Whole cell current and membrane potential regulation by a human smooth muscle mechanosensitive calcium channel

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Mechanotransduction is required for a variety of biological functions, such as touching, hearing, balance, regulation of blood flow, cardiovascular function, regulation of hollow organ volume, and regulation of bone and muscle growth (6, 12–13). The unitary element that underlies mechanotransduction is the ion channel. Mechanosensitive ion channels, also known as stretch-activated and stretch-inactivated ion channels, are characterized by a change in open probability ($P_o$) on membrane deformation. Mechanosensitive ion channels are found in a large variety of vertebrate and nonvertebrate cells, including smooth muscle (6, 8, 12–13). A mechanosensitive, stretch-activated, L-type Ca$^{2+}$ channel has been characterized in human jejunal circular smooth muscle cells (3). Activation of this channel by positive pressure applied to the recording pipette, or by an increase in shear stress on the cell membrane, resulted in an increase in whole cell Ca$^{2+}$ current. The increase in whole cell Ca$^{2+}$ current is blocked by the L-type Ca$^{2+}$ channel blocker nifedipine. At a single channel level, negative pressure applied to an on-cell patch through the recording pipette resulted in activation of an ~16 pS nifedipine-sensitive Ca$^{2+}$ channel.

Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels is the major pathway through which Ca$^{2+}$ enters gastrointestinal smooth muscle cells to activate the contractile apparatus. In the presence of nifedipine, intestinal smooth muscle contractile activity is decreased markedly. Previous experiments on the mechanosensitive, stretch-activated, L-type Ca$^{2+}$ channel were carried out with Cs$^{+}$ in the recording pipette to block K$^+$ current and at a hyperpolarized holding voltage (~100 mV) to accentuate inward Ca$^{2+}$ current (3). Under these recording conditions, it was not possible to determine the effects of Ca$^{2+}$ entry through mechanostimulation of L-type Ca$^{2+}$ channels on membrane potential, outward current, and the contractile state of human jejunal circular smooth muscle cells. Therefore, the aims of this study were to determine the effects of mechanical stimulation of human jejunal circular smooth muscle cells on membrane potential and outward current using K$^+$-containing pipette solutions and less-hyperpolarized holding voltages.

METHODS

Use of human jejunum, approved by the Institutional Review Board, was obtained as surgical waste tissue during gastric bypass operations performed for morbid obesity. Tissue specimens were harvested directly in chilled buffer with warm ischemia times of ~30 s. Single, isolated, relaxed circular smooth muscle cells were obtained from the human jejunal specimens as previously described (4, 5).

Patch-clamp recordings. Whole cell patch-clamp recordings were made using standard and amphotericin perforated-patch-clamp whole cell techniques. Whole cell and single channel recordings were obtained using Kimble KG-12 glass pulled on a P-97 puller (Sutter Instruments, Novato, CA). Electrodes were coated with R6101 (Dow Corning, Midland, MI) and were fire polished to a final resistance of 3–5 MΩ. Currents were amplified, digitized, and processed using an

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Axopatch 200A amplifier, a Digidata 1200, and pCLAMP 8 software (Axon Instruments, Foster City, CA). Whole cell records were sampled at 2 kHz and filtered at 1 kHz with an eight-pole Bessel filter using the pulse protocols shown in Figs. 1–7. Single channel records were sampled for 60 s at 5 kHz and were filtered at 2 kHz with an eight-pole Bessel filter. The pulse protocols used are shown in Figs. 1–7. Drugs were applied by complete bath changes with the solution containing the drug. Bath perfusion at 10 ml/min for 30 s was used to create shear stress and activate the mechanosensitive L-type Ca\(^{2+}\) channels according to a previously established protocol (3). Of the three methods (perfusion, positive pressure, negative pressure in on-cell mode) previously used to activate mechanosensitive Ca\(^{2+}\) channels, perfusion was chosen, since it may most closely mimic the effects of movement of the extracellular matrix and adjacent smooth muscle on ion channels present on the cell surface. It was also used because of the marked repeatability of its effects. Cell length was determined from digitized images taken before, during, and after perfusion. Single channel records were obtained from on-cell patches with either normal Ringer solution or 150 mM K\(^+\) in the bath. Large-conductance Ca\(^{2+}\)-activated K\(^+\) channels were identified by their large conductance, voltage dependence, and charybdotoxin sensitivity. The voltage applied to the pipette \((V_{pipette})\) values were chosen so that only one to two channels in each patch were open at rest. All records were obtained at room temperature (22°C). Records were not leak subtracted because the mean input resistance at -80 mV was 19 ± 4 GΩ.

**Drugs and solutions.** The pipette solution contained (in mM) 150 K\(^+\), 20 Cl\(^-\), 2 EGTA, 5 HEPES, and 130 methanesulfonate. The bath solution contained (in mM) 146 Na\(^+\), 4.7 K\(^+\), 154.7 Cl\(^-\), 2 Ca\(^{2+}\), and 5 HEPES (normal Ringer solution) for whole cell records and 146 Na\(^+\), 4.7 K\(^+\), 154.7 Cl\(^-\), 2 Ca\(^{2+}\), and 5 HEPES or 150 K\(^+\), 154 Cl\(^-\), 2 Ca\(^{2+}\), and 5 HEPES for single channel records. Drugs were purchased from Sigma Chemicals (St. Louis, MO).

**Data analysis.** Data were analyzed using pCLAMP 8 software or custom macros in Excel (Microsoft, Redmont, WA). Whole cell currents were quantified at applied to the pipette (presented as means ± SE). Values in text are records were obtained at room temperature (22°C). Records only one to two channels in each patch were open at rest. All

**RESULTS**

*Perfusion activates an outward K\(^+\) current.* Outward current (measured at +60 mV) increased from 664 ± 57 pA before bath perfusion to 773 ± 72 pA \((n = 14, P < 0.0002)\) after bath perfusion (10 ml/min normal Ringer, Fig. 1) in both standard whole cell and perforated-patch experiments. No difference was noted in the increase in outward current in response to perfusion between standard whole cell and perforated-patch experiments \((644 ± 112 to 763 ± 137 pA, n = 7, and 683 ± 38 to 789 ± 59 pA, n = 7, respectively, \(P > 0.05\)). An increase in outward current was noted in 13 of 14 cells tested and at all voltages positive to -40 mV. The voltage at zero current (resting membrane potential) was -42 ± 4 mV and hyperpolarized to -45 ± 4 mV 1 min after bath perfusion and -48 ± 4 mV 2 min after perfusion \((P < 0.05)\). The mean maximal hyperpolarization was -50 ± 5 mV \((P < 0.05)\). At 4 min after perfusion, membrane potential depolarized back to preperfusion values \((-40 ± 7 mV)\). An initial increase in outward current was noted within 30 s of perfusion, reached maximal levels at 80 ± 20 s, and returned to baseline at 187 ± 30 s. In 7 of 14 cells, an inward current could be seen at the beginning of the current trace (Fig. 2). The maximal transient inward current was 18 ± 6 pA and increased (in all cells) to 30 ± 12 pA \((n = 7, P < 0.05)\) with perfusion, consistent with mechanoactivation of Ca\(^{2+}\) channels as previously reported (3). Maximal perfusion-activated outward current was recorded 30 ± 11 s after maximal inward Ca\(^{2+}\) current was measured. In a separate series of experiments, cells were initially perfused at 1 ml/min, and then the perfusion rate was increased to 10 ml/min. The outward current increased in a dose-dependent manner with an increase from 620 ± 191 to 669 ± 197 pA at 1 ml/min \((P < 0.05, n = 4)\) and to 716 ± 205 pA at 10 ml/min \((P < 0.05, n = 4, \text{data not shown})\).

Nifedipine (10 μM) was used to determine if the increase in outward current was secondary to increased Ca\(^{2+}\) entry through mechanosensitive L-type Ca\(^{2+}\) channels or due to activation of a second, mechanosensitive channel. On bath perfusion (10 ml/min) in the presence of nifedipine, there was no change in outward current \((694 ± 133 to 713 ± 132 pA, n = 9, P > 0.05)\) or change in membrane potential \((-47 ± 5 to -48 ± 6 mV, P > 0.5, \text{Fig. 3})\).

The Ca\(^{2+}\) dependence of the activated outward current and the “noisiness” of the current traces suggested involvement of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels. Charybdotoxin (100 nM), a Ca\(^{2+}\)-activated K\(^+\) channel blocker, was used to test this possibility in amphotericin perforated-patch experiments. In the presence of charybdotoxin, bath perfusion (10 ml/min) did not increase outward current \((550 ± 32 to 518 ± 49 pA, n = 7, P > 0.05, \text{Fig. 4})\) or change membrane potential \((-39 ± 2 to -42 ± 2 mV, n = 7, P > 0.5)\).

Charybdotoxin not only blocks large-conductance Ca\(^{2+}\)-activated K\(^+\) channels but also blocks intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels (1) and a limited subset of small-conductance Ca\(^{2+}\)-activated K\(^+\) channels (1). Therefore, the effects of ibeirotoxin, a specific blocker of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels on the perfusion-induced increase in outward current, were tested. In the presence of ibeirotoxin (200 nM), bath perfusion did not increase outward current \((687 ± 77 to 680 ± 63 pA, n = 6, P > 0.05)\) nor hyperpolarize the membrane potential \((-50 ± 5 to \text{Fig. 5})\).
in the presence of charybotoxin (100 nM, n = 4) or iberiotoxin (200 nM, n = 1), perfusion (10 ml/min) still evoked an increase in inward Ca$^{2+}$ current (49 ± 23% increase, n = 5, P < 0.05), suggesting that the changes in Ca$^{2+}$ current were independent of changes in K$^{+}$ current (data not shown).

Nifedipine has been shown to inhibit a K$^{+}$ channel, hKv1.5 (15). To determine if nifedipine had a direct effect on K$^{+}$ currents in human jejunal circular smooth

![Figure 1](http://ajpgi.physiology.org/)

**Fig. 1.** Effect of bath perfusion at 10 ml/min on outward K$^{+}$ current. Whole cell currents were recorded with K$^{+}$ in the pipette to record outward current and membrane potential (A) using the pulse protocol shown in the inset. B: bath perfusion increased outward K$^{+}$ current from 680 to 841 pA at +60 mV. C: 4 min after bath perfusion, outward current (at +60 mV) decreased to 719 pA. Bath perfusion hyperpolarized the membrane potential from −52 to −64 mV. D: membrane potential depolarized back to −53 mV 3 min after perfusion. ○, Control; □, perfusion; ◆, 4 min postperfusion. E: time course of the changes in membrane potential. The mean change in outward K$^{+}$ current (inset) was from 664 ± 57 to 773 ± 72 pA; n = 14 patches, *P < 0.0002. Open bars, control; filled bars, perfused. The membrane potential hyperpolarized from −42 ± 4 to −50 ± 5 mV (P < 0.01).

![Figure 2](http://ajpgi.physiology.org/)

**Fig. 2.** A: effect of bath perfusion at 10 ml/min on inward Ca$^{2+}$ current and outward K$^{+}$ current. Currents were recorded using the pulse protocol shown in the inset. In 50% of cells recorded with K$^{+}$ in the pipette, an inward Ca$^{2+}$ current was discernible. B: bath perfusion increased inward Ca$^{2+}$ current in this cell from 17 to 31 pA (inset; scale for insets same for both A and B). Outward current increased from 480 to 525 pA in the recording shown, which was obtained 30 s after initiation of perfusion, and peaked 90 s after initiation of perfusion.
Fig. 3. Effect of block of the mechanosensitive Ca$^{2+}$ channel by nifedipine (10 μM) in bath on outward K$^+$ current. Currents were recorded using the pulse protocol shown in the inset between A and B. In the presence of nifedipine (A), perfusion did not increase outward K$^+$ current (1,465 to 1,488 pA at +60 mV; B) and did not change membrane potential (−44 to −43 mV; C). Control in the presence of nifedipine; ○, perfusion. The mean change in outward current (inset in C) was from 694 ± 133 to 713 ± 132 (n = 9 patches, P > 0.05), and the mean change in membrane potential was from −47 ± 5 to −48 ± 6 mV (P > 0.05; inset in C). Open bars, control; filled bars, perfusion with nifedipine.

Fig. 4. Effect of block of large-conductance Ca$^{2+}$-activated K$^+$ channels on the perfusion-induced increase in outward current. Currents were recorded using the pulse protocol shown in the inset. Addition of charybdotoxin (100 nM) to the bath decreased outward current (A; control; B, charybdotoxin). In the presence of charybdotoxin, perfusion of the bath did not increase outward current (C; 573 to 561 pA) or change membrane potential (D; −38 to −39 mV). Control; ○, charybdotoxin; □, perfusion in the presence of charybdotoxin. The mean change in outward current in the presence of charybdotoxin was from 550 ± 32 to 518 ± 49 pA (inset in D), and the mean change in membrane potential was from −39.3 ± 2 to −42.0 ± 2 mV (n = 7, P > 0.05; inset in D). Open bars, control; filled bars, perfusion with charybdotoxin.
muscle cells, nifedipine (1 μM) was added to the bath after incubation with iberiotoxin (200 nM). Nifedipine had no effect on outward K⁺ current (654 ± 108 to 627 ± 95 pA, n = 4, P > 0.05, data not shown).

Perfusion increases in Pₒ of large-conductance Ca²⁺-activated K⁺ channels. Single channel recordings of large-conductance Ca²⁺-activated K⁺ channels were obtained from on-cell patches. Initial experiments were carried out with 150 mM K⁺ in the bath to control the membrane potential (0 mV). Under these recording conditions, no change in Pₒ of large-conductance Ca²⁺-activated K⁺ channels was seen after a 30-s perfusion of the bath with a solution containing 150 mM K⁺. This was likely secondary to L-type Ca²⁺ channel inactivation at membrane potentials ~0 mV. Therefore, subsequent experiments were carried out using normal Ringer solution in the bath and perfusate. Perfusion (10 ml/min for 30 s) of a human jejunal circular smooth muscle cell with normal Ringer solution increased NPₒ of large-conductance Ca²⁺-activated K⁺ channels from 0.01 to 0.08 (Fig. 5, Vpipette = −40 mV); the values were measured immediately after perfusion was stopped. The mean increase in NPₒ of the large-conductance Ca²⁺-activated K⁺ channels was 3.8-fold (n = 5 patches).

**Discussion**

The present study suggests a functional link between mechanooactivated L-type Ca²⁺ channels and large-conductance Ca²⁺-activated K⁺ channels. Large-conductance Ca²⁺-activated K⁺ channels in mesenteric artery smooth muscle cells (2) and in the ascending limb of the kidney (10) exhibit mechanosensitivity. The data presented in this report suggest that large-conductance Ca²⁺-activated K⁺ channels in human jejunal circular smooth muscle cells are not mechanosensitive themselves but are activated by an increase in...
intracellular Ca\(^{2+}\) that is modulated by mechanosensitive L-type Ca\(^{2+}\) channels.

Ca\(^{2+}\)-activated K\(^+\) channels can be divided into the following three main groups: voltage-insensitive small-conductance (~1–20 pS; SK), voltage-insensitive intermediate-conductance (~10–50 pS; IK), and voltage-sensitive large-conductance (~100–650 pS; BK) Ca\(^{2+}\)-activated K\(^+\) channels (1). SK and BK channels are often coexpressed in a variety of cells (1). SK channels are an order of magnitude more Ca\(^{2+}\) sensitive than IK or BK channels (1), suggesting that, if present in human jejunal circular smooth muscle cells, they too would be activated by Ca\(^{2+}\) entry through mechanosensitive L-type Ca\(^{2+}\) channels. IK channels have been described in murine ileal and colonic myocytes, and SK channels have been described in ileal myocytes (9, 14). However, it is unknown whether SK and IK channels are expressed in human jejunal circular smooth muscle cells. BK and some IK channels are known to be charybdotoxin sensitive, but most SK channels are charybdotoxin insensitive (1). In the present study, charybdotoxin and iberiotoxin completely blocked the charybdotoxin insensitive (1). In the present study, opening of L-type Ca\(^{2+}\) channels was shown to stimulate adjacent large-conductance Ca\(^{2+}\)-activated K\(^+\) channels by increasing Ca\(^{2+}\) concentration in a local submembrane Ca\(^{2+}\) pool dissociated from bulk cytosolic Ca\(^{2+}\). In the present study, a similar mechanism is proposed. The temporal relationship between the L-type Ca\(^{2+}\) current and the outward K\(^+\) current, with maximal inward Ca\(^{2+}\) current recorded ~30 s before maximal outward K\(^+\) current and the block of activation of adjacent large-conductance Ca\(^{2+}\)-activated K\(^+\) channels by block of L-type Ca\(^{2+}\) channels, supports this hypothesis. Additionally, a trigger for initial Ca\(^{2+}\) entry is proposed to be mechanical stimulation of mechanosensitive Ca\(^{2+}\) channels.

The present study suggests a signaling pathway involving interaction of a novel, mechanosensitive L-type Ca\(^{2+}\) channel, the contractile apparatus of the human jejunal circular smooth muscle cell, and large-conductance Ca\(^{2+}\)-activated K\(^+\) channels. Interaction between these signaling elements (Fig. 7), as may occur during normal digestive activity or in pathobiologic obstructive disorders, results in transduction of mechanical energy into Ca\(^{2+}\) influx through mechanosensitive Ca\(^{2+}\) channels and subsequent contraction. Mechanostimulation of L-type Ca\(^{2+}\) channels may provide a mechanism by which the myocyte can act as both a motor and sensory organ. Contraction is limited by activation of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels.

![Diagram](http://ajpgi.physiology.org/)

**Fig. 7.** Proposed model. Mechanostimulation of L-type Ca\(^{2+}\) channels (Ca\(_L\)) in human jejunal circular smooth muscle cells increases Ca\(^{2+}\) entry (A and B) and activates the contractile apparatus (C). Ca\(^{2+}\) entry subsequently activates large-conductance Ca\(^{2+}\)-activated K\(^+\) channels (K\(_{Ca}\)), resulting in an increase in outward K\(^+\) current (D), membrane hyperpolarization (E), and relaxation (F).
channels, membrane hyperpolarization, and L-type Ca$^{2+}$ channel inactivation. Membrane hyperpolarization results in a decrease in the $P_o$ of L-type Ca$^{2+}$ channels, a decrease in intracellular Ca$^{2+}$, and muscle relaxation. Whether release of intracellular Ca$^{2+}$ also participates in this signaling pathway remains to be determined.

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