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Mechanical Hyperalgesia Induced by Blocking Calcium-activated Potassium Channels on Capsaicin-sensitive Afferent Fiber

Kyung Hee Lee, and Hong Kee Shin

Department of Physiology, College of Medicine, Hanyang University, Seoul 133-791, Korea

Small and large conductance Ca^{2+}-activated K^+ (SK_{Ca} and BK_{Ca}) channels are implicated in the modulation of neuronal excitability. We investigated how changes in peripheral K_{Ca} channel activity affect mechanical sensitivity as well as the afferent fiber type responsible for K_{Ca} channel-induced mechanical sensitivity. Blockade of SK_{Ca} and BK_{Ca} channels induced a sustained decrease of mechanical threshold which was significantly attenuated by topical application of capsaicin onto afferent fiber and intraplantar injection of 1-ethyl-2-benzimidazolinone. NS1619 selectively attenuated the decrease of mechanical threshold induced by charybdotoxin, but not by apamin. Spontaneous flinching and paw thickness were not significantly different after K_{Ca} channel blockade. These results suggest that mechanical sensitivity can be modulated by K_{Ca} channels on capsaicin-sensitive afferent fibers.

Key Words: Ca^{2+}-activated K^+ channel, Mechanical sensitivity, Capsaicin-sensitive primary afferent fiber

INTRODUCTION

Small- and medium-conductance Ca^{2+}-activated potassium (SK_{Ca} & IK_{Ca}) channels are expressed in the superficial layers of spinal cord and small dorsal root ganglion (DRG) neurons in rats (Sailer et al, 2004; Bahia et al, 2005; Mongan et al, 2005) and human (Boettger et al, 2002). The subtypes of SK_{Ca}, SK1 and SK2 colocalize with both calcitonin gene-related peptide (CGRP) and isolectin B4, known nociceptor subpopulation markers (Sailer et al, 2004). Apamin blocks slow afterhyperpolarizations (sAHP) recorded in small capsaicin-sensitive rat DRG neurons, and increases the action potential firing rate and ectopic spontaneous discharges in rats and humans with peripheral nerve injury (Chabal et al, 1989; Gold et al, 1996; Xing & Hu, 1999). Apamin-sensitive component of Ca^{2+}-dependent afterhyperpolarization is not recorded in CA1 hippocampal neuron of mice lacking SK2 subunit (Bond et al, 2004). SK_{Ca} and BK_{Ca} channel currents are coupled to the activation of different types of voltage-gated Ca^{2+} channels in a cell-type-specific manner. For example, SK_{Ca} and BK_{Ca} channel currents have been reported to be induced by the activation of L-type and N-type Ca^{2+} channel in CA1 hippocampal neurons, respectively, and Ca^{2+} released from the intracellular Ca^{2+} stores is involved in the activation of SK_{Ca} and BK_{Ca} channels (Marrion & Tavalin, 1998; Tanabe et al, 1998). However, the types of Ca^{2+} channels which contribute to SK_{Ca} and BK_{Ca} channel activation in the spinal dorsal horn neurons and peripheral sensory afferent fibers need to be clearly elucidated. Intrathecal (i.t.) administration of SK_{Ca} channel blockers also increased the responses of spinal wide dynamic range (WDR) dorsal horn neurons to peripheral inputs (Bahia et al, 2005) and the spinal ascending axon responses to sural nerve stimulation (Jurna & Habermann, 1983). On the other hand, K_{Ca} channel activator, 1-ethyl-2-benzimidazolinone (1-EBIO), inhibits high-threshold C-fiber-induced dorsal root-evoked ventral root potential (DR-VRP) more than low threshold A-fiber-induced DR-VRP (Bahia et al, 2005).

Blockade of large-conductance K_{Ca} (BK_{Ca}) channel has been reported to prolong action potential duration and to increase firing frequency in small rat DRG neurons (Scholz et al, 1998; Zhang et al, 2003). Neurotransmitter release is increased after blocking of BK_{Ca} channel in frog and cultured embryonic *Xenopus* neuromuscular junction ,and changes in presynaptic Ca^{2+} current and neurotransmitter release have the same time courses (Robitaille & Charlton, 1992; Yazejian et al, 1997). These experimental findings suggest that changes in peripheral afferent nerve SK_{Ca} and BK_{Ca} channel activity may contribute to mechanical hyperalgesia. The present study was carried out to investigate the effect of SK_{Ca} and BK_{Ca} channel blockers on peripheral afferent fibers, and to elucidate the afferent fiber type affected by K_{Ca} channel blockers.

Corresponding to: Hong Kee Shin, Department of Physiology, College of Medicine, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea. (Tel) 82-2-2220-0612, (Fax) 82-2-2281-3603, (E-mail) shinhg@hanyang.ac.kr

ABBREVIATIONS: 1-EBIO, 1-ethyl-2-benzimidazolinone; i.t., intrathecal; BK_{Ca} channel, large conductance Ca^{2+}-activated K^+ channel; CGRP, calcitonin gene-related peptide; ChTx, charybdotoxin; DRG, dorsal root ganglion; DR-VRP, dorsal root-evoked ventral root potential; K_{Ca} channel, Ca^{2+}-activated K^+ channel; sAHP, slow afterhyperpolarizations; PWT, paw withdrawal mechanical threshold; SK_{Ca} channel, small conductance Ca^{2+}-activated K^+ channel; VR, vanilloid receptor; WDR, wide dynamic range.

METHODS

Sprague-Dawley male rats (250~300 g) were used. The Animal Care and Use Committee at Hanyang University approved all experimental protocols. 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. A von Frey filament consists of a series of filament with a different bending force. In the present study, a von Frey filament was applied vertically to the mid-plantar surface of the hind paw with an increasing intensity order from underneath the floor. A bending force able to evoke a brisk paw withdrawal in more than 3 of 6 trials was expressed as the paw withdrawal mechanical threshold (PWT, g). A 26 gram bending force of a von Frey filament was selected as the upper limit, because stiffer filaments with a bending force greater than 10% of body weight tend to passively raise the entire limb, rather than to cause an active brisk withdrawal (Chaplan et al, 1994). Rats that sharply withdrew paws from a von Frey filament stimulation with a bending force less than 26 g were not used in this experiment. A mirror was placed below the mesh floor at a 30° angle to allow an unobstructed counting of flinching. Changes in paw thickness were measured with a caliper and expressed as a percentage change over the control state. After exposing the sciatic nerve at mid-thigh level in rats anesthetized with enflurane (4%), a small piece of cotton (5×5 mm) soaked in capsaicin solution (1%) was placed on the sciatic nerve for 30 min about 24 h before testing (Fitzgerald & Woolf, 1982). In this experiment, rats used showed thermal latencies which were increased by > 100% after capsaicin treatment. Changes in mechanical sensitivity were determined in capsaicin-treated rats. Capsaicin (Sigma, USA) was dissolved in 10% Tween 80 and 10% ethanol in saline. Changes in the mechanical threshold, flinch number and paw thickness were measured after an intraplantar apamin (0.75 & 1.5 μg, Sigma, USA) or charybdotoxin (0.75 & 1.5 μg, ChTx, Sigma, USA) injection into the hind paw of normal rats. The effects that NS1619 and 1-ethyl-2-benzimidazolinone (1-EBIO, Tocris, UK) had on apamin- and ChTx-induced changes in mechanical threshold were studied. NS1619 and 1-EBIO were intraplantarly injected 10 min before apamin or ChTx administration. All drugs were dissolved in saline. The data are expressed as mean±SE and analyzed using analysis of variance (ANOVA) followed by the Newman-Keuls test. p values less than 0.05 were considered significant. Rats were euthanized by an overdose of pentobarbital sodium following completion of the experiments.

RESULTS

PWTs were markedly decreased to 8.6±2.9 g and 6.5±0.7 g at 10 min after intraplantar injection of 0.75 μg (n=9) or 1.5 μg (n=10) selective SK_{Ca} channel inhibitor, apamin, respectively (Fig. 1). The PWTs remained low until 90~120 min after apamin injection, and gradually increased thereafter. The potency of apamin in reducing PWT was significantly attenuated after pretreating rats with 1-EBIO, K_{Ca} channel activator. In rats pretreated with 5 μg (n=10) and 10 μg (n=9) of 1-EBIO, PWTs were decreased to 15.5±3.0 g and 19.3±3.3 g, respectively, 10 min after apamin injection. These values are significantly higher than PWTs for rats injected with apamin only at the same time point ($p<0.01$). By 120 min after apamin injection, there was no difference in PWTs between the control and 10 μg 1-EBIO-treated rats. NS1619, a selective BK_{Ca} channel activator, did not have a significant inhibitory effect on the apamin-induced PWT decrease (n=10). The rate of PWT recovery tended to increase more rapidly until 3 hr after apamin injection, however, this difference was not significant.

Intraplantar injection of 0.75 μg (n=10) or 1.5 μg (n=8) of selective BK_{Ca} channel inhibitor, ChTx, also decreased PWTs to 8.8±2.2 g and 5.9±0.9 g, respectively, 10 min after injection. The PWTs in 0.75 μg ChTx treated rats increased much more quickly than 1.5 μg-treated rats (Fig. 2). In rats pretreated with NS1619 (5 μg/paw, n=11) and 1-EBIO (5 μg/paw, n=10), PWTs were 17.2±3.0 g and 14.3±3.1 g 10 min after ChTx injection. These values were significantly higher

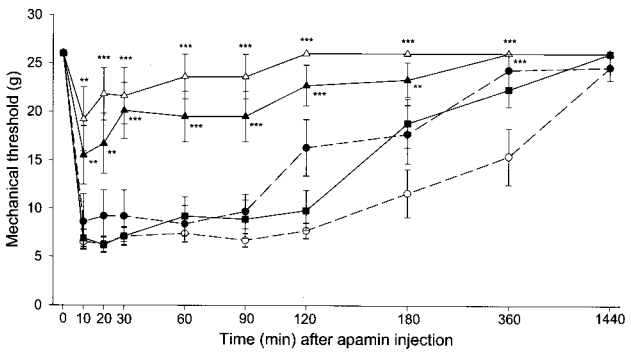

Fig. 1. Intraplantar apamin injection decreases the mechanical threshold (0.75 μg/paw, -●- 1.5 μg/paw, -○-). Apamin-induced (1.5 μg/paw) PWT decreases were significantly attenuated by 1-EBIO (5 μg/paw, -▲-; 10 μg/paw, -△-), but not NS1619 (2.5 μg/paw, -■-). * $p<0.05$, ** $p<0.01$, *** $p<0.001$; statistically significant difference from the apamin-induced mechanical threshold decrease.

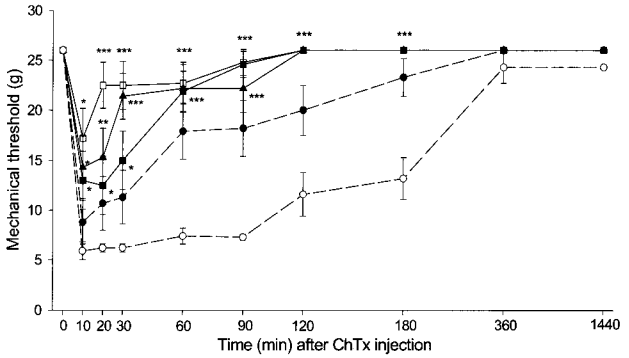

Fig. 2. Intraplantar charybdotoxin (ChTx) injection reduced mechanical threshold (0.75 μg/paw, -●-; 1.5 μg/paw, -○-), which was significantly inhibited by intraplantar 1-EBIO (5 μg/paw, -▲-) or NS1619 injection (2.5 μg/paw, -■-; 5 μg/paw, -□-). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, statistically significant difference from the charybdotoxin-induced mechanical threshold decrease (1.5 μg/paw, -○-).

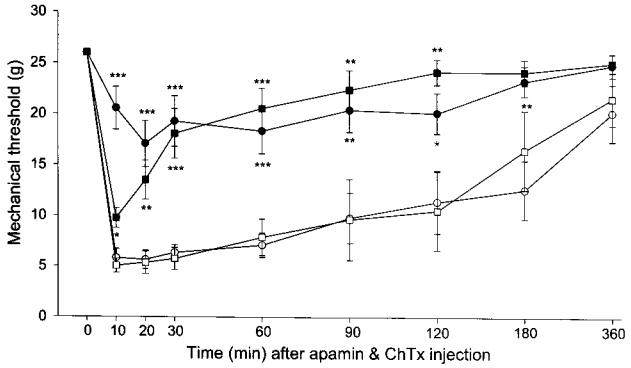

Fig. 3. Apamin and ChTx-induced changes in mechanical threshold were measured in control and capsaicin-treated rats (1% capsaicin+apamin, -●- or ChTx, -■-). The ability of apamin (1.5 μg/paw, -○-) or ChTx (1.5 μg/paw, -□-) to decrease mechanical threshold was significantly reduced in the capsaicin-treated rat. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, statistically significant difference from the apamin or ChTx-induced mechanical threshold decrease.

than PWTs for rats injected with ChTx alone ($p<0.05$). The reduced PWTs recovered completely to the baseline 120 min after ChTx injection.

The potencies of apamin and ChTx to reduce PWT were profoundly attenuated in capsaicin-treated rats (Fig. 3). PWTs at 10 min and 30 min after apamin injection (1.5 μg/paw, n=14) were 20.5±2.1 g and 19.2±2.5 g, respectively, in capsaicin-treated rats, which were significantly higher than rats injected with apamin only (5.8±0.9 g, $p<0.001$ and 6.3±0.7 g, $p<0.001$). Capsaicin-pretreatment also attenuated the ChTx potency to reduce PWT (n=12). The blocking effect of capsaicin on ChTx in the initial 10~20 min was weaker than on apamin. In capsaicin-pretreated rats, PWTs were 9.7±0.9 g ($p<0.05$) and 18.0±2.4 g ($p<0.001$) 10 min and 30 min after ChTx injection, respectively, and there was no difference in PWTs between rats treated with apamin or ChTx from 30 min after injections.

Intraplantar apamin or ChTx injection did not induce any significant change in paw thickness 30 min after apamin (n=10) or ChTx (n=8) injection (1.5 μg/paw; thickness, 102.9±1.7% and 100.7±1.3% of baseline, respectively). A 0.75 μg dose of apamin (n=9) or ChTx (n=10) did not cause a significant change in paw thickness either. A 0.75 μg (n=11) and 1.5 μg (n=14) intraplantar apamin injections caused small increase of flinching which was 2.1±0.6 and 2.7±1.3 during the first 30 min after injection. The flinching induced by 0.75 μg (n=9) and 1.5 μg (n=9) ChTx was 2.6±1.3 and 2.8±1.6, respectively, during the first 30 min after injection.

DISCUSSION

The present experiment shows that SK_{Ca} and BK_{Ca} channel blockage on afferent fibers induced a sustained decrease in mechanical threshold. Furthermore, this decrease was significantly blocked by an intraplantar administration of 1-EBIO and NS1619. Also, a conduction block of afferent fibers by topical capsaicin application significantly attenuated the ability of apamin and ChTx to reduce the mechanical threshold. There were no significant changes in spontaneous flinching and paw thickness. Mechanical hyperalgesia observed in this study appears to be primary hyperalgesia, because changes in mechanical threshold were measured only within the injected area of hindpaw.

SK_{Ca} channel subunits are highly expressed in the superficial laminae of spinal dorsal horn. Many nociceptive afferent fibers terminate in this region. SK_{Ca} channels are also localized to small DRG neurons, which have nociceptor characteristics (Sailer et al, 2004; Bahial et al, 2005; Mongan et al, 2005). Apamin-sensitive AHPs have been recorded from small and capsaicin-sensitive DRG neurons (Gold et al, 1996), and are dependent on the extracellular and intracellular calcium concentrations (Tanabe et al, 1998). Apamin-sensitive AHPs, however, have not been recorded from hippocampal neurons in mice lacking SK_{Ca} channel subunit, SK2 (Bond et al, 2004). In rats with peripheral nerve injury, K^+ current recorded from DRG neurons was greatly decreased, and the rates of ectopic spontaneous discharge and action potential generation were increased, which were further increased by apamin (Gold et al, 1996; Xing & Hu, 1999). In the spinal dorsal horn, the WDR neuron responses to electrical, thermal and mechanical stimuli are potentiated by intrathecal administration of an SK_{Ca} channel blocker. The SK_{Ca} channel activator, 1-EBIO, inhibits these WDR cell responses (Bahia et al, 2005), consistent with our data. These findings suggest that increased neuronal excitability resulting from SK_{Ca} channel blockade appears to have a close relationship with the mechanical threshold decrease in the current study. Chen et al (2006) also reported thermal and mechanical hypersensitivity induced by apamin subfraction which was prepared from whole bee venom by reverse-phase high pressure liquid chromatography (RP-HPLC). The increase in thermal and mechanical sensitivity was relatively small and significant only at 4h and 2h after apamin subfraction injection, respectively. However, their results are different from ours. In our experiment, mechanical threshold decreased strongly and maximally within 10 min and remained low until 90~120 min after apamin injection. Although we could not offer any definitive explanation for these differences, one plausible reason could be that because Chen et al (2006) used subfraction of which the amount of pure apamin was unknown, absolute amount of injected apamin might be very low whereas we administered apamin with 95% purity supplied by Sigma.

We could not find any immunocytochemical study on the BK_{Ca} channel distribution on sensory neurons or in the spinal dorsal horn. A few electrophysiological studies described BK_{Ca} channel function in the rat DRG neurons. Activation of BK_{Ca} channels in small- and medium-sized DRG neurons results in an increased repolarization speed, a shorter action potential duration and prolonged refractory period, which may decrease the neuronal excitability and firing rate. On the other hand, BK_{Ca} channel inhibitors such as iberiotoxin and ChTx induce a prolonged action potential duration, which may increase the amount of calcium entering neurons and the firing frequency (Scholz et al, 1998; Zhang et al, 2003). In the frog neuromuscular junction and glutamatergic hippocampal neurons, BK_{Ca} channel blockade increased neurotransmitter release and excitatory postsynaptic potential (Robitaille & Charlton, 1992; Hu et al, 2001). In the present study, ChTx-induced decrease in mechanical threshold was attenuated by 1-EBIO as well as NS1619, a selective BK_{Ca} channel activator. Although 1-EBIO has been known to selectively activate SK_{Ca} and

IK_{Ca} channels, Adeagbo (1999) reported that 1-EBIO induced the relaxation of rat mesenteric artery which was blocked by maxi-K^+ channel blocker, penitrem, but not by apamin. This experimental finding suggests that BK_{Ca} channel can be activated by 1-EBIO, and that ChTx-induced mechanical hyperalgesia may be inhibited through BK_{Ca} channel activation by 1-EBIO. These experimental findings also provide an evidence that the mechanical threshold could be changed by blocking or activating BK_{Ca} channels on sensory neurons.

SK_{Ca} and BK_{Ca} channels have been implicated in the peripheral and spinal antinociceptive action of a few analgesics. The antiallodynic effect of gabapentin was significantly suppressed in rats with L5/L6 spinal nerve ligation by the intrathecal apamin and ChTx administration (Mixcoatl-Zecuatl et al, 2004) A cyclooxygenase-2 inhibitor (meloxicam) and μ-opioid receptor agonist (fentanyl) dose-dependently induced antinociceptive actions in formalin and tail flick tests, which were also significantly attenuated by SK_{Ca} and BK_{Ca} channel blockade (Yamazumi et al, 2001; Ortiz et al, 2005).

The capsaicin (vanilloid) receptor (VR) is a non-selective cation channel with a high Ca^{2+} permeability, expressed in primary sensory neurons in the pain pathway. This receptor is activated by vanilloid compounds, protons, chemical and thermal stimuli (Caterina et al, 1997; Tominaga et al, 1998). VR1-null mice show a marked reduction in nociceptive behavioral responses to noxious heat and inflammatory thermal hyperalgesia (Caterina et al, 2000; Davis et al, 2000). Systemic neonatal capsaicin administration results in selective destruction of unmyelinated sensory fibers (Jancsó et al, 1977). Topical capsaicin application onto a peripheral nerve in adults induces a selective block of C-fiber afferent volleys (Chung et al, 1985). In the present study, apamin- and ChTx-induced decreases in mechanical threshold were markedly reduced in rats pretreated with capsaicin. Thus, the present result suggests that a great portion of the decrease in mechanical threshold results from SK_{Ca} and BK_{Ca} channel blockade on unmyelinated C-fibers.

In conclusion, an increased afferent nerve excitability, especially in unmyelinated C-fibers, resulting from SK_{Ca} and BK_{Ca} channel blockade contributes to a decreased mechanical threshold, which may lead to the development of mechanical hyperalgesia.

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