Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 360 (2007) 212–218
BBRC
www.elsevier.com/locate/ybbrc

Kcnq1 contributes to an adrenergic-sensitive steady-state K^+ current in mouse heart

Bjorn C. Knollmann [a,b,1,2], Syevda Sirenko [a,2], Qi Rong [a,2], Alexander N. Katchman [a], Mathew Casimiro [c], Karl Pfeifer [c], Steven N. Ebert [a,*]

[a] Department of Pharmacology, Georgetown University Medical Center, Washington, DC, USA
[b] Department of Medicine, Georgetown University Medical Center, Washington, DC, USA
[c] Laboratory of Mammalian Genes and Development, National Institute of Child Health and Development, The National Institutes of Health, Bethesda, MD, USA

Received 6 June 2007
Available online 15 June 2007

Abstract

It has been suggested that Kcne1 subunits are required for adrenergic regulation of Kcnq1 potassium channels. However, in adult mouse hearts, which do not express Kcne1, loss of Kcnq1 causes a Long QT phenotype during adrenergic challenge, raising the possibility that native Kcnq1 currents exist and are adrenergically regulated even in absence of Kcne1. Here, we used immunoblotting and immunohistochemical staining to show that Kcnq1 protein is present in adult mouse hearts. Voltage-clamp experiments demonstrated that Kcnq1 contributes to a steady-state outward current (I_{SS}) in wild-type ($Kcnq1^{+/+}$) ventricular myocytes during isoproterenol stimulation, resulting in a significant 7.1% increase in I_{SS} density (0.43 ± 0.16 pA/pF, $p < 0.05$, $n = 15$), an effect that was absent in Kcnq1-deficient ($Kcnq1^{-/-}$) myocytes (-0.14 ± 0.13 pA/pF, $n = 17$). These results demonstrate for the first time that Kcnq1 protein is expressed in adult mouse hearts where it contributes to a β-adrenergic-induced component of I_{SS} that does not require co-assembly with Kcne1.

Keywords: Potassium channel; Transgenic mouse; Electrophysiology; Adrenergic; Heart

The *KCNQ1* gene encodes a 6-transmembrane domain α-subunit of a K^+ channel protein that can either form a homomeric channel or partner with single transmembrane domain β subunits encoded by the KCNE family of genes to form heteromeric channels [1–8]. The biophysical properties of the KCNQ1 channel differ greatly depending upon the β-subunit with which it is co-expressed.

In the human heart, KCNQ1 is thought to primarily partner with KCNE1 to form a heteromeric channel protein that produces the slow component of the delayed rectifier current, I_{Ks} [2], whose amplitude is markedly increased by adrenergic stimulation [1]. Mutations in both the *KCNQ1* and *KCNE1* genes have been linked to Long QT Syndrome (LQTS), a disorder that predisposes individuals to increased risk of torsade de pointes ventricular arrhythmias and sudden cardiac death [3,9–11].

The role of KCNE1 in mediating β-adrenergic regulation of KCNQ1 is controversial: In one study [12], co-assembly of KCNE and KCNQ1 was required, while other reports showed that heterologously expressed KCNQ1 channels

* Corresponding author. Present address: Burnett College of Biomedical Sciences, Biomolecular Science Center, University of Central Florida, BMS Building, Room 321/321A, Orlando, FL 32816-2364, USA. Fax: +1 407 823 0956.
E-mail address: ebert@mail.ucf.edu (S.N. Ebert).
[1] Present address: Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA.
[2] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.038

were responsive to adrenergic stimulation in the absence of KCNE1 [3,13]. In mice, *Kcne1* expression is strongly down-regulated during postnatal development such that little or no Kcne1 remains in the adult mouse heart [14,15]. Correspondingly, cellular electrophysiological studies did not find I_{Ks} in adult mouse cardiomyocytes [16,17].

Despite the near-absence of *Kcne1* expression and I_{Ks} in adult mouse hearts, *Kcnq1* mRNA expression remains relatively robust in the heart throughout development and into adulthood [15,18], suggesting that Kcnq1 might play a Kcne1-independent role in cardiac function.

Here, we compare Kcnq1-null mice with wild-type littermates to test the hypothesis that Kcnq1 channels are responsive to adrenergic stimulation in native ventricular myocytes even in the absence of Kcne1 and to resolve the issue of Kcnq1 function in the adult mouse heart. Specifically, Kcnq1 protein expression was examined in adult mouse hearts using immunoblotting and immunofluorescent histochemical staining techniques, where we show that Kcnq1 protein is present in both atria and ventricles. To determine which currents were influenced by Kcnq1, we examined outward K^+ currents in isolated wild-type and Kcnq1-deficient adult cardiomyocytes. We hypothesized that since Kcne1 is nearly absent in adult murine myocardium, any Kcnq1-mediated current would contribute to the steady-state outward current (I_{SS}) because the biophysical properties of I_{SS} resemble those described for Kcne1-independent Kcnq1 currents described in heterologous expression systems [1,2,4,6,5].

Our results show that the β-adrenergic agonist, isoproterenol, significantly enhances I_{SS} in wild-type ventricular myocytes but has no significant effect on this current in Kcnq1-deficient myocytes. Thus, our data suggest that Kcnq1 is expressed in the adult murine heart where it contributes to a β-adrenergic-sensitive component of the outward steady-state K^+ current, I_{SS}.

Materials and methods

Materials. The anti-Kcnq1 antibody AB5932 was obtained from Chemicon International (Temecula, CA). The anti-Dihydropyridine Receptor α2 (DHPα2) subunit antibody was obtained from Sigma Chemical Co. (St. Louis, MO). Fluorescent secondary antibodies were obtained from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA). All other drugs and chemicals were purchased from Sigma Chemical Co.

Animals. Kcnq1-deficient mice were maintained as previously described [19]. *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mice were produced by breeding heterozygous (*Kcnq1*$^{+/-}$) mating pairs. All animal procedures were performed in accordance with protocols approved by the Georgetown University or by the National Institute for Child Health and Development Intramural Research Program Animal Care and Use Committees. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Immunoblotting. Preparation of membrane-enriched extracts and Western blotting procedures was performed essentially as described by Pond et al. [20]. Briefly, twenty micrograms of membrane extract were separated by SDS–polyacrylamide gel electrophoresis using pre-packaged Tris–Glycine (10%) gels from Invitrogen (Carlsbad, CA). The proteins were transferred to Invitrolon™ PVDF membrane (Invitrogen), and the blots were blocked, incubated with antibody solution, and developed as described previously [21].

Immunofluorescent histochemical staining. Single and double immunofluorescent histochemical staining was performed as described previously [22,23].

Whole-cell patch-clamp recordings. Adult mouse cardiomyocytes were isolated and whole-cell patch-clamp recordings were performed at 36 °C as described previously [24]. Briefly, pipettes with tip resistances of 2–3 MΩ were filled with solution containing (in mmol/L): KCl 155, EGTA 14, $CaCl_2$ 1, Hepes 10, MgATP 5, pH 7.2. Whole-cell recordings were performed in control Tyrode's solutions containing (in mmol/L): NaCl 140; KCI, 5.4; glucose, 10; $MgCI_2$, 1; $CaCI_2$, 2.0; and Hepes, 10; pH 7.4. Only cells with resting potentials more negative than −70 mV were used. After whole-cell voltage clamp was established, the extracellular solution was quickly exchanged and the steady-state outward current, I_{SS}, recorded using specific voltage protocols indicated in the text. To optimize recording of I_{ss}, the extracellular solution contained 2 mM 4-aminopyridine (to block slow and fast transient outward currents, $I_{K,slow}$ and the ultra-rapid delayed rectifier, I_{Kur}), 1 μM E-4031 (to block the rapid component of the delayed rectifier, I_{Kr}), 5 μM nifedipine and low (0.1 mM) Ca^{++} (to block the L-type calcium current, $I_{Ca,L}$), and 0 mM Na^+ (to abolish I_{Na}). In some experiments, chloride-free solutions were used (gluconate salts) to minimize potential interference from Cl^- currents. Similar results were obtained in the absence and presence of Cl^- (not shown). Wherever indicated in the text, isoproterenol (1 μM) was applied to the external solutions and the recording protocol was replicated following baseline measurements.

Results

Kcnq1 protein expression in adult murine myocardium

Although it is well-established that Kcnq1 mRNA concentrations remain relatively robust in the adult mouse heart [15,18], protein expression has not been studied. To address this issue, we used an anti-Kcnq1 antibody to probe protein extracts from mouse hearts. The predicted molecular weight for Kcnq1 is approximately 70 kDa and anti-Kcnq1 antibody detected a band of this size in extracts prepared from *Kcnq1*$^{+/+}$ but not from *Kcnq1*$^{-/-}$ hearts (Fig. 1A). As a control, the blots were stripped and re-probed with an anti-DHPα2 antibody, which detected a 143 kDa band of similar intensity in both extracts. These results demonstrate that Kcnq1 protein is expressed in adult mouse hearts.

To determine where Kcnq1 is localized within the heart, we used the anti-Kcnq1 antibody to perform immunofluorescent histochemical staining. An example of these results is shown in Fig. 1, where Kcnq1 protein was detected in both atrial and ventricular myocytes in *Kcnq1*$^{+/+}$ (Fig. 1B) but not in *Kcnq1*$^{-/-}$ (Fig. 1C) heart sections. The atrial staining pattern typically appears to be both more intense and more uniform than that observed in the ventricular myocardium.

To explore the ventricular Kcnq1 expression in more detail, we performed co-immunofluorescent staining for sarcomeric α-actinin. As shown in Fig. 1D, Kcnq1 staining was apparent in a "ladder-like" pattern in ventricular cardiomyocytes. Co-staining of the same section for sarcomeric α-actinin enabled identification of the Z-bands (Fig. 1E). There was substantial co-expression of Kcnq1 and sarcomeric α-actinin in these sections, as confirmed

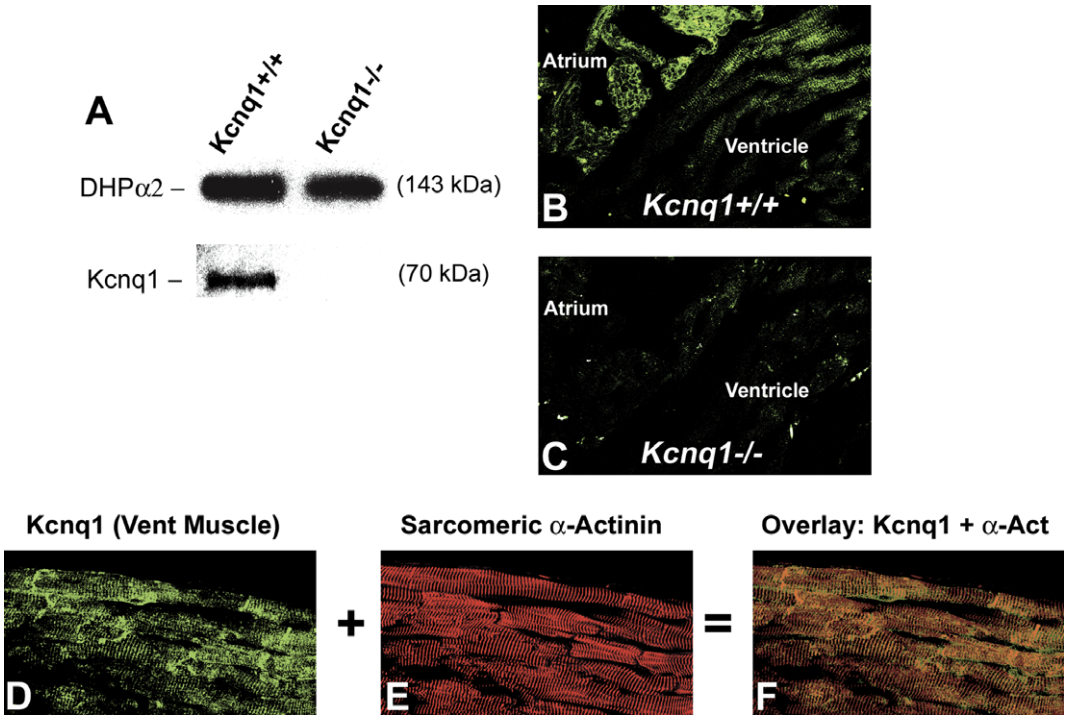

Fig. 1. Identification of Kcnq1 protein in adult mouse heart. (A) Western blot assays were performed using proteins extracted from *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ adult mouse hearts. The blot was initially probed with an anti-Kcnq1 antibody, and then stripped and re-probed with an anti-DHPα2 antibody. (B and C) Localization of Kcnq1 protein in adult mouse hearts using immunofluorescent histochemical staining. Sections from *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts are shown in (B and C), respectively. Atrial tissue is in the northwest portion while ventricular tissue is in the southeast portion of each panel. (D–F) Co-immunofluorescent staining in an adult mouse (*Kncq1*$^{+/+}$) ventricular muscle tissue section for (D) Kcnq1, (E) sarcomeric α-actinin, and (F) an overlay of these images. Co-expression is indicated by the yellow regions indicating overlapping staining for Kcnq1 and sarcomeric α-actinin in the same section.

by the yellow staining in Fig. 1F, which represents overlap of Kcnq1 and sarcomeric α-actinin expression. These results indicate that Kcnq1 protein is expressed in a sarcomeric-like pattern within ventricular myocytes.

Electrophysiological evaluation of I_{SS} in isolated wild-type and in Kcnq1-deficient ventricular myocytes

In the absence of Kcne1, Kcnq1 produces a rapidly activating time-independent current that has biophysical characteristics reminiscent of those described for the steady-state current, I_{SS}, in adult mouse ventricular cells [25]. I_{SS} appears to be generated by more than one type of K^+ channel protein [26]. Although Kcnq1 has not previously been identified as one of the channel proteins that contribute to I_{SS} [27], its electrophysiological characteristics in heterologous expression studies either as a homomeric channel protein or in partnership with other (non-Kcne1) subunits such as Kcne2 or Kcne3 [4,6,5] create a plausible scenario for Kcnq1 participation in I_{SS}.

To test the hypothesis that Kcnq1 contributes to I_{SS}, we prepared cardiomyocytes from adult *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ animals and recorded I_{SS}. To block potentially interfering currents, we isolated I_{SS} using inhibitors of I_{Ca}, I_{Na}, and I_K (see Materials and methods) in combination with the voltage-clamp protocol shown in Fig. 2A. I_{SS} was not different between *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ myocytes (6.2 ± 0.3 vs. 6.3 ± 0.3 pA/pF, $p =$ n.s., $n = 15$ and 17, respectively), suggesting that Kcnq1 contributes little or none to murine I_{SS} under basal conditions.

Since the Kcnq1 channel is known to produce enhanced current following β-adrenergic stimulation and subsequent phosphorylation by protein kinase A (PKA) in heterologous expression systems [3,16], we hypothesized that β-adrenergic stimulation would selectively increase a Kcnq1-dependent component of I_{SS}. To test this hypothesis, we repeated the protocol in the presence of the β-adrenergic agonist, isoproterenol (1 μM). Interestingly, I_{SS} increased in the presence of isoproterenol in *Kcnq1*$^{+/+}$ myocytes, whereas isoproterenol had no effect on I_{SS} in *Kcnq1*$^{-/-}$ myocytes (compare B and C, Fig. 2). As a result, average values of I_{SS} following isoproterenol challenge were significantly higher in *Kcnq1*$^{-/-}$ myocytes compared with *Kcnq1*$^{-/-}$ myocytes (6.2 ± 0.3 vs. 6.7 ± 0.3 pA/pF, $p < 0.05$, $n = 15$ and 17, Fig. 2D). These data suggest that the difference in I_{SS} (ΔI_{SS}) observed in the presence versus the absence of isoproterenol can be attributed to Kcnq1. Average ΔI_{SS} was 0.43 ± 0.16 pA/pF in *Kcnq1*$^{+/+}$ myocytes but -0.14 ± 13 pA/pF in *Kcnq1*$^{-/-}$ myocytes ($p < 0.05$, Fig. 2E). This translates into a net increase in I_{SS} density of about 7.1% in *Kcnq1*$^{+/+}$ myocytes and no significant change in *Kcnq1*$^{-/-}$ myocytes. Together, these data

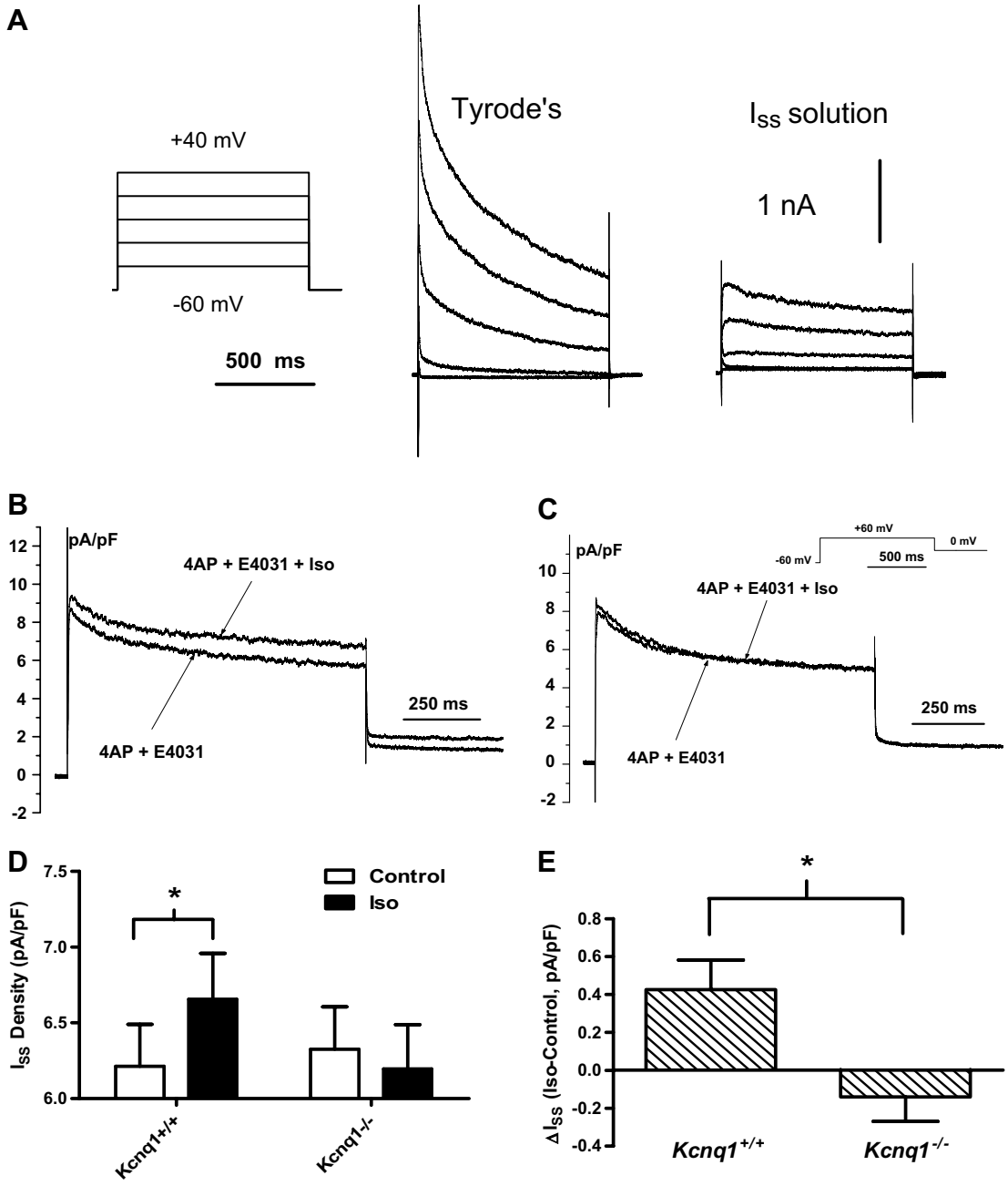

Fig. 2. Evaluation of the steady-state K^+ current, I_{SS}, in ventricular myocytes isolated from adult *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts before and after challenge with the β-adrenergic agonist, isoproterenol (Iso). (A) Voltage-clamp protocol used to record outward K^+ currents in these experiments. The left tracing shows control currents recorded without any drugs in normal Tyrode's solution. The tracing on the right shows I_{SS} recorded using the same voltage protocol following replacement of Tyrode's solution with "I_{SS} solution", a Tyrode's solution containing 4AP to block I_{to} and E4031 to block I_{Kr} (see Methods for details). (B and C) Examples of I_{SS} recorded in the absence and presence of Iso for *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ myocytes, respectively. Note that Iso enhances I_{SS} in *Kcnq1*$^{+/+}$ (B) but not *Kcnq1*$^{-/-}$ (C) myocytes. (D) Comparison of average I_{SS} densities for *Kcnq1*$^{+/+}$ ($n = 15$) and *Kcnq1*$^{-/-}$ ($n = 17$) myocytes in the absence (Control) versus the presence of Iso. (E) Comparison of the Iso-induced difference current (ΔI_{SS}) measured from the data sets shown in (D). $^*p < 0.05$ using Student's *t*-test for statistical evaluation of significance.

indicate that Kcnq1 contributes to a portion of I_{SS} that is selectively enhanced by β-adrenergic stimulation.

Discussion

The function of Kcnq1 in the adult mouse heart is controversial [16,19,28–30]. The major basis for this debate appears to be the well-established down-regulation of the Kcne1 subunit and consequent loss of I_{Ks} in adult murine myocytes compared to prenatal and early postnatal developmental stages [14,15]. Unlike mice, KCNE1 expression appears to remain relatively high in human hearts through adulthood, and I_{Ks} has been readily recorded in isolated human cardiomyocytes [31,32].

Nevertheless, we have shown that targeted disruption of *Kcnq1* [19] and introduction of a specific knock-in *Kcnq1* point mutations [30] lead to development of a Long QT phenotype in adult mice. Similar QT abnormalities have been observed in isolated perfused Kcnq1-deficient adult mouse hearts following challenge with sympathomimetic drugs such as nicotine, isoproterenol, and epinephrine [29], indicating this Long QT phenotype is intrinsic to the heart itself and does not reflect extra-cardiac factors.

Kcnq1 protein expression in adult mouse myocardium

To establish that Kcnq1 protein is actually expressed in the adult mouse heart, we first used an anti-Kcnq1 antibody to perform immunoblotting experiments. Our results show that a protein of approximately 70 kDa was specifically detected in extracts from $Kcnq1^{+/+}$ hearts, but was completely absent in $Kcnq1^{-/-}$ hearts. Thus, our data indicate that Kcnq1 protein is present in the adult murine heart.

Consistent with these immunoblotting results, we also detected Kcnq1 protein in the adult murine heart using immunofluorescent histochemical staining techniques. With this approach, we demonstrated that Kcnq1 protein is expressed in both atria and ventricles. The staining pattern was consistent with Kcnq1 expression in both atrial and ventricular working myocardium. Interestingly, immunostaining appeared more intense within the atria compared to the ventricles, suggesting that Kcnq1 channels may be more prevalent in atrial tissue. Indeed, Temple et al. [33] speculated that atrial fibrillation observed in the KCNE1-null mice could reflect a contribution of I_{KCNQ1} alone to atrial action potentials. Alternatively, the different subcellular patterns of Kcnq1 distribution in atrial versus ventricular myocytes may have contributed to the differential staining intensities. In ventricular myocytes, Kcnq1 staining patterns largely aligned with sarcomeric structures, similar to observations made with other cardiac ion channel distribution patterns in these cells [34–36], including Kcnq1 in rat ventricular myocytes [37]. The more diffuse pattern observed in atrial myocytes may reflect the lack of well-developed sarcomeric and t-tubule structures in these cells [38].

Kcnq1 contributes to a β-adrenergic-sensitive component of I_{SS}

Since there is no I_{Ks} present in adult murine ventricular myocytes, a role for Kcnq1 in these cells has not previously been identified. We hypothesized that Kcnq1 contributes to the steady-state outward K^+ current, I_{SS}, because of the similar electrophysiological properties of I_{SS} in isolated ventricular myocytes [27] and I_{KCNQ1} in transfected cells. Both currents display rapidly activating kinetics and do not inactivate. To test this hypothesis, we evaluated I_{SS} in ventricular myocytes isolated from $Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ hearts. Under control conditions, no significant differences in I_{SS} densities were observed between wild-type and mutant myocytes, thereby indicating that Kcnq1 may not contribute significantly to repolarization at baseline. In the presence of isoproterenol, however, a significant increase in I_{SS} density was observed exclusively $Kcnq1^{+/+}$ myocytes. Since the isoproterenol-induced increase in I_{SS} was dependent on the presence of Kcnq1, the logical conclusion is that endogenous Kcnq1 channels mediate the increased I_{SS} densities observed in the presence of isoproterenol.

It is clear from previous studies [3,13,16] that Kcnq1 itself is the target of PKA-mediated phosphorylation, and that I_{KCNQ1} can be enhanced by PKA or forskolin in the absence of KCNE1. However, because these previous studies were performed in heterologous expression systems, it has not been previously determined if endogenous Kcnq1 could be regulated by adrenergic hormones in the absence of Kcne1. At present, we cannot conclude that Kcnq1 is acting alone (i.e., homomeric Kcnq1 channels leading to I_{Kcnq1}) versus partnership with one or more non-Kcne1 subunits (e.g., Kcne2 or Kcne3) [6]. We can, however, conclude that Kcnq1 channel proteins are expressed in the adult mouse heart where they contribute to steady-state repolarizing currents during β-adrenergic receptor stimulation.

These results demonstrate for the first time that endogenously expressed Kcnq1 contributes to a β-adrenergic-responsive current other than I_{Ks} (and therefore independent of Kcne1) in ventricular cardiomyocytes.

Study limitations

One caveat of our work is that the findings may be specific to mice and other small rodents that display marked differences in cardiac electrophysiology compared to humans. The developmental down-regulation of Kcne1 in mice and rats likely contributes to the lack of I_{Ks} in the adult myocardium of these species, whereas I_{Ks} has been readily detected in larger mammalian species, including humans [31,32]. It is not clear, however, that I_{Ks} is the only important current to which KCNQ1 contributes to even in hearts where I_{Ks} is known to be present. Indeed, a recent study by Lundquist et al. [39] showed that multiple KCNE subunits are expressed in different regions of human myocyardium, and that co-expression of other KCNE [2–5] subunits can significantly affect KCNQ1 currents even when KCNE1 is also present. Furthermore, Dun and Boyden [40] recently showed that KCNQ1 contributed to steady-state-like (non-I_{Ks}) currents in canine ventricular myocytes isolated from post-infracted hearts. Clearly, additional work is needed to determine the molecular constituency and physiological correlates of cardiac channels containing Kcnq1 in humans and other species.

Acknowledgments

This work was supported by grants from the NHLBI (HL78716, S.N.E.; HL71670 and HL88635, B.C.K.) and intramural funds from the NICHD (K.P.).

References

[1] M. Sanguinetti, M. Curran, A. Zou, J. Shen, P. Spector, D. Atkinson, M. Keating, Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac IKs potassium channel, Nature 384 (1996) 80–83.

[2] J. Barhanin, F. Lesage, E. Guillemare, M. Fink, M. Lazdunski, G. Romey, KvLQT1 and IsK (minK) proteins associate to form the IKs cardiac potassium current, Nature 384 (1996) 78–80.

[3] W.P. Yang, P.C. Levesque, W.A. Little, M.L. Conder, F.Y. Shalaby, M.A. Blanar, KvLQT1, a voltage-gated potassium channel responsible for human cardiac arrhythmias, Proc. Natl. Acad. Sci. USA 94 (1997) 4017–4021.

[4] G.W. Abbott, S.A. Goldstein, A superfamily of small potassium channel subunits: form and function of the MinK-related peptides (MiRPs), Q, Rev. Biophys. 31 (1998) 357–398.

[5] B.C. Schroeder, S. Waldegger, S. Fehr, M. Bleich, R. Warth, R. Greger, T.J. Jentsch, A constitutively open potassium channel formed by KCNQ1 and KCNE3, Nature 403 (2000) 196–199.

[6] N. Tinel, S. Diochot, M. Borsotto, M. Lazdunski, J. Barhanin, KCNE2 confers background current characteristics to the cardiac KCNQ1 potassium channel, EMBO J. 19 (2000) 6326–6330.

[7] M. Grunnet, T. Jespersen, H.B. Rasmussen, T. Ljungstrom, N.K. Jorgensen, S.P. Olesen, D.A. Klaerke, KCNE4 is an inhibitory subunit to the KCNQ1 channel, J. Physiol. 542 (2002) 119–130.

[8] K. Angelo, T. Jespersen, M. Grunnet, M.S. Nielsen, D.A. Klaerke, S.P. Olesen, KCNE5 induces time- and voltage-dependent modulation of the KCNQ1 current, Biophys. J. 83 (2002) 1997–2006.

[9] Q. Wang, M.E. Curran, I. Splawski, T.C. Burn, J.M. Millholland, T.J. VanRaay, J. Shen, K.W. Timothy, G.M. Vincent, T. de Jager, P.J. Schwartz, J.A. Toubin, A.J. Moss, D.L. Atkinson, G.M. Landes, T.D. Connors, M.T. Keating, Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias, Nat. Genet. 12 (1996) 17–23.

[10] P. Duggal, M.R. Vesely, D. Wattanasirichaigoon, J. Villafane, V. Kaushik, A.H. Beggs, Mutation of the gene for IsK associated with 8both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome, Circulation 97 (1998) 142–146.

[11] C.E. Chiang, D.M. Roden, The long QT syndromes: genetic basis and clinical implications, J. Am. Coll. Cardiol. 36 (2000) 1–12.

[12] J. Kurokawa, H.K. Motoike, J. Rao, R.S. Kass, Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation, Proc. Natl. Acad. Sci. USA 101 (2004) 16374–16378.

[13] T. Yang, H. Kanki, D.M. Roden, Phosphorylation of the IKs channel complex inhibits drug block: novel mechanism underlying variable antiarrhythmic drug actions, Circulation 108 (2003) 132–134.

[14] E. Honore, B. Attali, G. Romey, C. Heurteaux, P. Ricard, F. Lesage, M. Lazdunski, J. Barhanin, Cloning, expression, pharmacology and regulation of a delayed rectifier K+ channel in mouse heart, EMBO J. 10 (1991) 2805–2811.

[15] M.D. Drici, I. Arrighi, C. Chouabe, J.R. Mann, M. Lazdunski, G. Romey, J. Barhanin, Involvement of IsK-associated K+ channel in heart rate control of repolarization in a murine engineered model of Jervell and Lange-Nielsen syndrome, Circ. Res. 83 (1998) 95–102.

[16] S.O. Marx, J. Kurokawa, S. Reiken, H. Motoike, J. D'Armiento, A.R. Marks, R.S. Kass, Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel, Science 295 (2002) 496–499.

[17] T.C. Chiello, C. Cabo, J. Coromilas, J. Kurokawa, R.S. Kass, A.L. Wit, Electrophysiological consequences of human IKs channel expression in adult murine heart, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H168–H175.

[18] T. Gould, K. Pfeifer, Imprinting of mouse Kvlqt1 is developmentally regulated, Hum. Mol. Genet. 7 (1998) 483–487.

[19] M. Casimiro, B.K. Knollmann, S.N. Ebert, J. Vary, A. Grinberg, K. Pfeifer, Targeted disruption of the Kvlqt1 gene produces a mouse model of Jervell and Lange-Nielsen syndrome, Proc. Natl. Acad. Sci. USA 98 (2001) 2526–2531.

[20] A.L. Pond, B.K. Scheve, A.T. Benedict, K. Petrecca, D.R. Van Wagoner, A. Shrier, J.M. Nerbonne, Expression of distinct ERG proteins in rat, mouse, and human heart. Relation to functional I(Kr) channels, J. Biol. Chem. 275 (2000) 5997–6006.

[21] J.J. Yeh, R.P. Yasuda, M.I. Davila-Garcia, Y. Xiao, S. Ebert, T. Gupta, K.J. Kellar, B.B. Wolfe, Neuronal nicotinic acetylcholine receptor alpha3 subunit protein in rat brain and sympathetic ganglion measured using a subunit-specific antibody: regional and ontogenic expression, J. Neurochem. 77 (2001) 336–346.

[22] S.N. Ebert, R.P. Thompson, Embryonic epinephrine synthesis in the rat heart before innervation: association with pacemaking and conduction tissue development, Circ. Res. 88 (2001) 117–124.

[23] S.N. Ebert, Q. Rong, S. Boe, R.P. Thompson, A. Grinberg, K. Pfeifer, Targeted insertion of the Cre-recombinase gene at the phenylethanolamine *n*-methyltransferase locus: a new model for studying the developmental distribution of adrenergic cells, Dev. Dyn. 231 (2004) 849–858.

[24] B.C. Knollmann, B.E. Knollmann-Ritschel, N.J. Weissman, L.R. Jones, M. Morad, Remodelling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin, J. Physiol. 525 (Pt. 2) (2000) 483–498.

[25] H. Xu, W. Guo, J. Nerbonne, Four kinetically distinct depolarization-activated K+ currents in adult mouse ventricular myocytes, J. Gen. Physiol. 113 (1999) 661–678.

[26] W. Guo, H. Xu, B. London, J.M. Nerbonne, Molecular basis of transient outward K+ current diversity in mouse ventricular myocytes, J. Physiol. 521 (Pt. 3) (1999) 587–599.

[27] H. Xu, W. Guo, J.M. Nerbonne, Four kinetically distinct depolarization-activated K+ currents in adult mouse ventricular myocytes, J. Gen. Physiol. 113 (1999) 661–678.

[28] M.P. Lee, J.D. Ravenel, R.J. Hu, L.R. Lustig, G. Tomaselli, R.D. Berger, S.A. Brandenburg, T.J. Litzi, T.E. Bunton, C. Limb, H. Francis, M. Gorelikow, H. Gu, K. Washington, P. Argani, J.R. Goldenring, R.J. Coffey, A.P. Feinberg, Targeted disruption of the Kvlqt1 gene causes deafness and gastric hyperplasia in mice, J. Clin. Invest. 106 (2000) 1447–1455.

[29] T. Tosaka, M.C. Casimiro, Q. Rong, S. Tella, M. Oh, A.N. Katchman, J.C. Pezzullo, K. Pfeifer, S.N. Ebert, Nicotine induces a long QT phenotype in Kcnq1-deficient mouse hearts, J. Pharmacol. Exp. Ther. 306 (2003) 980–987.

[30] M. Casimiro, B. Knollmann, E. Yamoah, L. Nie, J. Vary, S. Sirenko, A. Greene, A. Grinberg, S. Huang, S. Ebert, K. Pfeifer, Targeted point mutagenesis of mouse Kcnq1: phenotypic analysis of mice with point mutations that cause Romano-Ward syndrome in humans, Genomics 84 (2004) 555–564.

[31] G.R. Li, J. Feng, L. Yue, M. Carrier, S. Nattel, Evidence for two components of delayed rectifier K+ current in human ventricular myocytes, Circ. Res. 78 (1996) 689–696.

[32] Z. Wang, B. Fermini, S. Nattel, Rapid and slow components of delayed rectifier current in human atrial myocytes, Cardiovasc. Res. 28 (1994) 1540–1546.

[33] J. Temple, P. Frias, J. Rottman, T. Yang, Y. Wu, E.E. Verheijck, W. Zhang, C. Siprachanh, H. Kanki, J.B. Atkinson, P. King, M.E. Anderson, S. Kupershmidt, D.M. Roden, Atrial fibrillation in KCNE1-null mice, Circ. Res. 97 (2005) 62–69.

[34] S. Takeuchi, Y. Takagishi, K. Yasui, Y. Murata, J. Toyama, I. Kodama, Voltage-gated K(+)Channel, Kv4.2, localizes predominantly to the transverse-axial tubular system of the rat myocyte, J. Mol. Cell Cardiol. 32 (2000) 1361–1369.

[35] R.B. Clark, A. Tremblay, P. Melnyk, B.G. Allen, W.R. Giles, C. Fiset, T-tubule localization of the inward-rectifier K(+) channel in mouse ventricular myocytes: a role in K(+) accumulation, J. Physiol. 537 (2001) 979–992.

[36] W. Han, W. Bao, Z. Wang, S. Nattel, Comparison of ion-channel subunit expression in canine cardiac Purkinje fibers and ventricular muscle, Circ. Res. 91 (2002) 790–797.

[37] H.B. Rasmussen, M. Moller, H.G. Knaus, B.S. Jensen, S.P. Olesen, N.K. Jorgensen, Subcellular localization of the delayed rectifier K(+) channels KCNQ1 and ERG1 in the rat heart, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H1300–H1309.
[38] A.S. Ayettey, V. Navaratnam, The T-tubule system in the specialized and general myocardium of the rat, J. Anat. 127 (1978) 125–140.
[39] A.L. Lundquist, L.J. Manderfield, C.G. Vanoye, C.S. Rogers, B.S. Donahue, P.A. Chang, D.C. Drinkwater, K.T. Murray, A.L. George Jr., Expression of multiple KCNE genes in human heart may enable variable modulation of I(Ks), J. Mol. Cell Cardiol. 38 (2005) 277–287.
[40] W. Dun, P. Boyden, Diverse phenotypes of outward currents in cells that have survived in the 5 day infarcted heart, Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H667–H673.