Capsaicin Inhibits the Spontaneous Pacemaker Activity in Interstitial Cells of Cajal From the Small Intestine of Mouse

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Background/Aims
Capsaicin (8-methyl-N-vanillyl-6-ninenamide), a compound found in hot peppers, has been reported to have different physiological actions on different cell types. Not much work has been done about the effect of capsaicin on the function of interstitial cells of Cajal (ICC). In the present study, we examined the action of external application of capsaicin on pacemaker activity in the cultured ICC from the small intestine of mouse.

Methods
We investigated the effect of capsaicin on pacemaker currents in cultured ICC from the small intestine of mouse using a whole cell patch-clamp technique and Ca2+-imaging analysis.

Results
When capsaicin was applied externally to the pacemaker generating ICC, it completely inhibited the pacemaker potential under current-clamp mode (I = 0) and the pacemaker current under voltage-clamp mode at a -70 mV of holding potentials. The effect of capsaicin on pacemaker activity in ICC was shown dose dependently. The effect of capsaicin was not through the transient receptor potential of the vanilloid type 1 (TRPV1) channel as capsazepine did not block the effect of capsaicin. L-NAME, an inhibitor of nitric oxide synthase, also did not block the capsaicin-induced effects. When the action of capsaicin was examined in the intracellular calcium oscillation, it completely abolished the calcium oscillation.

Conclusions
These results prove that the capsaicin has the inhibitory effects on the ICC which is carried out neither through TRPV channel nor the nitric oxide production. Intracellular Ca2+ was also an important target for actions of capsaicin on ICC.

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Key Words
Capsaicin, Interstitial cells of Cajal, Pacemaker current, Gastrointestinal motility
Introduction

Capsaicin (8-methyl-N-vanillyl-6-ninenamide) is a compound which is available in various types of hot peppers and is famous for its pungent taste, burning sensation, and various effects on body temperature and gastric acid secretion. Because of its analgesic and anti-inflammatory activities, topical application of capsaicin has been used in clinical practice for the treatment of a variety of neuropathic pain conditions. The characteristic effects of capsaicin are known to be mediated by stimulating the polymodal vanilloid receptors of type 1, also termed as transient receptor potential of the vanilloid type 1 (TRPV1) and it is well known that capsaicin is a selective stimulant for mammalian afferent neurons of dorsal root ganglia with C and A thin fibers, which in large doses produce desensitization to the drug itself and to other stimuli of sensory neurons. Capsaicin is also involved in the subsequent release of several neuroregulatory peptides, including substance P and calcitonin-gene related peptide, from the nerve terminals of thin afferent fibers.

Capsaicin, as demonstrated in many reports, exerts many biological activities on different cells. It has been found to act on the capsaicin-sensitive afferent nerves and has been extensively studied in vivo and in vitro for the treatment of migraine, chronic cough, overactive bladder and diabetes, and also as a potent analgesic and anti-cancer agent. Interestingly, many studies have demonstrated capsaicin to have effects on gastrointestinal (GI) motility. For example, capsaicin induces contractions in the guinea-pig’s ileum and relaxations in human small and large intestines.

Interstitial cells of Cajal (ICC) are the pacemaking cells in the GI muscles which generate the rhythmic oscillations in the membrane potential known as slow waves. Auto-rhythmicity in the GI muscles is an exclusive property of ICC and the spread of slow waves through the GI muscles occurs electrically, rather than by active regeneration. Pacemaking ICC are organized into electrically coupled networks within discrete areas of the stomach, small bowel, and colon.

It has been established that capsaicin activates afferent fiber endings and capsaicin-sensitive primary afferent neurons participate in the regulation of GI motility. However, there are no reports on the effect of capsaicin on ICC which plays important roles for the regulation of GI motility. In the present study, we tried to examine the role of capsaicin on ICC in the small intestine of mouse.

Materials and Methods

All experiments were performed according to the Guiding Principles for the Care and Use of Animals approved by the Ethics Committee of Chosun University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize both the number of animals used and their suffering.

1. Preparation of cells

Balb/C mice (8-13 day old) of either sex were anesthetized with diethyl ether and killed by cervical dislocation. The small intestines from 1 cm below the pyloric ring to the cecum were removed and were opened along the mesenteric border. The luminal contents were washed away with Krebs-Ringer bicarbonate solution. The tissues were pinned to the base of a Sylgard dish and the mucosa was removed by sharp dissection. Small strips of intestinal muscle were equilibrated in Ca2+-free Hank’s solution containing KCl 5.36 mM, NaCl 125 mM, NaOH 0.336 mM, Na2HCO3 0.44 mM, glucose 10 mM, sucrose 2.9 mM, and HEPES 11 mM, adjusted to pH 7.4 with Tris for 30 minutes. After incubation for 15 minutes at 37°C with an enzyme solution containing collagenase 1.3 mg/mL (Worthington Biochemical, Lakewood, NJ, USA), bovine serum albumin 2 mg/mL (Sigma, St. Louis, MO, USA), trypsin inhibitor 2 mg/mL (Sigma), and ATP 0.27 mg/mL, the cells were dispersed. Cells were plated onto sterile glass coverslips coated with Poly-L-lysine solution (Sigma) in 35 mm culture dishes.

2. Patch-clamp experiments

The patch-clamp experiments of ICC were performed on cells cultured for 2-3 days. The whole-cell configuration of the patch-clamp technique was used to record the membrane currents (voltage clamp) and membrane potentials (current clamp) from cultured ICC. Currents or potentials were amplified using Axopatch 1-D (Axon Instruments, Foster City, CA, USA). Command pulse was applied using an IBM-compatible personal computer and pClamp software (version 9.2, Axon Instruments). The data were filtered at 5 KHz. All experiments were carried out in...
Effect of Capsaicin on Pacemaker Activity in ICC

Figure 1. Effects of capsaicin on pacemaker potentials in cultured interstitial cells of Cajal (ICC) from the small intestine of mouse. (A) Pacemaker potentials of ICC exposed to capsaicin (50 μM) in the current clamping mode (I = 0). The dot lines indicate the control resting membrane potentials levels. It shows the capsaicin effect on resting membrane potential (B), frequency (C) and amplitude (D) respectively at the current clamp mode (I = 0). The horizontal solid line scales duration of time. *Significantly different from the untreated Control (p < 0.05). RMP, resting membrane potential. The bars represent mean values ± SE.
Results

1. Effects of capsaicin on membrane potentials and pacemaker currents in ICC

The effect of capsaicin on the cultured ICC was evaluated in both the current and voltage clamp. Spontaneous pacemaker potentials were generated when recordings were done on the cultured ICC in current clamp mode ($I = 0$). Under the current clamp condition, capsaicin (50 μM) inhibited the spontaneous pacemaker potentials (Fig. 1A). The resting membrane potential, frequency and the amplitude of ICC without capsaicin were -68.2 ± 3.1 mV, 14.7 ± 1.6 cycles/min and 31.0 ± 3.3 mV respectively. After the treatment of capsaicin (50 μM), the resting membrane potential slightly depolarized to -64.1 ± 3.4 mV and the frequency and amplitude decreased to 0.9 ± 0.2 cycles/min and 3.0 ± 0.5 mV (n = 5, Fig. 1B-D).

![Image](image-url)

**Figure 2.** The dose-dependence of capsaicin on pacemaker currents in cultured interstitial cells of Cajal from the small intestine of mouse. There is the effect of capsaicin on pacemaker current at the concentration of 10 μM, 20 μM, and 50 μM respectively at a holding potential of -70 mV (A-C). The dot lines indicate the control resting current levels. It shows the inhibitory action (D-F). *Significantly different from the untreated Control (p < 0.05). The bars represent mean values ± SE.
2. Dose dependent effect of capsaicin on ICC pacemaker currents

Under the voltage clamp mode at a holding potential of -70 mV, different concentrations of capsaicin were applied on the cultured ICC to check if the effects were in dose dependent manner. Spontaneous inward currents also known as “pacemaker currents” were generated by ICC when the amplifier was converted to voltage clamp mode at a holding potential of -70 mV. Since many reports have used 1 to 100 μM of capsaicin in cellular level,21,22 firstly we tested 10 μM of capsaicin. But 10 μM capsaicin could not show any influence on pacemaker current of ICC (n = 7, Fig. 2A). When the concentration was increased to 20 μM, a little decrement in the frequency and amplitude was noticed (n = 9, Fig. 2B). With the concentration of capsaicin further increased up to 50 μM (n = 11, Fig. 2C), the pacemaker current was completely attenuated and the resting current was slightly changed to an inward direction but when compared to the control it was negligible. In the presence of capsaicin, the corresponding values of resting current, frequency and amplitude are shown in Figure 2D, E and F and the values at 50 μM were significantly different from control values.

3. No involvement of TRPV1 receptor on capsaicin-induced action and the effects of reduced external Na⁺ on pacemaker activity in ICC

Many experiments on different cells reported capsaicin to show effects on those cells through the TRP vanilloid receptor type1. As we could see the effect of capsaicin on ICC, we needed to know if this effect was through the TRPV1 channel. We incubated the ICC with the TRPV1 receptor antagonist capsazepine at a concentration of 10 μM prior to the treatment of capsaicin. The inhibitory effect of capsaicin (50 μM) was not blocked even with the pretreatment of TRPV1 antagonist in the voltage clamp mode at a holding potential of -70 mV (Fig. 3A).
35.97 pA and 0.85 ± 0.59 cycles/min, 94.85 ± 28.54 pA, and -124.0 ± 42.05 pA respectively (n = 7, Fig. 3B-D). The above data indicate that there were no significant differences in the values between capsaicin alone and with capsazepine.

4. No involvement of nitric oxide on capsaicin-induced action

The effects of L-NAME were examined to investigate the possible regulation of pacemaker currents by capsaicin. L-NAME (10 μM) was pretreated for 10 minutes before application of capsaicin (50 μM). The inhibitory effect of capsaicin was not blocked even with pretreatment of L-NAME in the voltage clamp mode at a holding potential of -70 mV (Fig. 4A). In the presence of L-NAME, the resting currents by capsaicin was -116.6 ± 32 pA, and the frequency and amplitude of the pacemaker currents were 0.58 ± 2.7 cycles/min and 69.4 ± 32.6 pA, respectively (n = 4, Fig. 4B-D). L-NAME itself had no effect on pacemaker currents.

5. Effect of capsaicin in the intracellular calcium oscillation

As many reports suggested that [Ca^{2+}]_i oscillations in ICC were considered to be the primary mechanism for the pacemaker activity in gastrointestinal activity, we examined the effects of capsaicin on [Ca^{2+}]_i oscillations in ICC. In this study, we measured the spontaneous [Ca^{2+}]_i oscillations of ICC which were connected with cell clusters. Spontaneous [Ca^{2+}]_i oscillations were observed in many ICC loaded with fluo-4. Figure 5A shows images of basal (F0) and peak point (F1/F0) of Ca^{2+} oscillations. In the presence of 50 μM capsaicin, [Ca^{2+}]_i oscillations in ICC declined rapidly (Fig. 5B). The data of time series are summarized in Figure 5C.

Discussion

In the present study, we have shown the capsaicin to have in-
Figure 5. Effects of capsaicin on intracellular Ca\(^{2+}\) oscillation in cultured interstitial cells of Cajal. (A) Sequential fluorescence intensity images of fluo-4-loaded cultured ICC in normal condition. (B) Sequential fluorescence intensity images of fluo-4-loaded cultured ICC in the presence of capsaicin (50 \(\mu\)M). The representative frames indicate in (C) basal 1, 2, peak 1, and 2. And the exposure time of each frame is 500 ms. (C) Fluorescence intensity change plotted in (A) and (B) red marker in the absence and presence of capsaicin. The horizontal solid line scales duration of fluorescence intensity change (sec).

Inhibitory actions on the spontaneous pacemaker current generated by the cultured ICC.

Previous reports have shown that ICC generate spontaneous pacemaker currents that depolarize membrane, which then spread to smooth muscles via gap junctions, resulting in the depolarization of smooth muscle cell membrane.\(^{23}\) In our study, the ICC showed spontaneous depolarization under current clamp mode and also this activity was mirrored by spontaneous inward currents under voltage clamp mode at a holding potential -70 mV. These results indicated that our cultured ICC were useful in investigation of the electrical rhythm and frequency regulation of pacemaker potentials and pacemaker currents in small intestine.

The TRPV1 channel is referred to as the capsaicin receptor and capsaicin stimulates the TRPV1 channels, usually followed by the stimulation of primary afferent neurons. Immunohistochemical studies have demonstrated the presence of TRPV1 channels in various layers of the GI tract.\(^{24-26}\) Therefore, TRPV1 has become an important target for pharmacological research in GI research and capsaicin has been used to stimulate the afferent fiber to investigate the TRPV1 effects in GI tract. To investigate whether the effects of capsaicin are through the TRPV1 receptor, we pretreated the cultured ICC with capsazepine, a selective TRPV1 channel antagonist, but the effects of capsaicin were present. In our previous study, we have already tested the distribution of TRPV1 channels in the small intestine of mouse using immunohistochemistry and the action of capsaicin at low concentration (10 \(\mu\)M) on pacemaker activity from small intestinal ICC.\(^{27}\) TRPV1-immunopositive cells were not co-localized with c-Kit positive tissue layer and 10 \(\mu\)M did not show any influence on pacemaker activity. These results clarified that the action of capsaicin on ICC was not through the TRPV1 channel and high concentration of capsaicin could act on ICC. It was reported that the canonical transient receptor potential channels 4 (TRPC4) and melastatin type transient receptor potential channel 7 (TRPM7) are the molecular candidate for the non-selective cation channels responsible for pacemaker activity in ICC.\(^{28}\) When activation of non-selective cation channels generate net inward current carried predominantly by \(\text{Na}^+\) under physiological condition, it leads to excitatory effects on GI smooth muscles.\(^{29}\) Although TRPV1 is a non-selective cation channel activated by...
capsaicin, capsaicin in ICC can modulate TRPC4 or TRPM7 channels with other signaling pathway. To elucidate how capsaicin act on TRPC4 or TRPM7 channels in ICC, further studies should be done.

It is well known capsaicin-sensitive sensory nerves to be present in GI tract and capsaicin to release many substances from capsaicin-sensitive sensory nerves of various tissues. There are some reports that capsaicin induced a relaxation via nitric oxide released from the capsaicin-sensitive sensory nerves in the stomach and ileum of guinea-pig. Furthermore, it was reported that ICC had the nitric oxide synthase and nitric oxide signaling could be amplified by ICC from canine colon. Taken together, these results and previous reports suggest capsaicin to regulate the pacemaker activity in small intestinal ICC which might be by the release of endogenous substance. However, we were not able to see any influence on capsaicin-induced action by L-NAME, an inhibitor of nitric oxide synthase in this study which means that the effects of capsaicin on ICC are not involved in the release of nitric oxide.

Next, with regard to the regulation of non-selective cation channels by capsaicin in ICC, we examined which mechanisms are involved in the actions of capsaicin. We focused on the periodic pacemaker activity of ICC which is dependent on \([\text{Ca}^{2+}]\) oscillations. It is well known that the pacemaker mechanism is initiated by release of \([\text{Ca}^{2+}]\) from the endoplasmic reticulum and is followed by reuptake of \([\text{Ca}^{2+}]\) into the mitochondria. Their results suggest that \([\text{Ca}^{2+}]\) plays a crucial role in pacemaking and \([\text{Ca}^{2+}]\) imaging at the tissue level to be a useful technique to investigate slow wave propagation in GI muscle. In this study, we could see the spontaneous \([\text{Ca}^{2+}]\) oscillations using Ca 2+ live cell imaging and \([\text{Ca}^{2+}]\) oscillations was diminished by capsaicin which returned back to normal condition after the washout showing similar effect on electrophysiological studies. The actions of capsaicin on \([\text{Ca}^{2+}]\) oscillations in ICC are in accordance with the suggestion that \([\text{Ca}^{2+}]\) oscillations are important for the pacemaker activity. This means that the inhibitory action of capsaicin on pacemaker activity in ICC is closely related to the modulation of \([\text{Ca}^{2+}]\).

In conclusion, the present results indicate that capsaicin attenuates the pacemaker current generated by ICC of the small intestine of mouse. These actions are carried out neither through the TRPV1 channel nor nitric oxide releasing, but the modulation of \([\text{Ca}^{2+}]\), by capsaicin is important for regulating the pacemaker activity in ICC. Further studies are needed to find out the exact signaling mechanism in the future.

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