Cardiac-enriched LIM domain protein fhl2 is required to generate $I_{K_s}$ in a heterologous system

Sabina Kupershmidt, Iris C.-H. Yang, Margaret Sutherland, K. Sam Wells, Tao Yang, Ping Yang, Jeffrey R. Balser, Dan M. Roden

Department of Pharmacology, 561A PRB, Division of Clinical Pharmacology, Vanderbilt University School of Medicine, 532C Robinson Research Building, 23rd Avenue South at Pierce Avenue, Nashville, TN 37232-6602, USA

Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN 37232-6602, USA

Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232-6602, USA

Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232-6602, USA

Received 12 December 2001; accepted 17 May 2002

Abstract

Objective: Co-expression of the KvLQT1 and minK potassium channel subunits is required to recapitulate $I_{K_s}$, the slow component of the cardiac delayed rectifier current, and mutations in either gene cause the congenital Long QT syndrome. It is becoming increasingly well-recognized that multiprotein channel complexes containing proteins capable of modulating channel function assemble at the plasma membrane. Thus, the aim of our study was to identify proteins involved in $I_{K_s}$ modulation. Methods and results: Using a yeast-two-hybrid screen with the intracytoplasmic C-terminus of minK as bait, we identified the cardiac-enriched four-and-a-half LIM domain-containing protein (fhl2) as a potential minK partner. We show interaction between the two proteins in GST pulldown assays and demonstrate overlapping subcellular localization using immunocytochemistry of transfected cells supporting a potential interaction. At the functional level, expression of KvLQT1 and minK in HEK cells, which lack endogenous fhl2 protein, generated $I_{K_s}$ only when fhl2 was co-expressed. By contrast, in CHO-K1 cells, which express fhl2 endogenously, $I_{K_s}$ was suppressed by anti-fhl2 antisense which did not affect the currents generated by KvLQT1 alone. Conclusion: These data indicate that at least in heterologous cells, the generation of $I_{K_s}$ requires fhl2 as an additional protein component.

Keywords: Arrhythmia (mechanisms); Ion channels; K-channel; Long QT syndrome; Ventricular arrhythmias

1. Introduction

Voltage-gated potassium channels comprise a large family of membrane proteins that regulate the membrane potential of excitable cells in the heart, central nervous system, and other organs. Functional potassium channel diversity originates in part by the expression of a large number of different, pore-forming α subunit proteins, and in part by the interaction of α subunit multimers with β subunits (reviewed in Ref. [1]). The delayed rectifier $I_{K_s}$, which plays an important role during repolarization of the cardiac action potential, illustrates this paradigm: co-expression of the minK (KCNE1) and KvLQT1 (KCNQ1) gene products is required to recapitulate $I_{K_s}$ in heterologous systems [2,3]. KvLQT1 acts as the α subunit and can generate a current (activating much more rapidly than $I_{K_s}$) when expressed by itself [4]. MinK is a small protein with a single transmembrane domain and no classical pore region. It does not generate a current autonomously, but functions to increase the amplitude of the KvLQT1-generated currents and to alter gating [2–4], unitary current [5,6] and pharmacological properties [7,8] to recapitulate $I_{K_s}$.

*Corresponding author. Tel.: +1-615-936-2586; fax: +1-615-322-4707. E-mail address: sabina.kupershmidt@vanderbilt.edu (S. Kupershmidt).

Present address: Department of Pharmacology, George Washington University, Washington, DC 20037, USA.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Ion channels; K-channel; Long QT syndrome; Ventricular arrhythmias

Time for primary review 27 days.
Loss of function mutations in either KCNQ1 or KCNE1 produce defective repolarization, syncpe, and sudden death due to arrhythmias in the congenital Long QT syndrome [9].

It is well established that potassium channels are associated with modulating proteins that are not directly involved in pore formation, but serve other functions such as signaling [10,11] or anchoring to specific loci within the cell [12–14]. In order to refine the molecular basis of $I_{Ks}$ physiology, we used the minK intracytoplasmic C-terminus as ‘bait’ in a yeast-two hybrid screen, and evaluated the functional consequences of a potential minK partner in cultured cells. Our studies demonstrate that the cardiac-enriched protein fhl2 (named after its ‘four and a half LIM’ domains) [15] interacts with minK and is required to recapitulate $I_{Ks}$. The LIM structural motif is composed of two adjacent zinc fingers that are involved in protein–protein interactions [16]. Proteins in this family may also contain functional domains such as homeodomains [17], PDZ [18], or kinase domains [19], but others, including fhl2, consist of LIM domains only. Several LIM proteins associate with cytoskeletal elements [20–22] and in human cardiac myocytes fhl2 has been shown to co-localize with α-actinin at the Z-lines [23] where minK has recently also been demonstrated [24]. The present study identifies fhl2 as an additional player in the $I_{Ks}$ complex and will allow us to further define its role in cardiac physiology.

2. Methods

2.1. Yeast two hybrid screening

To generate a bait plasmid, we cloned the 63 C-terminal amino acids of minK into the vector pGBDU-C2 [25], which carries a URA3 selection marker generating an in frame fusion with the GAL4 DNA binding domain (BD) of this vector. The resulting clone, MinK C-term, was sequenced to verify orientation of the insert and its correct reading frame. MinK C-term was transformed into yeast strain PJ69-4A (a kind gift from Dr P. James, University of Wisconsin Medical School) and grown on Synthetic Complete Dropout media lacking Uracil (SD/-ura) as a negative control and MinK C-term as a positive control. A well as assayed on SD/-ura/ -leu (leucine). The experimental enhanced green fluorescent protein (EGFP) cistron, and a poliovirus internal ribosome entry site (IRE).

2.2. Sequencing of inserts

Candidate yeast colonies were lysed in 10 μl of yeast lysate buffer (1.2 mol/l sorbitol, 100 mmol/l sodium phosphate, pH 7.4, and 2.5 mg/ml zymolyase (ICN, Canada)) for 10 min at 37 °C. Then 2 μl of the lysate was used as a template in a PCR reaction with pACT2-specific primers flanking the cDNA inserts. PCR fragments were sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech, Cleveland, OH).

2.3. RNA analysis

Northern hybridization was performed on total cell RNA as described previously [28] using a full-length fhl2 cDNA probe.

2.4. Constructs

The GST-minK fusion plasmid was constructed by ligating a PCR fragment coding for the last 63 amino acids of mouse minK in frame with the GST open reading frame into the EcoRI site of vector pGEX-2TK (Pharmacia, Amersham). EcoRI linkers and a stop codon were incorporated into the PCR primers. The entire fhl2 coding region was subcloned into pBKCMV (Stratagene, La Jolla, CA) following PCR amplification. Restriction enzyme sites as well as a stop codon were incorporated into the PCR primers. For sense- and antisense-fhl2 expression experiments, the entire fhl2 coding region was inserted in both orientations into the EcoRI site of vector GFP-Ire (a kind gift of Dr David Johns, Johns Hopkins School of Medicine) placing it downstream of the CMV promoter, the enhanced green fluorescent protein (EGFP) cistron, and a poliovirus internal ribosome entry site (IRE).

2.5. Cell culture and transfections

Chinese hamster ovary K1 (CHO-K1) cells were cultured in Ham’s F-12 media and HEK 293 and tsA201 cells in DMEM. Cells were transiently transfected using the
lipofectamine transfection reagents and method (Gibco-BRL). A GFP expressing vector was co-transfected and cells displaying green fluorescence 48–72 h after transfection were used for electrophysiologic experiments.

2.6. Preparation of protein extracts

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mouse hearts were harvested and minced in lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 150 mmol/l NaCl, 50 mmol/l Tris, 1 mmol/l EDTA). The homogenates were then passed through a Centricron Plus-20 column (Millipore, Bedford, MA).

2.7. GST pulldown assays

A GST–minK fusion plasmid containing the C-terminal 63 amino acids of minK in vector pGEX-2TK was transformed into BL-21 E. coli. Expression and purification of the fusion protein was carried out according to the manufacturer’s recommendations (Amersham Pharmacia Biotech) and linked to glutathione Sepharose 4B beads. Total mouse heart protein extracts were incubated with bacterially expressed GST–minK or GST alone for 1 h at RT. The complexes were then washed three times in RIPA buffer (0.1% SDS, 0.5% sodium deoxycholate and 0.5% NP-40 in PBS), boiled for 5 min in SDS gel loading buffer and run on a 10% SDS PAGE for Western analysis.

2.8. Western blotting

Membranes were blocked overnight in TS (50 mmol/l Tris, 150 mmol/l NaCl) plus 10% goat serum (S1) at 4 °C. They were then incubated in primary antibody (1:500 for anti-fhl2) in TS plus 5% goat serum plus 0.05% Tween 20 (S2) and washed two times in S3 (50 mmol/l Tris, 0.5 mol/l NaCl, 5% goat serum, 0.05% Tween 20) followed by incubation with secondary donkey anti-rabbit horseradish peroxidase (HRP)-linked antibody (1:10 000, Amersham Pharmacia Biotech) and linked to glutathione Sepharose 4B beads. Total mouse heart protein extracts were incubated with bacterially expressed GST–minK or GST alone for 1 h at RT. The complexes were then washed three times in RIPA buffer (0.1% SDS, 0.5% sodium deoxycholate and 0.5% NP-40 in PBS), boiled for 5 min in SDS gel loading buffer and run on a 10% SDS PAGE for Western analysis.

2.9. Immunocytochemistry

Transfected HEK 293 cells grown on 35-mm Mat-Tek dishes were fixed in 4% paraformaldehyde/PBS, washed, and preincubated in 5% goat serum, 2% BSA and 0.1% Triton X-100. This was followed by incubation with the anti-FLAG M2 (1:200, Sigma) and anti-fhl2 (1:50) primary antibody in 2% BSA/PBS overnight. Cells were then incubated with cy3-conjugated goat anti-mouse IgG and cy3-conjugated goat anti-rabbit IgG (1:400, Jackson Immuno Research) secondary antibody, respectively. The cells were imaged on a Zeiss LSM410 confocal imaging system using a laser for the excitation of cy3 (567 nm) and cy5 (647 nm).

2.10. Antibody production

A fhl2 immunogenic peptide was generated, linked to a KLH carrier, and rabbits were immunized (Quality Controlled Biochemicals, Hopkinton, MA). The peptide used was Ac-CGSSLVDKPFAAKEQL-amide. Reactive antisera were immunoaffinity purified using a thiol coupling gel.

2.11. Patch-clamp analysis

Potassium currents were recorded at room temperature (RT, 20–22 °C) using the whole cell patch clamp technique. Electrode resistances were 1–2 MΩ when filled with a pipette intracellular solution containing: 110 mmol/l KCl; 5 mmol/l K₃ATP, 2 mmol/l MgCl₂; 10 mmol/l Hepes; and 5 mmol/l K₃ATP, pH 7.2. The bath solution for all experiments contained: 145 mmol/l NaCl; 4 mmol/l KCl; 1.8 mmol/l CaCl₂; 1.0 mmol/l MgCl₂; 10 mmol/l HEPES; and 10 mmol/l glucose, pH 7.35. Iᵥ or IᵥKᵢ-LQT, were activated from a holding potential of −80 mV with 4- or 1-s depolarizations, respectively, to voltages ranging from −60 to +60 mV in 20-mV increments. The membrane potential was then stepped to −50 mV and tail currents recorded. Patch clamp data were acquired using pCLAMP6 software (v6.0.4; Axon Instruments, Foster City, CA). Currents were filtered at 5 kHz (−3 dB, four-pole Bessel filter) and recorded using an Axopatch 200 integrating patch clamp amplifier (Axon Instruments) with 80% series resistance compensation. Pooled data are presented as means and standard errors, and statistical comparisons were made by Student’s t-test with P<0.05 considered significant.

3. Results

3.1. Identification of fhl2 as a potential minK partner

Using the 63 intracytoplasmic C-terminal amino acids of minK as bait in a yeast two-hybrid screen of a mouse brain cDNA library, we isolated the four-and-a-half LIM domain protein—fhl2. The interacting clone included the sequence of all but the 25 N-terminal amino acids of fhl2. It reacted positive in all three selection systems: positive growth on SD/ura/-leu/-his (Fig. 1), positive growth and generation of white colonies on SD/ura/-leu/-ade and a positive reaction in liquid β-gal assays.

The complete 1087 nucleotide cDNA, including the 829-nucleotide open reading frame (279 amino acids), was then amplified from mouse heart cDNA. Full-length fhl2...
Fig. 1. Interactions of the minK C-terminus with full-length fhl2 in yeast. The bait constructs were transformed into yeast and grown on SD/-ura. Resulting colonies were grown in liquid culture and then supertransformed with the prey constructs and plated on SD/-ura/-leu and SD/-ura/-leu/-his. Only those plasmid combinations expressing proteins capable of interaction (minK C-term and full-length fhl2) activate the His reporter gene and can grow on His-deficient media.

was required in conjunction with the minK C-terminus to activate the His reporter gene in yeast, whereas LIM domains 1/2 to 2 or LIM domains 3 and 4 by themselves were unable to do so (Fig. 1). This indicates that LIM domains 1–4 are required for the interaction with the minK C-terminus or that the junction between LIM domains 2 and 3 must be intact. Similarly, the fhl2 prey construct or the minK bait constructs were not able to interact with a KvLQT1 bait construct and a HERG (another K⁺ channel 3.2.1) prey construct, respectively, indicating that the observed interaction is specific for minK and fhl2.

Fhl2 expression is known to be enriched in the heart [29] and Northern blotting revealed abundant fhl2 mRNA in heart. With prolonged exposure, expression could be detected in uterus and kidney, where minK is also expressed [30,31], as well as in brain, where our unpublished data indicate minK expression (Fig. 2).

To test for an association of minK with fhl2, we generated a peptide-specific antibody against fhl2. This antibody detects a single band of the expected molecular weight (32 kDa) in protein extracts derived from mouse heart (Fig. 3A, lane 3). We tested the total mouse heart extract in a glutathione S-transferase (GST) pulldown assay with a bacterially expressed minK–GST protein fusion. A protein co-migrating with fhl2 from mouse heart extracts associated with GST–minK-linked beads (Fig. 3A, lane 1), whereas it did not associate with GST-linked beads alone (Fig. 3A, lane 2). FLAG-tagged minK and fhl2 also showed overlap of subcellular localization when assayed by confocal microscopy of HEK 293 cells transfected with the two cDNAs (Fig. 3B). Neither minK nor fhl2 were distributed uniformly, but rather were preferentially expressed within discrete foci. Despite this heterogeneous intracellular distribution and a relatively broader expression pattern of fhl2, areas of overlap (yellow regions) were detected and minK staining was generally not observed in areas where fhl2 expression was absent.

3.2. Functional interactions of fhl2 and minK

Northern blot analysis readily identified fhl2 mRNA transcripts in five commonly used laboratory cell lines (COS, Ltk⁻, CHO-K1, HeLa, HEK 293) and in AT-1 cells (cardiac cells derived from a murine atrial tumor [32], Fig. 2B). However, we determined that HEK293 cells, in which fhl2 mRNA was least abundant (Fig. 2B), produced undetectable amounts of fhl2 protein, whereas protein was easily detected in CHO cells (Fig. 4A). HEK cells contain an endogenous outward current that can be readily distinguished from $I_{Ks}$ because of its rapid activation and inactivation kinetics [33]. However, within our experimental paradigm, this background current can not be distinguished from the current evoked by KvLQT1 alone. When HEK293 cells were co-transfected with KvLQT1, minK and a third plasmid expressing the green fluorescent protein (GFP), $I_{Ks}$ was not detected in 9/10 fluorescent cells. We detected a small tail current in a single transfected HEK cell and surmise that it may have been caused by the expression of KvLQT1 alone (Fig. 4B). The other nine transfected HEK cells showed outward current identical to...
Fig. 2. Expression of minK and fhl2 in the adult murine CNS and heart. (A) Northern blot using total RNA from various mouse tissues and the fhl2 cDNA probe. The same blot was stripped and re-probed using a rat cyclophilin cDNA (lower portion) to allow for comparison of loading. RNA from brain is present in relatively greater amounts. (B) Northern blot analysis using the fhl2 cDNA probe on 20 μg of total RNA isolated from commonly used laboratory cell lines demonstrates that fhl2 RNA is widely expressed.

the one shown in Fig. 4B, but no tail currents. By contrast, $I_{Ks}$ was recorded in 9/13 transfected with KvLQT1, minK, and a bicistronic vector expressing GFP and fhl2 (Fig. 4C, $P=0.06$ for 1/10 vs. 9/13, or $P=0.04$ for 1/11 vs. 9/13 cells (chi-square analysis)).

When the same set of experiments was performed in CHO-K1 cells, typical $I_{Ks}$ was recorded from cells transfected with minK and KvLQT1 alone (Fig. 5A), and was indistinguishable from that recorded from cells transfected with minK, KvLQT1 and the fhl2 sense construct (Fig. 5C, circles vs. squares). By contrast, $I_{Ks}$ amplitude was reduced by ~35% ($P<0.05$) in cells transfected with minK, KvLQT1 and an anti-fhl2 antisense construct (Fig. 5B and C, filled triangles). Neither sense nor antisense cDNA constructs altered $I_{Ks}$ voltage dependency of activation (Table 1).

Expression of the KvLQT1 cDNA alone generated a voltage-dependent, rapidly activating and slowly deactivating outward potassium current, as previously reported (Fig. 5D) [2–4]. Panels D–F of Fig. 5 show, that in contrast to
A.

<table>
<thead>
<tr>
<th>GST-minK</th>
<th>GST</th>
<th>Heart extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

KDa

- 41
- 33

B.

[Images of microscopic analysis with arrows indicating specific areas]
measured in Fig. 5 also remained unchanged (Fig. 5A: $V_{1/2} = 9.9 \pm 7.6$ mV, $n = 8$; Fig. 5B: $V_{1/2} = 18.3 \pm 3.7$ mV, $n = 14$; $P = 0.83$. Fig. 5D: $V_{1/2} = -4.6 \pm 3.3$ mV, $n = 10$; Fig. 5E: $V_{1/2} = -8.03 \pm 1.4$ mV, $n = 13$; $P = 0.31$). All paired current recordings were performed on the same day using cells transfected under identical conditions.

4. Discussion

Membrane bound proteins such as ion channels are affected by a multitude of proteins during their life cycle. Well-recognized examples include cis- and/or trans-acting transcriptional and translational regulators, and function-modulating β-subunits, such as minK (reviewed in Ref. [34]). Other interacting proteins target channels to specific loci such as postsynaptic densities [35], traffic them to the membrane [36], or serve as scaffolds to anchor modulators such as kinases/phosphatases [37,38]. Indeed, preliminary data indicate that $I_{KS}$ too is modulated by a macromolecular signaling complex [24,39,40]. Here we identify fh2 as a functionally important partner of the minK potassium channel subunit. This protein is enriched in heart, and interacts with minK both functionally and biochemically. Moreover, the interaction was specific for minK and did not extend to KvLQT1: we did not observe an effect of fh2 antisense treatment on current generated by the KvLQT1 cDNA alone (Fig. 5E,F) supporting the idea of a direct interaction effect of fh2 with minK. When anti-fh2 antisense was coexpressed with KvLQT1 and minK, activation was still $I_{KS}$-like (i.e. slow, Fig. 5, top), indicating that KvLQT1 and minK remain associated. The major effect of anti-fh2 antisense in this experiment was a reduction in current amplitude; this result suggests that the inclusion of fh2 in the channel complex stabilizes it at the cell surface.

Our data show that expression of $I_{KS}$ in HEK 293 cells, which do not contain endogenous fh2, is dependent upon fh2 addition. Freeman and Kass [33] have recorded $I_{KS}$ after minK expression in 18% of 367 transfected HEK 293 cells. However, $I_{KS}$ expression in this system was not dependent on the $I_{KS}$ subunit KvLQT1, raising the possibility that at least a subset of these HEK cells express ion channel subunits. One study reported effects of transfected minK on the HERG protein in HEK cells [41] in the absence of exogenous fh2, implying that minK reaches the plasma membrane without fh2. More recently, in our lab we have been able to express $I_{KS}$ in HEK cells by expressing minK and KvLQT1 alone (i.e. without the

Fig. 4. The generation of $I_{KS}$ requires fh2 expression. (A) Western blot analysis of total cell extracts using the anti-fh2 polyclonal antibody. (B) Current tracing from HEK 293 cell transfected with minK and KvLQT1. (C) $I_{KS}$ recorded from HEK 293 cells transfected with fh2 (sense construct) and KvLQT1 plus minK.
Fig. 5. Antisense-fhl2 treatment suppresses whole-cell $I_{\text{k}}$ magnitude. Currents recorded in CHO-K1 cells coexpressing KvLQT1 and minK are shown without (A) and with (B) cotransfection of anti-fhl2 antisense. (C) Summary data showing the current–voltage relationship of KvLQT1 plus minK currents. Anti-fhl2 antisense caused voltage-independent suppression of the KvLQT1 + minK current magnitude (* $P < 0.05$), but did not alter $I_{\text{k}}$ activation or deactivation (see Table 1). The voltage clamp protocol is shown in the inset. Currents recorded from cells expressing KvLQT1 alone are shown without (D) and with (E) cotransfection of the antisense fhl2. Antisense had no effect on the deactivation kinetics or the current magnitude. (F) Current–voltage relationship of KvLQT1 currents. In contrast to the experiment that included minK, anti-fhl2 antisense had no effect on KvLQT1 alone current.
requirement for cotransfection of fhl2 cDNA). Although this seemingly contradicted our previous data, we were able to reconcile the results after performing anti-fhl2 Western blots on the more recent HEK cell extracts. Indeed, we found a fhl2-related band in these extracts, indicating that HEK cells variably express a fhl2-like protein which influences expression of $I_{\text{Ks}}$. An important implication of this finding is that variable expression of potential ion channel modifiers by laboratory cell commonly used for electrophysiology assays may be a complicating factor in many studies.

Fhl2 is composed of cysteine-rich motifs forming Zn$^{2+}$ fingers (LIM domains) thought to function as protein interaction modules in both nuclear and cytoplasmic proteins (reviewed in Ref. [16]). Li et al. [23] have shown by immunohistochemistry that fhl2 colocalizes with $\alpha$-actinin at the Z-lines of human cardiac myocytes. Thus, one possible role for fhl2 is to link minK to the cytoskeleton, given that Z-lines are known to play a crucial role in the establishment and maintenance of cardiomyocyte cytoarchitecture [42]. This is consistent with an emerging body of work indicating regulation of ion channel function by interaction with the cytoskeleton [43–48]. Possible roles for compartmentalization of ion channels by cytoskeletal anchoring include the regulation of channel activity by stabilizing the protein at the cellular surface, or increasing the efficiency by which intracellular signaling molecules (e.g. kinases/phosphatases) control the channel. Other LIM domain proteins have been described that influence the myocardial cytoskeletal architecture [49] or have regulatory roles in myogenic differentiation [50,51]. An accumulating body of evidence supports the view that proteins associated with the cytoskeleton serve important roles in modulating response of cardiac physiology to exogenous stress such as hypertrophy (reviewed in Ref. [52]), or drug exposure [53,54].

The generation of lines of mice homozygous for fhl2 deficiency [55,56] with no overt phenotype indicated that the gene is not essential for cardiac development and function, although in fhl2 knockouts the hypertrophic response to $\beta$-adrenergic stimulation was altered [56].

Further supporting a possible interaction between minK and fhl2 are reports showing the highest levels of fhl2 expression during murine development in cardiac septa and in the region adjacent to the atrioventricular bundle [29], a pattern which has also been noted for minK [57,58].

In summary, we have identified fhl2 as a potentially important minK interacting protein in heart. The data we report may constitute a link between the $I_{\text{Ks}}$ channel complex and the cytoskeleton via the modular fhl2 protein. Moreover, the identification of fhl2 as an $I_{\text{Ks}}$ modifier makes it a candidate modulator for arrhythmia syndromes such as the congenital Long QT syndrome.

Acknowledgements

We would like to thank Drs Christoph Fahlke and Andrew Tapper for helpful discussions. This work was supported by an American Heart Association Scientist Development Grant to Dr Kupershmidt and grants from the United States Public Health Service (HL49989 and HL46681 to Drs Roden and Balser). Dr Roden is the holder of the William Stokes Chair in Experimental Therapeutics, a gift from the Daïichi Corporation.

References


Table 1

Voltage dependency of tail currents

<table>
<thead>
<tr>
<th></th>
<th>$V_{\text{1/2}}$ (mV)</th>
<th>$k$ (mV)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KvLQT1 plus minK</td>
<td>13.41±4.10</td>
<td>12.60±1.08</td>
<td>8</td>
</tr>
<tr>
<td>KvLQT1 plus minK plus fhl2 (sense)</td>
<td>12.74±3.15</td>
<td>12.83±0.87</td>
<td>11</td>
</tr>
<tr>
<td>KvLQT1 plus minK plus fhl2 (antisense)</td>
<td>12.20±3.06</td>
<td>13.22±0.59</td>
<td>14</td>
</tr>
<tr>
<td>KvLQT1</td>
<td>-19.90±3.35</td>
<td>23.83±2.81</td>
<td>10</td>
</tr>
<tr>
<td>KvLQT1 plus fhl2 (antisense)</td>
<td>-20.70±1.59</td>
<td>23.12±2.09</td>
<td>13</td>
</tr>
</tbody>
</table>

Relative activation curves were determined by fitting a Boltzman function: $y=[1+\exp((V-V_{\text{1/2}})/k)]^{-1}$ to the peak values of tail currents (see Section 2) vs. test potential. The voltage at which the current was half-maximally activated ($V_{\text{1/2}}$), and the slope factor ($k$) were calculated from these data and averaged values are reported. Statistically significant differences were not observed (Student’s $t$-test).
Gong J, Xu J, Bezanilla M et al. Differential stimulation of PKC
Sugimoto T, Tanabe Y, Shigemoto R et al. Immunohistochemical
Chu P, Ruiz-Lozano P, Zhou Q, Cai C, Chen J. Expression patterns
Warmke JW, Ganetzky B. A family of potassium channel genes
James P, Halladay J, Craig EA. Genomic libraries and a host strain
Li HY, Kotaka M, Kostin S et al. Translocation of a human focal
Horio Y, Hibino H, Inanobe A et al. Clustering and enhanced activity of
Sheng M, Kim E. Ion channel associated proteins. Curr Opin
Chan KK, Tsui SK, Lee SM et al. Molecular cloning and characteri-
Xia H, Winokur ST, Kuo WL, Altherr MR, Bredt DS. Actinin-
potassium channel beta-subunit minK with the sarcomeric protein
Bichet D, Cornet V, Geib S et al. The I–II loop of the Ca++ channel
Ratcliffe CF, Qu Y, McCormick KA et al. A sodium channel
cystin binding LIM protein, maps to mouse chromosome 19 and
Bichet D, Cornet V, Geib S et al. The I–II loop of the Ca++ channel
Petet F, Scott JD, Mohammad-Panah R, Escande D, Baro I, AKAP
Tsuonoda S, Sierralta J, Sun Y et al. Multivalent PDZ-domain
Way JC, Chalfie M. mec-3, a homeobox-containing gene that spec-
Kim AC, Peters LL, Knoll JH et al. Limatin (LIMAB1), an actin-
Beckerle MC, Zyxin zinc fingers at sites of cell adhesion. Bioessays
FHL2, two members of a LIM motif-containing protein kinase family.
Nunoe K, Ohashi K, Okano I, Mizuno K. LIMK-1 and LIMK-2, two
Beckerle MC. Zyxin: zinc fingers at sites of cell adhesion. Bioessays
Horio Y, Hibino H, Inanobe A et al. Clustering and enhanced activity of
Nehring RB, Wischmeyer E, Doring F et al. Neuronal inwardly rectifying K(+) channels differentially couple to PDZ proteins of
An WF, Bowly MR, Betty M et al. Modulation of A-type potassium
Gong J, Xu J, Bezanilla M et al. Differential stimulation of PKC
Sugimoto T, Tanabe Y, Shigemoto R et al. Immunohistochemical
Chu P, Ruiz-Lozano P, Zhou Q, Cai C, Chen J. Expression patterns
Warmke JW, Ganetzky B. A family of potassium channel genes


