Cytoskeletal modulation of sodium current in human jejunal circular smooth muscle cells

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Strege, Peter R., Adrian N. Holm, Adam Rich, Steven M. Miller, Yi JUN Ou, Michael G. Sarr, and Gianrico Farrugia. Cytoskeletal modulation of sodium current in human jejunal circular smooth muscle cells. Am J Physiol Cell Physiol 284: C60–C66, 2003; 10.1152/ajpcell.00532.2001.—A Na⁺ current is present in human jejunal circular smooth muscle cells. Am J Physiol Cell Physiol 284: C60–C66, 2003; 10.1152/ajpcell.00532.2001.—A Na⁺ current is present in human jejunal circular smooth muscle cells. The aim of the present study was to determine the role of the cytoskeleton in the regulation of the Na⁺ current. Whole cell currents were recorded by using standard patch-clamp techniques with Cs⁺ in the pipette to block K⁺ currents. Cytochalasin D and gelsolin were used to disrupt the actin cytoskeleton and phalloidin to stabilize it. Colchicine was used to disassemble the microtubule cytoskeleton (and intermediate filaments) and paclitaxel to stabilize it. Acrylamide was used to disrupt the intermediate filament cytoskeleton. Perfusion of the recording chamber at 10 ml/min increased peak Na⁺ current recorded from jejunal smooth muscle cells by 27 ± 3%. Cytochalasin D and gelsolin abolished the perfusion-induced increase in Na⁺ current, whereas incubation with phalloidin, colchicine, paclitaxel, or acrylamide had no effect. In conclusion, the Na⁺ current expressed in human jejunal circular smooth muscle cells appears to be regulated by the cytoskeleton. An intact actin cytoskeleton is required for perfusion-induced activation of the Na⁺ current.

small intestine; patch clamp; actin; microtubules

OPENING OF Na⁺ channels provides for a large change in membrane permeability for Na⁺, resulting in entry of Na⁺ into the cell with subsequent membrane depolarization (12, 16). The amount of Na⁺ entering the cell, and hence the degree of depolarization, is regulated by mechanisms and pathways that determine channel gating. In epithelial, skeletal, and cardiac Na⁺ channels, channel gating appears to be altered by the cytoskeleton (5, 11, 15, 23), although the exact mechanisms are still controversial (17). The cytoskeleton is made up of three major components: microfilaments, of which actin is the major component (size ≈60 Å), microtubules (size ≈150–250 Å), and intermediate filaments, which, as indicated by their nomenclature, are thicker than microfilaments but thinner than microtubules (2–4, 22, 24).

Patch-clamp experiments on dissociated human jejunal circular smooth muscle cells show that a tetrodotoxin (TTX)-resistant Na⁺ current is present, and the activation-inactivation kinetics suggest steady-state Na⁺ entry at physiological voltages (13). The channel shows close homology with the TTX-resistant cardiac Na⁺ channel NaH1/NaSkM2 (13), and electrophysiological and molecular biology evidence (13) suggests that both tissues express the same Na⁺ channel. The molecular sequence of the pore-forming subunit (SCN5A) of NaH1/NaSkM2 and human jejunal SCN5A contains the consensus sequence (S/T)XV-COOH, which binds to PDZ domains found on the cytoskeletal component syntrophin (11, 20). The aim of this study was to examine the effects of cytoskeletal modulation on the human jejunal circular smooth muscle Na⁺ current. We found that Na⁺ current is modified by disruption of the actin cytoskeleton by cytochalasin D and gelsolin, suggesting a link between the channel and the smooth muscle cytoskeleton.

METHODS

The use of human jejunum, obtained as surgical waste tissue during gastric bypass operations performed for morbid obesity, was approved by the Institutional Review Board. Tissue specimens with warm ischemia times of ~30 s were harvested directly into chilled buffer. Single isolated, relaxed circular smooth muscle cells were obtained from the human jejunal specimens as described previously (8, 9).

Patch-clamp recordings. Whole cell patch-clamp recordings were made with standard and amphotericin-perforated patch-clamp whole cell techniques. Whole cell recordings were obtained with Kimble KG-12 glass pulled on a P-97 puller (Sutter Instruments, Novato, CA). Electrodes were coated with R6101 (Dow Corning, Midland, MI) and fire polished to a final resistance of 3–5 MΩ. Currents were amplified, digitized, and processed with an Axopatch 200A amplifier, a Digidata 1200, and pCLAMP8 software (Axon Instruments, Foster City, CA). Whole cell records were sampled at 10 kHz and filtered at 4 kHz with an eight-pole Bessel...
filter with the pulse protocols shown in Figs. 1, and 3–8. Seventy to seventy-five percent series resistance compensation (lag of 10 μs) was applied during each recording. The cell capacitance (Cm) was 40–70 pF, and the access resistance (Ra) was 5–10 MΩ. All records were obtained at room temperature (22°C).

**Drugs and solutions.** The pipette solution contained (in mM) 145 CsCl, 2 CaCl2, 5 HEPES, and 130 methanesulfonate. The bath solution contained normal Ringer solution (in mM: 149.2 Na+, 4.74 K+, 156.5 Cl−, and 2.54 Ca2+) buffered with 5 mM HEPES and nifedipine (1 μM) unless indicated otherwise. Drugs were purchased from Sigma (St. Louis, MO). Nifedipine stock solution was made up in 100% ethanol. The final dilution of alcohol applied was <1.1,000. At this concentration, preliminary experiments showed that ethanol had no effect on currents recorded (data not shown).

Cytochalasin D and gelsolin were used to depolymerize the actin cytoskeleton and phalloidin to stabilize it (2, 14, 21). Colchicine was used to disassemble the microtubule cytoskeleton (4) and paclitaxel to stabilize it (4). Collapse of the microtubule cytoskeleton is associated with concomitant collapse of the intermediate filament cytoskeleton (2). Acrylamide was used to disrupt the intermediate filament cytoskeleton (3). The optimal dose and specificity of each agent were determined by staining for each component of the cytoskeleton in the presence and absence of each drug. Mouse anti-α-smooth muscle actin (Sigma A2547) primary antibodies were used to stain the actin (microfilament) cytoskeleton, and rabbit anti-desmin (Sigma D8281) antibodies were used to stain the intermediate filament cytoskeleton. The primary antibodies were visualized with affinity-purified secondary antibodies conjugated to rhodamine or CY3 (Chemicon, Temecula, CA). Primary antibodies were diluted 1:100 in 0.1 M PBS containing 5% normal donkey serum and 0.3% Triton X-100. Secondary antibodies were diluted 1:100 in 0.1 M PBS containing 2.5% normal donkey serum and 0.3% Triton X-100. Cells were examined with a laser scanning confocal microscope (Zeiss LSM 510). On the basis of these preliminary experiments, cells were incubated with the respective drugs for 10–15 min, except for acrylamide, which was incubated for 30 min. The following concentrations were used: cytochalasin D, 5 μg/ml; gelsolin, 1 μM; phalloidin, 25 μM; colchicine, 10 μM; paclitaxel, 25 μM; and acrylamide, 5 mM.

Drugs were applied by complete bath changes with the solution containing the drug, except for gelsolin, which is not membrane permeant. The extent of membrane permeability of phalloidin is uncertain. Preliminary experiments were performed with cells incubated with tetramethylrhodamine isothiocyanate (TRITC)-labeled phalloidin. These experiments showed that phalloidin can cross the intact cell membrane. Experiments using phalloidin were carried out with both intracellular (delivered via pipette) and extracellular (in separate experiments) phalloidin. No difference was noted between the two sets of experiments; therefore, the data were combined. Gelsolin was also placed in the recording pipette, and the data obtained with gelsolin and phalloidin were compared with separate controls. For drugs applied to the bath, comparisons were made with the peak control Na+ current before application of the drug, in which every cell acted as its own control. Records were obtained in the presence of nifedipine (1 μM) to block Ca2+ currents, except for the acrylamide experiments because the duration of the incubation time was considerably longer for this drug and we did not want to expose the cells to nifedipine for prolonged periods.

Perfusion was used to mechanically perturb the cell membrane. Perfusion and positive pressure in the whole cell mode and negative pressure in the on-cell mode have been used to mechanoactivate ion channels (7). Of the three methods, perfusion was chosen in this study because 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 and because of the marked repeatability of its effects (7).

**Data analysis.** Electrophysiological data were analyzed with pCLAMP8 software or custom macros in Excel (Microsoft, Redmond, WA). Voltages were adjusted for the junction potential. Paired Student’s t-test or ANOVA with Tukey correction was used to evaluate statistical significance. A P value of <0.05 was considered significant. Values in the text are presented as mean ± SE maximal peak inward currents. The mean percent changes in current reported reflect the mean of the percent increase in current for each experiment. Data in Figs. 1 and 3–8 are presented as normalized values with the preperfusion current normalized to 100% for the bar graphs and the current-voltage relationships normalized to the maximal inward current of the control current set at 1.

**RESULTS**

*Activation of Na+ current by perfusion.* Initial experiments showed that the Na+ current increased in size when the bath was perfused with Ringer solution, suggesting that the jejunal circular smooth muscle channel was shear stress sensitive. Therefore, the recording chamber was perfused for 30 s at 10 ml/min to mechanically perturb the cellular surface. Perfusion increased peak Na+ current in human jejunal circular smooth muscle cells by 27 ± 3% (147 ± 21 to 175 ± 23 pA, n = 41, P < 0.01; Fig. 1). Thirty-nine of the forty-one cells studied showed an increase in current with perfusion. Time to peak inward Na+ current (activation) was faster at voltages ranging from −50 to −2 mV (n = 13, P < 0.05). Inactivation, measured as a single tau, of the currents after perfusion was not different from before perfusion (n = 6; Fig. 2). Activation kinetics of the difference current, that is, the current selectively activated by perfusion, were faster at all voltages measured, as was inactivation of the difference current, again measured as a single tau (Fig. 2). The increase in Na+ current with perfusion was used to evaluate the effect of the cytoskeleton-altering drugs. In cells perfused with N-methyl-D-glucamine (NMDG) in the bath to replace Na+, no effect of perfusion was seen at all voltages tested (−90 to +30 mV, n = 3), suggesting that the effects of perfusion were on a Na+ current.

*Actin cytoskeleton-altering drugs.* Cytochalasin D was used to disassemble the actin cytoskeleton. Cells were patch clamped, and nifedipine (1 μM) was added to the bath to block Ca2+ current. After a 5-min equilibration period, cells were perfused for 30 s with NaCl Ringer solution alone. Cytochalasin D (10 μM) and nifedipine (1 μM) were then added to the bath, and after a 15-min incubation period the bath was perfused for 30 s. The mean maximal peak inward Na+ current was 158 ± 45 pA before and 163 ± 44 pA (n = 6) after incubation with cytochalasin D (P > 0.05). The perfusion-induced increase of Na+ current was markedly reduced from 21 ± 3% (P < 0.01, n = 6) before cytochalasin D to 6 ± 2% (P > 0.05, n = 6) after incubation.
with cytochalasin D (Fig. 3). Control experiments with two perfusions of Ringer solution 15 min apart did not show any decrease in the perfusion-induced increase in Na\textsuperscript{+} current between the first and second perfusion (data not shown).

The actin-severing protein gelsolin was used to further investigate the effects of altering the actin cytoskeleton on the perfusion-induced increase in Na\textsuperscript{+} current. Intracellular application of gelsolin (1 \mu M) markedly decreased the perfusion-induced increase in Na\textsuperscript{+} current from 27 ± 3% in control cells to 5 ± 2% (P > 0.05, n = 7) in the presence of gelsolin (Fig. 4).

Phalloidin was used to stabilize the actin cytoskeleton. Phalloidin had no effect on the maximal peak Na\textsuperscript{+} current (160 ± 46 pA before and 161 ± 46 pA after incubation with phalloidin, n = 7, P > 0.05). In contrast to cytochalasin D, phalloidin did not affect the perfusion-induced increase in Na\textsuperscript{+} current. The perfusion-induced increase in Na\textsuperscript{+} current in phalloidin-exposed cells was 25 ± 3% (P < 0.01, n = 7, Fig. 5) before and 21 ± 3% (P < 0.01, n = 7) after incubation with phalloidin in same-cell controls (P > 0.05 between the 2 perfusions).

Microtubular cytoskeleton-altering drugs. Colchicine was used to disassemble the microtubule (and intermediate filament) cytoskeleton and paclitaxel to stabilize it. Both colchicine and paclitaxel had no effect on either peak maximal Na\textsuperscript{+} current or perfusion-induced increase in Na\textsuperscript{+} current. After a 15-min incubation with colchicine (10 or 100 \mu M), maximal peak current was 207 ± 72 pA compared with 191 ± 67 pA (n = 10, P > 0.05) before addition of the drug. The perfusion-induced increase in Na\textsuperscript{+} current was 33 ± 8% (P < 0.05, n = 10) before colchicine and 18 ± 2% (P < 0.05, n = 10) after incubation with colchicine (Fig. 6; P > 0.05 between the 2 perfusions). Similarly, after a 15-min incubation with paclitaxel (25 \mu M) maximal peak current was 142 ± 25 pA compared with 150 ± 26 pA (n =...
6, \( P > 0.05 \)) before addition of the drug. The perfusion-induced increase in Na\(^+\) current was 21 \( \pm \) 3\% (\( P < 0.01, n = 6 \)) before paclitaxel and 17 \( \pm \) 4\% (\( P < 0.05, n = 6 \)) after incubation with paclitaxel (Fig. 7; \( P > 0.05 \) between the 2 perfusions).

Intermediate filament cytoskeleton-altering drugs. Acrylamide was used to disrupt the intermediate filament cytoskeleton. Human jejunal circular smooth muscle cells were incubated with acrylamide for 30 min. Because of the length of incubation, nifedipine was not added to the bath solution and same-cell control perfusions were not obtained. The perfusion-induced increase in Na\(^+\) current was not affected by incubation with acrylamide. After incubation with acrylamide, perfusion induced a 38 \( \pm \) 9\% (\( P < 0.01, n = 8 \); Fig. 8) increase in maximal current compared with 27 \( \pm \) 3\% in control cells (\( P > 0.05 \) between acrylamide and controls).

The L-type Ca\(^{2+}\) channel current present in human jejunal circular smooth muscle cells is also activated by perfusion. Experiments similar to those described above were carried out on the L-type Ca\(^{2+}\) current.

Fig. 3. Cytochalasin D inhibits the perfusion-induced increase in peak Na\(^+\) current. A and B: representative Na\(^+\) current recordings obtained with the pulse protocol in the inset. A: recordings were taken of a cell incubated in a solution of NaCl Ringer with 1 \( \mu \)M nifedipine, and then the cell was perfused at 10 ml/min with NaCl Ringer solution. B: recordings from the same cell after a 15-min incubation in NaCl Ringer solution containing 10 \( \mu \)M cytochalasin D and 1 \( \mu \)M nifedipine and then perfusion with Ringer solution at 10 ml/min. C: normalized current-voltage-relationships. D: mean \% increase in maximal peak Na\(^+\) current evoked by perfusion (\(*P < 0.01\)). Cytochalasin inhibited the perfusion-induced increase in Na\(^+\) current.

Fig. 4. Intracellular gelsolin inhibits perfusion-induced increase in Na\(^+\) current. A and B: representative Na\(^+\) current recordings with the pulse protocol in the inset. A: currents obtained from a cell patch-clamped with 1 \( \mu \)M gelsolin in the intracellular solution and NaCl Ringer solution in the bath. B: recording from the same cell perfused at 10 ml/min with NaCl Ringer solution 15 min after break-in. C: normalized current-voltage relationships. D: mean \% increase in maximal peak Na\(^+\) current evoked by perfusion. Perfusion did not induce an increase in maximal peak Na\(^+\) currents in the presence of gelsolin.
None of the cytoskeleton-modifying agents used had any effect on the perfusion-induced increase in Ca$^{2+}$ current (data not shown).

DISCUSSION

The main finding of the present study was that the actin cytoskeleton appears to modulate the increase in Na$^+$ current in human jejunal circular smooth muscle cells evoked by perturbation of the cell membrane. Baseline unstimulated Na$^+$ current was not altered by agents altering the cytoskeletal structure, suggesting that gating of unstimulated Na$^+$ channels mediating the inward Na$^+$ current is not dependent on the cytoskeleton but activation of jejunal circular smooth muscle Na$^+$ channels by membrane perturbation is. The luminal diameter of the gastrointestinal tract is constantly changing as a result of digestive and interdigestive contractile activity and secondary to the passage of food boluses. The gastrointestinal smooth muscle cell membrane is subsequently under a constantly varying amount of shear stress as it is sandwiched between the extracellular matrix and the rigid cytoskeleton. Regulation of Na$^+$ entry by shear stress may alter the contractile activity of intestinal smooth muscle.

Fig. 5. Phalloidin does not alter perfusion-induced increase in maximal peak Na$^+$ current. A and B: representative Na$^+$ current recordings with the pulse protocol in the inset. A: control recordings of a cell in NaCl Ringer solution subsequently perfused with the same solution at 10 ml/min. B: recordings of the same cell incubated for 15 min in Ringer solution with 25 μM phalloidin and then perfused at 10 ml/min with NaCl Ringer solution alone. C: normalized current-voltage relationships. D: mean % increase in maximal peak Na$^+$ current evoked by perfusion (*$P < 0.01$). Phalloidin incubation did not alter the perfusion-induced increase in Na$^+$ current.

Fig. 6. Colchicine does not inhibit the perfusion-induced increase in Na$^+$ current. A and B: representative Na$^+$ current recordings obtained with the pulse protocol in the inset. A: control recordings of a cell in NaCl Ringer solution that was then perfused with the same solution at 10 ml/min. B: recordings of the same cell incubated for 15 min in NaCl Ringer solution with 10 μM colchicine and then perfused at 10 ml/min. C: normalized current-voltage relationships. D: mean % increase in maximal peak Na$^+$ current evoked by perfusion (*$P < 0.05$).
muscle. Block of the intestinal smooth muscle Na\(^+\)/H\(^+\) channel results in membrane hyperpolarization (13), and an increase in Na\(^+\) entry in response to increased shear stress would be expected to depolarize intestinal smooth muscle, bringing the membrane potential closer to the contractile threshold. The actin cytoskeleton may serve as a mechanism to transmit force to the Na\(^+\)/H\(^+\) channel by constraining the lipid bilayer or by directly interacting with the Na\(^+\)/H\(^+\) channel.

Cytoskeletal modifiers have been shown to alter Na\(^+\) channels in cardiac, skeletal, and epithelial tissues (5, 11, 15, 23). Cytochalasin D, a disrupter of the actin cytoskeleton, is most commonly linked to modulation of Na\(^+\) current, but microtubular cytoskeletal modifiers such as colchicine and paclitaxel have also been shown to modulate Na\(^+\) current (19).

In skeletal muscle, voltage-gated Na\(^+\) channels are concentrated at postsynaptic membrane sites (6). A family of intracellular membrane-associated proteins called syntrophins (1, 2, 10, 18) are partly responsible for the aggregation of Na\(^+\) channels. Syntrophins associate with dystrophin, which in turn is linked to the cell membrane and extracellular matrix through actin and dystroglycans (2, 20). Thus syntrophin links sig-

Fig. 7. Paclitaxel does not inhibit the perfusion-induced increase in Na\(^+\) current. A and B: representative Na\(^+\) current recordings with the pulse protocol in the inset. A: control recordings from a cell in NaCl Ringer solution subsequently perfused with the same solution at 10 ml/min. B: recordings of the same cell incubated for 15 min in NaCl Ringer solution with 25 \(\mu\)M paclitaxel and then perfused at 10 ml/min with NaCl Ringer solution alone. C: normalized current-voltage relationships. D: mean % increase in maximal peak Na\(^+\) current evoked by perfusion (*\(P < 0.05\)).

Fig. 8. Acrylamide does not alter the perfusion-induced increase in Na\(^+\) current. A and B: representative Na\(^+\) current recordings obtained with the pulse protocol in the inset. Note the slower Ca\(^2+\) current present in these cells because nifedipine was not added to the bath. A: recordings from a cell incubated in NaCl Ringer solution with 5 mM acrylamide for 30 min. B: recording from the same cell perfused at 10 ml/min with NaCl Ringer solution. C: normalized current-voltage relationships. D: mean % increase in maximal peak Na\(^+\) current evoked by perfusion (*\(P < 0.01\)).
naling proteins to the actin cytoskeleton and the extracellular matrix. Syntrophins have a PDZ domain that binds to the consensus sequence (S/T)TXV-COOH (11, 20). Previous work showed that the molecular sequence of the α-subunit of the TTX-resistant Na⁺ channel (SCN5A) in human jejunal circular smooth muscle (13) and cardiac muscle is similar (GenBank accession numbers AY038064 and NM000335). The electrophysiological and pharmacological properties of native TTX-resistant Na⁺ channels in human jejunal circular smooth muscle and cardiac muscle are also similar (13). Both the human jejunal circular smooth muscle and the cardiac TTX-resistant Na⁺ channel α-subunit contain the sequence DRESIV, which binds strongly to syntrophins (11, 20), suggesting syntrophins as a possible link between the Na⁺ channel and the actin cytoskeleton. Human jejunal circular smooth muscle cells also express a mechanosensitive L-type Ca²⁺ channel that is activated when the actin-syntrophin cytoskeleton link to Na⁺ channels rather than a nonspecific effect on the cytoskeleton.

The results obtained in the present study suggest that the cytoskeleton may not only serve to anchor Na⁺ channels to the membrane but may also affect function. Recent work reported the effects of cytoskeletal modulators on the α-subunit rSkM1 expressed in CHO cells (17). The investigators reported minimal effects on peak current, current-voltage relationships, and kinetic properties and suggested that the cytoskeleton did not directly interfere with Na⁺ channel function (17). Our data suggest that such an interaction may not be apparent under unstimulated conditions but may become apparent when the membrane and attached cytoskeleton are deformed, as presumably occurs with perfusion.

In summary, human jejunal circular smooth muscle cells express a Na⁺ channel that is activated when the cell membrane is mechanically perturbed. The increase in Na⁺ channel current evoked by perfusion was abolished by agents that disrupt the actin cytoskeleton but not by other cytoskeleton-altering agents, suggesting that the actin-related cytoskeleton complex may specifically alter the regulation of Na⁺ channel current and subsequent Na⁺ entry into the cell in response to membrane perturbation.

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