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Actin cytoskeletons regulate the stretch-induced increase of Ca^{2+} current in human gastric myocytes

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Abstract

Using the whole-cell and single channel recording techniques, the influence of actin cytoskeletons on L-type Ca^{2+} current was investigated in human gastric smooth muscle cells. In isotonic condition, an actin depolymerizer cytochalasin D (Cyt-D) markedly decreased the whole-cell current (I_{Ba}) without changing steady-state voltage dependency and single channel conductance. Intracellular dialysis of phalloidin, an actin polymerizer, significantly increased the I_{Ba}. Hypotonic stretch (222 mOsm/L) of the myocytes increased the I_{Ba}, and Cyt-D significantly inhibited the I_{Ba} increase by the stretch. Phalloidin was without effect on the I_{Ba} increase by the stretch. Phalloidin antagonized the Cyt-D inhibition of the stretch-induced I_{Ba} increase. Neither heterotrimeric G protein modifiers (GTPγS and GDPβS) nor *rho* GTPase inhibitor (C3 exoenzyme) influenced the stretch-induced responses. These results reveal that the integrity of the actin cytoskeleton is an important factor which determines the activity of L-type Ca^{2+} channels and a response to stretch.

Keywords: L-type Ca^{2+} channels; Actin cytoskeletons; Human stomach; Smooth muscle; Stretch; Swelling; Cytochalasin D; Phalloidin

The Ca^{2+} influx via voltage-dependent L-type Ca^{2+} channels during the slow waves controls the magnitudes of the phasic contraction of gastrointestinal smooth muscles [1]. Mechanical strain is an important physiological stimulus that regulates the contractile properties of the smooth muscles. For example, distension of gastric wall by the ingested food elicits mechanical strain on the gastric smooth muscles. It has been demonstrated that, stretching smooth muscles depolarizes membrane potential and increases the frequency of action potentials and subsequent contraction [2]. Although the role of gastrointestinal pacemaker cells (interstitial cell of Cajal) in the mediation of the contractile response to the stretch has been recently suggested [3], an intrinsic property of smooth muscle's response to the stretch should be considered with a great importance.

The actin cytoskeleton is a network of proteins located under the plasma membrane and it provides structural rigidity, integrity, and shape to the cells [4,5]. The mechanical strain applied on the plasma membrane is transmitted to the cytoskeleton, and the tension generated by the actin rearrangement is delivered to the plasma membrane. Therefore, the reorganization of actin cytoskeletons and subsequent changes of the strain may affect the activity of the mechano-sensitive ion channels on the plasma membrane. We have previously reported that, L-type Ca^{2+} channels in human gastric smooth muscle cells are significantly activated by osmotic stretch of the membrane [6]. In the present study, therefore, we further questioned whether the stretch activation of L-type Ca^{2+} channels is influenced by the disturbance of the actin cytoskeleton. Using patch clamp techniques, we demonstrate that the integrity of actin filaments determines not only the basal Ca^{2+} channel activity but also the stretch-induced increase of L-type Ca^{2+} current.

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Materials and methods

Isolation of gastric myocytes. Experimental protocol using human stomach was approved by the Institutional Review Board for Clinical Research of Samsung Medical Center, Korea. Gastric antral smooth muscles were obtained from the surgical waste tissue during gastrectomy surgery. The procedures for digestion and isolation of single smooth muscle cells (SMCs) were previously described [6]. Isolated SMCs were stored in Ca^{2+}-free KB solution at 4 °C until use, and all experiments were performed within 10 h after cell dispersion.

Patch clamp. The procedures for the recording of whole-cell Ba^{2+} current (I_{Ba}) through L-type Ca^{2+} channels were previously described [6]. In brief, the myocytes were placed on an inverted microscope and superfused with bath solution through the chamber by gravity. The voltage clamp (Axopatch-1D, Axon Instruments, USA) was performed at room temperature (~ 24 °C) with patch pipettes of 2–4 MΩ. The liquid junction potentials were corrected and the currents were filtered at 5 kHz (–3 dB frequency) and sampled at 1 kHz. Single Ca^{2+} channels were recorded in cell-attached configuration with electrodes coated with Sylgard. Single channel activities were obtained using a series of depolarizing step pulses (400 ms duration, 20 sweeps) from a holding potential of −70 mV. A 5-s interval was used between sweeps to avoid channel inactivation. Ba^{2+} (80 mM) was used as a charge carrier in the pipette solution, and 0.5 μM Bay-K 8644 was included in pipette solution in order to facilitate the occurrence of fully resolved single-channel openings. Bath solution contained 140 mM KCl to maintain the cell membrane potential to 0 mV. Single channel currents were filtered at 1 kHz (–3 dB frequency) and sampled at 5 kHz. Single-channel current amplitude was measured from multiple Gaussian fits to the all-points amplitude histogram as well as from well-resolved openings.

Solutions and chemicals. Whole-cell Ca^{2+} channel current was recorded in isotonic solution (80 mM NaCl, 5 mM KCl, 10 mM Hepes, 10 mM $BaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, pH 7.35, with TRIZMA; 290 mOsm/L with sucrose). Hypo-osmotic stretch of the membrane was challenged with sucrose-free solution (222 mOsm/L). The internal pipette solution for whole-cell recording contained: 110 mM CsCl, 20 mM tetraethylammonium chloride, 3 mM Na_2ATP, 3.5 mM $MgCl_2$, 10 mM EGTA, and 10 mM Hepes (pH 7.25 with TRIZMA). The bath solution for single channel recording contained: 140 mM KCl, 10 mM glucose, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.4 with TRIZMA). The pipette solution for single channel recording contained: 80 mM $BaCl_2$, 70 mM sucrose, 10 mM tetraethylammonium chloride, and 10 mM Hepes (pH 7.4 with TRIZMA). Modified KB solution contained: 50 mM L-glutamic acid, 40 mM KCl, 20 mM taurine, 20 mM KH_2PO_4, 3 mM $MgCl_2$, 10 mM glucose, 10 mM Hepes, and 0.5 mM EGTA (pH 7.35 with KOH). The following chemicals were used: (±) Bay K 8644 (RBI), cytochalasin D (Sigma), phalloidin (Sigma), and others (Sigma).

Statistics. The values given in the text are means ± SEM with n, the sample size. Significant differences were detected using Student's paired or unpaired t test ($P < 0.05$).

Results and discussion

Inhibition of L-type Ca^{2+} current by Cyt-D

An actin depolymerizing agent Cyt-D (30 μM) decreased the whole-cell Ba^{2+} current (I_{Ba}) under the isotonic condition (Fig. 1A). *I–V* curves of I_{Ba} obtained 15 min after the treatment of Cyt-D are illustrated in Fig. 1B. Cyt-D (30 μM) reduced the I_{Ba} amplitudes in a whole test voltage range, and the peak current at 0 mV was reduced from -527 ± 156 to -339 ± 102 pA ($n = 6$). Fig. 1C shows the inhibition of single channel activities by Cyt-D. Inclusion of Bay K 8644 (Bay-K, 0.5 μM) in the recording pipette fully activated the single channel openings (Fig. 1C, left panel). As clearly shown from the ensemble-averaged currents, Cyt-D (30 μM for 15 min) significantly reduced channel openings. The opening probability of the channels at +10 mV decreased from 0.144 to 0.029 by 30 μM Cyt-D. Single channel conductance values yielded from the *I–V* curves were 23.9 ± 0.6 pS in control (without Bay-K, $n = 6$), 22.8 ± 0.8 pS in 0.5 μM Bay-K ($n = 9$), and 24.6 ± 0.4 pS in 0.5 μM Bay-K + 30 μM Cyt-D ($n = 4$), and they are statistically insignificant ($P > 0.05$) (Fig. 1D).

As a result, it is revealed that Cyt-D reduces L-type Ca^{2+} current in human gastric myocytes by a decrease of opening probability without changes of the channel conductance. The present data are consistent with previous studies that show the inhibition of L-type Ca^{2+} current by Cyt-D in cardiac myocytes and cultured vascular smooth muscles [7–9]. On the contrary, Xu et al. [10] show that Cyt-D is without effect on L-type Ca^{2+} current of guinea-pig gastric myocytes. They show that 20 min incubation of the guinea-pig myocytes with Cyt-D (20 μM) does not inhibit the I_{Ba}, though the treatment significantly prevented the stretch-induced activation of the current. Therefore, this discrepancy between human and guinea-pig gastric myocytes remains to be explained.

Effect of Cyt-D on the I_{Ba} increase by osmotic stretch

It has been postulated that the volume expansion increases mechanical tension of the plasma membrane and the underlying actin filaments. As previously shown in guinea-pig and human gastric myocytes [6,11], cell swelling by a hypotonic solution reversibly increased cell diameter (>30% increase in 1 min) and the I_{Ba}. As shown in Fig. 2, hypotonic superfusate (222 mOsm/L) increased the I_{Ba} by $39 \pm 6\%$ ($n = 11$). Then, we hypothesized that the stretch-induced responses can be modified by disrupting actin filament. As expected, Cyt-D strongly inhibited the I_{Ba} increase by the stretch, and the increase was $8 \pm 2\%$ ($n = 4$) and $2 \pm 0.3\%$ ($n = 7$) in presence of 20 and 30 μM Cyt-D, respectively ($P < 0.01$ compare to control) (Fig. 2B). In presence of Cyt-D, the *I–V* curves and the steady-state voltage dependences of the channels were not changed by the stretch (Fig. 2C and D). The half activation voltages were -11.01 ± 0.76 and -10.60 ± 0.72 mV ($P > 0.05$), with k of 4.76 ± 0.72 and 5.29 ± 0.66 mV ($P > 0.05$) for 290 and 222 mOsm, respectively ($n = 5$). The half inactivation voltages were -22.12 ± 1.04 and -22.22 ± 1.15 mV ($P > 0.05$), with k of 8.11 ± 1.04 and 9.74 ± 1.15 mV ($P > 0.05$) for 290 and 222 mOsm, respectively ($n = 3$).

Increase of L-type Ca^{2+} current by phalloidin

Under the isotonic condition, intracellular dialysis of an actin polymerizer phalloidin (30 μM) through the patch pipette markedly increased the I_{Ba} (Fig. 3A). This is in accordance with the increase of cardiac Ca^{2+} current by phalloidin [7]. In addition, a morphological change was noticed from the phalloidin-dialyzed cells, i.e., they were