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Original Article

Expression of multiple KCNE genes in human heart may enable variable modulation of I_{Ks}

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Abstract

Voltage-gated potassium (K_V) channels are modulated by at least three distinct classes of proteins including the KCNE family of single transmembrane accessory subunits. In the human genome, KCNE proteins are encoded by five genes designated *KCNE1* through *KCNE5*. KCNE1 associates with KCNQ1 in vitro to generate a potassium current closely resembling the slowly activating delayed rectifier (I_{Ks}). Other KCNE proteins also affect the activity of heterologously expressed KCNQ1. To investigate the potential physiological relevance of this gene family in human heart, we examined the relative expression of *KCNQ1* and all five KCNE genes in samples derived from normal tissues representing major regions of human heart by real-time, quantitative RT-PCR. KCNE genes are expressed in human heart with a relative abundance ranking of *KCNE1* > *KCNE4* > *KCNE5* ~ *KCNE3* >> *KCNE2*. In situ hybridization revealed prominent expression of *KCNE1* and *KCNE3–5* in human atrial myocytes. In cardiomyopathic hearts, expression of *KCNE1*, *KCNE3*, *KCNE4*, and *KCNQ1* was significantly increased, while *KCNE2* and *KCNE5* exhibited reduced expression. In a cell line stably expressing KCNQ1 and KCNE1, transient expression of KCNE3, KCNE4, or KCNE5 significantly altered I_{Ks} current profiles. Even in the presence of additional KCNE1, KCNE4 and KCNE5 exert dominant effects on I_{Ks}. Although KCNE1 is the predominant KCNE family member expressed in human heart, the abundance of other KCNE transcripts including potential KCNQ1 suppressors (KCNE4 and KCNE5) and their altered expression patterns in disease lead us to speculate that a balance of KCNE accessory subunits may be important for cardiac K_V channel function.

Keywords: Potassium channel; *KCNQ1*; *KCNE1*; *KCNE2*; *KCNE3*; *KCNE4*; *KCNE5*

1. Introduction

Voltage-gated potassium (K_V) channels are heteromultimeric protein complexes essential for a variety of physiological processes involved in normal cardiac function, especially myocardial repolarization. In addition to the pore-forming subunits encoded by a large number of distinct potassium channel genes, at least three distinct classes of accessory proteins have been identified. These include the K_V channel β subunits (Kvβ) [1–4], potassium channel interacting proteins (KChiPs) [5,6] and the KCNE family of single transmembrane proteins including minK and minK-related peptides (MiRPs) [7,8].

In the human genome, KCNE proteins are encoded by five genes designated *KCNE1* through *KCNE5*. The physiological importance of the KCNE gene family is illustrated by associations with inherited disorders of cardiac and skeletal muscle excitability. Mutations in *KCNE1* have been associated with congenital long QT syndrome (LQTS) [9–11] while *KCNE2* variants have been identified in cases of sporadic and drug-induced LQTS [10,12–14]. Familial and thyrotoxic periodic paralysis, two related skeletal muscle disorders, have been associated with a mutation in *KCNE3* [15,16]. Finally, dele-

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tion of a genomic region (Xq22.3) containing *KCNE5* results in the AMME contiguous gene syndrome featuring Alport syndrome, Mental retardation, Midface hypoplasia and Elliptocytosis [17]. Mild cardiac abnormalities (short PR interval, left axis deviation, bicuspid aortic valve) and skeletal muscle hypotonia, which have also been described in this syndrome, have uncertain relationships to *KCNE5* deletion. There are currently no disease associations with *KCNE4*.

Mutation of *KCNE1* impairs generation of the slowly activating cardiac delayed rectifier (I_{Ks}) [9,18], a critical potassium current contributing to myocardial repolarization that can be reconstituted in vitro by heterologous co-expression of recombinant KCNQ1 potassium channels with KCNE1 [19,20]. The other KCNE proteins have also been demonstrated to exert diverse functional effects on heterologously expressed KCNQ1 [12,21–27]. However, the potential physiological relevance of these in vitro observations is uncertain because it is not clear which KCNEs are expressed in human heart.

In this study, we examined the expression of each KCNE gene using qualitative and quantitative methods. Prominent expression of two alternatively spliced *KCNE1* transcripts was observed in all cardiac regions examined, while *KCNE2* exhibited the lowest level of cardiac expression among the KCNE genes. Interestingly, *KCNE4* and *KCNE5* which encode subunits that suppress KCNQ1 current in heterologous systems, are highly expressed in an overlapping pattern with *KCNE1*, the most probable partner for KCNQ1 in the native I_{Ks} channel complex. In cardiomyopathic human left ventricle, we observed divergent changes in the relative expression patterns of KCNE genes. We also assessed the functional interaction of each KCNE individually in a cell line stably expressing I_{Ks}. Our data provide a more complete knowledge of KCNE gene expression in the heart. Furthermore, the expression patterns observed for *KCNE4* and *KCNE5* coupled with their in vitro effects on I_{Ks} lead us to speculate that these putative inhibitory subunits may have relevance in cardiac physiology.

2. Materials and methods

2.1. Real-time quantitative RT-PCR

Transcription start sites were mapped for all KCNE genes in human ventricular muscle using ribo-oligonucleotide ligation modified rapid amplification of cDNA ends (RLM-RACE, Ambion, Austin, TX, USA) [28] followed by nucleotide sequencing of subcloned products. We identified exons containing the 5′-untranslated region (5′-UTR) of each KCNE gene by alignment of RLM-RACE sequences with publicly available human genome sequence. Two transcripts having identical coding sequences were identified for KCNE1, denoted KCNE1a (short 5′-UTR) and KCNE1b (long 5′-UTR), that arise by alternative splicing of exons containing portions of the 5′-UTR (GenBank accession numbers: AY789479, AY789480).

Fluorescently labeled TaqMan (Applied Biosystems, Foster City, CA, USA) probes for KCNQ1 and KCNE1-4 were designed to span an exon–exon boundary within the coding and 5′-untranslated regions, respectively, while a probe for KCNE5 targeted a region of the coding exon (KCNE5 is intronless). Primer sets that amplified 99–104 bp products encompassing each probe annealing site were designed using PrimerExpress (Applied Biosystems). Sequences for all amplification primers and gene-specific probes are presented in Table 1. None of the KCNE-specific primer and probe sets cross-reacted with the other KCNE sequences after 40 cycles of PCR, and no amplification was observed after 45 cycles of PCR in control reactions containing water as the template. For each tissue, results were compared to a gene-specific standard curve and normalized to expression of the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), in the same samples. Template used for determining standard curves consisted of plasmid DNA containing the expected target sequence quantitated by PicoGreen fluorescence (Molecular Probes, Eugene, OR, USA). Statistical comparisons were made using Student's *t*-test and significance was assumed for $P < 0.05$.

Table 1
TaqMan probe and primer information

mRNA	Forward primer	Reverse primer	Probe[a]
KCNE1a	TaqE1aF: GGAGGAAGGCATTATCTGTATCCA	TaqE1aR: TCCTGGGCATTAAGGTTCCA	E1aProbe: FAM-ATATTCAGAGGTGTGCCTG-MGBQ
KCNE1b	TaqE1bF: TCACTGTGCAAGCTACAAAAGCT	TaqE1bR: TCACCGCTGTGGTGTTAGACA	E1bProbe: FAM-CATATTTAGAGGTGTGCCTG-MGBQ
KCNE2	TaqE2F: CAGAACAGCCTGGCTTTGGA	TaqE2R: TCCAGCGTCTGTGTGAAATTG	E2Probe FAM-CTGCATAGCAGGAGGGA-MGBQ
KCNE3	TaqE3F: ACTGAGAGCCAGTGGATTTGC	TaqE3R: AGGTCTCCGTTCCATTGGTAGTC	E3Probe: FAM-AGCAGTCTGAGCTTC-MGBQ
KCNE4	TaqE4F: AACCCTCTTGGACTGGACGAT	TaqE4R: AGGCTCCATTTTCAGCATTGA	E4Probe: FAM-TCAGCCTTGCCCCTG-MGBQ
KCNE5	TaqE5F: CCCCTACCCCGCACATC	TaqE5R: TTGGACGTGTTGGATTCAGTTC	E5Probe: FAM-TGCACTAAACTGCCTCTG-MGBQ
KCNQ1	TaqQ1F: CCCTGAAGGTGCAGCAGAA	TaqQ1R: GCCTTCCGGATGTAGATCTTCC	Q1Probe: FAM-TCACTCATTCAGACCGCAT-MGBQ

[a] FAM, 6-carboxy-fluorescein; MGBQ, minor groove binding non-fluorescent quencher.