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Calcium Ions are Involved in Modulation of Melittin-induced Nociception in Rat: I. Effect of Voltage-gated Calcium Channel Antagonist

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Melittin-induced nociceptive responses are mediated by selective activation of capsaicin-sensitive primary afferent fibers and are modulated by excitatory amino acid receptor, cyclooxygenase, protein kinase C and serotonin receptor. The present study was undertaken to investigate the peripheral and spinal actions of voltage-gated calcium channel antagonists on melittin-induced nociceptive responses. Changes in mechanical threshold and number of flinchings were measured after intraplantar (i.pl.) injection of melittin (30 μg/paw) into mid-plantar area of hindpaw. L-type calcium channel antagonists, verapamil [intrathecal (i.t.), 6 or 12 μg; i.pl.,100 & 200 μg; i.p., 10 or 30 mg], N-type calcium channel blocker, ω-conotoxin GVIA (i.t., 0.1 or 0.5 μg; i.pl., 5 μg) and P-type calcium channel antagonist, ω-agatoxin IVA (i.t., 0.5 μg; i.pl., 5 μg) were administered 20 min before or 60 min after i.pl. injection of melittin. Intraplantar pre-treatment and i.t. pre- or post-treatment of verapamil and ω-conotoxin GVIA dose-dependently attenuated the reduction of mechanical threshold, and melittin-induced flinchings were inhibited by i.pl. or i.t. pre-treatment of both antagonists. P-type calcium channel blocker, ω-agatoxin IVA, had significant inhibitory action on flinching behaviors, but had a limited effect on melittin-induced decrease in mechanical threshold. These experimental findings suggest that verapamil and ω-conotoxin GVIA can inhibit the development and maintenance of melittin-induced nociceptive responses.

Key Words: Melittin, Hyperalgesia, Spontaneous pain, Voltage-gated calcium channel antagonist

INTRODUCTION

Lariviere and Melzack (1996) first introduced bee venom (BV)-induced tonic pain model which was characterized by local inflammation, edema and tonic pain responses. Increasing dose of BV produced stronger pain and enhanced the duration of pain responses. In the subsequent studies, BV injection into the plantar surface of hind paw has been reported to cause spontaneous nociceptive behaviors such as flinching, licking and lifting, and to reduce mechanical and thermal threshold that may result in hyperalgesia and allodynia (Chen et al, 1999a; 1999b; You et al, 2002). BV injection also induces heat hyperalgesia in the hind paw opposite to the BV-injected paw (Chen et al, 2000). This contralateral heat hyperalgesia and spontaneous flinchings are suppressed in the rat with bilateral lesions of rostral medial medulla (Chen et al, 2003), suggesting that descending facilitatory inputs contribute to BV-induced nociception, and referred mirror heat hyperalgesia.

Intraplantar (i.pl.) injection of BV caused an increase of c-Fos protein expression in deep and superficial dorsal horn (Luo et al, 1998) and increased monophasically the firings of wide dynamic range (WDR) neurons (Chen et al, 1998; 1999a; 1999b; You et al, 2002). The time-courses of spinal c-Fos expression and change in the firing rate of WDR neurons paralleled those of hyperalgesia and allodynia (Luo et al, 1998). BV injection into i.pl. surface did not cause any increase in the firing rate of WDR neurons that received only A-fibers, but not C-fiber, inputs from the periphery (Chen et al, 1998). Topical application of lidocaine on the sciatic nerve completely blocked BV-induced increase in the firing of WDR neurons (Chen et al, 1998), and BV-induced spontaneous nociception, and thermal and mechanical hyperalgesia were strongly suppressed in the rat whose sciatic nerves were blocked by topical application of capsaicin (Chen & Chen, 2001; Shin & Kim, 2004). These experimental findings suggest that BV-induced increase in discharges of WDR neurons has peripheral origin and is mediated by the selective activation of capsaicin-sensitive primary afferent fibers.

BV-induced nociceptive responses have been known to be

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ABBREVIATIONS: BV, bee venom; ω-Aga, ω-agatoxin IVA; ω-CTx, ω-conotoxin GVIA; EP receptor, E-type prostaglandin receptor; EPSC, excitatory postsynaptic current; 5-HT, 5-hydroxytryptamine; IP_3, inositol 1,4,5-trisphosphate; i.pl., intraplantar; i.t., intrathecal; MEL, melittin; NMDA, N-methyl-D-aspartate; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PWT, paw withdrawal mechanical threshold; VER, verapamil; WDR, wide dynamic range.

modulated by peripheral and/or spinal factors that include N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Chen et al, 1999a; You et al, 2002; Kim & Shin, 2005), protein kinase A and C (Li et al, 2000; Li & Chen, 2003), P_{2x} purinoreceptor (Zheng & Chen, 2000), neurokinin receptor (Zheng & Chen, 2001) and serotonin (5-HT) receptors (Liu et al, 2005).

Major component of whole BV is melittin which comprises more than 50% of whole BV. Subcutaneous injection of melittin has been reported to induce pain in human and animals and to cause almost the same pain responses as those induced by whole BV (Sumikura et al, 2003; Li & Chen, 2004; Shin et al, 2004). Intraplantar injection of melittin induced spontaneous flinchings and edema, markedly reduced mechanical threshold, and monophasically increased the discharges of WDR neurons which were strongly blocked by topical application of lidocaine onto sciatic nerves (Li & Chen, 2004; Shin & Kim, 2004; Shin et al, 2004). Selective blockade of capsaicin-sensitive primary afferent fibers almost completely prevented the melittin-induced decrease in mechanical threshold (Shin & Kim, 2004). Intrathecal (i.t.) as well as i.pl. injection of melittin decreased in mechanical threshold and increased spontaneous flinchings in rat (Shin et al, 2004). Inactivation of peripheral and/or spinal NMDA, non-NMDA, serotonin and E-type prostaglandin (EP) receptors and activation of spinal serotonin receptors suppressed melittin-induced nociceptive responses in the behavioral tests of rat (Kim & Shin, 2005; Lee et al, 2005; Kim et al, 2006). In the recent study, Yu and Chen (2005) reported that activation of spinal extracellular signaling-regulated kinase contributed to melittin-induced spontaneous pain, and mechanical and thermal hyperalgesia.

Melittin has been known to have direct depolarizing effect by increasing Na^+ influx in Swiss 3T3 cell (Rozengurt et al, 1981), to increase phospholipase A_2 (PLA_2)-mediated excitatory postsynaptic current (EPSC) in substantia gelatinosa neurons (Yue et al, 2005) and to increase Ca^{2+} influx in rat PC12 cells which was blocked by L-type calcium channel blockers such as nifedipine, verapamil and diltiazem (Choi et al, 1992). Acute nociception, inflammatory and neuropathic pains are significantly relieved by the peripheral and i.t. administration of various types of calcium channel antagonists (Neugebauer et al, 1996; Diaz & Dickenson, 1997; Shin et al, 1997; White & Cousins, 1998; Todorovic et al, 2004). Mice deficient in $\alpha 1\beta$ subunit of N-type calcium channel showed reduced pain responses to i.pl. injection of formalin, L5/L6 spinal nerve ligation and noxious mechanical stimulation (Hatakeyama et al, 2001; Kim et al, 2001; Saegusa et al, 2001), whereas peak Ca^{2+} current was increased in small dorsal root ganglion neurons, and mechanical allodynia, thermal hyperalgesia and increased responses of spinal dorsal horn neurons to noxious thermal and mechanical stimuli were observed in the rat overexpressed with $\alpha 2\delta 1$ subunit of voltage-gated Ca^{2+} channel (Li et al, 2001). These studies indicate that calcium channels play a crucial role in nociceptive transmission. The present study was undertaken to elucidate the role of peripheral and spinal calcium channels in the melittin-induced nociceptive responses.

METHODS

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

All rats were placed on an elevated metal mesh floor 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. A bending force being able to evoke a brisk paw withdrawal in more than 50% of 6 trials was expressed as the paw withdrawal mechanical threshold (PWT, g). 26 g of bending force of von Frey filament was selected as the upper limit for testing, since stiffer filaments with bending force of more than 10% of body weight tends to passively raise the entire limb rather than to cause an active brisk withdrawal (Chaplan et al, 1994). Rats that sharply withdrew their paws, when von Frey filament with weak bending force below 26 g was applied,were not used in the experiment. A mirror was placed below the metal mesh floor at a 30' angle to allow an unobstructed counting of flinching behaviors. Changes in PWT at a given time-point and total number of flinchings for the initial 30 min were measured after the injection of melittin into the mid-plantar area of the hindpaw. We measured the total number of flinchings for the first 30 min, because more than 95% of flinchings were observed within the first 30 min after i.pl. injection of melittin. To observe the effects of voltage-gated calcium channel antagonists on melittin-induced nociceptive responses, verapamil (i.t., 6 or 12 μg; i.pl., 100 or 200 μg; i.p., 10 or 30 mg), ω-conotoxin GVIA (i.t.,0.1 or 0.5 μg; i.pl., 5 μg) and ω-agatoxin IVA (i.t., 0.5 μg; i.pl., 5 μg) were administered 20 min before or 60 min after i.pl. injection of melittin. Intrathecal administration of each antagonist was followed by additional flush of 10 μl saline to ensure complete movement of each antagonist into intrathecal space. For i.t. administration of calcium channel antagonists, chronic i.t. catheters were inserted under the enflurane anesthesia by passing PE-10 tubing through an incision in the atlanto-occipital membrane to a position 8.0 to 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. All rats showing motor defects were not used in the experiment. All drugs were dissolved in 10 μl of saline. In the preliminary experiments, i.t. or i.pl. injection of 10 μl saline and i.p. administration of the highest dose of each antagonist did not induce any changes in PWT and spontaneous flinchings. Each rat was tested for a single antagonist.

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

RESULTS

Intraplantar injection of melittin induced flinching behaviors which were frequent immediately after melittin injection and, thereafter, decreased gradually (data not shown). Flinching behaviors almost completely disappeared about 30 min after melittin injection. In the rat pre-treated with verapamil, melittin-induced flinchings (61.1±5.1/30 min) were significantly reduced by i.t., i.pl., or i.p. pre-administration of verapamil (Fig. 1). After i.t. (12 μg, n=16)

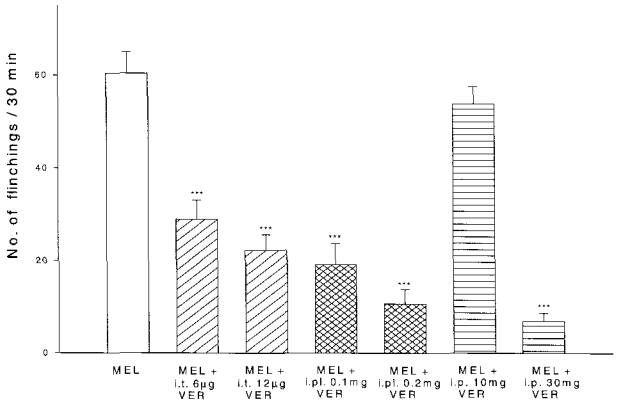

Fig. 1. Flinching behaviors of verapamil. Intrathecal (i.t.), intraplantar (i.pl.) and intraperitoneal (i.p.) administration of verapamil (VER) 20 min before injection of melittin reduced flinching behaviors induced by i.pl. injection of melittin (MEL, 30 μg/paw). Data are expressed as mean±S.E. ***$p<0.001$, significant differences from the melittin-induced flinchings.

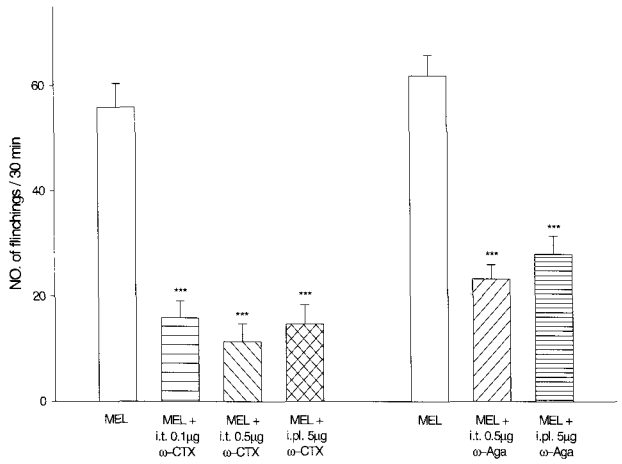

Fig. 2. Melittin-induced flinchings. Of ω-CTx & ω-Aga. Intrathecal (i.t.) and intraplantar (i.pl.) administration of ω-conotoxin-GVIA (ω-CTx) and ω-agatoxin-IVA (ω-Aga) 20 min before injection of melittin reduced flinching behaviors induced by i.pl. injection of melittin (MEL, 30 μg/paw). Data are expressed as mean±S.E. ***$p<0.001$, significant differences from the melittin- induced flinchings.

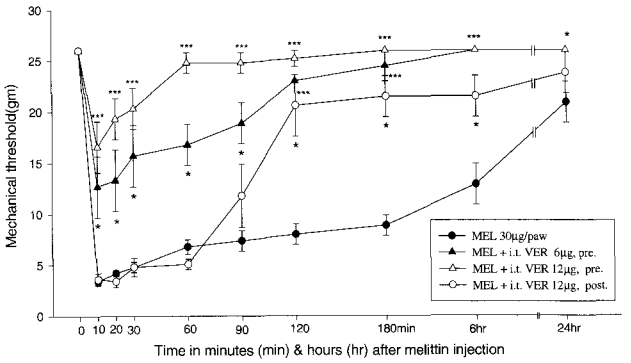

Fig. 3. Mechanical threshold of verapamil (i.t.). Intraplantar injection of melittin (MEL, 30 μg/paw) dramatically reduced mechanical threshold in the rat hind paw. Intrathecal (i.t.) pre- and post-treatment of verapamil (VER) 20 min before and 60 min after injection of melittin strongly attenuated melittin-induced reduction of mechanical threshold. Data are expressed as mean±S.E. *$p<0.05$, ***$p<0.001$ significant differences from the melittin-induced reduction of mechanical threshold.

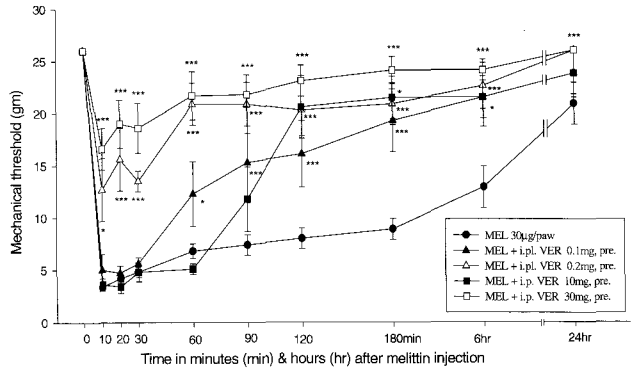

Fig. 4. Intraplantar (i.pl.) and the intraperitoneal (i.p.) injection of verapamil (VER). Melittin-induced reduction of mechanical threshold was significantly attenuated by the intraplantar (i.pl.) and the intraperitoneal (i.p.) injection of verapamil (VER) 20 min before intraplantar injection of melittin (MEL). Data are expressed as mean±S.E. *$p<0.05$, ***$p<0.001$ significant differences from the melittin-induced reduction of mechanical threshold.

or i.pl. (0.2 mg, n=8) injection of verapamil, melittin-induced flinching behaviors decreased to 22.3±3.4/30 min and 10.7±3.1/30 min, respectively ($p<0.001$). Intraperitoneal administration of verapamil at dose of 10 mg (n=10) did not have any significant inhibitory action on flinching behaviors, but flinchings were greatly suppressed to 6.9± 1.8/30 min after i.p. administration of 30 mg verapamil (n=14, $p<0.001$).

N-type and P-type calcium channel antagonists also inhibited melittin-induced flinching behaviors (Fig. 2). Intrathecal (0.5 μg, n=8) or i.pl. (5 μg, n=8) administration of ω-conotoxin GVIA inhibited melittin-induced flinching behaviors (55.9±4.6/30 min) to 11.4±3.4/30 min and 14.7± 3.7/30 min, respectively ($p<0.001$). ω-Agatoxin IVA also suppressed melittin-induced flinchings (62.0±3.9/30 min) which were decreased to 23.3±2.7/30 min and 28.0±3.5/30 min ($p<0.001$), by i.t. (0.5 μg, n=9) or i.pl. (5 μg, n=10) administration of ω-agatoxin IVA, respectively.

PWT was dramatically reduced to 3.4±0.3 g 10 min after i.pl. injection of melittin (Fig. 3). The reduced PWT slowly increased to 6.8±0.7 g and 12.9±2.2 g 60 min and 6 h after melittin injection, respectively. Melittin-induced decrease in PWT was markedly attenuated by i.t. pre- or post-administration of verapamil. In the rat i.t. pre-treated with 6 μg (n=9) and 12 μg (n=16) verapamil, PWTs measured 10 min after melittin injection were 12.7±3.4 g and 16.6±2.5 g, respectively, which were significantly high, compared to PWT of the rat injected with melittin alone ($p<0.05$ & $p<0.001$). PWT of rat pre-treated with 12 μg verapamil was increased to 25.4±1.2 g 60 min after melittin injection and PWT of 6 μg verapamil-treated rat was 23.6±1.1 g 120 min after melittin injection. No significant differences were

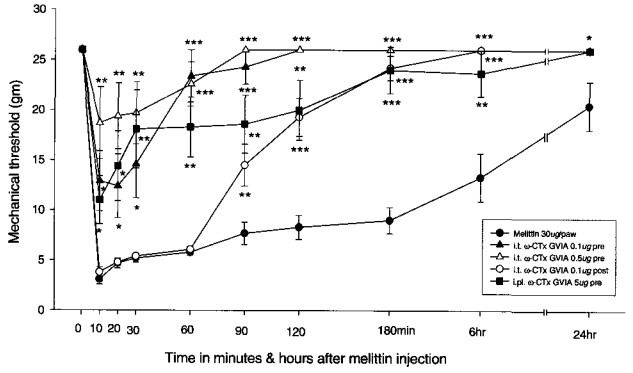

Fig. 5. ω-conotoxin GVIA (ω-CTx). Intraplantar injection of melittin (MEL, 30 μg/paw) significantly reduced mechanical threshold in the rat hind paw. Intrathecal (i.t.) and intraplantar (i.pl.) pre- or post-treatment of ω-conotoxin (CTx) GVIA 20 min before and 60 min after injection of melittin strongly attenuated melittin-induced reduction of mechanical threshold. Data are expressed as mean±S.E. *$p<0.05$, **$p<0.01$, ***$p<0.001$, significant differences from the melittin- induced reduction of mechanical threshold.

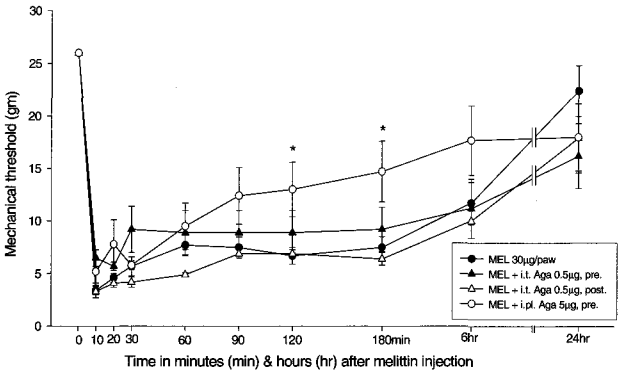

Fig. 6. ω-Agatoxin IVA (ω-Aga). Intrathecal (i.t.) administration of ω-Agatoxin (Aga) IVA 20 min before and 60 min after injection of melittin (MEL, 30 μg/paw) did not have any significant effect on melittin-induced nociceptive behavior. Intraplantar (i.pl.) pre-treatment of ω-Agatoxin IVA significantly increased the recovery of reduced mechanical threshold only 120 min and 180 min after melittin injection. Data are expressed as mean±S.E. *$p<0.05$, significant differences from the melittin-induced reduction of mechanical threshold.

observed in PWTs between the rats injected with melittin alone and the rats post-treated with verapamil until 60 min after i.pl. melittin injection. However, i.t. post-administration of verapamil (n=9) profoundly attenuated an ability of melittin to reduce PWT. The decreased PWT (3.5±0.5 g) 10 min after melittin injection was markedly increased to 20.6±3.4 g 60 min after i.t. injection of verapamil (120 min after melittin injection) which was significantly high, compared to PWT of melittin-injected rat (8.0±1.1 g, $p<0.05$).

Intraplantar injection of verapamil dose-dependently prevented PWT from decreasing (Fig. 4). PWT of the rat i.pl. pre-treated with 0.2 mg verapamil (n=8) was 12.7±3.1 g 10 min after melittin injection and already increased to 20.9±2.5 g 60 min after melittin injection ($p<0.05$ & $p<0.001$). Low dose of verapamil (0.1 mg, i.pl., n=7) did not affect melittin-induced decrease in PWT until 30 min after melittin injection. PWTs of low dose verapamil group were significantly higher than PWTs of the rat injected with melittin alone at all time-points from 60 min after melittin injection ($p<0.05$ & $p<0.001$).

Melittin-induced decrease in PWTs was also attenuated by i.p. injection of verapamil (Fig. 4). PWTs of the rat i.p. pre-treated with 30 mg (n=14) verapamil were 16.6±2.0 g and 21.7±2.3 g 10 min and 60 min after melittin injection, respectively, which were significantly high, compared to PWT of melittin group ($p<0.001$). There were no substantial differences in PWTs of the rat pre-treated with low dose of verapamil (10 mg, n=10) and of the rat injected with melittin alone for initial 60 min after melittin injection. However, PWTs of the rat pre-treated with low dose of verapamil were significantly high, compared to PWTs of the rat injected with melittin alone at all time-point from 90 min after melittin injection ($p<0.05$).

Intrathecal or i.pl. pre-administration of ω-conotoxin GVIA strongly attenuated the decrease of PWTs induced by i.pl. injection of melittin (Fig. 5). PWTs of the rat i.pl. injected with melittin was 3.1±0.5 g 10 min after melittin injection, but PWTs of the rat pre-treated with 0.1 μg (n=10) or 0.5 μg (n=9) ω-conotoxin GVIA were 12.9±3.0 g and 18.7±3.6 g at same time-point and increased almost to normal PWT 60 to 90 min after melittin injection. These PWTs and recovery rate of ω-conotoxin GVIA- treated rat were significantly high, compared to those of melittin-injected group ($p<0.05$ or $p<0.001$). An ability of melittin to reduce PWT was markedly attenuated by post-administration of ω-conotoxin GVIA 60 min after melittin injection. PWT of the rat i.t. post-treated with 0.1 μg ω-conotoxin GVIA (n=8) was 14.5±2.1 g 30 min after ω-conotoxin GVIA (90 min after melittin injection) which was significantly high, compared to PWT of melittin-injected rat ($p<0.01$), and increased almost to normal PWT about 180 min after i.pl. melittin injection. Intraplantar as well as i.t. pre-administration of ω-conotoxin GVIA profoundly attenuated an ability of melittin to reduce PWT at all time-points after melittin injection. In the rat i.pl. pre-administered with 5 μg ω-conotoxin GVIA (n=8), PWT was decreased to 11.0±2.4 g 10 min after i.pl. melittin injection which was significantly high, compared to PWT of the rat injected with melittin alone (3.1±0.5 g) ($p<0.05$). The reduced PWT returned almost to normal level 180 min after i.pl. melittin injection which was significantly high, compared to PWT of melittin group ($p<0.001$).

In general, P-type calcium channel antagonist, ω-agatoxin IVA, did not have significant modulatory effect on melittin-induced reduction of PWT (Fig. 6). No significant difference in PWT was found between the rat injected with melittin alone and the rat with i.t. pre- and post-administration of ω-agatoxin IVA plus melittin at all time-points (n=8 & n=7, respectively). We tried to increase the dose of ω-agatoxin IVA more than 0.5 μg, but it was impossible to increase the dosage because of side effects. On the other hand, in the rat i.pl. pre-administered with 5 μg ω-agatoxin IVA (n=9), there was a tendency for PWTs to be higher than PWT of the rat injected with melittin at later time-points. However, significant differences were observed between PWTs of the rat treated with ω-agatoxin IVA plus melittin and treated with melittin only 120 min and 180 min after melittin injection ($p<0.05$).

DISCUSSION

Intrathecally or intraplantarly administered N-type Ca^{2+} channel antagonist, ω-conotoxin GVIA, and L-type Ca^{2+} channel antagonist, verapamil, had a strong inhibitory effect on the action of melittin to induce spontaneous flinchings and to reduce mechanical threshold. On the other hand, P-type Ca^{2+} channel antagonist, ω-agatoxin IVA, suppressed only spontaneous flinchings. These present results indicate that melittin-induced spontaneous flinchings and decrease in mechanical threshold could be modulated by the changes in the activities of the voltage-gated calcium channels in the spinal cord and peripheral site.

In the peripheral site, melittin appears to induce a long-lasting increase in neural inputs to the spinal dorsal horn neurons by a direct activation of primary afferent fibers and by producing various inflammatory mediators (Calixto et al, 2003). To the best of our knowledge, there is no direct evidence for melittin to induce membrane depolarization of peripheral sensory neurons. However, melittin dose-dependently increased the frequency of spontaneous excitatory postsynaptic currents in substantia gelatinosa neurons of adult rat spinal cord (Yue et al, 2005) and Na^+ influx in Swiss 3T3 cell (Rozengurt et al, 1981), and caused depolarization in skeletal and cardiac muscles (Habermann, 1972). Intraplantar injection of melittin also caused an increase in the firing of WDR neurons which was completely blocked by local application of lidocaine to sciatic nerves (Shin et al, 2004). In the rat whose sciatic nerves were pre-treated with capsaicin 24 h before testing, melittin-induced spontaneous flinchings and decrease in mechanical threshold were almost completely blocked (Shin & Kim, 2004). All these experimental findings suggest that melittin may directly activate capsaicin-sensitive primary afferent fibers by increasing Na^+ influx.

In the present study, i.pl. administered N-type and L-type calcium channel antagonists strongly suppressed spontaneous pain and mechanical allodynia. These findings indicate that increase in the intracellular calcium concentration might contribute to the induction of serial pain reactions in the peripheral nerves. Evidence in support of this proposal is that in the rat pheochromocytoma PC12 cells, melittin has been reported to dose-dependently increase Ca^{2+} influx which was significantly blocked by administration of L-type calcium channel antagonist and to stimulate phospholipase C (PLC)-mediated formation of inositol 1,4,5-trisphosphate (IP_3) which increases the release of Ca^{2+} from the intracellular Ca^{2+} stores (Choi et al, 1992). Resulting increase in the intracellular Ca^{2+} concentration activates PLA_2 and protein kinase C (PKC), and to increase neurotransmitter release which are major factors to induce pain reaction (Coderre, 1992). In addition to Ca^{2+} influx through voltage-gated calcium channels, Ca^{2+} influx through melittin-activated NMDA receptors may further increase nociceptive responses in the neurons (MacDermott et al, 1986; Kim & Shin, 2005). In a few studies, it was reported that L-type calcium channel antagonist did not have any antinociceptive actions (Chaplan et al, 1994; Diaz & Dickenson, 1997; White & Cousins, 1998) but, in the present study, i.t. or i.pl. injection of verapamil induced strong antinociception. Behavioral responses induced by acute noxious stimuli and the responses of WDR neurons to i.pl. injection of formalin, C-fiber stimulation, noxious mechanical and thermal stimuli were significantly suppressed by L-type as well as N-type Ca^{2+} channel antagonists in inflammatory and neuropathic model of pain (Xiao & Bennett, 1995; Neugebauer et al, 1996; Shin et al, 1997; Matthews & Dickenson, 2001; Todorovic et al, 2004). Putting these experimental findings together, it appears that suppression of melittin-induced increase in Ca^{2+} influx into peripheral nerve may result in the inhibition of pain reaction and resulting decrease in neuronal input signals to nociceptive dorsal horn neurons.

In the present study, P-type Ca^{2+} channel antagonist, ω-agatoxin IVA, had a limited effect on the melittin-induced nociception. Intrathecal or i.pl. injection of ω-agatoxin IVA significantly inhibited flinching behaviors, but without any marked effect on the melittin-induced decrease in mechanical threshold. We tried to increase the dosage of ω-agatoxin IVA, however it was impossible due to side effects. Chaplan et al (1994) and White and Cousins (1998) also reported that P-type calcium channel antagonist did not have any antinociceptive effect on mechanical hyperalgesia and allodynia in the rat model of neuropahtic pain. On the other hand, there are a number of reports indicating that ω-agatoxin IVA has a significantly inhibitory effect on the inflammatory and neuropathic pain (Malmberg & Yaksh, 1994; Diaz & Dickenson, 1997; Matthews & Dickenson, 2001). The reason for this discrepancy from the present experiment remains unclear.

On the basis of the results obtained from this and other experiments, it is reasonable to believe that i.pl. injected melittin can increase Na^+ influx, Ca^{2+} influx through voltage-gated calcium channels, PLA_2-catalyzed arachidonic acid production, PLC-catalyzed IP_3 formation, activation of NMDA receptors and production of various inflammatory mediators in the peripheral site (Rozengurt et al, 1981; Choi et al, 1992; Calixto et al, 2003: Kim & Shin, 2005). All these factors act together and activate capsaicin-sensitive primary afferents which may transmit the sustained neuronal signals to nociceptive dorsal horn neurons. Sustained inputs from the periphery can induce a sensitization in the dorsal horn by increasing neurotransmitter release from the central endings of afferent fibers and by inducing serial pain reactions in the dorsal horn neurons. Calcium ions are prerequisite factor for these pre- and postsynaptic pain mechanisms.

Calcium influx into presynaptic nerve terminals in the dorsal horn induces the release of nociceptive neurotransmitters such as excitatory amino acids, substance P, calcitonin gene-related peptide and others (Sluka et al, 1992; Teoh et al, 1996). It has been reported that a common feature of these pronociceptive neurotransmitters is to increase calcium ion concentration and IP_3 formation in the nociceptive dorsal horn neurons (MacDermott et al, 1986; Womack et al, 1988; Parsons et al, 1995). Increase of calcium ion concentration in the nociceptive dorsal horn neuron can activate protein kinase C (PKC), PLA_2 and nitric oxide synthase. The activated PKC further increases the activity of NMDA receptors and voltage-gated calcium channels, and neurotransmitter release, which may result in the additional increase in Ca^{2+} influx into the dorsal horn neurons and PKC activation (Chen & Mae Huang, 1992; Yang & Tsien, 1993; Barber & Vasko, 1996). This positive vicious cycle can induce sensitization of nociceptive dorsal horn neurons characterized by a dramatic decrease in mechanical threshold and spontaneous pain. Although we do not know whether i.t. administered verapamil and

ω-conotoxin GVIA acted on the pre- and/or postsynaptic site, i.t. injected antagonists appear to have both pre- and postsynaptic actions in the present experiment. Melittin-induced decrease in mechanical threshold and spontaneous flinchings were strongly reduced after i.t. administration of verapamil and ω-conotoxin GVIA. Pre-treatment as well as post-treatment of both antagonists had profound inhibitory effect on the melittin-induced nociceptions, indicating that voltage-gated calcium channels are involved in both production and maintenance of melittin-induced nociception in the spinal cord. The results in the present study provide further evidence that the peripheral and spinal modulation of calcium channel activity, especially N-type and L-type channel, can be very useful for the management of inflammatory pains.

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