Sodium current in human intestinal interstitial cells of Cajal

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INTERSTITIAL CELLS OF CAJAL (ICC) are central in the control of gastrointestinal (GI) motility. ICC generate spontaneous rhythmic electrical oscillations, or “slow waves,” which cause smooth muscle contractions predominantly via activation of voltage-gated Ca2+ channels in smooth muscle (reviewed in Refs. 3 and 9). Furthermore, ICC serve as the connection between enteric nerves and smooth muscle cells of the GI tract. These conclusions about ICC function originate from work correlating the loss of ICC in the myenteric plexus region of the small intestine with the absence of the slow wave from recorded tissue (4, 34) and loss of effective neurotransmission in animal models lacking ICC (38). Mutations and point mutations (WWγ/Wγ, Wδ/Wδ) in the gene encoding the tyrosine kinase receptor c-Kit, expressed on ICC (22, 39), lead to reduced or absent ICC in the myenteric plexus region and the loss of slow waves in the murine small intestine despite otherwise normal-appearing enteric nerves (11, 12, 14, 26, 27, 39). In humans, decreased ICC numbers and volume have been associated with several GI disorders, including slow-transit constipation (6, 20), diabetic gastroparesis (7), pseudoobstruction (13), hypertrophic pyloric stenosis (37), and paraneoplastic dysmotility (25). Functioning ICC appear to be essential for generation of pacemaker activity and for normal GI motility.

Generation of the electrical slow wave requires coordinated ion channel activity initiated by a pacemaker current. Despite the central role of ICC in GI motility, the ionic conductances expressed in human intestinal ICC have not been studied. Recent reports on human jejunal circular smooth muscle cell physiology have shown that a Na+ current is expressed in these cells (8, 24). This Na+ current is of particular interest because the pore-forming α-subunit is encoded by SCN5A (23). SCN5A also encodes for the α-subunit of the tetrodotoxin-resistant cardiac Na+ channel responsible for the rapid depolarization of the cardiac action potential. Mutations in SCN5A lead to cardiac arrhythmias (2), raising the possibility that a similar mechanism may be operative in the intestine. The Na+ current recorded from human intestinal circular smooth muscle cells is regulated by the actin cytoskeleton and by syntrophin (24, 31) and is mechanosensitive (31). A window current is present at the resting smooth muscle membrane potential, suggesting that there is Na+ entry through the channel at this voltage range (8). Intestinal smooth muscle and ICC develop from the same progenitor cell, and it is suggested that one cell type can revert to the other under appropriate conditions (reviewed in Ref. 41). These observations suggest that a Na+ current may also be expressed in human intestinal ICC and may play a role in the control of intestinal motor function. The aims of this study were to determine if a Na+ current is present in human intestinal ICC and to determine the effects of the Na+ current on the electrical slow wave.

METHODS

The Institutional Review Board approved the use of human jejunal circular smooth muscle cells obtained as surgical waste tissue during gastric bypass operations performed for morbid obesity. Tissue speci-
imens were harvested directly into chilled buffer with warm ischemia times of ∼30 s. Cells from human jejunal circular smooth muscle strips were dissociated as previously described (8). The dissociation procedure resulted in a mixture containing a majority of isolated smooth muscle cells and a few cells with a distinctive ICC-like morphology (3 or more well-defined primary processes originating from the cell body).

**Single-Cell RT-PCR**

Immunohistochemical identification of freshly dissociated ICC is difficult because c-kit expression is often lost during the dissociation procedure, making immunohistochemistry unreliable. Therefore, confirmation that the cells identified visually were in fact ICC was obtained by collecting individual cells and amplifying c-kit message with single-cell RT-PCR. Single putative ICC were collected by applying negative pressure to a cell in contact with a recording pipette, lifting the cell out of the bath, and immediately placing it in a 200-µl tube on dry ice. A cDNA library constructed from human jejunal circular smooth muscle strips was used as a positive control for c-kit mRNA. Visually identified single human jejunal circular smooth muscle cells were collected as negative controls. RT-PCR was performed within 1 h of cell collection by using a one-step RT-PCR kit (Qiagen). Gene-specific first-round RT-PCR was performed with 35 cycles at an annealing temperature of 55°C using the forward and reverse primers TGT GAT GAT TCT GAC CTA CA and GAA TCA GTT TTT CTC CTA A, respectively, for c-kit. Second-round PCR used 2 µl of reaction mixture from the first round as template, a forward primer GGA AGG TTG TTG AGG AGA, and a reverse primer CTT TGA TGT CAG AGC TGA GG TA. For SCN5A, a single round of PCR was performed with a forward primer GTG ACC ATG ATG GTG GAG ACA and a reverse primer PRIMER TGC CAG CCT CCC ACT TGA CAT.

**Patch-Clamp Recordings**

Whole cell currents were recorded from freshly dissociated ICC by using standard whole cell patch-clamp techniques. All cells were held at −100 mV between pulse protocols, as shown in the figure insets. Kimble KG-12 glass electrodes were 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 by using an Axopatch 200A amplifier, a Digidata 1200, and pCLAMP 8 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 by using the pulse protocols shown in the figures. The pulse protocols used in Figs. 2, 3, 4, 5, and 7 were designed to show all inward currents and were not designed to determine the reversal potential for the Na+ current. The protocol in Fig. 6 was designed to maximally open Na+ channels and to determine Na+ reversal potentials. In the absence of N-methyl-D-glucamine (NMDG), there are at least three residual currents contributing to the reversal potential: an L-type Ca2+ current (reversal positive to 0 mV), a nonselective cation current (reversal at ~0 mV), and the Na+ current (reversal positive to 0 mV). Block of either the Ca2+ or the Na+ current will shift the reversal potential to the left, because now the contribution of the nonselective current is relatively larger. As in the standard protocols used in Figs. 2, 3, 4, 5, and 7, the Na+ current is very small at positive voltages compared with the nonselective cation current; the latter current dominates the reversal potentials. A 70–75% series resistance compensation (lag of 60 μs) was applied during each recording. The ICC capacitance was from 60 to >100 pF, and the access resistance was 5–10 MΩ. Because the Axopatch amplifier only allows capacity compensation to 100 pF, the capacity transient in some records was not completely compensated for (e.g., Fig. 3). All records were obtained at room temperature (21°C).

**Intracellular Recordings**

Muscle strips were cut from human jejenum and placed in a recording chamber with the mucosa removed and with the circular muscle facing upward. One end of the muscle strip was pinned down to a Sylgard-coated (Dow Corning) chamber to record intracellular electrical activity, and the other end was attached to an isometric force transducer to record circular smooth muscle contractile activity of the entire muscle strip. The chamber (3 ml total volume) was perfused with warmed (37°C) and oxygenated Krebs’ solution at a constant flow of 3 ml/min. After an equilibration period of at least 2 h, the muscle strips were stretched to an initial tension of 50–100 mg above the baseline tension. Recordings of intracellular electrical activity from smooth muscle cells were obtained by using glass capillary microelectrodes filled with 3 M KCl and with resistances ranging from 30 to 80 MΩ. Intracellularly recorded potentials were amplified by using a WPI M-707 amplifier (WPI, New Haven, CT) and were displayed on an oscilloscope (Tektronix 5113, Beaverton, OR). Force was measured isometrically and amplified with a bridge circuit amplifier. Both electrical signals and mechanical activity were digitally recorded and also recorded on chart paper (Gould 220; Gould, Cleveland, OH). Drugs were applied by perfusion into the recording chamber, and weights were applied to one end of the muscle strip after an impalement of a cell to alter the degree of stretch of the muscle strip.

**Drugs and Solutions**

Patch-clamp records. Intracellular solution for all but reversal potential experiments contained (in mM) 145 Cs+ (to block outward K+ current), 20 Cl−, 2 EGTA, 5 HEPES, and 130 methanesulfonate, resulting in a final osmolality of 285 mosM. In reversal potential experiments, the intracellular solution contained (in mM) 50 Na+, 95 Ca2+, 20 Cl−, 2 EGTA, 5 HEPES, and 130 methanesulfonate, for a final osmolality of 292 mosM. The extracellular solution contained (in mM) 149.2 Na+, 4.74 K+, 156.5 Cl−, 2.54 Ca2+, 5 HEPES (normal Ringer solution, 299 mosM). Na+ was partially or entirely replaced with equal concentrations of NMDG (1, 5, 50, or 149.2 mM) when indicated, keeping Cl− concentrations unaltered. Intracellular and extracellular solutions were adjusted to pH 7.0 and 7.35, respectively.

Nifedipine (L-type Ca2+ channel blocker; Sigma, St. Louis, MO) was reconstituted in 100% ethanol and diluted in normal Ringer solution to 1 µM. The final dilution of ethanol applied, <1:1,000, had no effect on control currents. Lido- caine-derivative QX-314 (a selective Na+ channel blocker; Alomone Labs, Jerusalem, Israel) was dissolved in normal Ringer solution and diluted to a working concentration of 500 µM. The QX-314 experiments were performed in the presence of nifedipine (1 µM) to block Ca2+ currents.

Muscle strip experiments. Records were obtained with atropine, phentolamine, propranolol, and tetrodotoxin (all 1 µM) in the bath to block neuronal activity, including neuronal tetrodotoxin-sensitive Na+ channels. Electrical field stimulation under these conditions did not generate an excitatory or inhibitory junction potential, suggesting that neurotransmission was effectively blocked. Nifedipine (1 µM)
was also added to the bath to block smooth muscle mechanosensitive L-type Ca\(^{2+}\) channels. Nifedipine blocked phasic contractile activity but did not alter electrical slow wave activity.

Data Analysis

Electrophysiological data were analyzed by using pCLAMP 8 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’s correction was used to evaluate statistical significance. A P value of <0.05 was considered significant. Values in the text are presented as mean maximal peak inward current ± SE. Reversal potentials for instantaneous current-voltage relationships were determined by using the Nernst equation.

RESULTS

Correctly Identifying ICC for Patch Clamping

Putative small intestinal circular layer ICC (Fig. 1, A and B) were collected and processed by one round of single-cell RT-PCR and a second amplification using nested primers specific for c-kit mRNA. A 258-base product of the appropriate size was amplified from collected ICC (Fig. 1C, lanes 1 and 2) and the cDNA library positive control (Fig. 1C, lane 4), indicating the presence of c-kit mRNA. A larger fragment (623 bp) was also observed that matched the predicted size from genomic amplification in lanes 1 and 2 but, as expected, not in lane 4. A cell visually identified as a human jejunal circular smooth muscle cell was negative for c-kit mRNA but did reveal the 623-bp genomic amplification fragment (Fig. 1C, lane 3). The identity of the bands was confirmed by sequencing.

Two Transient Inward Currents in ICC

Cells matching the morphology of the ICC identified by RT-PCR were patch clamped, and transient inward currents were measured with Cs\(^{+}\) in the pipette to block K\(^{+}\) currents. Whole cell recordings from patch-clamped human small intestinal ICC under these conditions revealed two distinct transient components of inward current, one with peak inward current at −30 mV and the other with peak inward current at 0 mV (Fig. 2). The component peaking at −30 mV displayed faster inactivating kinetics than the slower component peaking at 0 mV (Fig. 2A).

To determine the ionic contribution to the slow component, nifedipine (1 μM) was added to the extracellular solution. Nifedipine blocked the slow component (−105 ± 33 to −14 ± 6 pA; n = 6; P < 0.05) but not the fast component (−168 ± 70 to −157 ± 67 pA; n = 8; P > 0.05; Fig. 3C), suggesting that the slow component was carried by L-type Ca\(^{2+}\) channels (Fig. 3A) similar to human jejunal circular smooth muscle cells (8). Ni\(^{2+}\) (40 μM) added to the bath with 1 μM nifedipine had no further effect on the peak negative value of the fast component (analyzed by subtraction), suggesting that the majority of the fast component of the inward current was not carried by T-type Ca\(^{2+}\) channels (data not shown).

Identifying the Ion Contributing to the Fast Component of the Inward Current

Substitution of bath solution Na\(^{+}\) with NMDG was used to test for contribution of Na\(^{+}\) to the fast component. Replacement of Na\(^{+}\) with NMDG abolished the fast component of inward current from a peak of −177 ± 50 to −12 ± 2 pA (n = 13; P < 0.01; Fig. 4). NMDG reduced the magnitude of maximal peak Ca\(^{2+}\) current from −76 ± 17 to −63 ± 16 pA (n = 7; P < 0.05).

The lidocaine-derivative drug QX-314 blocks both cardiac and neuronal Na\(^{+}\) channels when applied intracellularly but only blocks the cardiac/intestinal type

Fig. 1. Single-cell RT-PCR amplifies c-kit message from cells visually identified as interstitial cells of Cajal (ICC). A and B: examples of single cells displaying characteristic ICC morphology (ICC). Single-cell RT-PCR was performed in 2 rounds by using nested primer pairs specific for c-kit mRNA. Amplification products are shown for 2 collected ICC (lanes 1 and 2), 1 smooth muscle cell (lane 3), and a human jejunal cDNA library (lane 4). A band of the correct size, confirmed by sequencing, was present in lanes 1, 2, and 4. No product for c-kit was present in lane 3, the smooth muscle cell. The ~600-bp band is the result of genomic amplification.
when applied extracellularly (23, 33). When applied extracellularly to small intestinal ICC, QX-314 inhibited the fast inward component. The amplitude of Na⁺ current remained unchanged after 20 min for control ICC (-148 ± 55 pA at t = 0 to -136 ± 47 pA at t = 20 min; 1 μM nifedipine in the bath to block Ca²⁺ current; n = 3; P > 0.05), whereas extracellular QX-314 (500 μM) with nifedipine (1 μM) exponentially decreased the Na⁺ current by 65% at 20 min (-143 ± 36 to -51 ± 11 pA; n = 4; P < 0.05; Fig. 5).

To test the selectivity of the fast component to Na⁺, ICC were patch clamped with 50 mM intracellular Na⁺ and with several concentrations of Na⁺ plus nifedipine (1 μM) in the bathing solution; 50 mM intracellular Na⁺ was used to move the expected reversal potentials for a Na⁺-selective channel away from those for a Ca²⁺-selective channel and a nonselective cation channel, allowing a clear distinction between the currents. Recorded reversal potentials from instantaneous current-voltage relationships tracked the equilibrium potential for Na⁺ (Fig. 6), suggesting that a Na⁺-selective channel carried the fast component of the inward current.

Mechanosensitivity of the Na⁺ Current

To test for mechanosensitivity of the Na⁺ current, ICC were patched and then perfused with equiosmolar NaCl Ringer solutions at a rate of 10 ml/min for 30 s (21). The amplitude of the Na⁺ current increased from -146 ± 30 pA in the control to -191 ± 45 pA in the perfused treatment (n = 7; P < 0.05; Fig. 7). The inward Ca²⁺ component did not increase (-120 ± 34 to -131 ± 30 pA; n = 3; P > 0.05; Fig. 7).

Molecular Identity of the Na⁺ Channel

The electrophysiological and pharmacological properties of the ICC Na⁺ current were indistinguishable from the human jejunal circular smooth muscle Na⁺ current. The smooth muscle Na⁺ channel α-subunit is SCN5A (23). Single-cell PCR was used to determine if SCN5A is also expressed in human intestinal ICC. Single intestinal ICC were collected and processed by one round of single-cell RT-PCR and a second amplification using nested primers specific for SCN5A. SCN5A was successfully amplified with SCN5A primer sets designed to span an intron (n = 4 experiments; Fig. 8). The correct-size band was recovered from the agarose gel conventional techniques, and results were confirmed by sequence analysis. There were no products in negative controls (4 μl of bath solution aspirated just above an ICC).

Effect of Sodium Removal and Sodium Channel Blockers on the Slow Wave and Membrane Potential

To determine if the Na⁺ current expressed in human intestinal ICC played a role in the generation of the slow wave, intracellular recordings from circular smooth muscle cells in muscle strips and mechanical activity were recorded before, during, and after application of the Na⁺ channel blocker lidocaine (200 μM) or the more selective Na⁺ channel blocker QX-314 (500 μM). Tetrodotoxin (1 μM) was present in the bath to block neuronal activity. Lidocaine altered the slow wave. The rate of rise (measured as τ) of the slow wave slowed from 0.89 ± 0.1 to 1.1 ± 0.1 s (n = 6 tissues; P < 0.05), and the duration of each slow wave cycle increased from 6.5 ± 0.2 to 7.1 ± 0.3 s (n = 6 tissues; P <
0.01), resulting in a decrease in the slow wave frequency from 8.1 ± 0.2 to 7.2 ± 0.3/min (n = 6 tissues; P < 0.05). Lidocaine also hyperpolarized the membrane potential by 4 ± 2 mV (n = 6; P < 0.05; Fig. 9). Similarly, in separate experiments, the more selective Na\(^+\) channel blocker QX-314 also slowed the rate of rise of the slow wave and decreased the slow wave frequency. The rate of rise (measured as τ) of the slow wave slowed from 0.7 ± 0.05 to 0.53 ± 0.08 s (n = 3 tissues; 13 cells for control, 8 for drug; P < 0.01), and the slow wave frequency decreased from 7.3 ± 0.2 to 6.2 ± 0.05 waves/min (n = 3 tissues; 20 cells for control, 11 for drug; P < 0.01). Application of QX-314 also hyperpolarized the membrane potential from 57 ± 2 mV (n = 18) to 63 ± 3 mV (n = 10; P < 0.05). Nifedipine (1 μM) had no effect on slow wave frequency or rate of rise (data not shown).

In separate experiments, Na\(^+\) was removed from the bath and substituted with NMDG. Removal of Na\(^+\) resulted in an immediate hyperpolarization from −60 ± 2 to −66 ± 3 mV (n = 6; P < 0.05; Fig. 10), and the slow wave amplitude decreased over the next minute until the slow wave was no longer discernible after 243 ± 29 s. These effects were reversible when Na\(^+\) was reintroduced into the bath.

**Effect of Stretch**

The lidocaine and QX-314 experiments suggested that Na\(^+\) current contributed to the generation of the electrical slow wave. Since the Na\(^+\) current was found to be mechanosensitive, then stretch of human circular smooth muscle strips would be expected to activate the Na\(^+\) channel. Smooth muscle cells were impaled to record the electrical slow wave in the presence of tetrodotoxin (1 μM) and nifedipine (1 μM, to block smooth muscle L-type mechanosensitive Ca\(^{2+}\) channels) in the bath. After impalement the slow wave was recorded, then 1.5–3 g of tension were applied to the muscle strip. Higher tension was not applied because it usually resulted in loss of the slow wave. This tension resulted in a change in frequency of the slow wave from 8.2 ± 0.2 to 8.5 ± 0.15/min (n = 7; P < 0.05; Fig. 11).

**DISCUSSION**

The present study identifies the presence of a Na\(^+\) current in ICC isolated from the human intestinal circular smooth muscle layer. This Na\(^+\) current bears strong electrophysiological, pharmacological, and...
mechanosensitive similarities to the Na⁺ current carried by the tetrodotoxin-insensitive Na⁺ channel in human jejunal circular smooth muscle cells and in cardiac myocytes (8, 23, 24, 31). The molecular identity of the pore-forming α-subunit of the tetrodotoxin-insensitive Na⁺ channel in both human intestinal smooth muscle and cardiac myocytes is encoded by SCN5A. Message for SCN5A was also identified in single ICC, suggesting that the same Na⁺ current is present in both human circular smooth muscle cells and intestinal ICC.

Fig. 4. Effect of N-methyl-D-glucamine (NMDG) on the fast component of the transient inward current. A: representative ICC current recordings using the pulse protocol in the inset with nifedipine (1 µM) in the bath to block L-type Ca²⁺ current. Replacement of bath solution Na⁺ with NMDG completely blocked the fast component of inward current. B: current-voltage relationships. C: mean maximal peak current of the fast component (n = 13; *P < 0.01).

Fig. 5. Effect of the selective Na⁺ channel blocker QX-314 on the fast inward component. A: typical Na⁺ current recordings from an ICC patched using the pulse protocol in the inset with nifedipine (1 µM) in the bath to block L-type Ca²⁺ current. Top records show currents recorded at t = 0 and t = 20 min from a control cell in NaCl Ringer bath. Bottom records show currents from an ICC 0 and 20 min after exposure to 500 µM QX-314. B: amplitude of normalized peak Na⁺ currents over time in the control (●) and QX-314-exposed cell (○). C: mean normalized peak Na⁺ currents at 0 and 20 min for control (n = 3; P > 0.05) and QX-314-treated ICC (n = 4; P < 0.05).
The role of the tetrodotoxin-insensitive Na\(^+\) channel is well defined in cardiac muscle. Opening of the channel leads to rapid depolarization and is responsible for the upstroke of the cardiac action potential. The role of a Na\(^+\) channel is less well defined in smooth muscle and ICC of the GI tract. Action potentials are superimposed on the slow wave generated by ICC. Removal of Na\(^+\) generally has no effect on the size and shape of action potentials (10, 29, 30), suggesting that the ac-

Fig. 6. Reversal potential of instantaneous current-voltage relationships from the fast component of the inward current. Reversal potentials for the fast component of the inward current were recorded from ICC patched in the presence of 50 mM intracellular Na\(^+\) and 150, 50, 5, and 1 mM extracellular Na\(^+\), with NMDG substituting Na\(^+\). Nifedipine (1 μM) was present in the bath to block the L-type Ca\(^{2+}\) current. Reversal potentials (○) were plotted against the expected reversal potential of a perfectly selective Na\(^+\) channel current (solid line).

Fig. 7. Effect of perfusion on transient inward currents. Representative inward currents are displayed from an ICC before and during perfusion with NaCl Ringer solution. A: individual sweeps at −30 and 0 mV are shown to highlight the separate effects of perfusion on the Na\(^+\) and Ca\(^{2+}\) currents, respectively. B: current-voltage relationship for the peak inward currents. C: mean maximal peak current values for the Na\(^+\) (n = 7; P < 0.05) and Ca\(^{2+}\) (n = 3; P > 0.05) currents before and during perfusion.

Fig. 8. SCN5A is expressed in single human intestinal ICC. Single ICC were collected from dissociated human jejunal circular smooth muscle strips. Single-cell RT-PCR was performed using primer pairs specific for SCN5A and designed to span an intron to detect genomic sequence. A band of the right product size was identified and sequenced (lane 2) to confirm that it was SCN5A. No band was seen from bath solution aspirated just above the cells (lane 1).
tion potential does not require participation of Na\(^+\) channels. The contribution of Na\(^+\) channels to the slow wave has also been unclear because Na\(^+\) removal from the bath does not immediately abolish the slow wave, suggesting that Na\(^+\) channels are not the pacemaker channel. The present finding that ICC express a Na\(^+\) channel therefore raises the question of what role Na\(^+\) channels may play in ICC electrical activity. Block of Na\(^+\) channels open at the hyperpolarized portion of the slow wave, expressed on either ICC or smooth muscle, would be expected to depolarize the baseline membrane potential. If Na\(^+\) channels contribute to the ICC-generated slow wave, then block of Na\(^+\) channels expressed on ICC would be expected to decrease the rate of depolarization of the membrane potential and thereby slow the rate of rise of the slow wave and delay repolarization. Our results are consistent with these expected findings, suggesting a role for Na\(^+\) channels in the generation of the electrical slow wave. Removal of Na\(^+\) from the muscle strip bath resulted in an immediate hyperpolarization and the subsequent disappearance of the slow wave. Although it is possible that Na\(^+\) removal decreases inward current through a nonselective cation channel and thereby alters the electrical slow wave through this mechanism, experiments carried out with Na\(^+\)-selective pharmacophores support a direct role for Na\(^+\) channels. Lidocaine, a nonselective Na\(^+\) channel blocker, and QX-314, a selective cardiac/intestinal-type Na\(^+\) channel blocker, when applied externally also hyperpolarized smooth muscle cell membrane potential. These experiments were carried out in the presence of tetrodotoxin to block neuronal tetrodotoxin-sensitive Na\(^+\) channels. Hyperpolarization of the membrane potential with lidocaine or QX-314 implies that there is steady-state Na\(^+\) influx through Na\(^+\)-selective ion channels at the hyperpolarized portion of the electrical slow wave. Because we recorded from smooth muscle cells, it was not...
possible to separate out smooth muscle vs. ICC hyperpolarization. The blockers used would block both smooth muscle and ICC Na\(^+\) channels, making it likely that the changes in membrane potential seen were due to an effect on both cell types. In contrast, the chronotropic effects seen on the slow wave are likely due to a direct effect on ICC because slow wave generation and chronotropicty are thought to be a property of ICC, not of smooth muscle. Both QX-314 and lidocaine slowed the rate of rise of the slow wave and decreased slow wave frequency. The observed change in the rate of rise of the electrical slow wave suggests that Na\(^+\) channels contribute to this portion of the human intestinal slow wave, but the relatively small changes in the rate of rise suggest that more than one ion channel type contributes the upstroke of the electrical slow wave or that there was inadequate Na\(^+\) channel blockade. Irrespective, the observed Na\(^+\) channel block was sufficient to decrease electrical slow wave frequency, suggesting a physiological relevance. The muscle strip stretch experiments are also consistent with a role for Na\(^+\) channels in regulating the slow wave and suggest that the regulation of electrical slow wave frequency is mechanosensitive. Gentle stretch aimed at activating the Na\(^+\) channel was performed with nifedipine in the bath to block the mechanosensitive L-type Ca\(^{2+}\) channel. Of interest, the L-type Ca\(^{2+}\) channel, known to be mechanosensitive in human intestinal smooth muscle, was not stimulated by perfusion in single ICC experiments, perhaps reflecting a different interaction between the ion channel and membrane and cytosolic proteins. However, the small sample size for the L-type Ca\(^{2+}\) current makes it difficult to come to any definitive conclusion in this regard. Stretch of the tissue resulted in an increase in slow wave frequency. The mechanosensitive Na\(^+\) channel expressed in human intestinal ICC and smooth muscle cells may therefore provide a dual mechanism for ICC to both sense intestinal stretch and to respond to the stretch stimulus with a change in slow wave frequency.

Cyclic oscillations in membrane potential were not recorded from the human intestinal ICC patch clamped. This was likely due to theionic composition of the pipette solution; Cs\(^+\) was used to replace K\(^+\) to block K\(^+\) currents and to thereby unmask inward currents of interest. Under these recording conditions, rhythmic electrical oscillations cannot be generated.

Similar experiments have not been performed on freshly dissociated murine ICC because it is not possible to visually identify murine ICC after cell dissociation. In contrast, the dissociation procedures used in this study on human intestinal tissue preserve ICC processes, making it possible to visually identify and patch clamp freshly dissociated human intestinal ICC. Confirmation that the cells visually identified were ICC was obtained by showing that the message for c-Kit was present in single ICC using single-cell PCR.

The ionic mechanisms that give rise to the initiation and maintenance of electrical slow waves in intestinal muscle determine smooth muscle ionotropicity and chronotropicity. Understanding the ionic conductances increases our understanding of the basic mechanisms that control intestinal contractility and may provide a potential therapeutic target. Nonhuman animal models have provided important insights into the ion channels expressed in small intestinal and colonic ICC. Initial patch-clamp recordings from canine colonic ICC identified inward L-type and possibly T-type Ca\(^{2+}\) channels (18), whereas subsequent patch-clamp recordings demonstrated Ca\(^{2+}\)-activated K\(^+\) channels and spontaneously generated slow waves (17). Delayed rectifier-type K\(^+\) channels and inward rectifier K\(^+\) channels have been described in murine ICC (1, 5). Regular slow waves were also identified in cultured murine ICC (16, 19, 35), and nonselective cation channels that carry voltage-independent inward currents are thought to act as the pacemaker signal and initiate the generation of the slow wave (28). Changes in submembrane Ca\(^{2+}\) concentrations, including uptake into mitochondria, may serve as the stimulus to activate pacemaker currents (40). Cl\(^-\) channels may also participate in pacemaker currents, because a Cl\(^-\) channel blocker (4-acetamido-4-(isothiocyanatostilbene-2,2'-disulfonic acid, 300 \(\mu\)M) inhibited rhythmically oscillating current in 2-day-old murine cultured cells (36). A recent study examined inward Ca\(^{2+}\) current from cultured murine small intestinal ICC (15). A nifedipine-sensitive and a nifedipine-insensitive component was found. The nifedipine-insensitive component was blocked by Ni\(^{2+}\) and by mibebradil. Both components inactivated on a several-hundred-millisecond time scale. The Na\(^+\) current expressed in human ICC described in this report and the Na\(^+\) current in human intestinal smooth muscle (8) inactivate with a 5-ms time constant.
and were not recorded in cultured murine ICC. Similarly, we were able to record a Na\(^+\) current in only 1 of 23 freshly dissociated murine small intestinal smooth muscle cells and not in porcine small intestinal smooth muscle cells (32), suggesting a possible species-dependent variation in the expression of SCN5A. These differences imply possible variations in the contribution of Na\(^+\) channels to the electrical slow wave across species and possibly anatomic sites.

In summary, a mechanosensitive Na\(^+\) current is present in human intestinal ICC. The channel may play an important role in the normal physiological control of human intestinal motor function by contributing to the setting of the membrane potential, the rate of rise of the slow wave, and mechanosensitive regulation of slow wave frequency.

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DISCLOSURES

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REFERENCES

32. Streve PR, Oy Y, Lim JF, Miller SM, Gibbons SJ, Sarr MG, Szurszewski JH, and Farrugia G. Species dependent expres-

33. Sunami A, Glaaser IW, and Fozzard HA. A critical residue for isoform difference in tetrodotoxin affinity is a molecular determinant of the external access path for local anesthetics in the cardiac sodium channel. Proc Natl Acad Sci USA 97: 2326–2331, 2000.


