Suppression of Slow Delayed Rectifier Current by a Truncated Isoform of KvLQT1 Cloned from Normal Human Heart

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It has been suggested that the cardiac slow delayed rectifier channel is formed by the association of two subunits: IsK (also called minK) and KvLQT1. N-terminal splice variants of the human KvLQT1 gene have been identified, but the functional roles of different KvLQT1 isoforms are not clear. Using the nested 5’-rapid amplification of cDNA ends technique, we obtained a truncated isoform of KvLQT1 (termed tKvLQT1) that lacks the N-terminal cytoplasmic domain and the initial one-third of the first transmembrane domain. The function of tKvLQT1 was tested by oocyte expression, alone or together with the full-length KvLQT1 or a human IsK clone (hIsK). tKvLQT1 alone did not generate functional channels. However, it suppressed the KvLQT1 current when coexpressed with the full-length isoform. It also suppressed the slow delayed rectifier current induced by hIsK, probably by competing with the KvLQT1 subunit endogenous to Xenopus oocytes in coassembly with the hIsK subunit. On the other hand, tKvLQT1 did not suppress the expression of Kv1.4, Kv4.3, or hERG. Using the reverse transcription-polymerase chain reaction technique, we further show that the truncated and full-length isoforms are coexpressed in different regions of human heart. Therefore, tKvLQT1 may modulate the function of IKs in human cardiac myocytes.

KvLQT1 was first identified as a gene involved in the long-QT syndrome by positional cloning (1). Hydropathy analysis revealed a structure of six putative transmembrane a-helices and a “pore” region homologous to those of known potassium channels. Subsequently, KvLQT1 was cloned from human (2, 3) and other species (mouse (4), Xenopus oocytes (2)). The KvLQT1 channel is produced (2, 4). These results suggest that KvLQT1 subunits probably do not form homomultimer channels in the heart, but are associated with IsK subunits to form functional IKs channels. Expressing IsK alone in oocytes can induce an IKs current, because oocytes have an endogenous KvLQT1 subunit (2). Coexpression of exogenous KvLQT1 with IsK in oocytes greatly increases the amplitude of IKs, probably by augmenting the KvLQT1 protein level in the oocytes (2–4). The linkage between KvLQT1 mutations and abnormal QT prolongation in long-QT patients clearly indicates the importance of KvLQT1 subunits (and thus IKs channels) in action potential repolarization in the heart (1, 5–7). KvLQT1 and IsK are coexpressed not only in heart but also in other organs (e.g. inner ear (6) and kidney and pancreas (2, 8)). Therefore, they may form IKs channels that serve a variety of physiological functions in different organs.

It has been shown that there are alternative splice variants in the 5’-end of the human KvLQT1 gene (9). Therefore, there may be KvLQT1 isoforms that have different gating properties or serve various functions. However, the roles of splice variants of KvLQT1 proteins have not been examined.

EXPERIMENTAL PROCEDURES

Cloning of tKvLQT1—The technique of rapid amplification of 5’-complementary DNA ends (5’-RACE) was used to amplify KvLQT1 N-terminal sequences from cDNAs of normal human hearts (CLONTECH Marathon Ready cDNA, Lot number 60050449). Two 5’-RACE primers were synthesized based on the partial KvLQT1 cDNA sequence (GeneBank accession number U40990 (1)). Primer A corresponds to nucleotides 427 to 401 of U40990: 5’-CAGGAAAGCCGATGTACAGGGTG-3’. Primer B corresponds to nucleotides 351–326 of U40990: 5’-CTCCTCCTGCGCAGCTGACAGGTGGTTA-3’. These primers were combined with CLONTECH RACE adapter primers for two sequential nested PCR amplifications (30 cycles of 95 °C/30 s, 70 °C/1 min, and 72 °C/1 min, beginning with hot start and ending with an additional 72 °C/7 min extension). PCR products were fractionated on 1% agarose gel. One major band of approximately 650 bp was consistently seen in multiple PCR reactions. This band was purified and cloned into a TA-cloning vector (pCR2.1, Invitrogen). Six 5’-RACE clones were sequenced for both strands. They have the same sequence except minor variations in the 5’-ends (denoted by open circles in Fig. 1). Two of the 5’-RACE clones were selected for transfer of the 5’ differentially spliced exon into the human KvLQT1 cDNA clone (a gift of Dr. M. T. Keating (2), using a unique restriction enzyme site, NcoI, that is shared between the 5’-RACE clones and the KvLQT1 clone. The complete construct, termed tKvLQT1, was subcloned into a vector, pCDNA3 (+) (Invitrogen), for expression and functional analysis. DNA sequencing was carried out by an ABI373 automated DNA sequencer and the dye-termination method. Computer software used for DNA sequence analysis included Sequencher (GeneCodes), Lasergene
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Electrophysiological Experiments—Whole-cell currents were recorded using the two-microelectrode voltage clamp technique. The current-passing and voltage-recording electrodes were made of “agar-cushioned” pipettes (14) of tip resistance 0.1–0.3 MΩ. Currents were recorded at room temperature (22°C); oocytes were bathed in a symmetrical solution (see the figure legend or text).

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alone at a 10-fold higher amount of cRNA (investigators (2–4). On the other hand, tKvLQT1 expressed oocytes with properties similar to those described by other investigators (1b) recently identified in the human KvLQT1 gene (9). There-

-KvLQT1 cDNA sequence (1). The remaining 5th-terminal sequence from the 3'-end of the RACE clone is identical to the 5' end sequence is reportedly in KvLQT1 appear to be different from those in channels of other Kv gene families (16). This is also suggested by the lack of a sequence “EFYFDV” in the N terminus of KvLQT1. This sequence is conserved in potassium channels of Kv1 to Kv4 subfamilies and is found in the N-terminal domains that are crucial for subfamily-specific subunit coassembly (16).

In some uninjected oocytes, we could resolve a small, time-
dependent outward current (Fig. 2A). This current appeared to be similar to that induced by the full-length human KvLQT1 in showing a mild inward rectification upon strong depolarization (data not shown). Therefore, it might originate from the KvLQT1 isoform endogenous to Xenopus oocytes (2). At +40 mV, this time-dependent outward current amounted to 0.13 ± 0.01 μA on day 1 after oocyte isolation (n = 5), and 0.09 ± 0.01 μA on day 2 (n = 10). These current amplitudes are significantly larger than those seen in oocytes coinjected with tKvLQT1 and KvLQT1 at +40 mV, 0.06 ± 0.004 μA on day 1, p < 0.05, and 0.02 ± 0.007 μA on day 2, p < 0.001). Therefore, tKvLQT1 disrupted the channel function of not only the exogenously expressed full-length human KvLQT1, but also the endogenous Xenopus KvLQT1.

RESULTS AND DISCUSSION

Cloning of a Truncated Isoform of KvLQT1 (tKvLQT1) — Fig. 1 illustrates the nucleotide sequence of the 5'-RACE clone we obtained from normal human heart cDNA. The 351-bp sequence from the 3'-end of the RACE clone is identical to the KvLQT1 cDNA sequence (1). The remaining 5'-end sequence is completely different from that of KvLQT1 (5), but is identical to the available sequence of a differentially spliced KvLQT1 exon (1b) recently identified in the human KvLQT1 gene (9). Therefore, with 5'-RACE, we cloned an N-terminal splice variant of KvLQT1. The deduced amino acid sequence indicates that this KvLQT1 isoform is shorter than the full-length isoform by 127 amino acids (lacking the cytoplasmic N terminus and the initial part of the first transmembrane domain, S1) (Fig. 1). Therefore, we call this truncated isoform “tKvLQT1.” In the following experiments testing the functional role of tKvLQT1, cRNAs from three independent clones were used, and the results were the same. Therefore the data were pooled.

tKvLQT1 Alone Does Not Form Functional Channels but Can Suppress the Function of the Full-length KvLQT1 — The full-length KvLQT1 encodes a voltage-gated potassium channel in oocytes with properties similar to those described by other investigators (2–4). On the other hand, tKvLQT1 expressed alone at a 10-fold higher amount of cRNA (n = 30) did not give any currents similar to those recorded from KvLQT1-expressing oocytes. There are two possible explanations for these re-

FIG. 3. Specificity of suppression of channel function by tKvLQT1 coexpression. Shown are normalized current amplitudes plotted against the types of cRNAs injected. Each oocyte received 2 ng of cRNA of KvLQT1, Kv1.4, Kv4.3, or hERG or 2 ng of KvLQT1 plus 0.2 ng of hIsK (hIsK + KvLQT1), without (white bars) or with (black bars) 8 ng of tKvLQT1. Another batch of oocytes received 10 ng of KvLQT1 cRNA each (gray bar). The current amplitude was measured as current level 1 s after depolarization to +60 mV (KvLQT1), peak current at +60 mV (Kv1.4 and Kv4.3), peak tail current at −60 mV after a 1-s depolarization pulse to +60 mV (hERG) or time-dependent current during a 5-s depolarization pulse to +40 mV (hIsK + KvLQT1). For each channel type, the current amplitudes were normalized by the mean current amplitude recorded from oocytes not receiving KvLQT1 cRNA. For oocytes receiving 10 ng of KvLQT1, the current amplitudes were normalized by the mean current amplitude recorded from oocytes injected with 2 ng of KvLQT1. Recordings were made 36–60 h after injection. Silkolyzed results were obtained using oocytes from two frogs, and the data were combined (n = number of oocytes tested). *, p < 0.001 by unpaired t test between current amplitudes from oocytes without or with tKvLQT1 coinjection or between oocytes receiving 2 or 10 ng of KvLQT1.

μg of poly(A) RNA was used in a 20-μl reaction mix, and a combination of oligo(dT) and random hexamer primers was used for the initiation of cDNA synthesis. For negative controls, the reactions were run in the absence of reverse transcriptase. Each of the 20-μl cDNA product was divided into two halves and subjected to PCR amplification using the primer pair specific for tKvLQT1 or for KvLQT1. PCR protocol was 30 cycles of 94 °C/30 s, 60 °C/45 s, and 72 °C/45 s, with a 94 °C min hot start in the beginning of the cycles and a 72 °C/5 min extension at the end. The PCR products were size-fractionated by electrophoresis on a 2% gel of 3:1 (w/w) agarose:synergel (Diversified Biotech).

Coexpression of tKvLQT1 and KvLQT1 in human heart revealed by RT-PCR reactions. Tissues from the following regions of a nonfailing human heart were used for poly(A) RNA preparation: left and right atrial appendages (LA and RA), epicardium from left and right ventricles (LV/Epi and RV/Epi). “Heart 1” and “heart 2” are two independent lots of normal human heart poly(A) RNA from CLON-TECH. Reverse transcription reactions were run in the absence (“−RT,” as negative control) or presence (“+RT”) of reverse transcriptase. For each of the poly(A) RNA samples, products from the two RT-PCR reactions with tKvLQT1- and KvLQT1-specific primer pairs were combined for electrophoresis. The lanes marked by “tKvLQT1” and “KvLQT1” are products from parallel PCR reactions using plasmid DNAs of the tKvLQT1 and KvLQT1 clones as PCR DNA templates. They serve to illustrate the specific sizes produced from the two isoforms (tKvLQT1, 298 bp; KvLQT1, 348 bp).
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When hlsK was expressed in oocytes, it induced an IKs current similar to the cardiac IKs (Fig. 2B). It has been suggested that hlsK subunit coassembles with the endogenous Xenopus KvLQT1 subunit to form functional IKs channels (2). When coexpressing tKvLQT1 with hlsK at a cRNA ratio of 10:1 (30 ng of tKvLQT1 plus 3 ng of hlsK), the amplitude of IKs was consistently and significantly reduced (Fig. 2B). The time-dependent current during a 5-s depolarization pulse to +40 mV was 2.39 ± 0.18 μA in oocytes injected with hlsK alone (n = 10), but only 0.66 ± 0.06 μA in oocytes injected with hlsK along with tKvLQT1 (n = 12, p < 0.001). These data suggest that tKvLQT1 could suppress the function of IKs channels induced by hlsK, probably by competing with endogenous Xenopus KvLQT1 subunits in coassembly with the hlsK subunits.

Specificity of the Suppressing Effects of tKvLQT1 on Channel Function—There remain two questions regarding the “specificity” of tKvLQT1’s effects on channel expression. First, in the experiments shown in Fig. 2, 30 ng of tKvLQT1 cRNA was coinjected with 3 ng of KvLQT1 or hlsK cRNA. Could such an amount of tKvLQT1 cRNA saturate the protein translation machinery of oocytes, causing a nonspecific reduction of expression of other channel subunits? Second, are the suppressing effects of tKvLQT1 specific for KvLQT1 and IKs, or can it suppress the expression of other seemingly unrelated potassium channel subunits? These two questions are addressed in the experiments shown in Fig. 3. Coinjection of tKvLQT1 with KvLQT1 at a cRNA ratio of 4:1 (8 ng of KvLQT1 plus 2 ng of KvLQT1) reduced the KvLQT1 current amplitude by 56% (p < 0.001). In oocytes injected with 2 ng of KvLQT1 plus 0.2 ng of hlsK, coinjection of 8 ng of tKvLQT1 reduced the IKs current amplitude by 56 ± 7% (p < 0.001). On the other hand, increasing the amount of KvLQT1 cRNA injected from 2 to 10 ng (equal or similar to the total amounts of cRNAs received by the tKvLQT1-coinjected oocytes) augmented the current amplitude by 228 ± 43%, suggesting that 8 ng of tKvLQT1 cRNA probably did not saturate the translation machinery. The same amount of tKvLQT1 (8 ng) did not significantly affect the expression of Kv1.4, Kv4.3, or hERG (induced by 2 ng of channel cRNA, p ≥ 0.2). Therefore, tKvLQT1 reduced KvLQT1 or IKs current amplitude not by a nonspecific effect of reducing subunit translation and was not seen with three other potassium channel subunits. The lack of effects of tKvLQT1 coexpression on Kv4.3 and hERG function is significant, because these two subunits are important for the transient outward and rapid delayed rectifier currents in the human heart, respectively (17, 18).

Identification of KvLQT1 Isoforms in Different Regions of Human Heart—We used the RT-PCR technique to see if the two KvLQT1 isoforms can be detected in the same anatomical regions of human heart. KvLQT1- and tKvLQT1-specific PCR primer pairs were used to amplify the respective isoform sequences from mRNAs isolated from four different regions of a nonfailing human heart. As shown in Fig. 4, both isoform-specific bands were detected in the left and right atria and the left and right ventricular epicardium. Fig. 4 also shows that both isoform specific bands were detected from two independent CLONTECH mRNA preparations from normal human heart. It is important to note that these results are similar to two recent reports, both of which show that human heart is unique among many human tissues in coexpressing the full-length and the truncated KvLQT1 isoforms (3, 9). This, in conjunction with data presented in Fig. 3, indicates that the suppressing effect of tKvLQT1 on channel function is likely to be specific for IKs in the heart. Since the tKvLQT1 isoform was cloned from and identified in normal human hearts, the observations we report here (i.e. coexpression of tKvLQT1 with full-length KvLQT1 in different regions of the human heart and a suppressing effect of tKvLQT1 on the function of the slow delayed rectifier channel) are of physiological significance. Coexpression of tKvLQT1 with KvLQT1 in cardiac myocytes at different ratios may be one contributing factor to the observed regional heterogeneity in the IKs current amplitude in the heart (19). It has been suggested that, under the influence of drugs or pathological conditions, regions of the ventricle that have a low IKs current density tend to develop abnormally prolonged action potentials and may form the foci of arrhythmogenesis due to early or delayed afterdepolarizations (20). Therefore, it is important to pinpoint the cellular distribution of tKvLQT1 versus KvLQT1 in the heart and to correlate this expression pattern with the IKs current density.

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