[3] Behavioral studies for models of neuropathic and inflammatory pain, spinal mGluRs can be important for the development of pain behaviors.[20-22] However, the plantar incision itself for postoperative pain does not involve nerve injury. In addition, inflammation may be important for the development of some models of persistent pain, and this can be modified by spinal modulation of mGluRs. But inflammatory reaction to the surgical tissue damage is insensitive to spinal group II and group III mGluRs unlike other persistent pain models. Finally, the extent of injury in the plantar incision may not be sufficient to activate spinal group II and group III mGluRs. It is possible that mGluRs could contribute to the development or maintenance of pain after more extensive surgery. Indeed, spinal mGluRs are expressed after midline laparatomy of adult sheep.[6]

On the other hand, intrathecal morphine resulted in a dose-dependent increase in the withdrawal threshold on the incised paw, corroborating previous result.[23]

In the current study, coadministration of intrathecal ACPT-III, being ineffective for postoperative pain by itself, with the ED_{50} of intrathecal morphine strengthened the antinociceptive action of morphine after paw incision. These results indicate that the spinal combination of group III mGluRs agonist (ACPT-III) with morphine is able to augment the antinociceptive effect of morphine in a postoperative pain state evoked by plantar incision. Although a pharmacological interaction between two kinds of drugs is most likely complicated to characterize, several explanations may be possible for this potentiation. First, drugs may interact by altering each others kinetics. One agent may alter the actions of the other agents at a receptor or channel. Secondly, such interaction may occur when both drugs affect different critical points along a common pathway.[24] Both the metabotropic glutamate receptors and opioid receptors are linked with G-proteins. Hence, the action of ACPT-III and morphine may independently alter intracellular second messenger systems coupled with G-proteins activation and result in potentiative interaction.[25] On the other hand, APDC, unlike to ACPT-III, did not increase the effect of morphine. Although the exact reason on this different effect may not been understood in this study, we added APDC to only ED_{50} of morphine, which may underestimate the effect of APDC.

Clinically, spinal group III mGluRs agonist has not yet

been made available. However, selective group III mGluRs agonist may be used in combination with morphine in the treatment of postoperative pain in the future.

In summary, intrathecal group II and group III mGluRs agonist themselves are not active to postoperative pain, but the addition of ineffective ACPT-III (group III mGluRs agonist) to effective morphine in the spinal cord increases the antinociception of morphine alone at a rat plantar incisional pain model.

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