Expression and function of KCNH2 (HERG) in the human jejunum

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Farrelly, A. M., S. Ro, B. P. Callaghan, M. A. Khoyi, N. Fleming, B. Horowitz, K. M. Sanders, and K. D. Keef. Expression and function of KCNH2 (HERG) in the human jejunum. Am J Physiol Gastrointest Liver Physiol 284: G883–G895, 2003; 10.1152/ajpgi.00394.2002.—Previous studies suggest that ether-a-go-go related gene (ERG) KCNH2 potassium channels contribute to the control of motility patterns in the gastrointestinal tract of animal models. The present study examines whether these results can be translated into a role in human gastrointestinal muscles. The KCNH2 protein was detected immunohistochemically in circular and longitudinal smooth muscle and enteric neurons but not in interstitial cells of Cajal. In the presence of TTX (10^-6 M), atropine (10^-6 M), and L-nitroarginine (10^-4 M) human jejunal circular muscle strips contracted phasically (9 cycles/min) and generated slow waves with superimposed spikes. Low concentrations of the KCNH2 blockers E-4031 (10^-6 M) and MK-499 (3 x 10^-8 M) increased phasic contractile amplitude and the number of spikes per slow wave. The highest concentration of E-4031 (10^-6 M) produced a 10–20 mV depolarization, eliminated slow waves, and replaced phasic contractions with a small tonic contracture. E-4031 (10^-6 M) did not affect [14C]ACh release from enteric neurons. We conclude that KCNH2 channels play a fundamental role in the control of motility patterns in human jejunal muscle through their ability to modulate the electrical behavior of smooth muscle cells.

MOTILITY PATTERNS IN MOST gastrointestinal (GI) tissues are intimately related to the level of resting membrane potential set by potassium channels in the smooth muscle and to the fluctuations in potential (i.e., slow waves) generated by interstitial cells of Cajal (ICC). Recently, studies have suggested that KCNH2 potassium channels encoded by ether-a-go-go related gene (ERG) (28) importantly contribute to the control of resting membrane potential in the GI muscles of two animal models and by extension, the motility patterns of these tissues (17). The present study examines whether these results can be translated into a functional role for KCNH2 channels in the control of motility patterns in human GI muscle.

Cisapride (Propulsid) is an effective oral prokinetic agent that increases lower esophageal sphincter tone, accelerates gastric emptying, and increases small-bowel motility. It has proven useful in the treatment of dyspepsia, gastroparesis, and gastroesophageal reflux (13). It has also been shown to have utility in the treatment of pseudoobstruction, peptic ulcer, irritable bowel syndrome, and postoperative ileus (9). Cisapride was removed in 2000 from general usage in the United States by the FDA due to reports of heart rhythm abnormalities, including 80 deaths (11). This side effect of cisapride is due to blockade of human cardiac KCNH2 channels leading to long QT syndrome (16, 21). That cisapride also depolarizes and contracts the guinea pig Taenia coli and isolated smooth muscle cells of the rat stomach (17) and that message for KCNH2 channels has been detected in GI muscles of laboratory animals (1, 17) have led to the suggestion that the actions of cisapride on human GI motility may be mediated, in part, by blockade of KCNH2 channels in the smooth muscle. The present study addresses the potential role of KCNH2 channels in human GI smooth muscle by: 1) characterizing the transcriptional expression of KCNH2 in human jejunum, 2) investigating whether KCNH2 channels are expressed at the protein level in jejunal smooth muscle cells, and 3) characterizing the actions of KCNH2 blockers on membrane potential and contractile activity in smooth muscle and [14C]ACh release from nerves in human jejunum. Our results suggest that KCNH2 channels are present in human jejunal muscles and play a fundamental role in the control of electrical and contractile activities of this muscle.

METHODS

Human jejunal segments were obtained as surgical waste tissues during gastric bypass operations performed for morbid obesity. The protocol was approved by the University of Nevada, Reno and the University of California, Davis Human Subjects Research Committees.

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Structure of the KCNH2 gene. The human KCNH2 gene (NT_007914) is a single copy gene on chromosome 7. This gene contains 15 exons and 14 introns spanning 33,129 bp (Fig. 1). Two discrete transcripts have been completely sequenced and characterized (i.e., NM_000238, AB044806) (13, 28). A third transcript closely related to AB044806 has recently been sequenced (BC001914) but not characterized. When the amino acid sequences of these three splice variants are aligned, it is apparent that they contain a different exon at the NH2 and/or the COOH terminus. By comparing these sequences back to the known KCNH2 genomic DNA sequence, it is apparent that these variants arise from alternative splicing of the gene at exon(s) 1 and/or 9 (Fig. 1). The splice variant AB044806 begins at exon 1, whereas the alternative splice variant BC001914 begins at exon 2. These two variants differ significantly from NM_000238 at the COOH-terminal end. Exon 9 contains a stop codon that can lead to a truncated COOH-terminal end. Both AB044806 and BC001914 contain this truncation, and therefore, we have designated both as KCNH2 V2, because the primers used in this study cannot distinguish between them. The proteins represented by KCNH2 V2 are predicted to have amino acid lengths of either 888 or 772 (i.e., configuration AB044806 or BC001914, respectively). The other splice variant we investigated (NM_000238) is the one that encodes for a protein that has been sequenced in human brain and was first

Fig. 1. Alternative splice variants of the human KCNH2 gene. Schematic diagram of the human KCNH2 gene obtained from GenBank genomic sequences. This gene is alternatively spliced at exon 1 and 9. Alternative splicing at exon 9 introduces a stop codon that leads to a truncated protein. Stop codons (3) in exons 15 and 9 V2, respectively are indicated. The primers for this study are shown under the exons. The table indicates the nucleotide sequences of primers, their gene specificity, and the relevant accession numbers. Erg1-sm refers to a unique smooth muscle isoform (24). Accession numbers shown are as follows: a, NM_000238; b, AB044806; c, BC001914; d, NM_013569.
designated as the human ERG (HERG) channel (28). NM_000238 begins at exon 1. Thus the N-\textsubscript{2}-terminal end of NM_000238 is the same as AB044406. At the COOH-terminal end, the second half of exon 9 is ligated to exon 10, yielding an elongated protein 1,159 amino acids long. We designated this splice variant as KCNH2 V1 (see Fig. 1). The variants KCNH2 V1 and KCNH2 V2 are of particular interest, because they contain a different cyclic nucleotide binding domain (cNBBD) at the COOH terminus. In Fig. 1 we have also summarized the oligonucleotides synthesized for use in RT-PCR (Bio-source, Camarillo, CA) designed to analyze the expression of KCNH2 V1 and KCNH2 V2.

**RT-PCR.** Human brain poly A\(^+\) RNA obtained from normal whole brains pooled from three male and female Cauca-sians and human genomic DNA obtained from human whole blood were commercially purchased (Clontech, Palo Alto, CA). Human colon tissues were obtained during elective cervical dislocation. The mucosa was removed from samples of jejunum and colon and the muscularis was cut into strips. Total RNA was extracted from brain, colon, and jejunum strips, or from 20 freshly dispersed smooth muscle cells as described previously (7). First-strand cDNA was synthesized by using 200 units of SuperScript II RNase H\(^-\)RT (GIBCO-BRL) at 42°C for 50 min in the presence of 1 \(\mu\)g total RNA or 25 ng poly A\(^+\) RNA in a 20-\(\mu\)l reaction volume. PCR reactions were performed by using a GeneAmp PCR System 2400 (Perkin-Elmer) by adding 12.5 \(\mu\)l of AmpliTaq Gold PCR master mix (Applied Biosystems, Branchburg, NJ), 1 \(\mu\)l of the synthesized cDNA or 100 ng of human genomic DNA, and 10 \(\mu\)M primers in a 25-\(\mu\)l reaction volume. The amplification procedure was as follows: 1 cycle at 95°C for 10 min; 35 cycles for 15 s at 95°C and for 1 min at 60°C. After PCR, 5 \(\mu\)l of the RT-PCR product was analyzed on a 1.5% agarose gel. The fragments amplified by RT-PCR were eluted by using a PCR purification kit (QIAGEN, Valencia, CA) and analyzed by digesting restriction enzymes or direct sequencing.

**Quantitative RT-PCR.** cDNAs transcribed from RNAs of jejunum tissues or cells were prepared as described in RT-PCR. Real-time quantitative PCR was performed by using Syber Green chemistry on an ABI Prism 5700 sequence detector (PE Biosystems, Foster City, CA). Regression analysis of the mean values of eight multiplex RT-PCR for log\(_{10}\)-diluted cDNA was used to generate standard curves. Unknown quantities relative to the standard curve for a set of KCNH2 primers were calculated yielding the transcriptional quantification of KCNH2 relative to the endogenous GAPDH standard. The data were plotted by using Excel Chart Wizard and graphed by using the GraphPad Prism. The PCR samples were analyzed on a 1.5% agarose gel and confirmed by analyzing restriction enzyme digestion.

**Immunohistochemistry.** Tissue samples were pinned onto a Sylgard dish with the mucosal side up and fixed in 4% paraformaldehyde (wt/vol) made up in 0.1 M PBS. Once fixed, the tissue was washed for 30 min in PBS (0.1 M, pH 7.4). Small muscle strips were cut by using a razor blade and cryoprotected in a series of graded sucrose solutions. Tissues were then embedded overnight in a solution containing tissue Tek (Miles, IL) and 20% sucrose in PBS (1:2 parts; vol/vol) and then were rapidly frozen in isopentane precooled liquid nitrogen. Cryosections were cut to a thickness of 8 \(\mu\)m and collected on Vectabond (Vector Laboratories, Burlingame, CA)-treated slides. Nonspecific binding was reduced by incubating the slides in 1% BSA for 1 h at room temperature. For the double immunolabeling, tissues were incubated in each of the primary antibodies for 48 h in a sequential manner. Antibodies used were polyclonal rabbit anti-HERG antibody (6 \(\mu\)g/ml) directed toward the COOH terminus of KCNH2 V1 (GenBank accession no. Q12809; Alalone Labs, Jerusalem, Israel) and made up in PBS (pH 7.2), c-kit (CD117–104 D2 mouse monoclonal antibody to human c-kit; DAKO, Kyoto Japan) diluted to 1:400 (3 \(\mu\)g/ml), protein gene product (PGP) 9.5 (1:100; Biogenesis, United Kingdom). The secondary antibodies (Vector Laboratories, Burlingame, CA) were diluted 1:150 in PBS. The secondary antibody used to detect Kit labeling was FITC-coupled to human anti-mouse, and for the detection of HERG labeling, Texas-red-coupled streptavadin was used. All the secondary antibody incubation was performed for 30 min at room temperature. Tissues were examined with a confocal microscope (model MRC 600; Bio-Rad Hercules, CA) with an excitation wavelength appropriate for FITC (494 nm) and Texas Red (595 nm). For negative controls, primary antibody was omitted, and PBS was added in its place to control for nonspecific binding of the secondary antibody. Control predesorbed Tissue Tek slides were exposed to 10 \(\mu\)g/ml of the antibodies used, and PBS was added in place to control for nonspecific binding of the secondary antibody.

**Contractile activity and membrane potential.** Muscle samples were pinned in a dissecting dish containing oxygenated Krebs-Ringer bicarbonate solution (KRB) of the following composition (in mM): 118.5 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgCl\(_2\), 23.8 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), and 11.0 dextrose. This solution had a pH of 7.4 at 37°C when bubbled to equilibrium with 95% O\(_2\)-5% CO\(_2\). Unless otherwise specified, all experiments were performed in the presence of 100 \(\mu\)M N\(^\text{\text{-}}\)nitro-L-arginine (L-NNA) and 1 \(\mu\)M atropine. In some experiments, TTX (1 \(\mu\)M) was included as well.

Muscle strips (15-mm long) for intracellular and contractile recording were cut parallel to the circular muscle fibers with a knife consisting of a pair of parallel scalpel blades set 1.5 mm apart. Mucosa was removed from strips by sharp dissection. For contractile recordings, muscle strips were attached to a force transducer in an organ bath, and a resting force of 1 g was applied. These muscles began phasic contractions 30–45 min after applying 1 g of tension to the strips.

For intracellular recordings, tissue strips were pinned in the recording chamber to expose the entire circular muscle thickness. One end of the muscle was attached to a tension transducer, and experiments began by warming the tissue and waiting for the onset of spontaneous rhythmic contractions to ensure the health of the tissue. Thereafter, tissues were exposed to 10–25 \(\mu\)M wortmannin for 20 min and were then washed for at least 30 min in regular KRB solution before beginning experiments. This treatment greatly reduced or abolished spontaneous contractions, making it possible to maintain long-term impalement that would otherwise be impossible during ongoing phasic contractions. We have previously used this procedure in studies of the human colon (20) and dog colon (4) and found that the electrical activity of the tissue remains unchanged, whereas contractions are greatly reduced due to irreversible binding of wortmannin to myosin light-chain kinase. In addition, the frequency and amplitude of slow waves in our study are the same as those previously described Hara et al, (10) in studies of human jejunum before wortmannin was available. Muscle cells were impaled with glass microelectrodes filled with 3 M KCl and having resistances ranging from 60 to 100 M\(\Omega\). Membrane potential was measured with a high input impedance electrometer (model Duo 773; World Precision Instruments, New Haven, CT), and outputs were displayed on an oscilloscope (Hitachi). Analog electrical signals were digitized and recorded on videotape (Vetter 875) and AcqKnowl-
edge version 3.2.4 software (Biopac System) for later data analysis.

Transmitter release experiments. Strips of jejunal muscularis (1.5 cm × 2 mm strips) were secured between platinum plate electrodes and incubated at 37°C in modified Krebs solution of the following composition (in mM): 140 NaCl, 5 KCl, 1 MgCl₂, 1.5 CaCl₂, 5 HEPES, 0.03 EDTA, 0.06 ascorbic acid, 10 glucose, containing [¹⁴C]choline chloride (2.5 μCi/ml; New England Nuclear-Life Science Products). Tissues were initially stimulated with electrical field stimulation (EFS) (1-Hz, 1-ms pulses) for 40 min to allow uptake of [¹⁴C]choline chloride. Tissues were then placed into 300-μl perfusion chambers and superfused (1.8 ml/min) with Krebs solution containing hemicholinium-3 (10⁻⁶ M). After 50-min superfusion, the tissues were stimulated (5 Hz, 1 ms, 1 min) and samples were collected every minute in scintillation vials. The first stimulus (S1) was delivered at minute 50. S2, S3, and S4 were delivered at minutes 90, 130, and 170, respectively. Ecolume scintillant (4.8 ml; ICN Biomedicals, Cleveland, OH) was added to each vial and [¹⁴C] was counted (model LS6001C; Beckman). The tissues were transferred to scintillation vials, solubilized overnight in 1 ml of 10% NaOH, neutralized with HCl and buffered with HEPES before counting. Overflow of [¹⁴C] was calculated as a fraction of the entire [¹⁴C] content of the tissue at the time of stimulation. This technique has been used frequently to study the release of ACh from the tissue (2).

Statistics. Significant differences between means were calculated by a two-tailed paired or unpaired Student’s t-test, and values were considered significantly different when P < 0.05; n values represent the number of patients. Significant differences between [¹⁴C]ACh transmitter release groups was determined by first normalizing each S3 response to the S2 response in the same tissue. Groups were then compared by using ANOVA followed by all pairwise multiple comparison (Tukey’s test).

Drugs. 1-[2-(6-Methyl-2-pyridinyl)ethyl]-4-(4-methylsulphonylaminobenzoyl)piperidine (E-4031) was obtained from Wako. N-{1’-(6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl)-3,4-dihydro-4-hydroxyspiro-2H-1-benzopyran-2,4’-piperidine]-6-yl-(+)-methanesulfonamide monohydrochloride (MK-499) was kindly donated by Merck, and [¹⁴C]choline chloride was obtained from New England Nuclear-Life Science Products. 4-Aminopyridine (4-AP), TTX (atropine), nifedipine, t-NNA, hemicholinium-3, and wortmannin were obtained from Sigma.

RESULTS

Comparison of KCNH2 transcripts in human and murine tissues. Recent studies (24) suggest that there is a unique smooth muscle form of KCNH2 expressed in human colon (erg1-sm). Surprisingly, the erg1-sm transcript, as reported, shares 99% homology with murine KCNH2. We designed primers specific for erg1-sm (m-2 and m-2r) to determine whether this transcript is present in human jejunum. These primers were designed to amplify a region of erg1-sm spanning exons 6 and 7 and encoding domains S3 to S5 of erg1-sm channels (24). The same primers would also amplify this region in murine KCNH2. We also designed primers (h-2 and h-2r) spanning the same region of the human brain isoform of KCNH2 (see Fig. 2A). These primers, lacking sufficient homology with erg1-sm, would not be expected to amplify erg1-sm, but would amplify both hKCNH2 V1 and V2 (see Fig. 1).

Expression of KCNH2 mRNAs in murine and human brain, colon, and jejunum were determined by RT-PCR. Each set of primers detected amplicons of 273 bp (Fig. 2B). However, the primers were species specific and showed no cross-reactivity between mouse and human. Thus erg1-sm primers amplified murine KCNH2 transcripts but did not amplify transcripts from human tissues. All amplicons were free of genomic DNA contamination, because genomic DNA would contain intron 6 and result in an amplicon of 468 bp for mouse (NW_0002925) and 590 bp for human (NT_007914). Two other pairs of gene-specific primers were also tested to confirm these results (primer pairs m-1, m-1r and m-3, m-3r; see Fig. 1), and each of these pairs also detected species-specific amplicons of the expected sizes in murine and human brain, colon, and jejunum (data not shown).

All amplicons of the 273-bp fragments in Fig. 2 were sequenced. The cDNA sequences of the amplicons of mouse and human tissues show 100% homology with those previously reported for mKCNH2 and hKCNH2, respectively. The 273-bp fragment of mouse KCNH2 cDNA has 91% homologous with that of hKCNH2. However, because all differences in base pairs through this region are silent, the amino acid sequences of the two species are identical in mKCNH2 and hKCNH2. Thus the mRNA sequence of domain S3 to S5 in human jejunum and colon are identical to published sequences of hKCNH2 V1 and V2. Significantly, this base-pair sequence will not give rise to the alanine to valine substitution (erg1-sm) reported by Shoeb et al. (24).

Splice variants of KCNH2 in human jejunum. RT-PCR detected splice variants KCNH2 V1 and V2 in human brain and jejunum and in isolated smooth muscle cells (Fig. 3A). Use of specific primers for KCNH2 V1 (h-5 and h-5r V1) and V2 (h-5 and h-5r V2) produced amplicons of predicted sizes (i.e., 140 and 158 bp, respectively) in brain and jejunal muscles and smooth muscle cells. We excluded the possibility of contamination from genomic DNA by testing the same primers against human genomic DNA. Primers h-4 on exon 8 and h-4r on exon 9 generated a 645-bp fragment from genomic DNA (due to the presence of intron 8). The same primers produced 144-bp fragments from cDNAs from brain, jejunal muscles, and jejunal smooth muscle cells (Fig. 3A). These sizes agreed with those predicted from the genomic DNA sequence (NT_007914) and the cDNA sequences reported for hKCNH2.

Quantitative RT-PCR revealed that KCNH2 V2 was the dominant variant expressed in human brain and jejunal muscles. Both V1 and V2 were expressed in greater quantity in brain than in jejunum. The relative transcriptional expressions of KCNH2 V1 and V2 are shown in Fig. 3B. KCNH2 expression (relative to GAPDH in arbitrary units; means ± SE; n = 3) in brain was 0.0023 ± 0.0006 for both V1 and V2 and 0.00046 ± 0.000046 for V1 and 0.0011 ± 0.00035 for V2; in jejunum expression of KCNH2 was 0.0014 ± 0.00043 for both V1 and V2 and 0.00016 ± 0.000022 for V1 and 0.0007 ± 0.00035 for V2.
KCNH2-like immunoreactivity in human jejunum smooth muscle. We then performed immunohistochemistry to determine whether transcriptional expression of KCNH2 resulted in protein expression in jejunal muscles. These studies utilized a polyclonal antibody directed toward amino acids 1106–1159 of hKCNH2 (GenBank accession no. Q12809). This antibody is highly selective for KCNH2 VI over KCNH2 V2, because the epitope is missing from KCNH2 V2.

KCNH2 VI-LI (KCNH2 VI-LI) was identified in both longitudinal and circular muscle cells (Fig. 4A). KCNH2 VI-LI was not resolved in ICC, which were identified in double-labeling experiments with a monoclonal antibody for Kit receptors (Fig. 4C). KCNH2 VI-LI was also observed in enteric neurons identified by double labeling with a monoclonal antibody for PGP (9.5) (Fig. 4, D–F). KCNH2 has previously been localized to some neuronal preparations (23) but to our knowledge this is the first time it has been identified in enteric neurons.

Effects of KCNH2 (HERG) channel blockers on contractions of jejunal muscles. Isolated strips of human jejunum contracted rhythmically at 9.1 ± 0.2 (n = 22) cycles/min in the absence of external stimuli (Fig. 5A). The neural blocker TTX (1 μM) did not alter the pattern of spontaneous contractile activity. We tested the methanesulfonide compound, E-4031 (Class III antiarrhythmic), a specific blocker of KCNH2 (HERG) channels (25) to investigate the role of this conductance in regulating contractile activity. Low concentrations of E-4031 (10^{-9} M to 10^{-8} M) enhanced the amplitude of phasic contractions (Fig. 5Aa). Above 10^{-8} M, the amplitude and frequency of contractions decreased (Fig. 5Ab), and at 10^{-6} M, phasic contractions were nearly abolished and the muscles generated tone (Fig. 5Ac).

Comparison of effect of high KCl and E-4031 on contractile activity. Cause of the inhibitory effects of blockers on phasic contractions was unclear, because blocking K^+ channels might be expected to depolarize the tissue and increase contractions. Therefore, we compared the effects of nonspecific depolarization by raising extracellular potassium concentration ([K_o]) to the effects of KCNH2 channel blockers. When [K_o] was
raised above 20 mM, phasic contractile amplitude was reduced, and at the highest concentration tested (i.e., 45 mM \([K_0]\)), phasic contractions were blocked. This was accompanied by the development of tone (Fig. 7). The amplitude of tonic contraction with 45 mM \([K_0]\) was not different than that observed with 10^{-4} \text{M} E-4031 (i.e., 27 ± 7.7 vs. 21.3 ± 9.3% of the phasic contractile amplitude, respectively; \(P = 0.1313\)). These data suggest that depolarization per se is capable of producing the kind of inhibitory contractile effects observed with KCNH2 channel blockers in human jejunal muscles.

Effects of KCNH2 blocker E-4031 on the electrical activity in the human jejunum. We also studied the effects of KCNH2 blockers on the membrane potentials of circular muscle cells to directly test the effects of these compounds on electrical parameters. Under control conditions, resting membrane potential averaged \(-61 ± 4.3\) mV (\(n = 30\)) and slow waves were observed with a mean amplitude and frequency of \(8.6 ± 0.6\) mV and \(10.3 ± 0.9\) cycles/min (\(n = 35\)), respectively. One to six APs (2.2 ± 0.2, \(n = 33\)) were typically superimposed on slow waves (Fig. 8B). The addition of low concentrations (e.g., \(10^{-9}\) to \(10^{-8}\)M) of E-4031 led to an increase in the amplitude and frequency of slow waves. This effect was concentration-dependent, with the highest concentration tested (45 mM \([K_0]\)) leading to complete block of the slow waves. These results suggest that depolarization per se, in addition to inhibiting phasic contractions, can also affect the electrical activity of circular muscle cells in the human jejunum.
in the number of spikes per slow wave without depolarization in the resting membrane potential (defined as the maximal level of polarization between slow waves; see Fig. 8C). Intermediate concentrations (3 × 10⁻⁸ to 3 × 10⁻⁷ M) of E-4031 depolarized the resting potential and reduced slow-wave frequency (Fig. 8D). Higher concentrations (10⁻⁶ M) resulted in continuous spiking from a potential similar to the peak of slow waves (Fig. 8E). Removal of E-4031 reversed these effects, but the reversal required ≤1 h in the case of higher concentrations of E-4031. MK-499 had similar effects, including depolarization and continuous spiking at high concentrations. The mean concentration-response relationships for membrane potential with E-4031 and MK-499 are shown in Fig. 9.

**Effects of KCNH2 blocker E-4031 in the presence of L-type Ca²⁺ channel blockade.** Increased generation of APs after KCNH2 blockers would be expected to increase cell Ca²⁺. Because Ca²⁺-activated inward currents (e.g., Cl⁻ conductances) are known to be present in smooth muscle cells (14), it is possible that the depolarization caused by KCNH2 blockers could be secondary to Ca²⁺ entry. Therefore, we carried out additional experiments in the presence of nifedipine to reduce Ca²⁺ entry in response to depolarization. As expected, exposure to nifedipine (10⁻⁶ M) completely abolished phasic contractile activity in this tissue. It also abolished the APs superimposed on slow waves and significantly reduced the amplitude and duration of slow waves. In the presence of nifedipine, E-4031 still produced significant depolarization of membrane potential (e.g., 8.3 ± 1.1 mV; n = 8). An example of this depolarization is shown in Fig. 10.

**Effect of E-4031 on [¹⁴C]ACh release from enteric neurons in the human jejunum.** Because immunohistochemical studies revealed KCNH2-LI in enteric neurons, additional experiments were performed to determine whether KCNH2 blockers affected release of the excitatory neurotransmitter ACh. Strips of human jejunum were loaded with radiolabeled [¹⁴C]ACh and transmitter release was measured in response to 5-Hz EFS. Electrical stimulation enhanced release of [¹⁴C]ACh in a TTX-sensitive manner (Fig. 11, A and B). The pattern of release was not modified by either E-4031 (10⁻⁶ M, n = 4; Fig. 11C) or MK-499 (n = 1; data not shown). As a positive control, we tested the...
effects of the K⁺ channel blocker, 4-AP (10⁻³ M), which enhances transmitter release from enteric neurons (6). In contrast to E-4031, 4-AP (10⁻³ M; n = 3) enhanced the release of [¹⁴C]ACh from tissues (Fig. 11D).

**DISCUSSION**

GI smooth muscle cells respond to the depolarization/repolarization cycles (i.e., slow waves) generated by ICC, and the extent of smooth muscle depolarization in response to these events affects mechanical responses. By regulating excitability and responsiveness of smooth muscle cells to slow-wave depolarizations, the K⁺ channels expressed by these cells play an important role in controlling the motility patterns of the GI tract. A variety of K⁺ channels have been identified that contribute to the excitability of GI muscles (8, 12).
Recently, studies in animal models suggest that KCNH2 channels contribute to the regulation of membrane potential in GI muscles (1, 17). In the present study, we show transcriptional expression of KCNH2 in muscles of human jejunum and colon. Sequencing of the region spanning the S3 to S5 domains indicate that this region of jejunal and colonic KCNH2 channels are 100% homologous with previously published sequences for human KCNH2. This conductance appears to play an active role in the control of membrane potential in

Fig. 7. Effect of raised extracellular potassium concentration ([K]o) on the pattern of contractile activity in the human jejunum. Raising [K]o above 21 mM dramatically reduced the amplitude of phasic contractions. At 45 mM [K]o, phasic contractions ceased and only a small tonic contracture remained.

Fig. 8. Effect of E-4031 on electrical activity. A: continuous recording from a single cell over a 37-min time period. E-4031 (10⁻⁸ M) was added at the arrow. The concentration was increased to 4 × 10⁻⁸ M at the 2nd arrow. Note that membrane potential significantly depolarized after the addition of 4 × 10⁻⁸ M E-4031. B–E: excerpts of the trace in A displayed at faster sweep speeds. The time at which each sample recording was taken is indicated the letters above the trace in A. The first trace (B) shows control activity with ongoing slow waves and superimposed spikes. The second trace (C) shows activity recorded in the presence of 10⁻⁸ M E-4031. Note that the number of spikes per slow wave has increased and the amplitude of the spikes increased. D: electrical activity in the presence of 4 × 10⁻⁸ M E-4031. This increased the duration of slow waves and greatly increased the number of spikes per minute. E: development of the response to 4 × 10⁻⁸ M E-4031. At this point no discernible slow wave was apparent and there was nearly continuous spiking. F: greatly expanded trace of the slow wave and spikes denoted by an F in C. G: spikes during the continuous activity in E (denoted above the trace by G).
human jejunal smooth muscle, because inhibitors of KCNH2 channels depolarized membrane potential and increased contractions. Thus conclusions drawn from studies of animal models can be translated into a role for KCNH2 channels in the control of human small bowel motility. Our data also suggest that the effects of cisapride, a benzamide derivative used as a prokinetic agent to treat various motility disorders in human patients, may be partially mediated through block of KCNH2 channels, because compounds of this group block this conductance (16, 21).

KCNH2 (HERG1) was originally cloned from human brain cDNA library (NM_000238) (28) using probes on the basis of the Drosophila eag. The human KCNH2 gene (NT_007914) is a single-copy gene on chromosome 7. This gene contains 15 exons and 14 introns spanning 33,129 bp. A recent study suggests that a unique isoform of KCNH2 (i.e., erg1-sm) is present in human (GenBank accession no. AY130462) and rabbit colonic smooth muscles (AF439342) (24). The reported cDNA sequences of human and rabbit colonic smooth muscles (AF439342) (24).

The reported cDNA sequences of human and rabbit erg1-sm are 99% homologous to that of mouse KCNH2 (NM_013569), but human and rabbit erg1-sm are 99% homologous to that of mouse KCNH2. The reported cDNA sequences of human and rabbit erg1-sm are 99% homologous to that of mouse KCNH2.

It was also reported that the erg1-sm isoform contained an alanine-to-valine substitution in the S4 segment of the channel. This region is important for voltage sensing. The substitution was shown to lead to faster activation and deactivation kinetics when erg1-sm channels were expressed in Xenopus laevis oocytes compared with hKCNH2 channels (24).

It is possible that the sequence published for erg1-sm cDNAs is an error due to accidental contamination with a mouse KCNH2 cDNA clone. All of the reported mutations in the erg1-sm of rabbit and human colon, including a single nucleotide deletion resulting in erg1-sm truncation by 101 amino acids in the COOH terminus, could be due to Taq DNA polymerase error when the erg1-sm cDNAs of 3.5 kb were amplified by PCR. In general, all commercial Taq DNA polymerases, including proofreading Taq DNA polymerases, have an error rate of 2.6–16% of mutated PCR products (1-kb target, over 20 cycles) depending on PCR conditions (Stratagene).

In 1998, Kupershmidt et al. (13) described a COOH-terminal splice variant of KCNH2 and termed it HERGUSO (KCNH2 V2). Message for this variant was detected in human heart, brain, uterus, and lymphocytes (13), and greater expression for HERGUSO has been observed than KCNH2 V1 (HERG1) in some tissues. KCNH2 V2 (HERGUSO) is identical to KCNH2 V1 (HERG1) up to the region of the cNBD at the COOH-terminal end where it is truncated at the end of exon 9. Despite the greater amount of HERGUSO message, expression studies with HERGUSO in Ltk− cells failed to give rise to significant current. Although transfection of GFP was noted in these studies, there was no direct evidence that channels were trafficked to the membrane surface (13). Coexpression of HERGUSO with HERG1 resulted in a current with properties more similar to those of native cardiac rectifier K+ current (I_Kr) than HERG1 channels alone, suggesting that the endogenous HERG conductance may be com-

![Fig. 9. Summary of the effects of E-4031 on resting membrane potential (defined as the most negative potential achieved between slow waves or between spikes when no slow wave was resolved). Both KCNH2 blockers depolarized muscle cells.](image)

![Fig. 10. Effects of E-4031 on membrane potential in the presence of nifedipine. In this trace, nifedipine was present throughout. Nifedipine abolished spike potentials that are normally superimposed on slow waves. The addition of E-4031 (10^-6 M) in the continued presence of nifedipine depolarized membrane potential.](image)

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posed of HERG1 and HERGUSO subunits. However, others have failed to find expression of KCNH2 V2 (HERGUSO) with HERG1 in human atrial and ventricular cells (19). We found greater expression of KCNH2 V2 (HERGUSO) message than KCNH2 V1 (HERG1) message in human jejunal muscles, but at present, we do not know the consequences of HERGUSO expression on the current density or composition of native KCNH2 channels in GI muscles.

KCNH2 channels are best known for their contribution to the cardiac AP. During the repolarization phase of the cardiac AP, KCNH2 channels recover from inactivation and remain open long enough to significantly contribute to further repolarization (15, 22). Mutations in KCNH2, lead to the chromosome 7-linked form of congenital long QT syndrome, a lengthening of the cardiac AP that can give rise to fatal arrhythmias (5). KCNH2 channels do not appear to play a significant role in the maintenance of resting membrane potential in cardiac muscles. KCNH2 channels have also been identified in other cell types, and in some tissues, the role of these channels differs significantly from cardiac muscle. For example, in rat lactotrophs, rabbit carotid glomus cells, mammalian neuroblastoma cells, rat microglia, and rat stomach myocytes there is evidence that KCNH2 channels significantly contribute to the setting of resting membrane potential (3, 17, 18, 29). Contributions of this conductance to resting potential are due to overlap of the voltage dependence of activation and steady-state inactivation that results in significant “window current” in the range of the resting potential. Studies of KCNH2-like currents in opossum esophagus suggest that window current contributes to the resting membrane potentials of GI muscles (1). Our data on human jejunal muscles are consistent with this role of KCNH2. Thus subtle changes in the conductiv- ity or availability of KCNH2 channels in GI muscles.

Fig. 11. Effect of various blockers on [14C]ACh release in human jejunal strips. A: control [14C]ACh release as a function of time. Samples were taken at 1-min intervals. Nerves were stimulated for 1 min with electrical field stimulation (EFS; 5 Hz, 1 ms duration) at minute 5. The maximum release of transmitter occurred 2 min after the start of EFS. Because the content of [14C]ACh in the tissue declines with time, the third stimulus (S3) is always smaller than the second stimulus (S2). B: neural blocker TTX (10⁻⁶ M) greatly reduces [14C]ACh release during S3, but [14C]ACh release returns with S4 after washout of TTX. C: relationship between S2 and S3 is unchanged in the presence of E-4031 (10⁻⁶ M). D: In contrast to E-4031, the amplitude of S3 is significantly greater (ANOVA, P < 0.05) than S2 in the presence of 4-aminopyridine (4-AP; 10⁻³ M).
of blockers was to reduce both contractile amplitude and frequency. This was accompanied by slow waves of longer duration and reduced amplitude spikes. At the highest concentrations of channel inhibitors we used, slow waves were abolished and membrane potential was relatively steady at a depolarized potential. Small amplitude spikes were often superimposed on the tonic depolarization. The decrease in contractile amplitude at higher concentrations of KCNH2 blockers was likely due to the decrease in AP amplitude that resulted from depolarization. APs and phasic contractions in human jejunal muscles appeared to be due to Ca$^{2+}$ entry via voltage-dependent (L-type) Ca$^{2+}$ channels, because both responses were entirely abolished by nifedipine. Depolarization of membrane potential by KCNH2 blockers would be expected to reduce AP amplitude due to the reduction in driving force for Ca$^{2+}$ and the development of voltage-dependent inactivation of Ca$^{2+}$ channels that would accompany depolarization. A similar phenomenon is predicted to occur if smooth muscle cells are tonically depolarized by increasing [K]o. Indeed, we found that raising [K]o to 45 mM mimicked the effects of high concentrations of KCNH2 blockers.

In immunohistochemical studies, we used a polyclonal antibody directed toward amino acids 1106–1159 in the COOH terminus of KCNH2 V1. KCNH2 V1-like immunoreactivity (KCNH2 V1-LI) was observed in longitudinal and circular muscle cells. KCNH2 V1-LI was also found in enteric neurons, but we did not resolve immunoreactivity in ICC. At present, there are no antibodies available specific for the COOH terminus of KCNH2 V2, so we cannot confirm that transcriptional expression of KCNH2 V2 resulted in functional protein.

All mechanical and electrophysiological studies were done in the presence of antagonists to block nerve conduction and postjunctional effects due to the major excitatory and inhibitory motor neurotransmitters. Thus our study focused particularly on the role of KCNH2 channels in smooth muscle. It is possible that additional effects of KCNH2 blockers on enteric neurons could add to the overall changes in motility produced by these blockers. This topic requires further study. However, we did note that E-4031 was without effect on the release of [14C]ACh from strips of the tunica muscularis suggesting effects on the smooth muscle may predominate.

There is ample evidence that the prokinetic drug cisapride can inhibit KCNH2 channels in both native cells and expression systems (16, 21, 26, 27). In 2000, cisapride was removed from common usage in the United States because of deleterious effects on cardiac KCNH2 channels leading to long QT syndrome (16). Our data suggest that in addition to 5-HT receptors, human GI smooth muscle KCNH2 channels are also a likely target for this drug. Further elucidation of the complete sequence of KCNH2 channels and the subunit composition of these channels in human GI muscles may ultimately lead to the development of new drug therapies with greater specificity for GI dysmotilities.

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