Isolation and characterization of the human gene encoding I_{to}: further diversity by alternative mRNA splicing

WEI KONG,1 SUNNY PO,1 TOSHIO YAMAGISHI,1 M. DOMINIQUE ASHEN,1 GAIL STETTEN,2 AND GORDON F. TOMASELLI1
1Department of Medicine, Section of Molecular and Cellular Cardiology, and 2Department of Gynecology and Obstetrics, Johns Hopkins University, Baltimore, Maryland 21205

Kong, Wei, Sunny Po, Toshio Yamagishi, M. Dominique Ashen, Gail Stetten, and Gordon F. Tomaselli.


The transient outward K\(^+\) current (I_{to}) in the heart is responsible for the initial phase of repolarization and for setting the plateau voltage of the ventricular action potential. Recently, Kv4.3 has emerged as the leading candidate K\(^+\) channel gene that underlies I_{to} in larger mammals such as dogs and humans. We have cloned the human Kv4.3 homolog and describe a carboxyl-terminal splice variant that inserts 19 amino acids with a consensus protein kinase C (PKC) phosphorylation site into the protein after the last membrane-spanning segment. The coding region of Kv4.3 is comprised of at least five exons and is located on chromosome 1p13.3. In the basal state the basic biophysical properties of both of the splice variants are identical.

potassium channel; Kv4.2; Kv4.3; Xenopus oocytes; heterologous expression; fluorescence in situ hybridization; chromosome 1

THE CARDIAC ACTION POTENTIAL is generated by the composite activity of many ion channels, pumps, and transporters. Voltage-dependent K\(^+\) channels are particularly prominent in repolarization of cardiac myocytes. K\(^+\) channels exhibit extreme physiological diversity, which arises from the large number of K\(^+\) channel genes, alternative RNA splicing, heteromultimeric assembly of channel proteins, and the existence of accessory subunits (for review see Ref. 3). The determination of the molecular basis of K\(^+\) currents resident in excitable tissue is complicated by the diversity of K\(^+\) channel genes.

The transient outward K\(^+\) channel (I_{to}) is especially important in the early phase of repolarization in many species, including human (5, 10, 14, 17, 21, 23–25, 31, 37, 40, 41). Recently, the gene encoding a K\(^+\) channel subunit that underlies the calcium-independent I_{to} has been described. On the basis of analysis of mRNA transcripts in mammalian ventricles (12) and correlation of the mRNA level with that of I_{to} current density in human ventricular myocytes (20), Kv4.3 has emerged as the leading candidate K\(^+\) channel gene encoding the cardiac I_{to} in large mammals such as dogs and humans.

Physiological and pathophysiological stimuli can alter the level of expression of K\(^+\) channel genes, protein, and current (16, 20, 32, 36, 38). The first step in understanding the molecular basis of the regulation of K\(^+\) currents in response to biological stimuli is the cloning of the cDNA and genomic DNA encoding relevant K\(^+\) channel genes. We describe the cloning, functional expression, and chromosomal location of the human Kv4.3 (hKv4.3) K\(^+\) channel. The hKv4.3 gene has two alternatively spliced forms, with the long version containing a 19-amino acid insert after the S6 membrane-spanning region. Both variants are present in human atrial and ventricular myocardium.

METHODS

Cloning of human Kv4.3 cDNA and genomic DNA. Standard hybridization screening of a random primed human cDNA library in X-Zap (Uni-Zap, Stratagene) was performed to obtain partial cDNAs encoding hKv4.3. Degenerate oligonucleotide primers designed to the Shal (Kv4) family of K\(^+\) channels were used in the polymerase chain reaction with reverse transcribed cDNA (RT-PCR) from total human ventricular RNA as a template (12). A 290-bp fragment of the hKv4.3 channel in the amino terminus immediately preceding the first membrane-spanning segment was amplified. This fragment was used to screen the cardiac library, and four identical clones 5 kb in length without the 3'-end of hKv4.3 cDNA were identified. This 5-kb cDNA was subcloned into pBluescript II SK (Stratagene). The 3'-end of the coding region was obtained by RT-PCR of human cardiac RNA using rat-specific primers. The upstream primer located at the S2 transmembrane segment had the sequence 5'-CCCTCTCTCTGCTGGACAC-3' encoding the amino acid sequence FFCLD. The downstream primer encodes the last seven amino acids of the rat channel protein (VVVKSVL) with the nucleotide sequence 5'-CAAGACAGAGACCTTGACAAC-3'. The 3'-RT-PCR fragment was cloned into pGEM-T (Promega). The sequence of the 3'-end of the cDNA was confirmed by sequencing the corresponding human genomic DNA.

The genomic clone designated p54 (clone address PAC-137-07, Genome Systems) was one of three unique clones obtained by hybridization screening of a human PAC library with a cDNA containing the entire coding region of rat Kv4.2. The p54 clone contains a large 5'-untranslated region and the 5'-end of the hKv4.3 coding region.

A 1-kb Nael-I-Nar I fragment was excised from the 5'-end of hKv4.3 cDNA in pBluescript II SK. The plasmid pGEM-T containing the PCR product encoding the 3'-end of hKv4.3 was cut by Sac II, blunted with the Klengow fragment of DNA polymerase I, and then subsequently digested with Nar I. The 1-kb Nael-I-Nar I fragment was ligated to the pGEM-T vector to generate the full-length hKv4.3 cDNA (pGEM-T-hKv4.3). pGEM-T-hKv4.3 was cut by Apa I-Not I to get a 2-kb fragment that contains the entire coding region that was subcloned into pcDNA 3.1– (Invitrogen). The pRES-GFP-hKv4.3 expres-
sion vector was constructed by digesting pCDNA3.1(–)-hKv4.3 with Apa I and EcoR V. The 2-kb Apa I-EcoR V fragment was cloned into pIRE-GFP (Clontech, Palo Alto, CA) digested with Apa I and Sma I. The entire cDNA was sequenced on both strands with an ABI 3100 Sequencer (Perkin-Elmer).

Northern and Southern blots, ribonuclease protection assay, and PCR. Human ventricular myocardium was obtained from explanted failing hearts or donor hearts unsuitable for transplantation. The tissue was excised from the left ventricular free wall between the left anterior descending and left circumflex coronary arteries. The samples used for RNA isolation were transversial including both the epicardium and endocardium.

The tissue was quick frozen in liquid N2 within 10–15 min after tissue harvesting and stored at –80°C until further processing. Total RNA for RT-PCR was prepared either by using TRIzol reagent (GIBCO BRL) according to the manufacturer’s instructions or by centrifugation through a CsCl cushion (34). The integrity of all RNA samples was confirmed by analysis on a denaturing agarose gel and quantified by optical density measurements at 260 nm.

A multiple human tissue Northern blot was obtained from Invitrogen (catalog no. 11101-01). The blot was probed with a 480-bp fragment of hKv4.3 from nucleotides 1,485 to 1,965, corresponding to amino acids P496–L655. The blot was developed on a storage phosphor screen and then scanned on a phosphomager (Molecular Dynamics).

RT-PCR was performed on 5 µg of total RNA isolated from the left ventricular free wall. RT was performed with a poly-dT primer and Superscript II RT (GIBCO BRL) according to the manufacturer’s instructions. The primers 5′-CTCTGGAGCTGACGCGACC-3′ (F1) and 5′-GGGAGCAG-CAGGTTGGTGTTGG-3′ (R) span the insert and were used to amplify the short and long variants of hKv4.3. These primers generate PCR products of 231 and 288 bp in length. In addition, a forward primer in the insert, 5′-CCCCGTGGTGTGTACGACCCCTC-3′ (Fins) and the reverse primer were used to generate a 160-bp RT-PCR product.

Ribo-nuclease protection assays (RPA) were performed as described previously (11, 12, 20). All probes contained regions of plasmid sequence at one or both ends of the transcript, permitting easy distinction between any remaining undigested probe and the shorter, specifically protected region of the probe. Ten micrograms of yeast tRNA were used as a negative control to test for the presence of probe self-protection. For each sample point, 10 µg of total RNA were used in the assay. Determinations were performed in duplicate for each sample. Steady-state mRNA levels were quantified by exposing the gels on a storage phosphor screen and then scanning on a phosphomager (Molecular Dynamics).

Genomic DNA was prepared from white blood cells using a standard protocol (34). Genomic and p54 DNA were cut with EcoR V, BamHI I, and Bgl II, separated on 1% agarose, and transferred to nitrocellulose. The same fragment that was used to screen the cDNA library was used to probe the Southern blot.

Chromosomal localization. Fluorescence in situ hybridization was employed to confirm the chromosomal location of hKv4.3. One microgram of the p54 clone containing the genomic sequence was labeled with digoxigenin (Boehringer Mannheim) in a nick-translation reaction (GIBCO BRL), used as a probe, and hybridized to metaphase spreads from peripheral blood lymphocytes (29). Two hundred nanograms of labeled probe were precipitated with 5 µg of Cot-1 DNA; resuspended in 70% formamide, 2× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 10% dextran sulfate, pH 7.0; denatured for 5 min at 75°C; and preannealed for 1 h at 37°C. Slides were prepared from phytohemagglutininstimulated human blood by standard methods and denatured in 70% formamide for 5 min at 75°C before hybridization at 37°C for 39 h. The slides were washed twice in 50% formamide-2× SSC and then twice in 2× SSC at 42°C. The probe was detected with rhodamine-labeled anti-digoxigenin according to the manufacturer’s instructions (Oncor). Chromosomes were counterstained with the A-T binding fluorophore 4′,6-diamidino-2-phenylindole (DAPI) and viewed with a Zeiss Axioskop equipped with a SenSys cooled charge-coupled device camera (Photometrics) and Smart Capture imaging software (Vysis).

Heterologous expression and electrophysiology. Full-length cDNAs encoding both splice variants of hKv4.3 were cloned into the pIRES-GFP vector for bicistronic expression of the channel and green fluorescence protein driven by the human cytomegalovirus (CMV) major immediate-early promoter/enhancer in cultured mammalian cells. The expression cassette contains the hKv4.3 cDNA with an artificial intron upstream from the encephalomyocarditis that permits the translation of two proteins from a single RNA. Mouse Ltk- fibroblasts were used for channel expression. The culture and transfection conditions were as previously described (44). Transient transfection was performed by calcium phosphate precipitation for 6–12 h with 2.5 µg of the hKv4.3-containing expression plasmids per 35-mm dish.

Transfected cells were transferred to the stage of an inverted microscope (Nikon Diaphot). Cells expressing the channel were identified by epifluorescence. The cells were perfused with a bath solution containing (in mM) 140 NaCl, 5 KCl, 1 MgCl2, 2 CaCl2, 10 HEPEs, and 10 glucose, pH 7.4, at 3 ml/min. To determine the selectivity of the currents for K reveal, NaCl was replaced with an equimolar amount of KCl. Current were recorded with the whole cell configuration of the patch clamp at room temperature (22–23°C) using an Axopatch 200A (Axon Instruments). Pipettes were pulled from borosilicate glass with tip resistances of 2–3 MΩ when filled with the internal solution containing (in mM) 110 KCl, 1 MgCl2, 2 MgATP, 1 EGTA, and 10 HEPEs, pH 7.2 (final K concentration ~112 mM). Cell capacitance was estimated by integrating the area under an uncompensated 10-mV depolarizing voltage step from 80 mV; the cell capacitance was 8.6 ± 0.7 pF (n = 17). Series resistance was compensated as much as possible (generally 80–90%) such that the maximal uncompensated voltage error was <5 mV. Currents were low-pass filtered at 2 kHz and digitized at 10 kHz through a Digidata 1200 analog-to-digital interface (Axon Instruments) for offline analysis. Currents were corrected for small (<5 mV) liquid junction potentials (Axoscape, Axon Instruments) (4).

Pooled data are presented as means ± SE. Statistical comparisons were made using an ANOVA with P < 0.05 considered to be significant.

RESULTS

Isolation of Kv4.2 and Kv4.3 cDNA and genomic clones. The full-length cDNAs encoding hKv4.3 were obtained by a combination of screening of a cardiac cDNA library and RT-PCR of human ventricular RNA. The cDNA contains an open reading frame of 1,911 nucleotides encoding a 637-amino acid channel protein. The human Kv4.3 is highly homologous to the rat homolog (92 and 99% at the nucleotide and amino acid levels, respectively). The hKv4.3 shares 95% amino acid identity in the transmembrane regions and overall 76% identity with the related hKv4.2 channel (unpub-
lished data). RT-PCR of the carboxyl terminus of hKv4.3 reproducibly produced two bands that differed in size by 57 bp (Fig. 1A). Cloning and sequencing the PCR products from the carboxyl-terminal end revealed a short product that was homologous to the previously described rat Kv4.3 cDNA and a longer product containing a 19-amino acid insert after S6 (1,968 nucleotides encoding a 656-amino acid protein). This insert contains a consensus protein kinase C (PKC) phosphorylation site of the form RXXT*XK, where T* is the phosphorylated amino acid (28). The relative location and sequence of the splice insert are shown in a schematic of the predicted transmembrane topology (Fig. 1B).

We examined the tissue distribution of hKv4.3 using Northern blot analysis. The hKv4.3 transcript is large, ~8.5 kb, and is found in abundance in heart and brain, with no detectable transcript in kidney, liver, lung, pancreas, spleen, or skeletal muscle (Fig. 1C). The related K⁺ channel hKv4.2 is not present in the ventricle but is demonstrated by RPA to be present in abundance in the human brain (Fig. 1D).

Gene structure and chromosomal location of Kv4.3. We used the full-length rat Kv4.2 cDNA to screen a human genomic PAC library and obtained three unique clones. The clones designated p54 and p56 contain the genes encoding hKv4.3 and hKv4.2, respectively. The genomic clone p54 contains the 5' untranslated region and the coding region in the first exon of hKv4.3. There is an apparent splice donor site in the predicted P-region of the channel at the first glycine of the GYGD K⁺ channel signature motif (Fig. 2A). The long 5'-untranslated region is consistent with the size of the mRNA on the Northern blot (Fig. 1C).

Fig. 1. Expression of the K⁺ channel gene hKv4.3 in human heart. A: RT-PCR demonstrates that both splice variants of hKv4.3 are expressed in human ventricle. Two different forward primers, one 5' to the splice insert (F1) and another in the splice (Fins), are paired with a common 3' primer to amplify cDNA generated from RT of total human ventricular RNA. The primer pair that straddles the splice (F1-R, lane 1) generates 2 PCR products that differ in size by 57 bp (288 and 231 bp). The pair with forward primer in the splice insert (Fins-R, lane 4) generates a single band in the heart RNA lane of 160 bp. No PCR products are generated when RT is omitted from reaction (lanes 2 and 5) or when water is used as a template (lanes 3 and 6). M, 100-bp marker. B: schematic of topology of α-subunit of hKv4.3. Cylinders represent the 6 membrane-spanning repeats (S1–S6). The 19-amino acid insert of the long splice variant is shown after S6. The insert contains a protein kinase C consensus phosphorylation site of the form RXXT*XK, where T* is the phosphorylated amino acid. C: multiple tissue human Northern blot using a 480-bp fragment of hKv4.3 cDNA common to both splice variants. A major band of ~8.5–9 kb is seen in heart and brain lanes. The blot was developed for 4 days on a storage phosphor screen. D: ribonuclease protection assay for hKv4.2. Lane P contains undigested probes, and lane t is hybridization with yeast tRNA, which affords no specific protection. Ten micrograms of total human brain (lane b) or heart (lane h) RNA are incubated with riboprobes that recognize the I-II linker of the cardiac isoform of Na⁺ channel (hH1) and a region of the amino terminus of hKv4.2. A 404-bp fragment in cardiac RNA is protected by hH1 riboprobe, and a 271-bp fragment in brain RNA is protected by hKv4.2. There is no protection by the hH1 and hKv4.2 riboprobes in brain and heart RNA, respectively.
human genomic DNA by PCR and sequencing demonstrates at least four exons that encode the carboxyl terminus of the channel. The size of the second and third coding region-containing exons is uncertain. There are two large introns between the first and second exons and the second and third exons. PCR was used to define the downstream structure of hKv4.3. A 2.4-kb product was amplified using human genomic DNA, a forward primer that recognizes the sequence encoding the 19-amino acid insert, and a reverse primer that was complementary to the carboxyl terminus of the channel. This product contained the third through the fifth exons, which are separated by introns of 1,162 and 746 bp in length (Fig. 2A). A Southern blot of the genomic clone p54 and human genomic DNA reveals identical banding patterns with an hKv4.3 fragment from the 5'8-end of the cDNA used as a probe (Fig. 2B).

Fluorescence in situ hybridization was used to determine the chromosomal location of the hKv4.3 channel gene. Metaphase spreads revealed clear hybridization to the short arm of chromosome 1 at band p13.3 on both homologs (Fig. 3A), with no consistent signals seen on any other chromosome in the 20 spreads analyzed. The exact map location at 1p13.3 was determined from high-resolution chromosomes using simultaneous DAPI banding (Fig. 3A and B).

Functional expression of hKv4.3 splice variants. We cloned both splice variants of hKv4.3 into the expression vector pIRES-GFP, which drives bicistronic expression of the channel and reporter GFP genes by the CMV promoter/enhancer. Transient transfection into mouse Ltk⁻ cells results in the robust expression of inactivating K⁺-selective currents. The whole cell currents elicited by a family of depolarizing voltage steps are shown in Fig. 4, A and B. The current activates rapidly and decays to a steady-state level within 400 ms. The current-voltage relationships for each of the splice variants demonstrate that the currents activate over the same voltage range, between −40 and −30 mV, and monotonically increase in size at more positive voltages (Fig. 4C). The time course of inactivation was determined by fitting the decay of the whole cell current to a single exponential. The rate of inactivation was voltage dependent at less positive voltages and essentially voltage independent at test potentials above +10 mV (Fig. 4D). The long and short splice variants do not differ in either their voltage dependence or inactivation kinetics in the basal state.

The currents are selective for K⁺ over Na⁺. The reversal potential of the tail current elicited by a family of voltage steps from −120 to +60 mV after a test pulse to +60 mV was determined over a range of external K⁺ concentration ([K⁺]₀). The reversal potential as a func-

Fig. 2. A: low-resolution genomic map of hKv4.3. Shaded boxes represent exons, and lines represent introns. Locations of restriction enzyme sites, introns, and exons are relative and not drawn to scale. The coding region of hKv4.3 contains sequence from at least 5 exons. The first exon contains a long (−5 kb) 5'-untranslated (UT) region. A consensus splice donor site occurs after the codon for the first glycine in the K⁺ channel signature sequence GYGD. This splice site is followed by a large intron. Thick lines represent ambiguous exon-intron boundaries. Restriction sites are EcoRI (R), BamHI (Bm), HindIII (H), and BglII (Bg). The probe used in Southern blot is indicated above first exon. B: Southern blot of PAC clone designated p54, which contains hKv4.3 gene and human genomic DNA. DNA is restricted with BglII (lanes 1 and 2), BamHI (lanes 3 and 4), and EcoRI (lanes 5 and 6). U, uncut p54 genomic clone. A probe from 5'-end of hKv4.3 cDNA produces a similar pattern of bands in cloned and genomic DNA. The downstream BglII site is presumed to be in the unknown intronic sequence between first and second exons.

Fig. 3. Localization of hKv4.3 by fluorescence in situ hybridization. A: partial metaphase spread showing hybridization (white signal, arrows) to both homologs of chromosome 1. A reversed 4',6-diamidino-2-phenylindole-banded image of chromosome 1 shows hybridization in band p13.3 at the 850 band level. B: ideogram of chromosome 1 with an arrow marking the location of hybridization.
tion of $[K^+]_o$ is plotted in Fig. 4E. The dotted line is the reversal potential predicted by the Nernst equation. The solid line is the best fit of the data to the Goldman-Hodgkin-Katz voltage equation (19) with a relative permeability of $Na^+$ to $K^+$ of 0.01.

Sensitivity to block by 4-aminopyridine (4-AP) is a hallmark of the $Ca^{2+}$-independent $I_{to}$ (9, 19). The short and long splice variants of hKv4.3 exhibit a similar sensitivity to 4-AP; currents through both splice variants are ~50% blocked by 1 mM 4-AP. The dose-response curves are shown in Fig. 4F. The $IC_{50}$ values determined by fitting the data to a single binding site function are $1.3 \pm 0.3$ and $1.4 \pm 0.3$ mM for the short and long splice variants, respectively ($P = $ not significant (NS)).

The steady-state availability was determined using 1-s conditioning pulses to voltages between $-100$ and $+20$ mV in increments of 10 mV. The currents are half-maximally available at $-42.6 \pm 5.1$ and $-43.1 \pm 6.4$ mV with slope factors of $6.3 \pm 1.3$ and $6.3 \pm 0.6$ mV for the short and long splice variants, respectively ($P = $ not significant (NS)).

DISCUSSION

The transient outward $K^+$ current is a critical component of the normal action potential of atrial and ven-
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Fig. 5. Steady-state inactivation and recovery kinetics of hKv4.3. A: a 2-pulse protocol is used to generate steady-state inactivation curves for hKv4.3-1 (■ and solid line) and hKv4.3-2 (○ and dotted line). Data are fit to Boltzmann functions with half-maximal availability voltages (V_{0.5}) and slope factors of −42.6 ± 5.1 and 6.3 ± 1.3 mV, respectively, for hKv4.3-1 and −43.1 ± 6.4 and 6.3 ± 0.6 mV, respectively, for hKv4.3-2. B: cells expressing each variant of hKv4.3 were depolarized to +50 mV for 500 ms (P1) and then allowed to recover for variable durations at 100 mV before a second depolarizing pulse to +50 mV (P2). The time course of recovery was determined by plotting ratio of peak current amplitudes (P2/P1) at various recovery intervals. Curves were fit with a single exponential function. Most of the current recovers rapidly with time constants of 73.4 ± 32 and 7.5 ± 23 ms (P = not significant) for hKv4.3-1 and hKv4.3-2, respectively.

tricular myocytes of most species. In human ventricle, Kv4.3 is the gene that encodes the α-subunit of the K^+ channel that underlies I_o (12). The density of I_o is reduced in failing human ventricles (25), and this may be caused by altered transcription (20). We describe the cloning, genomic structure, and chromosomal localization of hKv4.3. Two alternatively spliced cDNAs are present in the ventricle; the long splice variant has a 19-amino acid insert that contains a consensus PKC phosphorylation site. Similar splice variants have been described in the rat ventricle (27, 36). Each of the hKv4.3 splice variants encode K^+-selective currents similar to native I_o when expressed in mammalian tissue culture cells.

The hKv4.3 cDNAs are 8.5–9.0 kb with a large 5′- and smaller 3′-untranslated region. The hKv4.3 gene spans at least 40 kb of the genome. Analysis of the genomic structure of hKv4.3 reveals the presence of five exons in the coding region; one splice site occurs at the K^+ channel signature sequence. Another pair of alternatively utilized exons determines the hKv4.3 variant. The gene is located on the short arm of chromosome 1 at 1p13.3. Notably, the human K^+ channel Shaker homolog KCNA3 (Kv1.3) (15) and the Shab homolog KCNC4 (Kv3.4) (33) have been localized to human chromosome 1p21, the Giemsa-positive band just distal to p13.3.

The voltage dependence of the hKv4.3 splice variants expressed in Ltk^- fibroblasts resembles that of native I_o in human ventricular myocytes recorded under similar conditions (1, 6, 25, 26, 40, 41). The activation voltage range is similar in both native currents and expressed channels; in both cases, activation occurs at test potentials greater than −40 mV and the half-maximal activation voltage is approximately +10 mV (1, 6, 25, 26, 40, 41). Steady-state inactivation characteristics of the expressed hKv4.3 channels are similar to native cardiac I_o (25, 26, 40, 41).

Regional differences in the biophysical characteristics of the cardiac I_o have been described. In cells isolated from the subepicardium or mid left ventricular wall, I_o recovers rapidly from inactivation (25, 26, 40, 41). In contrast, I_o from endocardium recovers from inactivation slowly (26, 41). Recovery from inactivation is no different in the two hKv4.3 splice variants; thus it is unlikely that differences in the expression of the splice variants mediates the regional differences in recovery in the basal state (Fig. 5B). It is conceivable that phosphorylation could differentially modulate the rate of recovery of the hKv4.3 splice variants.

Heteromultimerization of K^+ channel subunits can significantly change the electrophysiological features of the resulting current (e.g., see Ref. 30). Regional differences in recovery from inactivation of I_o are not likely to be the result of heteromultimerization of hKv4.3 with hKv4.2 because heterologously expressed hKv4.2 also recovers rapidly (data not shown) and is not expressed in the human ventricle (Fig. 1D). It may be that a more slowly recovering transient K^+ channel such as hKv1.4 underlies human endocardial I_o (7); however, it is uncertain whether hKv1.4 is expressed in adult human cardiomyocytes. Other explanations for the slowly recovering subendocardial I_o include differences in posttranslational modification or differences in regional expression of ancillary subunits or other modifying proteins.

The addition of a PKC phosphorylation site in the long splice variant raises the possibility of isoform-specific regulation of hKv4.3 by phosphorylation. Stimulation of α-adrenergic receptors enhances cardiac contractility largely because of prolongation of the action
potential duration that results from reduction of repolarizing K⁺ currents (13). The transient outward current is modulated by second messenger systems. Prominently, stimulation of α₁-adrenergic receptors has been shown to reduce Iₒ current density (2, 8). In some preparations, activation of PKC mimics the effect of α₁-adrenergic stimulation (2), whereas in others, different second messenger systems appear to mediate the effect of α₁-stimulation (8). The role of PKC in α₁-adrenergic regulation of the human cardiac Iₒ has not been defined; however, in cultured human atrial myocytes, Iₒ is inhibited by the PKC inhibitor staurosporine (18).

Transient outward current expression in the mammalian heart is dynamic. For example, the density and, in some cases, the kinetics of Iₒ change with development (22, 39, 43) and exposure to thyroid hormone (35, 42) in the rat ventricle. The changes in the action potential and outward current profile have suggested to some investigators an isoform switch from Kv1.4 to Kv4 channels during development and treatment with triiodothyronine (42). In the human ventricle there is also a reduction in the current density of Iₒ that accompanies heart failure (6, 25, 41). The molecular basis of this change in current density is uncertain, but we have observed a reduction in the steady-state level of hKv4.3 mRNA that correlates with current density in the same hearts (20). As a first step to try and understand the basis of changes in the expression of human Iₒ, we describe the cloning, genomic structure, and functional expression of the splice variants of hKv4.3. Understanding the regulation of expression of this gene may have important implications for changes in expression of this and other ion channels and transport proteins in human heart failure.

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