

## Spinal Metabotropic Glutamate Receptors (mGluRs) are Involved in the Melittin-induced Nociception in Rats

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Intraplantar injection of melittin has been known to induce sustained decrease of mechanical threshold and increase of spontaneous flinchings. The present study was undertaken to investigate how the melittin-induced nociceptive responses were modulated by changes of metabotropic glutamate receptor (mGluR) activity. Changes in paw withdrawal threshold (PWT), number of flinchings and paw thickness were measured at a given time point after injection of melittin (10  $\mu$ g/paw) into the mid-plantar area of rat hindpaw. To observe the effects of mGluRs on the melittin-induced nociceptions, group I mGluR (AIDA, 100  $\mu$ g and 200  $\mu$ g), mGluR<sub>1</sub> (LY367385, 50  $\mu$ g and 100  $\mu$ g) and mGluR<sub>5</sub> (MPEP, 200  $\mu$ g and 300  $\mu$ g) antagonists, group II (APDC, 100  $\mu$ g and 200  $\mu$ g) and III (L-SOP, 100  $\mu$ g and 200  $\mu$ g) agonists were intrathecally administered 20 min before melittin injection. Intraplantar injection of melittin induced a sustained decrease of mechanical threshold, spontaneous flinchings and edema. The effects of melittin to reduce mechanical threshold and to induce spontaneous flinchings were significantly suppressed following intrathecal pre-administration of group I mGluR, mGluR<sub>1</sub> and mGluR<sub>5</sub> antagonists, group II and III mGluR agonists. Group I mGluR antagonists and group II and III mGluR agonists had no significant effect on melittin-induced edema. These experimental findings indicate that multiple spinal mGluRs are involved in the modulation of melittin-induced nociceptive responses.

**Key Words:** Spinal metabotropic glutamate receptors, Melittin, Nociceptive responses

### INTRODUCTION

Intraplantar (i.pl.) injection of bee venom induces local inflammation and tonic pain (Lariviere & Melzack, 1996), and melittin, a major component of bee venom, also produces a sustained pain behaviors such as decrease of mechanical threshold and spontaneous flinchings in a dose-dependent manner in human (Sumikura et al, 2003) as well as in experimental animals (Li & Chen, 2004; Shin et al, 2004). The nociceptive responses induced by i.pl. injection of melittin have the same characteristics as those of bee venom-induced pain (Shin et al, 2004). Melittin has been shown to induce nociceptive responses by selective activation of capsaicin-sensitive primary afferent fibers (Shin & Kim, 2004). Melittin-induced nociceptive responses have been reported to be modulated by changes in the activities of voltage-sensitive Ca<sup>2+</sup> channels (Shin & Lee, 2006), multiple 5-hydroxytryptamine receptors (Shin & Lee, 2007), cyclooxygenase (Kim et al, 2006), extracellular signaling-regulated kinase (Yu & Chen, 2005), NMDA and non-NMDA receptors (Kim & Shin, 2005). All these findings suggest that melittin-induced pain responses can be modulated by multiple factors that are already known to be

involved in the development of pain.

Metabotropic glutamate receptors (mGluRs) have been classified into three groups, based on sequence homology, signal transduction mechanisms and pharmacologic characteristics: group I (mGluR<sub>1</sub> & mGluR<sub>5</sub>), group II (mGluR<sub>2</sub> & mGluR<sub>3</sub>) and group III (mGluR<sub>4</sub>, mGluR<sub>6</sub>, mGluR<sub>7</sub> & mGluR<sub>8</sub>) mGluRs. mGluRs except mGluR<sub>6</sub> are distributed in the superficial laminae of spinal dorsal horn and on the small isolectin B<sub>4</sub>-positive neurons of trigeminal and dorsal root ganglion (Ohishi et al, 1995; Li et al, 1997; Berthele et al, 1999; Jia et al, 1999; Alvarez et al, 2000; Azkue et al, 2000; Bhave et al, 2001; Carlton et al, 2001). mGluRs are reported to be localized both in pre- and post-synaptic sites in the spinal cord (Ohishi et al, 1995; Jia et al, 1999; Alvarez et al, 2000; Carlton et al, 2001). Group I mGluRs are present on the unmyelinated and small myelinated afferent fibers (Bhave et al, 2001; Zhou et al, 2001), and nociceptive C- and A $\delta$ -primary afferent terminals are in synaptic contact with or in direct apposition to mGluR<sub>5</sub>

**ABBREVIATIONS:** AIDA, (RS)-aminoinidan-1,5-dicarboxylic acid; AMPA, (RS)- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APDC, (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate; DHPG, (RS)-3,5-dihydroxyphenylglycine; EPSP, excitatory postsynaptic potential; i.pl., intraplantar; i.t., intrathecal; L-SOP, O-phospho-L-serine; mGluR, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl) pyridine hydrochloride; NMDA, N-methyl-D-aspartate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PWT, paw withdrawal threshold.

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neurons in the spinal cord (Tao et al, 2000). The expression of mGluRs, especially mGluR<sub>1</sub> and mGluR<sub>5</sub>, is increased in the superficial laminae of spinal cord following spinal cord injury, midline laparotomy, ultraviolet irradiation and chronic inflammation, and on the myelinated dorsal root ganglion neurons after sciatic nerve ligation (Boxall et al, 1998; Hudson et al, 2002; Mills & Hulsebosch, 2002; Dolan et al, 2003; Dolan et al, 2004). Intraplantar or i.t. injection of group I mGluR agonist induces mechanical hyperalgesia in behavior test, activates spinal wide dynamic range neurons, and potentiates the evoked responses of wide dynamic range neurons (Neugebauer et al, 1994; Budai & Larson, 1998). Nerve injury- or inflammation-induced hyperalgesias are suppressed by i.pl. or i.t. administration of group I mGluR antagonists (Neugebauer et al, 1994; Bhave et al, 2001; Zhou et al, 2001; Hudson et al, 2002). Group II and III mGluR agonists inhibit peripheral inflammation- and nerve injury-induced hyperalgesia as well as responses of spinothalamic tract cells to noxious mechanical stimulation and capsaicin (Dolan & Nolan, 2002; Fisher et al, 2002; Chen & Pan, 2005; Soliman et al, 2005). (2S, 1'R, 2'R, 3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (group II mGluR agonist) and (S)-2-amino-4-phosphonobutanoate (group III mGluR agonist) depress excitatory postsynaptic potential (EPSP) evoked by the stimulation of primary afferent fibers in the spinal cord dorsal horn (Gerber et al, 2000).

These experimental results indicate that mGluRs may contribute to nociceptive responses with different origins. However, there has been no study to elucidate the effects of changes in spinal mGluR activities on the melittin-induced nociceptive responses. Therefore, the present study was undertaken to investigate how the melittin-induced nociceptive responses were modulated by the changes of spinal mGluR activity.

## METHODS

### Animals

Male Sprague-Dawley rats (250~300 g) were used. The Animal Care and Use Committee at Hanyang University approved all experimental protocols, and algometric assays were conducted under the ethical guidelines set forth by the International Association for the Study of Pain.

### Behavioral test

All rats were placed on an elevated metal mesh floor of the transparent acrylic cage and allowed to acclimate for at least 30 min before behavioral testing. Von Frey filament was applied vertically to the mid-plantar surface of the hindpaw in an ascending intensity order from underneath the floor. The least bending force which was able to evoke a brisk paw withdrawal in more than 50% of 6 trials was expressed as the paw withdrawal mechanical threshold (PWT, g). Twenty six grams of bending force of von Frey filament was selected as the upper limit for testing, since the filaments with bending force of more than 10% of body weight tend to passively raise the entire limb rather than to cause an active brisk withdrawal (Chaplan et al, 1994). A mirror was placed below the metal mesh floor at a 30° angle to allow unobstructed counting of spontaneous flinchings. Spontaneous nociception was estimated by counting the total number of flinchings of injected paw for initial

30 min. Flinching behavior is a spontaneous brisk movement of hindpaw without an application of mechanical or any other type of stimulation to hindpaw. Changes in paw thickness were measured by using caliper and expressed as % changes of the control paw thickness measured in rats before melittin injection. Changes in PWT, number of flinching behaviors and paw thickness were measured at a given time point after the injection of melittin (10 µg/paw, Sigma, St Louis, Missouri, USA) into mid-plantar area of rat hindpaw.

### Placement of intrathecal catheter

For i.t. administration of mGluR agonist or antagonist, chronic i.t. catheters were inserted under the enflurane anesthesia by passing a PE-10 tubing through an incision in the atlanto-occipital membrane to a position 8.5 cm caudal to the cisterna at a level of the lumbar enlargement. Rats were allowed to recover for at least 5 days before being used in the experiment. Rats showing motor defects were not used in the experiment.

### Effects of drugs on the melittin-induced nociceptions

To observe the effects of mGluRs on the melittin-induced nociception, group I mGluR antagonists and group II and III mGluR agonists were intrathecally administered 20 min before melittin injection. Intrathecal administration of each mGluR antagonist or agonist was followed by an additional injection of 10 µl of saline to ensure complete flush of each drug into intrathecal space. Drugs tested in the present experiment were (RS)-1-aminoindan-1,5-dicarboxylic acid, (group I mGluR antagonist, AIDA, 100 µg & 200 µg), LY367385, (mGluR1 antagonist, 50 µg & 100 µg), 2-methyl-6-(phenylethynyl)pyridine hydrochloride, (mGluR5 antagonist, MPEP, 200 µg & 300 µg), (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate, (group II mGluR agonist, APDC, 100 µg & 200 µg) and O-phospho-L-serine, (group III mGluR agonist, L-SOP, 100 µg & 200 µg). All drugs were supplied by Tocris, Avonmouth, UK. In preliminary experiments, the intraperitoneally injected highest dose of each agonist or antagonist did not induce any changes in melittin-induced PWT and spontaneous flinchings, indicating that i.t. injected agonist or antagonist acted locally on the spinal cord. Each rat was tested for a single antagonist or agonist.

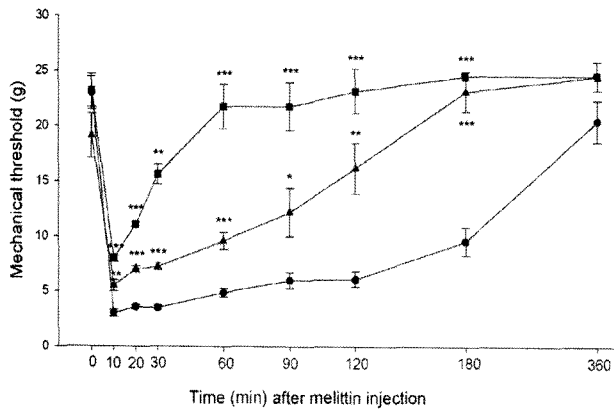
### Statistical analysis

The data are expressed as mean±SE and analyzed using ANOVA, followed by Newman-Keuls test. p values less than 0.05 were considered significant. When experiments were completed, rats were euthanized by an overdose of pentobarbital sodium.

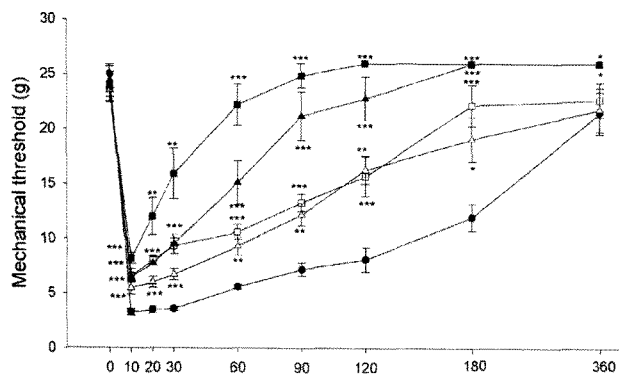
## RESULTS

### Effects of group I mGluR antagonist on melittin-induced decrease of mechanical threshold

Paw withdrawal mechanical threshold (23.0±1.5 g) was decreased to 3.0±0.3 g in 10 min after i.pl. injection of 10 µg of melittin and remained low until 30 min after melittin injection (3.5±0.2 g) (Fig. 1). The decreased mechanical threshold was slowly increased to 6.1±0.7 g and 9.6±1.3

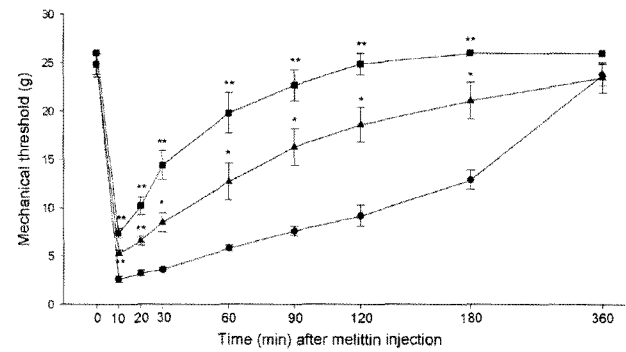


**Fig. 1.** Intraplantar injection of melittin ( $10 \mu\text{g/paw}$ ,  $\bullet$ ,  $n=12$ ) caused prolonged decrease of mechanical threshold which was significantly attenuated in the rats pretreated with group I metabotropic glutamate receptor (mGluR) antagonist, [(RS)-1-aminindan-1,5-dicarboxylic acid, AIDA,  $100 \mu\text{g}$ ,  $\blacktriangle$ ,  $n=10$ ;  $200 \mu\text{g}$ ,  $\blacksquare$ ,  $n=11$ ]. Data are expressed as mean $\pm$ S.E. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significant differences from melittin-induced decrease in mechanical threshold.



**Fig. 2.** Intrathecal injection of metabotropic glutamate receptor 1 (mGluR1) antagonist, (LY367385,  $50 \mu\text{g}$ ,  $\square$ ,  $n=10$ ;  $100 \mu\text{g}$ ,  $\blacksquare$ ,  $n=11$ ) and mGluR5 antagonist, [2-methyl-6 (phenylethynyl) pyridine hydrochloride, MPEP,  $200 \mu\text{g}$ ,  $\triangle$ ,  $n=9$ ;  $300 \mu\text{g}$ ,  $\blacktriangle$ ,  $n=10$ ] attenuated the effect of melittin ( $10 \mu\text{g/paw}$ ,  $\bullet$ ) to reduce mechanical threshold. Data are expressed as mean $\pm$ S.E. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significant differences from melittin-induced decrease in mechanical threshold.

g in 120 min and 180 min after melittin injection, respectively, suggesting that the sustained melittin-induced pain responses are a good model for the study of pain mechanism. In all the following experiments, mechanical thresholds were decreased to a similar extent after i.p.l. injection of melittin. Intrathecal pre-injection of AIDA, group I mGluR antagonist, dose-dependently reduced the decrease of mechanical threshold induced by melittin. Mechanical thresholds were  $5.5 \pm 0.5$  g and  $8.0 \pm 0.3$  g in 10 min after melittin injection in the rat pre-injected with  $100 \mu\text{g}$  or  $200 \mu\text{g}$  AIDA, respectively, which were significantly high ( $p < 0.01$  &  $p < 0.001$ ) compared to the decrease of threshold by melittin only. The mechanical thresholds were higher at all time points except 360 min in the rat pretreated with AIDA than in the rat injected with melittin only, and the



**Fig. 3.** The effect of melittin ( $10 \mu\text{g/paw}$ ,  $\bullet$ ) to reduce mechanical threshold was suppressed in the rats which received intrathecal pre-injection of group II metabotropic glutamate receptor (mGluR) agonist [(2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate, APDC,  $100 \mu\text{g}$ ,  $\blacktriangle$ ,  $n=9$ ;  $200 \mu\text{g}$ ,  $\blacksquare$ ,  $n=10$ ]. Data are expressed as mean $\pm$ S.E. \* $p < 0.01$ , \*\* $p < 0.001$ , significant differences from melittin-induced decrease in the mechanical threshold.

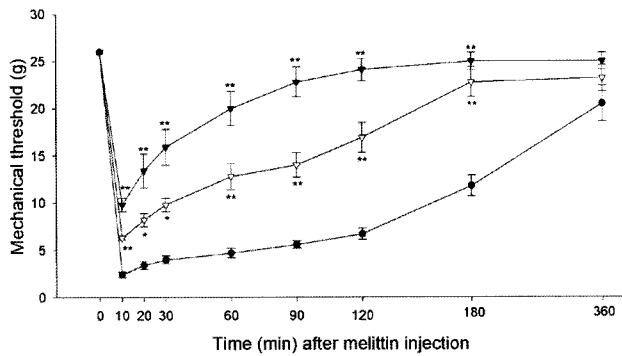
reduced mechanical threshold increased almost to the control level 60 min ( $21.8 \pm 2.0$  g,  $p < 0.001$ ) and 180 min ( $23.2 \pm 1.8$  g,  $p < 0.001$ ) after melittin injection in the rats pre-treated with  $200 \mu\text{g}$  and  $100 \mu\text{g}$  AIDA, respectively.

#### Attenuation of melittin-induced decrease of mechanical threshold by mGluR<sub>1</sub> and mGluR<sub>5</sub> antagonists

Intrathecal administration of selective mGluR<sub>1</sub> and mGluR<sub>5</sub> antagonists also attenuated the decrease of mechanical threshold induced by i.p.l. melittin injection (Fig. 2). Mechanical thresholds decreased to  $8.2 \pm 0.5$  g and  $6.5 \pm 0.3$  g in the rats pretreated with  $100 \mu\text{g}$  LY367385 and  $300 \mu\text{g}$  MPEP 10 min after i.p.l. melittin injection, respectively, whereas the mechanical threshold of the rat injected with melittin only was  $3.3 \pm 0.3$  g, and thereafter, mechanical thresholds of LY367385- and MPEP-pretreated rats increased over the control level 90 min and 180 min after melittin injection, respectively. Administration of low dose LY367385 ( $50 \mu\text{g}$ ) and MPEP ( $200 \mu\text{g}$ ) significantly attenuated the melittin-induced decrease of mechanical threshold and the mechanical thresholds of the rats pretreated with low dose and high dose of LY367385 and MPEP were significantly high at all time points except 360 min after melittin injection, compared to those of the rat injected with melittin only.

#### Effects of group II mGluR agonist on the melittin-induced decrease of mechanical threshold

Mechanical threshold was decreased to  $2.6 \pm 0.3$  g 10 min after i.p.l. injection of melittin alone, whereas in the rats i.t. pretreated with  $100 \mu\text{g}$  or  $200 \mu\text{g}$  of APDC (group II mGluR agonist) mechanical thresholds were decreased to  $5.3 \pm 0.4$  g and  $7.4 \pm 0.5$  g 10 min after melittin injection, respectively, which were significantly high compared to melittin-induced decrease in threshold ( $p < 0.001$ ) (Fig. 3). The reduced mechanical threshold in APDC- and melittin-treated rats recovered more rapidly at all time points than in the rats injected with melittin alone, and the mechanical threshold in the rats pretreated with  $200 \mu\text{g}$  of APDC recovered completely to the control level at 180 min after melittin injection.



**Fig. 4.** Melittin-induced decrease in the mechanical threshold ( $10 \mu\text{g/paw}$ ,  $\bullet$ ) was attenuated in the rats intrathecally administered with group III metabotropic glutamate receptor (mGluR) agonist, O-phospho-L-Serine (L-SOP,  $100 \mu\text{g}$ ,  $\nabla$ ,  $n=12$ ;  $200 \mu\text{g}$ ,  $\blacktriangledown$ ,  $n=12$ ). Data are expressed as mean $\pm$ S.E. \* $p < 0.01$ , \*\* $p < 0.001$ , significant differences from melittin-induced decrease in the mechanical threshold.

#### Modulation of melittin-induced decrease of mechanical threshold by group III mGluR agonist

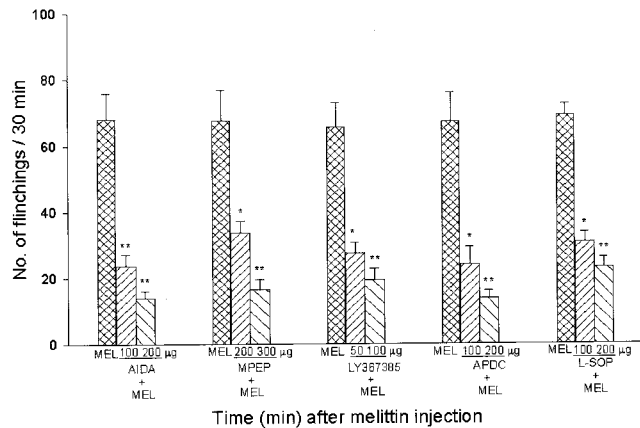
Mechanical thresholds were decreased to  $6.3 \pm 0.2 \text{ g}$  and  $9.8 \pm 0.7 \text{ g}$  in the rats i.t. pretreated with  $100 \mu\text{g}$  or  $200 \mu\text{g}$  of L-SOP (group III mGluR agonist) 10 min after melittin injection, respectively, which were significantly high compared to those of the rats injected with melittin alone ( $p < 0.001$ ). The decreased mechanical thresholds of the L-SOP- and melittin-treated rats recovered more rapidly at all time points except 360 min after melittin injection than those of the melittin-injected rats (Fig. 4). The mechanical thresholds of melittin- or melittin plus  $200 \mu\text{g}$  of L-SOP-treated rats were  $6.7 \pm 0.6 \text{ g}$  and  $24.1 \pm 1.2 \text{ g}$  120 min after melittin injection, respectively and they were significantly different from each other ( $p < 0.001$ ).

#### Inhibition of melittin-induced flinchings by group I mGluR antagonist, group II and III mGluR agonist

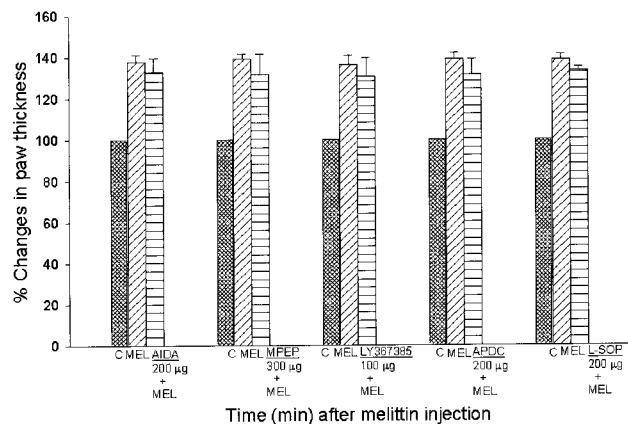
Intraplantar injection of melittin induced spontaneous flinchings, which were decreased gradually and almost not observed 30 min after melittin injection. Total number of spontaneous flinchings induced by  $10 \mu\text{g}$  of melittin injection was  $68.1 \pm 7.7/30 \text{ min}$ , which were significantly decreased to  $23.7 \pm 3.4/30 \text{ min}$  and  $13.9 \pm 2.1/30 \text{ min}$  in the rats pretreated with  $100 \mu\text{g}$  or  $200 \mu\text{g}$  AIDA, respectively ( $p < 0.001$ ), and LY367385 and MPEP antagonists also had inhibitory actions on melittin-induced flinching behaviors (Fig. 5). Melittin-induced flinching behaviors ( $67.1 \pm 8.9/30 \text{ min}$  in APDC-treated group and  $69.8 \pm 3.4/30 \text{ min}$  in L-SOP-treated group) were significantly inhibited to  $13.6 \pm 2.4/30 \text{ min}$  and  $23.1 \pm 2.8/30 \text{ min}$  in the rats pretreated with  $200 \mu\text{g}$  of APDC or L-SOP, respectively ( $p < 0.001$ ).

#### Effects of mGluR agonist and antagonist on the melittin-induced increase of paw thickness

Intraplantar injection of melittin ( $10 \mu\text{g}$ ) caused the increase of paw thickness which was  $137.5 \pm 0.4\%$  of the control paw thickness and similar in each experimental group (Fig. 6). Paw thickness increased to  $132.9 \pm 0.2\%$  in the rats treated with  $200 \mu\text{g}$  AIDA and melittin which was



**Fig. 5.** Melittin-induced ( $10 \mu\text{g/paw}$ ) spontaneous flinchings were dose-dependently inhibited in the rats pre-treated with group I metabotropic glutamate receptors (mGluRs) (AIDA,  $100 \mu\text{g}$  &  $200 \mu\text{g}$ ), mGluR<sub>1</sub> (LY367385,  $50 \mu\text{g}$  &  $100 \mu\text{g}$ ), and mGluR<sub>5</sub> (MPEP,  $200 \mu\text{g}$  &  $300 \mu\text{g}$ ) antagonist, group II (APDC,  $100 \mu\text{g}$  &  $200 \mu\text{g}$ ) and group III (L-SOP,  $100 \mu\text{g}$  &  $200 \mu\text{g}$ ) mGluR agonist. Data are expressed as mean $\pm$ S.E. \* $p < 0.01$ , \*\* $p < 0.001$ , significant differences from melittin-induced increase in flinching behaviors.



**Fig. 6.** Intraplantar injection of melittin ( $10 \mu\text{g/paw}$ ) caused increases of paw thickness which were not changed following intrathecal pre-administration of group I metabotropic glutamate receptor (mGluR) antagonist (AIDA,  $200 \mu\text{g}$ ; MPEP,  $300 \mu\text{g}$ ; LY367385,  $100 \mu\text{g}$ ), group II (APDC,  $200 \mu\text{g}$ ) and group III (L-SOP,  $200 \mu\text{g}$ ) mGluR agonist. Data are expressed as mean $\pm$ S.E.

not significantly different from the increase of paw thickness induced by melittin alone. Intrathecal administration of mGluR<sub>1</sub> (LY367385) and mGluR<sub>5</sub> (MPEP) antagonists, and group II (APDC) and group III (L-SOP) agonists also did not have any inhibitory effects on melittin-induced increase of paw thickness.

## DISCUSSION

Intraplantar injection of melittin induces nociceptive responses such as sustained decrease of mechanical threshold and spontaneous flinchings (Li & Chen, 2004; Shin et al, 2004) which are mediated by the activation of NMDA receptor, non-NMDA receptor,  $\text{Ca}^{2+}$  channels, serotonin

receptors and cyclooxygenase (Kim & Shin, 2005; Kim et al, 2006; Shin & Lee, 2006; Shin et al, 2006; Shin & Lee, 2007). Melittin-evoked selective excitation of capsaicin-sensitive primary afferent fibers increases the discharge rate of spinal nociceptive dorsal horn neurons with C-fiber inputs from the peripheral receptive field (Shin et al, 2004). The results obtained from the present study show that multiple spinal mGluRs are also involved in the melittin-induced nociceptive responses.

Iontophoretic or i.t. application of group I mGluRs directly excites spinal dorsal horn neurons and potentiates the responses of dorsal horn neurons to NMDA, kainite, AMPA [(RS)- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid] and mechanical stimulation (Neugebauer et al, 1994; Young et al, 1995; Budai & Larson, 1998). The hyperalgesia and allodynia induced by i.t. or i.pl. group I mGluR agonists, inflammation and nerve injury are suppressed by i.t. administration of selective group I mGluR antagonists and antisense oligonucleotide against mGluR1 (Neugebauer et al, 1994; Fundytus et al, 1998; Bhave et al, 2001; Fundytus et al, 2001; Zhou et al, 2001; Hudson et al, 2002; Noda et al, 2003).

Intrathecal administration of group II and III mGluR agonists inhibit the responses of sensitized spinothalamic tract cells, mechanical hypersensitivity and allodynia induced by nerve injury, interleukin-1 $\beta$ , capsaicin and carrageenan (Neugebauer et al, 2000; Dolan & Nolan, 2002; Fisher et al, 2002; Chen & Pan, 2005; Soliman et al, 2005; Jung et al, 2006). These experimental findings in the behavioral and electrophysiological studies indicate that group I, II and III mGluRs in the spinal cord may contribute to the nociceptive responses with a variety of other causes as well as inflammation and nerve injury. The involvement of spinal mGluRs in melittin-induced nociceptive responses was demonstrated in the present study in which i.t. administration of group I mGluR antagonists, and group II and III mGluR agonists significantly attenuated the decrease of mechanical threshold and spontaneous flinchings induced by i.pl. injection of melittin. However, melittin-induced increase in paw thickness was not affected by the changes in the spinal mGluR activity, suggesting that changes in paw thickness may be mediated by peripheral mechanisms.

Intrathecal administration of group I mGluR antagonist has been suggested to block C-fiber-induced spinal nociceptive responses, and this suggestion is consistent with the observation that melittin selectively activates primary afferent C-fibers (Shin & Kim, 2004) and that melittin-induced nociceptive responses were suppressed by i.t. group I mGluR antagonist in the present study. Subdermal capsaicin-induced hyperalgesia and mustard oil-induced responses of dorsal horn neurons are also suppressed following i.t. administration of mGluR<sub>1</sub>, mGluR<sub>5</sub> or group I mGluR antagonists (Young et al, 1995; Soliman et al, 2005). Iontophoretic application of antisense oligonucleotide against spinal mGluR1 also significantly inhibits wide dynamic range cell responses to mustard oil (Young et al, 1998). Spontaneous nociceptive behaviors induced by i.t. (RS)-3,5-dihydroxyphenylglycine ((RS)-DHPG) are markedly attenuated in the rats neonatally treated with capsaicin (Lefebvre et al, 2000). Furthermore, wind-up response of wide dynamic range neurons has been reported to be mediated by C-fiber activation and is suppressed by (S)-4-carboxy-3-hydroxyphenylglycine, group I mGluR antagonist (Budai & Larson, 1998).

Activation of primary afferent C-fibers by melittin may

cause the release of nociceptive neurotransmitters, including glutamate, into the spinal cord which can activate mGluR as well as ionotropic glutamate receptors. Activation of phospholipase C (PLC) by group I mGluR agonist stimulates phosphoinositide hydrolysis/[Ca<sup>2+</sup>]<sub>i</sub> mobilization signal transduction pathway, resulting in activation of protein kinase C (PKC). (S)-3,5-DHPG (group I mGluR agonist) increases [Ca<sup>2+</sup>]<sub>i</sub> in spinal substantia gelatinosa neurons and nucleus tractus solitarius cells which is blocked by the antagonists of group I mGluR, sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase, PLC, inositol trisphosphate receptor, L-type Ca<sup>2+</sup> channel and PKC (Endoh, 2004; Heinke & Sandkuhler, 2007). Intrathecal administration of group I mGluR antagonist and antisense oligonucleotide against mGluR<sub>1</sub> suppresses the increased phorbol 12,13-dibutyrate bindings in the dorsal horn of the rats with peripheral nerve injury (Fundytus et al, 2001; Yashpal et al, 2001). Finally, PKC inhibitors also inhibit i.t. DHPG-induced spontaneous nociceptive responses, (1S,3R)-ACPD-induced wide dynamic range neuronal responses and DHPG-induced extracellular signaling-regulated kinase activation in chinese hamster ovary cell (Young et al, 1995; Ferraguti et al, 1999; Fundytus et al, 2001).

Bath application of (S)-3,5-DHPG or (RS)-2-chloro-5-hydroxyphenylglycine (group I mGluR agonist) induces membrane depolarization and sustained potentiation of polysynaptic EPSP in the laminae I-V neurons of rat spinal cord slice, resulting in an increase of action potential firings (Zhong et al, 2000). In the trigeminal substantia gelatinosa neurons, long-term potentiation of EPSP induced by conditioning stimulation of A $\delta$ - or C-fibers is blocked by PLC inhibitor, PKC blocker, intracellular Ca<sup>2+</sup> depletion by thapsigargin and mGluR<sub>5</sub> antagonist (Liang et al, 2005). Long term potentiation is one of the mechanisms which may contribute to development and/or maintenance of nociceptive responses.

In contrast to group I mGluRs, however, activation of group II and III mGluRs has been known to induce antinociceptive effect in experimental studies. Intrathecal administration of (2S,3S,4S)-2-(carboxycyclopropyl)glycine (group II mGluR agonist) dose-dependently increases mechanical threshold. Furthermore, carrageenan- or interleukin 1 $\beta$ -induced inflammatory hyperalgesia, neuropathic mechanical hypersensitivity (Dolan & Nolan, 2000, 2002; Jung et al, 2006) and the sensitized responses of spinothalamic tract cell by capsaicin are suppressed following i.t. application of group II or III mGluR agonists (Fisher et al, 2002; Chen & Pan, 2005). These experimental findings are in agreement with the present data that APDC (group II mGluR agonist) and O-phospho-L-serine (group III mGluR agonist) dose-dependently attenuated melittin-induced decrease of mechanical threshold and inhibited spontaneous flinchings.

The mechanisms by which group II and III mGluRs induce antinociceptive effect are not clear. Activation of group II and III mGluRs inhibits the release of glutamate and aspartate from injured spinal cord (Mills et al, 2001) and EPSP recorded in nucleus tractus solitarius (Chen et al, 2002). Group II and III mGluRs have been reported to inhibit adenylyl cyclase and cAMP formation, resulting in the reduction of neuronal excitability and neurotransmitter release. Noxious inputs such as nerve injury, i.pl. formalin injection, and protein kinase A (PKA) activator cause an increase in phosphorylated cAMP response element-binding protein in the spinal cord which is suppressed by KT5720

(PKA inhibitor) (Ji & Rupp, 1997; Miyabe & Miletic, 2005), and i.t. administration of 8-bromo-cAMP induces dose-dependent hyperalgesia and allodynia (Sluka, 1997; Sluka & Willis, 1997). Intrathecal administration of PKA inhibitor (H89) has antiallodynic effect on neuropathic and capsaicin-induced mechanical allodynia (Sluka & Willis, 1997; Hua et al, 1999). Activation of PKA by 8-bromo-cAMP increases action potential frequency and decreases the threshold of spinal superficial dorsal horn and dorsal root ganglionic neurons (Hu & Gereau, 2003; Song et al, 2006).

In summary, the present results show that i.p. injection of melittin activates multiple spinal mGluRs and serial responses of PLC activation, inositol 1,4,5-trisphosphate formation and resultant activation of PKC induced by activated group I mGluR and/or cAMP inhibition by group II and III mGluR may contribute to the development of hyperalgesia.

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