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

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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 \(\beta\)-adrenergic-induced component of I\(_{SS}\) that does not require co-assembly with Kcne1.

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The KCNQ1 gene encodes a 6-transmembrane domain \(\alpha\)-subunit of a K\(^+\) channel protein that can either form a homomeric channel or partner with single transmembrane domain \(\beta\) 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 \(\beta\)-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 \(\beta\)-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...
were responsive to adrenergic stimulation in the absence of KCNE1 [3,13]. In mice, Kcnel expression is strongly down-regulated during postnatal development such that little or no Kcnel remains in the adult mouse heart [14,15]. Correspondingly, cellular electrophysiological studies did not find \( I_{\text{ks}} \) in adult mouse cardiomyocytes [16,17].

Despite the near-absence of Kcnel expression and \( I_{\text{ks}} \) 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 Kcnel-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 Kcnel 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 Kcnel is nearly absent in adult murine myocardium, any Kcnq1-mediated current would contribute to the steady-state outward current (\( I_{\text{ss}} \)) because the biophysical properties of \( I_{\text{ss}} \) resemble those described for Kcnel-independent Kcnq1 currents described in heterologous expression systems [1,2,4,6,5].

Our results show that the \( \beta \)-adrenergic agonist, isoproterenol, significantly enhances \( I_{\text{ss}} \) in wild-type ventricular myocytes but had 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 \( \beta \)-adrenergic-sensitive component of the outward steady-state \( K^+ \) current, \( I_{\text{ss}} \).

**Materials and methods**

**Materials.** The anti-Kcnq1 antibody AB5932 was obtained from Chemicon International (Temecula, CA). The anti-Dihydropyridine Receptor a2 (DHPa2) 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 were 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, CaCl₂ 1, Hepes 10, MgATP 5, pH 7.2. Whole-cell recordings were performed in control Tyrode’s solutions containing (in mmol/L): NaCl 140; KCl, 5.4; glucose, 10; MgCl₂, 1; CaCl₂, 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, \( I_{\text{ss}} \), recorded using specific voltage protocols indicated in the text. To optimize recording of \( I_{\text{ss}} \), the extracellular solution contained 2 mM 4-aminopyridine (to block slow and fast transient outward currents, \( I_{\text{Ks,slow}} \) and the ultra-rapid delayed rectifier, \( I_{\text{Kur}} \)), 5 µM nifedipine and low (0.1 mM) Ca²⁺ (to block the L-type calcium current, \( I_{\text{C,L}} \), and 0 mM Na⁺ (to abolish \( I_{\text{ks}} \)). 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 \( I_{\text{ss}} \) in adult mouse cardiomyocytes. To explore the ventricular Kcnq1 expression in more detail, we performed co-immunofluorescent staining for sarcomeric \( \alpha \)-actinin in these sections, as confirmed
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 \( I_{SS} \) (Δ\( I_{SS} \)) observed in the presence versus the absence of isoproterenol can be attributed to Kcnq1. Average Δ\( I_{SS} \) was 0.43 ± 0.16 pA/pF in Kcnq1\(^{+/+}\) myocytes but −0.14±0.13 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
indicate that Kcnq1 contributes to a portion of \( I_{SS} \) that is selectively enhanced by \( \beta \)-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 Kcn1 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].

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 \( \beta \)-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 (\( \Delta I_{SS} \)) measured from the data sets shown in (D). *\( p < 0.05 \) using Student’s \( t \)-test for statistical evaluation of significance.
Nevertheless, we have shown that targeted disruption of \textit{Kcnq1} [19] and introduction of a specific knock-in \textit{Kcnq1} point mutations [30] lead to development of a Long QT phenotype in adult mice. Similar QT abnormalities have been observed in isolated perfused \textit{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.

\textbf{\textit{Kcnq1} protein expression in adult mouse myocardium}

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

Consistent with these immunoblotting results, we also detected \textit{Kcnq1} protein in the adult murine heart using immunofluorescent histochemical staining techniques. With this approach, we demonstrated that \textit{Kcnq1} protein is expressed in both atria and ventricles. The staining pattern was consistent with \textit{Kcnq1} expression in both atrial and ventricular working myocardium. Interestingly, immunostaining appeared more intense within the atria compared to the ventricles, suggesting that \textit{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 \textit{I}_{\text{KCNOQ1}} alone to atrial action potentials. Alternatively, the different subcellular patterns of \textit{Kcnq1} distribution in atrial versus ventricular myocytes may have contributed to the differential staining intensities. In ventricular myocytes, \textit{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 \textit{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].

\textbf{\textit{Kcnq1} contributes to a \textbeta{}-adrenergic-sensitive component of \textit{I}_{SS}}

Since there is no \textit{I}_{Ks} present in adult murine ventricular myocytes, a role for \textit{Kcnq1} in these cells has not previously been identified. We hypothesized that \textit{Kcnq1} contributes to the steady-state outward \textit{K}⁺ current, \textit{I}_{SS}, because of the similar electrophysiological properties of \textit{I}_{SS} in isolated ventricular myocytes [27] and \textit{I}_{\text{KCNOQ1}} in transfected cells. Both currents display rapidly activating kinetics and do not inactivate. To test this hypothesis, we evaluated \textit{I}_{SS} in ventricular myocytes isolated from \textit{Kcnq1}\textsuperscript{+/+} and \textit{Kcnq1}\textsuperscript{-/-} hearts. Under control conditions, no significant differences in \textit{I}_{SS} densities were observed between wild-type and mutant myocytes, thereby indicating that \textit{Kcnq1} may not contribute significantly to repolarization at baseline. In the presence of isoproterenol, however, a significant increase in \textit{I}_{SS} density was observed exclusively \textit{Kcnq1}\textsuperscript{+/+} myocytes. Since the isoproterenol-induced increase in \textit{I}_{SS} was dependent on the presence of \textit{Kcnq1}, the logical conclusion is that endogenous \textit{Kcnq1} channels mediate the increased \textit{I}_{SS} densities observed in the presence of isoproterenol.

It is clear from previous studies [3,13,16] that \textit{Kcnq1} itself is the target of PKA-mediated phosphorylation, and that \textit{I}_{\text{KCNOQ1}} 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 \textit{Kcnq1} could be regulated by adrenergic hormones in the absence of KCNE1. At present, we cannot conclude that \textit{Kcnq1} is acting alone (i.e., homomorphic \textit{Kcnq1} channels leading to \textit{I}_{\text{Kcnq1}}) versus partnership with one or more non-\textit{Kcne1} subunits (e.g., \textit{Kcne2} or \textit{Kcne3}) [6]. We can, however, conclude that \textit{Kcnq1} channel proteins are expressed in the adult mouse heart where they contribute to steady-state repolarizing currents during \textbeta{}-adrenergic receptor stimulation.

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

\textbf{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 \textit{Kcnq1} in mice and rats likely contributes to the lack of \textit{I}_{Ks} in the adult myocardium of these species, whereas \textit{I}_{Ks} has been readily detected in larger mammalian species, including humans [31,32]. It is not clear, however, that \textit{I}_{Ks} is the only important current to which \textit{KCNOQ1} contributes to even in hearts where \textit{I}_{Ks} is known to be present. Indeed, a recent study by Lundquist et al. [39] showed that multiple KCNNE subunits are expressed in different regions of human myocardium, and that co-expression of other KCNNE [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-\textit{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 \textit{Kcnq1} in humans and other species.

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References


