Manipulation of KCNE2 expression modulates action potential duration, and $I_{to}$ and $I_K$ in rat and mouse ventricular myocytes

Short title: KCNE2 regulates cardiac electrical stability

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Abstract

Background In heterologous expression systems, KCNE2 has been demonstrated to interact with multiple α-subunits of voltage-dependent cation channels and modulate their functions. However, the physiological and pathological roles of KCNE2 in cardiomyocytes are poorly understood.

Objective This study aims to investigate the effects of bidirectional modulation of KCNE2 expression on action potential duration (APD) and voltage-dependent K⁺ channels in cardiomyocytes.

Methods Adenoviral gene delivery and RNA interference were used to either increase or decrease KCNE2 expression in cultured neonatal and adult rat or neonatal mouse ventricular myocytes.

Results Knockdown of KCNE2 prolonged APD in both neonatal and adult myocytes, whereas overexpression of KCNE2 shortened APD in neonatal but not adult myocytes. Consistent with the alterations in APD, KCNE2 knockdown decreased transient outward current (Iₒ) densities in neonatal and adult myocytes, whereas KCNE2 overexpression increased Iₒ densities in neonatal but not adult myocytes. Furthermore, KCNE2 knockdown accelerated the rates of Iₒ activation and inactivation, whereas KCNE2 overexpression slowed Iₒ gating kinetics in neonatal but not adult myocytes. The Iₖ densities were remarkably affected by manipulation of KCNE2 expression in mouse but not rat cardiomyocytes. Simulating AP of rat ventricular myocyte with a mathematical model shows that the alterations in Iₒ current densities and gating properties can result in similar APD alterations in KCNE2 overexpression and knockdown cells.

Conclusions Endogenous KCNE2 in cardiomyocytes is important in maintaining cardiac electrical stability mainly by regulating Iₒ and APD. Perturbation of KCNE2 expression may predispose the hearts to ventricular arrhythmia by prolonging APD.

Keywords KCNE2; action potential; transient outward current; delayed rectifier current; cardiomyocytes.

New & Noteworthy

Through bidirectional manipulation of KCNE2 expression in native rat or mouse cardiomyocytes, this study demonstrates that cardiac endogenous KCNE2 regulates the magnitude and gating properties of Iₒ and APD. Furthermore, it shows for the first time that KCNE2 heterologously regulates these electrophysiological properties in neonatal and adult rat cardiomyocytes.
Introduction

Voltage-dependent potassium (K_v) channels are multisubunit transmembrane protein complexes that are essential for repolarization of cardiomyocytes. Heterologous expression studies have demonstrated that the functions of α subunits of multiple K_v channels, including hERG, KCNQ1–3, K,3.1, K,3.2, K,4.2, K,4.3, K,1.5, K,2.1, and HCN (pacemaker) can be modulated by an auxiliary subunit KCNE2 (also called Mink-related peptide or MiRP1), a small peptide consisting of single transmembrane domain encoded by KCNE family gene(1, 7, 15, 16, 20, 22, 33). Despite the evidence in vitro, there are intense debates over the putative role of KCNE2 in native cardiac myocytes for two major reasons. First, reports on the expression level of KCNE2 protein in the ventricle are controversial, some studies reported low compared to others(4-6, 8, 14, 19, 21, 34). Second, evidence about KCNE2 regulating K_v channels are almost all from heterologous expression experiments. Native cardiomyocyte is a far more complicated system, where multiple ion channels and signaling pathways are present and their functions are interwoven to maintain cardiac electrical stability. What happens in heterologous expression system may just stay in it. Nevertheless, KCNE2 is important in maintaining cardiac electrical stability because mutations or a polymorphism in KCNE2 gene have been linked to long QT syndrome (LQT6) and familial atrial fibrillation/short QT syndrome (2, 24, 31). Furthermore, alterations in KCNE2 expression have been linked to cardiac arrhythmia in diseased hearts (8). To understand the role of KCNE2 in ventricular repolarization and arrhythmogenic mechanism(s) of LQT6 and diseased hearts, it is of particular importance to determine the precise roles of endogenous KCNE2 in native cardiomyocytes.

First investigation regarding the direct physiological role of KCNE2 in heart has been performed by Abbott and his colleagues in KCNE2 gene knockout mice. The authors demonstrated that targeted deletion of KCNE2 gene impaired ventricular repolarization via disruption of I_{K,slow}1 and I_{to,f} (22). The elegant work confirms that KCNE2 exists in ventricles and plays an important role in ventricular repolarization. Albeit gene knockout technique is strong in providing insight into physiological roles of the target gene, it has disadvantages. It should be taken into account that both direct and indirect actions of gene products during embryo development may contribute to the observed phenotypes. Furthermore, we may not deduce the roles of the altered gene in pathological conditions from the phenotypes of gene knockout.

For these considerations, we explored the effects of modulation of endogenous KCNE2 expression on action potential (AP) and the major K_v channels, including transient outward (I_o), delayed rectifier (I_k) and inward rectifier (I_{K1}) currents in native neonatal and adult rat ventricular myocytes. Here, we showed that bidirectional modulation of KCNE2 expression heterogeneously regulates AP duration (APD) in neonatal and adult rat ventricular myocytes. Concomitantly, I_o and I_k, the two major repolarization K_v channels in rat cardiomyocytes were heterogeneously regulated by bidirectional manipulation of KCNE2 expression.

Methods

Isolation and culture of adult rat ventricular epicardial myocytes

All experimental procedures and protocols on animals were approved by the Institutional Animal
Care and Use Committee of Shenzhen University and comply 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). Adult (2- to 3-month-old) Sprague-Dawley rats (200–250 g) were anesthetized with a mixture of 86 mg/100 g urethane and 3.3 mg/100 g chloralose (urethane and chloralose were dissolved in normal saline solution to make the final concentrations of 13.3 and 0.5%, respectively, 0.65 ml/100 g.ip). Single myocytes were isolated from the epicardial of left ventricles under sterile conditions as described previously with minor revision (32). In brief, the heart was removed from the chest, cleaned and flushed with nominally Ca²⁺-free Tyrode’s solution consisting of (in mM) 137 NaCl, 5.4 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 10 glucose, and 20 Hepes (pH 7.4), and perfused using a Langendorff apparatus. Perfusion began with nominally Ca²⁺-free Tyrode solution for 5 min at 37°C, and then switched to the enzyme solution with 0.5 mg/mL collagenase (Worthington, Type II) and 0.06 mg/mL protease (Sigma, Type XIV) for 20-25 min. The free wall of the left ventricle was dissected into outer (epicardial) and inner (endocardial) halves. The outer half was minced and gently triturated for 15 min to release single myocytes. The supernatant was collected and the cells were pelleted by low-speed centrifugation. Cell pellets were resuspended in Hepes-buffered solution containing (mM): 137 NaCl, 5.4 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 1 CaCl₂, 20 glucose and 20 Hepes (pH 7.4, adjusted with NaOH). For culture, the cells were seeded on 30 mm laminin-coated coverslip at a density of 10⁵ and cultured in medium 199 (Sigma) supplemented with (in mmol/L) creatine 5, L-carnitine 2, taurine 5, insulin/transferring/selenium-X 0.1%, penicillin-streptomycin 1%, and Hepes 20, pH adjusted to 7.2 with NaHCO₃ (27).

Isolation and culture of neonatal rat (or mouse) ventricular myocytes

Neonatal rat ventricular myocytes (NRVMs) were isolated from 2-day-old Sprague–Dawley rats and cultured as previously described (13). In brief, ventricles were obtained following decapitation and immersed in PBS and minced with scissors. The small pieces of ventricular tissue were digested with 0.25% Trypsin–EDTA in PBS at 37°C. The isolated cells were put in fetal bovine serum (FBS) and pelleted by centrifugation at 1000 rpm for 5 min. The pelleted cells were resuspended in DMEM containing 10% FBS, 1% penicillin–streptomycin and then preplated for 1.5 h at 37°C to allow fibroblasts to adhere to the plate. The un-adhered cells were pelleted again and resuspended in DMEM containing 10% FBS, 1% penicillin–streptomycin and bromodeoxyuridine (1:100, to inhibit fibroblast growth), which were finally plated at a concentration of about 10⁶ cells per 35mm plate.

Neonatal mouse ventricular myocytes (NMVMs) were isolated from 3-day-old C57BL/6 mice and cultured with the method described for culturing NRVMs.

Manipulating KCNE2 expression in cardiac myocytes

Cultured myocytes were infected with recombinant adenovirus vectors carrying human KCNE2 (Ad-KCNE2) and green fluorescent protein (GFP) at a multiplicity of infection (MOI) of 5, with GFP adenovirus (Ad-GFP) applied at the same MOI as the control. Recombinant adenovirus for silencing endogenous KCNE2 expression (KCNE2-shRNA, at a MOI of 10) was prepared with the BLOCK-iT
Adenoviral RNAi Expression system (Invitrogen). The sequences of the oligonucleotides for KCNE2 RNA interference were as follows: forward, CACCGGGATTGGCACGAGTGATAGCGAACTATACTTTCTGCTGCCAACATCC; reverse, AAAAGATTGGCAGCAGAAGTAGTTTCGCTATACTTTCTGCTGCCAACATCC. Adenoviral vectors containing a scrambled shRNA sequence (Ctrl-shRNA) at the same MOI served as the control. Experiments were performed 48 hr after adenoviral transfection.

Western blotting

Protein was extracted 48 hours after viral infection. Total cellular extracts were lysed in Laemmli buffer. The supernatant was ultracentrifuged to pellet membrane fractions and equal amount of proteins were loaded and separated on 10% SDS–PAGE before being transferred to polyvinylidene difluoride (PVDF) membranes. Then membranes were probed with primary antibody: anti-Kv4.3 (1:200, Alomone, Jerusalem, Israel), anti-Kv4.2 (1:200, Alomone), anti-Kv1.4 (1:200, Alomone), anti-KChIP2 (1:1000, NeuroMab) or anti-KCNE2 (1:200, Alomone) followed by horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, Ely, UK). Blots were then washed three times in TBST and the signal was recorded using the Kodak Image Station 2000R System (Kodak, USA). The density of target protein concentration was determined by using NIH Image J image software and normalized with α-actin.

Electrophysiological recording

The I_{to} and I_{K} current recordings were performed under the whole-cell patch-clamp configuration using a patch amplifier (Axoclamp-200B, Molecular Devices, Sunnyvale, CA) as described previously(32). The cells were superfused with a modified Tyrode’s solution of the following composition (in mM): NaCl 146, KCl 4, MgCl₂ 2.5, HEPES 5, dextrose 5.5, CdCl₂ 0.3, and MnCl₂ 2, tetrodotoxin (TTX) 0.02 (pH7.3 with NaOH). TTX and CdCl₂ were used to block sodium current (I_{Na}) and I_{Ca,L}, respectively. MnCl₂ was used to replace CaCl₂. The pipette solution contained (mM): KCl 135, EGTA 10, HEPES 10, dextrose 5, ATP (Mg salt) 3, GTP (Tris salt) 0.5, pH 7.2 with KOH. The recordings were conducted at room temperature (24-26°C). When filled with the pipette solution, pipettes (Garner Glass, Claremont, CA, USA) had resistance ranging from 3 to 5 MΩ. The junctional potential was corrected when pipette tip entered bath solution. After the cell membrane was broken by application of additional suction, cell capacitance and series resistance were electrically compensated. The liquid junction potential between the pipette solution and the bath solution was estimated to be about10 mV (pipette side negative) which was corrected during data analysis. For inhibition of outward I_{to} or I_{K} currents, 6 mM or 50 µM 4-aminopyridine (4-AP; Sigma, for inhibition of I_{to} in rat cardiomyocytes or I_{K,slow1} in mouse cardiomyocytes, respectively) or 20 mM or 25 mM tetraethylammonium (TEA; Sigma, for inhibition of I_{K} in rat cardiomyocytes or I_{K,slow2} in mouse cardiomyocytes, respectively)) or 300 nM heteropodatoxin 2 (HpTx2; Abcam; for inhibition of I_{K,slow1} in mouse cardiomyocytes) was added, respectively. TEA solution was made by replacing equimolar NaCl with TEACl.

Action potentials (APs) were recorded using the current-clamp mode of the whole cell patch-clamp technique as previously described (9). APs were stimulated with 5 ms square-wave pulses at 1-HZ
interval. The bath solution for AP recording was a modified Tyrode’s solution containing (in mmol/L): NaCl 136, KCl 5.4, NaH₂PO₄ 0.33, MgCl₂ 1.0, HEPES 10, glucose 10, and CaCl₂ 1.8 (pH 7.4). The pipette solution contained (in mmol/L): KCl 130, NaCl 5.4, MgCl₂ 1, HEPES 10, Mg-ATP 5, phosphocreatine 5, and cAMP 0.05 (pH 7.2).

**A mathematical model of AP in adult rat ventricular myocytes**

A mathematical model of AP was derived from a published left ventricular epicardial cell model. (18). All of current equations and parameters were from the study with minor modification based on the data obtained under our experimental condition. In the original model, 4-AP-sensitive component (I_{to}) is not distinguish from TEA-sensitive component (I_{k}) and the Ca²⁺-independent transient outward K⁺ current (I_t) applied in the modeling consists of these two components. In this study, we recorded I_{to} and I_{k} separately and I_t densities applied for the modeling was the sum of I_{to} and I_{k} densities.

**Statistics**

Data are presented as mean±SEM. Unpaired two-tailed t test or one-way ANOVA was used for statistical analysis when appropriate, with p<0.05 being considered significant.

**Results**

**Knockdown of KCNE2 prolonged action potential duration (APD) in neonatal and adult rat ventricular myocytes**

To investigate the role of endogenous cardiac KCNE2 in maintaining cardiac electrical stability, we investigated the effect of bidirectional modulation of KCNE2 on APD in rat ventricular myocytes. It is known that APD and ion channel components involved in shaping AP are varied between neonatal and adult rat ventricular myocytes. We found in this study that the protein level of KCNE2 in adult ventricular myocytes is much higher than that in neonatal, and it was further increased in aging (18-month-old) cardiomyocytes (Fig.1A). Considering that KCNE2 may differentially regulate APD and Kᵥ channels, we thus did the experiments in both neonatal and adult cardiomyocytes.

Knockdown of KCNE2 expression was performed by RNA interference. KCNE2-shRNA infection at MOI of 10 for 48 hrs reduced KCNE2 protein level by 71.3% and 56.6% in neonatal and adult rat cardiomyocytes, respectively, as compared with Ctrl-shRNA controls (Fig. 1B, C). In all experiments, APD was measured at 20% (APD20), 50% (APD50) and 90% repolarization (APD90). Knockdown of KCNE2 resulted in prolongation of APD in both neonatal and adult cardiomyocytes (Fig. 1D). With KCNE2 knockdown, APD20, APD50 and APD90 were increased by 17.3% (p<0.05), 34.9% (p<0.01) and 63.1% (p<0.01), respectively, in neonatal cardiomyocytes (Fig. 1E), and 38.8% (p<0.05), 87.7% (p<0.01) and 438.1% (p<0.01), respectively, in adult cardiomyocytes (Fig. 1F). The resting membrane potential (RMP) and the amplitude of AP overshoot were unaltered by knockdown of KCNE2 in neonatal and adult cardiomyocytes (Fig. 1G, H). The results collectively indicate that ventricular KCNE2 is important in shaping AP no matter in neonatal and adult rat ventricular myocytes and downregulation of KCNE2 can lead to APD prolongation.

**Upregulation of KCNE2 heterogeneously modulated APD in neonatal and adult rat ventricular
We next examined the effect of KCNE2 overexpression on APD for three considerations. First, KCNE2 expression is dynamic and increases with the growth of age (Fig. 1A). Second, it has been suggested that “gain of function” mutations of KCNE2, as well as “loss of function” mutations of KCNE2 cause cardiac arrhythmia (12, 28, 31). Overexpression of KCNE2 may to some extent reflect the effect of “gain of function”. Third, it’s interesting to know whether there is a redundance in KCNE2 regulation of APD and K_v channels. KCNE2 overexpression was performed by adenoviral gene delivery. Ad-KCNE2 infection at MOI of 5 for 48 hours led to about 93.1% and 67.0% increase in KCNE2 protein level, respectively, in neonatal and adult cardiomyocytes, as compared with Ad-GFP controls (Fig. 2A, B).

In neonatal cardiomyocytes, KCNE2 overexpression shortened APD. The values of APD20, APD50 and APD90 were decreased by 10.8%, 18.0% (p<0.05) and 16.9% (p<0.05), respectively (Fig. 2C, D). In adult cardiomyocytes, however, KCNE2 overexpression had no significant effects on APD (Fig. 2C, E). The resting membrane potential (V_{rest}) and amplitude of AP overshoot were unaltered by overexpression of KCNE2 in neonatal and adult cardiomyocytes (Fig. 2F, G).

**KCNE2 regulation of I_{to} current densities in rat cardiomyocytes**

To investigate the mechanisms underlying KCNE2 regulations of APD, we studied the effects of bidirectional modulation of KCNE2 on the two major repolarization K+ currents, I_{to} and I_K. In rat ventricular myocytes, at least two distinct components of Ca^{2+}-independent, depolarization-activated K+ currents have been identified previously (3, 32). We will use the same terms “delayed rectifier current” (I_K) denotes the slowly decaying component that is sensitive to TEA, and “transient outward current” (I_{to}) denotes the fast decaying component that is sensitive to 4AP as used by others before (32). In the measurement of I_{to}, the non-inactivating, steady-state outward currents (I_{ss}) recorded at the end of 2-s voltage steps were eliminated.

We first recorded outward current eliminating I_K in the presence of 20 mM TEA. The results show that knockdown of KCNE2 similarly decreased I_{to} in neonatal and adult cardiomyocytes, consistent to the alterations of APD (Fig. 3A). In neonatal cardiomyocytes, I_{to} densities were significantly decreased at the voltages ranging from 10 mV to 60 mV (p<0.05 at 10 mV, p<0.01 at 20-60 mV, Fig. 3D). In adult cardiomyocytes, I_{to} densities were significantly decreased at the voltages ranging from 20 mV to 60 mV (p<0.05 at 20-40 mV, p<0.01 at 50 and 60 mV, Fig. 3G).

However, KCNE2 overexpression heterologously regulated the magnitude of I_{to} in neonatal and adult cardiomyocytes, where I_{to} densities were significantly increased at the voltages ranging from 20 mV to 60 mV in neonatal cardiomyocytes (p<0.05 at 20-40 mV, p<0.01 at 50 and 60 mV, Fig. 4D), but unaltered at all voltages tested in adult cardiomyocytes (Fig. 4G). Neither overexpression nor knockdown of KCNE2 had any significant effect on I_{ss} in both types of cardiomyocyte (Fig. 3C, F, Fig. 4C, F).

To explore whether the changes of I_{to} density were related to alteration of the protein expression of α and β subunits of I_{to}, we examined the membrane protein levels of Kv4.2, Kv4.3, Kv1.4 and KChIP2 and found that neither up- nor downregulation of KCNE2 expression had any significant effect on protein levels of the subunits of I_{to} in neonatal (Fig. 5 A-C) and adult cardiomyocytes (Fig. 5 D-F). The result indicates that KCNE2 regulation of I_{to} is unrelated to alteration of protein levels of I_{to} subunits.
KCNE2 changed I_{to} gating kinetics

The changes in the rates of activation and inactivation of I_{to} were quantified by the time to peak (TTP) and time constant of inactivation (τ) estimated by a monoexponential function of the currents recorded at 0, 20, 40 and 60 mV. We first examined the effects of KCNE2 overexpression on I_{to} gating kinetics in adult cardiomyocytes. The results show that both TTP and τ of inactivation were not significantly changed in KCNE2 overexpression cells (Fig. 6A, C). In contrast, knockdown of endogenous KCNE2 resulted in acceleration of I_{to} activation and inactivation kinetics (Fig. 6C). TTP and τ of inactivation at 60 mV in KCNE2-shRNA group were 4.1±0.3 ms and 38.3±2.9 ms, significantly shorter than those in Ctrl-shRNA group (5.1±0.4 ms and 49.2±3.9 ms, p<0.05, Fig. 6B, C).

In neonatal cardiomyocytes, overexpression of KCNE2 slowed the rates of both activation and inactivation of I_{to}, where the TTP and τ of inactivation at 60 mV in Ad-KCNE2 group were 14.8±1.2 ms and 75.3±3.1 ms, much longer than those of 10.1±0.9 ms and 60.2±3.2 ms in Ad-GFP group (p<0.01, Fig. 6B). In contrast, knockdown of KCNE2 accelerated the rates of both activation and inactivation of I_{to}, where the TTP and τ of inactivation in KCNE2-shRNA group were 9.1±0.7 ms and 50.4±2.7 ms, significantly shorter than those in Ctrl-shRNA group (11.3±0.8 ms and 63.5±2.4 ms, p<0.05, Fig. 6B).

KCNE2 regulated the voltage-dependence of I_{to} activation, inactivation and recovery from inactivation

The curves of voltage-dependent activation and inactivation were constructed as previously described (13). In adult cardiomyocytes, upregulation of KCNE2 expression had no significant effect on the voltage-dependent activation and inactivation of I_{to} (Fig. 6D, F, G, I). The voltages at half-maximum activation (V_{0.5, act}) estimated by fitting the activation curve with Boltzmann function were 7.3±0.6 mV and 8.2±0.7 mV in Ad-GFP and Ad-KCNE2 groups, respectively (p>0.05, Fig. 6D, F). The voltages at half-maximum inactivation (V_{0.5, inact}) estimated by fitting the inactivation curve with Boltzmann function were -24.4±1.5 mV and -22.9±1.3 mV in Ad-GFP and Ad-KCNE2 groups, respectively (p>0.05, Fig. 6G, I). In contrast, knockdown of KCNE2 caused a mild negative shift of the voltage-dependent activation and inactivation (Fig. 6E, H). The V_{0.5, act} was 7.4±0.5 mV and 4.1±0.3 mV in Ctrl-shRNA group and KCNE2-shRNA group, respectively (p<0.05, Fig. 6E, F), and The V_{0.5, inact} was -24.9±1.4 mV and -29.1±1.2 mV in Ctrl-shRNA group and KCNE2-shRNA group, respectively (p<0.05, Fig. 6H, I).

Consistent with the alterations in adult myocytes, knockdown of KCNE2 similarly caused a slightly negative shift of voltage-dependent activation and inactivation of I_{to} in neonatal cardiomyocytes. V_{0.5, act} were 26.8±1.4 and 22.7±1.2 mV in Ctrl-shRNA and KCNE2-shRNA group, respectively (p<0.05), and V_{0.5, inact} were -31.3±1.3 and -35.6±1.6 mV in Ctrl-shRNA and KCNE2-shRNA group, respectively (p<0.05). However, in contrast to the results in adult cardiomyocytes, overexpression of KCNE2 positively shifted the voltage-dependent I_{to} activation and inactivation, where V_{0.5, act} were 27.9±1.1 and 31.8±1.5 mV in Ad-GFP and Ad-KCNE2 group, respectively (p<0.05), and V_{0.5, inact} were -32.8±1.3 and -29.1±1.7 mV in Ad-GFP and Ad-KCNE2 group, respectively (p<0.05).

The recovery from inactivation of I_{to} was determined with a double-pulse protocol with recovery
intervals from 3 to 1000 ms at -80 mV. Since $I_{to}$ densities in neonatal cardiomyocytes were very small, we only analyzed the recovery from inactivation of $I_{to}$ in adults. The result shows that upregulation of KCNE2 expression had no effect on, whereas knockdown of KCNE2 slowed the $I_{to}$ recovery from inactivation (Fig. 6J, K). The time constant ($\tau$) of recovery measured by fitting $I_{to}$ recovery from inactivation with a monoexponential function was $245.7 \pm 10.8$ ms and $233.1 \pm 8.5$ ms in Ad-GFP group and Ad-KCNE2 group ($p>0.05$), and $240.1 \pm 15.4$ ms and $270.7 \pm 13.3$ ms in Ctrl-shRNA and KCNE2-shRNA groups ($p<0.05$), respectively (Fig. 6L).

**KCNE2 regulation of delayed rectifier current $I_{K}$ and inward rectifier current $I_{K1}$ in rat cardiomyocytes**

We next explored the effect of bidirectional modulation of KCNE2 expression on $I_{K}$ current. Since the amplitudes of $I_{K}$ are too small to be recorded and accurately analyzed, we did the experiments in adult cardiomyocytes. Figure 7A shows that overexpression of KCNE2 reduced $I_{K}$ current densities at the voltages of 50 and 60 mV ($p<0.05$). The current density at 60 mV was decreased by 10.5 %, from $5.3 \pm 0.3$ pA/pF in Ad-GFP group to $4.8 \pm 0.2$ pA/pF in Ad-KCNE2 group ($p<0.05$) (Fig. 7B). However, knockdown of KCNE2 had no effect on $I_{K}$ current densities (Fig. 7C). The current densities at 60 mV were $5.1 \pm 0.3$ pA/pF in the KCNE2-shRNA group and $5.2 \pm 0.2$ pA/pF in Ctrl-shRNA group ($p>0.05$).

Fig. 7D illustrates typical current traces and the average current density-voltage relationships of the ‘background’ $K^+$ current in KCNE2 overexpression and knockdown cells and their respective controls in adult cardiomyocytes. Neither up- nor down-regulation of KCNE2 had any significant effect on $I_{K1}$ current in adult cardiomyocytes (Fig. 7D-F). The result is consistent with the above data that bidirectional modulation of KCNE2 expression did not affect resting membrane potential of cardiomyocytes.

**Effects of bidirectional modulation of KCNE2 expression on APD and $I_{to}$ in neonatal mouse ventricular myocytes (NMVMs)**

Previous work reports the effects of KCNE2 knockout on APD and $K_v$ currents in mouse ventricular myocytes(22). We also examined the regulation of endogenous KCNE2 on the electrophysiological properties in NMVMs by bidirectional manipulation of KCNE2 expression. Tab. 1 illustrates that bidirectional modulation of KCNE2 had similar effects on APD in NMVMs as in NRVMs, where overexpression of KCNE2 shortened and knockdown of KCNE2 prolonged APD.

It has been suggested that murine ventricular myocytes present four kinetically distinct voltage-gated $K^+$ currents, $I_{to,f}$ presents in myocytes from apex and some from the septum, $I_{to,s}$ presents in septal myocytes, $I_{K,slow}$ and $I_{K}$ present in all myocytes (30). In cultured NMVMs, we cannot distinguish the currents from which part of ventricular myocytes. We thus distinguished the currents, $I_{to,f}$, $I_{K,slow 1}$ and $I_{K,slow 2}$ with specific inhibitors as reported in previous study (22). The results show that the mean peak outward current densities were significantly decreased by KCNE2 overexpression, but increased by KCNE2 knockdown (Fig. 8A, B). In parallel, the HpTx2-sensitive $I_{to,f}$ current densities were significantly decreased by KCNE2 knockdown, but remarkably increased by KCNE2 overexpression (Fig. 8C, D). Analyzing the activation and inactivation kinetics reveal that overexpression of KCNE2 slightly but significantly slowed the kinetics of $I_{to}$ activation and inactivation, whereas knockdown of KCNE2 had the contrary effect. The TTP and $\tau$ of inactivation at
60 mV in Ad-KCNE2 group were 11.2±0.4 ms and 100.8±5.7 ms, much longer than those of 9.9±0.5 ms and 88.2±4.4 ms in Ad-GFP group (p<0.05). In contrast, the TTP and τ of inactivation in KCNE2-shRNA group were 7.6±0.3 ms and 70.1±2.8 ms, significantly shorter than 9.1±0.4 ms and 81.0±3.2 ms in Ctrl-shRNA group. The protein levels of Kv4.2 and Kv4.3 which mediate I_{to,f} remained unaltered (Fig. 8I, J). The results are in general agreement with KCNE2 regulation of I_{to} in NRVMs.

IK,slow in mouse ventricular myocytes has two pharmacologically distinct components: a 50 μM 4-AP-sensitive current mediated by Kv1.5 (IK,slow1) and a 25 mM TEA-sensitive current mediated by Kv2.1 (IK,slow2). The results show that knockdown of KCNE2 significantly decreased IK,slow1, whereas overexpression notably increased IK,slow1 (Fig. 8E, F). Neither overexpression nor knockdown of KCNE2 had any effect on IK,slow2 (Fig. 8G, H) and I_{ss} (data not shown). The results are consistent with previous study in KCNE2 knockout mice (22), but in contrast with the regulation of IK in rat ventricular myocytes. Given that Kv1.5 is exclusively present in the atria and responsible for IKur, except in mouse ventricles (23), it’s rational that KCNE2 had less effect on IK in rat ventricular myocytes than in mouse cells. Of note, we found that the protein level of Kv1.5 (~80 kDa, the mature form of Kv1.5) was not significantly affected by either KCNE2 overexpression nor knockdown (Fig. 8I, J). In KCNE2 knockout mice, the mature form of Kv1.5 had been shown to be decreased. The difference might be due to developmental compensation in KCNE2 knockout mice. Nevertheless, the modulation of KCNE2 on IK,slow1 found in our study can be explained by KCNE2 interacting with Kv1.5 and promoting its surface expression, as shown by previous study (22).

**A mathematical model of AP in adult rat cardiomyocytes with KCNE2 overexpression or knockdown**

To establish a quantitative link between alterations in I_{to} and APD in response to KCNE2 manipulation, we resorted to a mathematical model of AP derived from adult rat left ventricular myocytes (18). Since there is not a mathematical model of AP for neonatal cardiomyocytes available, the AP modeling was done only in adult myocytes. The original model was constructed based on the data obtained from the freshly isolated myocytes which are differed from the data obtained from cells cultured for 48 h under our experimental condition, we thus made modifications to the following ionic current properties based on our experimental data: I_{t} density was decreased by 19%, τ of I_{t} inactivation at voltages positive to 0 mV was changed to 52.0 ms (35.0 ms in the original model) and I_{Ca} density was decreased by 30%. The values of the simulated V_{rest}, peak overshoot of AP, APD50 and APD90 were shown in Tab.1. We found that APD90 in simulated control group was much shorter than that in experimental control group, which might be due to the alterations in many other factors, such as Na⁺-Ca²⁺ ion exchanger current, Na⁺-K⁺ pump current, sacolemmal Ca²⁺ pump current, etc, under our experimental condition.

In KCNE2 overexpressed cells, I_{t} density was decreased by 2.1%, while the other parameters were unaltered. The simulated AP shape and APD were almost the same as those in Ad-GFP control (Fig. 9A, Tab.2). In KCNE2 knockdown cells, I_{t} density was decreased by 13%, τ of I_{t} inactivation at voltages positive to 0 mV was changed to 38.2 ms and V_{0.5,inact} was negatively shifted by 4 mV. With these alterations, the simulated APD was prolonged, where APD50 and APD90 were increased by 53.4% and 44.9%, respectively (Fig. 9B, Tab.2). The simulated V_{rest} and peak overshoot of AP were unaltered in either KCNE2 overexpressed or knockdown cells, consistent to the results under our experimental condition (Tab.2).
Discussion

The present study provides the first comprehensive characterization of bidirectional modulations of the protein expression of endogenous KCNE2, the promiscuous auxiliary subunit, in regulation of APD and repolarization-related KV channels in native neonatal and adult rat or neonatal mouse ventricular myocytes. The major findings are 3 folds. (1) Knockdown of KCNE2 consistently prolonged APD in neonatal and adult cardiomyocytes. However, moderate overexpression of KCNE2 shortened APD in neonatal cardiomyocytes, but had no effect on APD in adult cardiomyocytes. (2) Bidirectional modulations of KCNE2 expression regulated Ito current densities in parallel to the alterations of APD. Knockdown of KCNE2 decreased Ito densities in both neonatal and adult cardiomyocytes, whereas overexpression of KCNE2 increased Ito densities in neonatal but not adult cardiomyocytes. (3) In neonatal cardiomyocytes, knockdown of KCNE2 expression accelerated the gating kinetics, and caused a positive shift of both voltage-dependent activation and inactivation of Ito, whereas overexpression of KCNE2 had the contrary effects on the gating properties of Ito. In adult cardiomyocytes, knockdown of KCNE2 had the same effects on the gating properties of Ito as in neonatal cardiomyocytes, but overexpression of KCNE2 again had no effects on them.

KCNE2 had little effect on IK in rat ventricular myocytes, but significantly upregulated K\textsubscript{\text{r1.5}}-mediated I_{K_{\text{slow}1}} current density in mouse ventricular myocytes. Since K\textsubscript{\text{r1.5}} is exclusively present in the atria and responsible for I_{K_{ur}}, except in mouse ventricles (23), the role of KCNE2 in regulation of Kv1.5-mediated current may play important role in maintaining electrical stability in atria rather than in ventricles in human heart.

KCNE2 modulation of I_{to} in cardiomyocytes

A number of studies showed that KCNE2 modulated I_{to}, but the results are controversial. Zhang et al reported that KCNE2 dose-dependently regulated the current amplitude of Kv4.2 expressed in *Xenopus* oocytes, and slowed the rates of Kv4.2 activation and inactivation, positively shifted the voltage dependent channel gating and accelerated the recovery of the channel from inactivation (33). KCNE2 regulation of Kv4.3 had also been investigated in mammalian cell lines, where KCNE2 similarly regulated the gating properties of Kv4.3 as of Kv4.2. However, KCNE2 significantly reduced Kv4.3 current densities (11, 28). Using KCNE2 knockout mice, Roepke et al demonstrated that deletion of KCNE2 significantly decreased I_{to} current densities in cardiomyocytes(22). In contrast to the findings in heterologous expression system, deletion of KCNE2 had no effect on I_{to} gating kinetics. The effects of deletion of KCNE2 on voltage-dependent activation and inactivation of I_{to} were not examined.

Resorting to adenoviral gene delivery and RNA interference to either increase or decrease KCNE2 expression in native neonatal and adult rat or neonatal mouse ventricular myocytes, we found in this study that bidirectional modulation of KCNE2 protein heterologously regulated I_{to} current densities in neonatal and adult cardiomyocytes. Knockdown of KCNE2 decreased I_{to} current densities in both neonatal and adult myocytes, which is in agreement with the findings in KCNE2 knockout mice (22). However, overexpression of KCNE2 increased I_{to} current densities in neonatal myocytes but not in adults. Two-fold implications can be derived from these findings. First, endogenously expressed KCNE2 plays an essential role in maintaining the physiological I_{to} current densities in ventricular...
myocytes. Thus, knockdown of KCNE2 results in decrease of I_{to} densities in both neonatal and adult myocytes. Second, KCNE2 contributes to the developmental increase of I_{to} current densities from neonatal to adult ventricular myocytes. It is known that I_{to} current densities increase with the growth of age. This has also been shown in our study that I_{to} current densities in adult cardiomyocytes were much bigger than those in neonatal cardiomyocytes. The bigger I_{to} in adults has been suggested to be related to the increase of the protein levels of I_{to} subunits. This study provides another possible explanation for larger I_{to} in adults, the gradual increase of KCNE2 expression from neonatal to adult contributes, as well, to the increase of I_{to} current densities. The result that moderate increase in KCNE2 had no further effect on I_{to} current densities in adults suggests that the stimulating effect of KCNE2 on I_{to} is limited and will be saturated when KCNE2 is increased to a certain level. Although the protein levels of I_{to} subunits are largely responsible for I_{to} current densities, this may not be the case for KCNE2 regulation of I_{to} densities, for neither increase nor decrease of KCNE2 interfered with Kv4.2, Kv4.3 and KChIP2 protein expression in cell membrane. In addition to the number of functional channels, the channel current densities can also be determined by the channel open probability (P_{o}) and single channel conductance. KCNE2 regulation of I_{to} densities may be attributed to alteration(s) of the channel open probability (P_{o}) and/or single channel conductance.

In addition to regulating I_{to} current densities, our data demonstrated that KCNE2 modulated I_{to} gating kinetics in native cardiomyocytes. Knockdown of KCNE2 consistently accelerated the gating kinetics in both neonatal and adult cardiomyocytes. Overexpression of KCNE2 slowed the rates of the activation and inactivation kinetics of I_{to} in neonatal cardiomyocytes, but had no effects on the gating properties in adult cardiomyocytes, which might also be due to the saturating effect of KCNE2 in adults. These findings are in general agreement with previous observations of KCNE2 regulation of I_{to} gating kinetics in heterologous expression system (33). However, bidirectional manipulation of KCNE2 had only slight regulatory effects on voltage-dependent activation and inactivation, and recovery from inactivation in cardiomyocytes, which is in contrast to the strong regulatory effects of KCNE2 on these properties in heterologous expression system (33). It is well documented that heterologously expressed KChIP2 strongly regulated these channel properties, similar to KCNE2. Our present findings suggest that these I_{to} properties may be dominantly modulated by native KChIP2 and less affected by KCNE2 in the context of cardiomyocytes.

Mechanisms underlying KCNE2 modulation of APD in cardiomyocytes

One of the most important findings in this study is that native cardiac KCNE2 differentially regulates APD in neonatal and adult myocytes. Knockdown of KCNE2 prolonged APD in both neonatal and adult cardiomyocytes. However, overexpression of KCNE2 shortened APD in neonatal cardiomyocytes but had no effect in adult cardiomyocytes. It is well known that I_{to} underlies the initial, rapid repolarization phase of the action potential (AP), and I_{K} is responsible for the subsequent, slower phase of AP repolarization back to the resting membrane potential (3). In adult rat ventricular myocytes, I_{K} is relatively small, so the larger I_{to} is responsible for the spike-like morphology of action potential (17, 25). The quantitative link between I_{to} and APD in response to KCNE2 manipulation was assessed by applying the alterations of I_{to} and I_{K} to a mathematical model of adult rat APD. In KCNE2 knockdown cells, the alterations in I_{to} current densities and gating properties resulted in prolongation of APD estimated by the mathematical model. The simulated APD50 and APD90 were increased by 53.4% and 44.9%, respectively, which were comparable to those (APD50 and APD90 were increased
by 87.7% and 38.2%) calculated from experimentally recorded APD. In KCNE2 overexpressed cells, $I_K$ current densities were slightly decreased and $I_{Io}$ remained unaltered, resulting in 2.1% decrease in $I_t$ (the sum of $I_{Io}$ and $I_K$). The simulated APD was less altered, which is also comparable to experimentally recorded APD. The data collectively indicate that $I_{Io}$ is a major contributor to KCNE2 modulation of APD.

Recently, we found that KCNE2 regulated L-type Ca$^{2+}$ current ($I_{Ca,L}$), but not sodium current ($I_{Na}$) in cardiomyocytes, in which upregulation of KCNE2 by 2.5 fold decreased the peak $I_{Ca,L}$ by 18%, whereas downregulation of KCNE2 by 79% increased the peak $I_{Ca,L}$ by 29% (12). The increase in $I_{Ca,L}$ by KCNE2 knockdown may explain the relatively larger experimental APD prolongation compared to simulated APD prolongation. Although a large increase in KCNE2 (by 2.5 fold) decreases $I_{Ca,L}$ amplitude and can shorten APD, this effect may not or only mildly affect APD by KCNE2 overexpression under our experimental condition because KCNE2 was only moderately increased (by 67%).

Previously, it has been reported that KCNE2 expression was decreased in acute myocardial infarction and infarct cardiomyopathy (8, 29). APD prolongation-related long QT is a prominent manifestation of the diseased hearts (10, 26). That knockdown of KCNE2 prolongs APD suggests that abnormal KCNE2 expression may contribute to the electrical disturbances in the diseased hearts.

Limitations

There is limitation in this study. We had only examined the regulatory effect of KCNE2 on AP and $K_V$ currents in neonatal and adult rat ventricular myocytes and neonatal mouse ventricular myocytes, where the action potential (AP) is triangle-shaped and $I_{Io}$ is the dominant repolarization current in adults. However, the shape of AP and the contribution of $I_{Io}$ to AP in big animal and human cardiomyocytes are quite different from those in rodent cardiomyocytes. In human ventricular myocytes, $I_{Io}$ contributes to the phase I repolarization and $I_K$ (including $I_{Kr}$ and $I_{Ks}$) plays an important role in determining phase II and phase III repolarization. Therefore, it should be cautious to translate the present findings to human diseases.

In summary, this study demonstrated for the first time that endogenous KCNE2 in cardiomyocytes differentially regulates APD in neonatal and adult cardiomyocytes, which is largely attributed to KCNE2 regulation of $I_{Io}$. These findings highlight the important role of physiological expressed KCNE2 in maintaining cardiac electrophysiological stability by homeostatic regulation of voltage-dependent channels in normal heart and may be a novel view for understanding the pathogenesis of cardiac arrhythmia in diseased hearts.

Acknowledgment

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Conflict of Interest
References


Figure Legends

**Fig. 1 Effects of knockdown of KCNE2 expression on action potential duration (APD) in neonatal and adult rat ventricular myocytes.** A, Representative images of western blots and statistics of KCNE2 and α-actin proteins in neonatal and 2-3-month-old (adult) and 18-month-old (aging) ventricular myocytes (n=4). α-Actin was used as an internal control. B, C, Representative images of western blots and statistics of KCNE2 and α-actin proteins in NRVM (B) and ARVM (C) cardiomyocytes without (Ctrl-shRNA) or with KCNE2 knockdown (KCNE2-shRNA, n=4 in each group, 4 rats). D, Representative traces of action potential at a 1-Hz stimulation in NRVM and ARVM without (Ctrl-shRNA) or with KCNE2 knockdown (KCNE2-shRNA). E, F, Averages of APD20, APD50 and APD90 in NRVM (E) (Ctrl-shRNA, n=35, 13 rats; KCNE2-shRNA, n=41, 13 rats) and ARVM (Ctrl-shRNA, n=38, 11 rats; KCNE2-shRNA, n=47, 11 rats) (F). G, H, Statistics of resting membrane potential (RMP, G) and amplitude of AP overshoot (H). *p<0.05, **p<0.01 vs Ctrl-shRNA.

**Fig. 2 Effects of overexpression of KCNE2 on action potential duration (APD) in neonatal and adult rat ventricular myocytes.** A, B, Representative images of western blots and statistics of KCNE2 and α-actin proteins in neonatal (NRVM, A) and adult (ARVM, B) cardiomyocytes without (Ad-GFP) or with KCNE2 (Ad-KCNE2) overexpression (n=4 in each group, 4 rats). C, Representative traces of action potential at a 1-Hz stimulation in neonatal (NRVM) and adult cardiomyocyte (ARVM). D, E, Averages of APD20, APD50 and APD90 in NRVMs (D) (n=33 and 39 in Ad-GFP and Ad-KCNE2 group, respectively, 13 rats) and ARVMs (E) (n=31 and 40 in Ad-GFP and Ad-KCNE2 group, respectively, 14 rats). F, G, Statistics of resting membrane potential (RMP, F) and overshoot values (G). *p<0.05, **p<0.01 vs Ad-GFP.

**Fig. 3 Effects of knockdown of KCNE2 expression on I\textsubscript{to} in neonatal and adult rat ventricular myocytes.** A, Representative current traces of transient outward K\textsuperscript{+} currents in neonatal (NRVM) and adult (ARVM) cardiomyocytes without (Ctrl-shRNA) or with KCNE2 (KCNE2-shRNA) knockdown. B-D, Average current density-voltage relationships of the peak current (B), I\textsubscript{ss} (C) and I\textsubscript{to} (D) in NRVMs without (Ctrl-shRNA) or with KCNE2 (KCNE2-shRNA) knockdown (n=19 and 23 in Ctrl-shRNA and KCNE2-shRNA group, respectively, 8 rats). E-G, Average current density-voltage relationships of the peak current (E), I\textsubscript{ss} (F) and I\textsubscript{to} densities (G) in ARVMs without (Ctrl-shRNA) or with KCNE2 (KCNE2-shRNA) knockdown (n=20 and 24 in Ctrl-shRNA and KCNE2-shRNA group, respectively, 14 rats). *p<0.05, **p<0.01 vs Ctrl-shRNA.
**Fig. 4 Effects of overexpression of KCNE2 on I_{to} in neonatal and adult rat ventricular myocytes.**
A, Representative current traces of transient outward K\(^+\) currents in neonatal (NRVM) and adult (ARVM) cardiomyocytes without (Ad-GFP) or with KCNE2 (Ad-KCNE2) overexpression. B-D, Average current density-voltage relationships of the peak current (B), I_{ss} (C) and I_{to} (D) in NRVMs without (Ad-GFP) or with KCNE2 (Ad-KCNE2) overexpression (n=22 and 17 in Ad-GFP and Ad-KCNE2 group, respectively, 7 rats). E-G, Average current density-voltage relationships of the peak current (E), I_{ss} (F) and I_{to} (G) in ARVMs without (Ad-GFP) or with KCNE2 (Ad-KCNE2) overexpression (n=24 and 28 in Ad-GFP and Ad-KCNE2 group, respectively, 8 rats). \(^*p<0.05, \quad \text{**p}<0.01\) vs Ad-GFP.

**Fig. 5 Measurement of the membrane protein levels of \(\alpha\) and \(\beta\) subunits of I_{to}.** A, D, Representative images of western blots of Kv4.2, Kv4.3, Kv1.4 and KChIP2 and \(\alpha\)-actin proteins in Ad-GFP and Ad-KCNE2 groups, and Ctrl-shRNA and KCNE2-shRNA groups in NRVMs (A) and ARVMs (D). B, E, Quantification of protein levels Kv4.2, Kv4.3, Kv1.4 and KChIP2 in Ad-GFP and Ad-KCNE2 groups in NRVMs (B) and ARVMs (E) (n=4 in each group, 4 rats). C, F, Quantification of protein levels Kv4.2, Kv4.3, Kv1.4 and KChIP2 in Ctrl-shRNA and KCNE2-shRNA groups (n=4 in each group, 8 rats) in NRVMs(C) and ARVMs (F). The abundance of the target protein was normalized by that in Ad-GFP or Ctrl-shRNA group.

**Fig. 6 Effects of KCNE2 on I_{to} gating kinetics and voltage-dependent gating properties.** A, Superimposed current traces were recorded at 60 mV from Ad-GFP and Ad-KCNE2 groups or Ctrl-shRNA and KCNE2-shRNA groups in neonatal and adult cardiomyocytes. The display gain was adjusted so that peak current amplitudes match each other. B, C, Statistics of time to peak (TTP, B) and time constant of inactivation (\(\tau\), C) of I_{to}. N=21 to 29 in each group. D, E, Voltage-dependent activation of I_{to} curves in Ad-GFP and Ad-KCNE2 groups (n=22 and 33 in Ad-GFP and Ad-KCNE2 group, respectively, 8 rats) (D) or Ctrl-shRNA and KCNE2-shRNA groups (E) (n=23 and 27 in Ctrl-shRNA and KCNE2-shRNA group, respectively, 14 rats). F, Average of \(V_{0.5}\) of activation. G, H, Voltage-dependent inactivation of I_{to} curves in Ad-GFP and Ad-KCNE2 groups (G) or Ctrl-shRNA and KCNE2-shRNA groups (H). I, Average of \(V_{0.5}\) of inactivation. J, K, Time courses of I_{to} recovery from inactivation in Ad-GFP and Ad-KCNE2 groups (J) (n=20 and 16 in Ad-GFP and Ad-KCNE2 group, respectively, 9 rats) or Ctrl-shRNA and KCNE2-shRNA groups (n=12 and 16 in Ctrl-shRNA and KCNE2-shRNA group, respectively, 14 rats) (K). Inset shows the voltage-clamp protocol. L, Average of time constant (\(\tau\)) of I_{to} recovery from inactivation(n=7,10,10 and 11 in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA group, respectively, 5 rats). * \(p<0.05\) vs Ad-GFP, \(\text{**p}<0.01\) vs Ctrl-shRNA.

**Fig. 7 KCNE2 modulated I_{K} and I_{K1} in adult rat ventricular myocytes.** A, Representative I_{K} current traces in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA groups. B, C, Average current density-voltage relationships of I_{K} in Ad-GFP and Ad-KCNE2 groups (B) or Ctrl-shRNA and KCNE2-shRNA groups (C, n=13,10, 11 and 10 in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA group, respectively, 6 rats). D, Representative I_{K1} current traces in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA groups. E, F, Average current density-voltage
relationships of $I_{K1}$ in Ad-GFP and Ad-KCNE2 groups (E) or Ctrl-shRNA and KCNE2-shRNA groups (F, n=13, 10, 11 and 10 in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA group, respectively; 5 rats).* $p<0.05$ vs Ad-GFP.

**Fig. 8** Effects of knockdown of KCNE2 expression on $I_{to}$, $I_{K,slow1}$ and $I_{K,slow2}$ in neonatal mouse ventricular myocytes. A, C, E, G, Representative current traces of transient outward K$^+$ current (A), HøTX-sensitive $I_{to}$ (B), 4-AP-sensitive $I_{K,slow1}$ (E) and TEA-sensitive $I_{K,slow2}$ (G) in Ad-GFP, Ad-KCNE2, Ctrl-shRNA and KCNE2-shRNA groups. B, D, F, H, Average current density-voltage relationships of the peak current (B), $I_{to}$ (D), $I_{K,slow1}$ (F) and $I_{K,slow2}$ (H) in four groups (n=19-24 in each group). I, J Representative images of western blots (I) and statistics (J) of Kv4.2, Kv4.3, Kv1.5 and KChIP2 and $\alpha$-actin proteins in four group (n=4 in each group). * $p<0.05$, ** $p<0.01$ vs Ad-GFP; # $p<0.05$, ## $p<0.01$ vs Ctrl-shRNA.

**Fig. 9** Model generated action potentials in Ad-GFP (solid line) and Ad-KCNE2 (dashed line) myocytes (A), and in Ctrl-shRNA (solid line) and KCNE2-shRNA (dashed line) myocytes (B).

Table 1 Characteristics of action potential in neonatal mouse ventricular myocytes (NMVMs) with or without KCNE2 overexpression (or KCNE2 knockdown). N=15-28 in Ad-GFP, Ad-KCNE2, Ctrl-shRNA or KCNE2-shRNA group, respectively. * $p<0.05$, vs Ad-GFP; # $p<0.05$ vs Ctrl-shRNA.

Table 2 Simulated and experimental action potential characteristics in control and KCNE2 overexpression (or knockdown) adult rat ventricular myocytes (ARVMs), ## $p<0.05$ vs Ctrl-shRNA.
Fig. 2

A  
KCNE2
α-actin

Fold change of KCNE2 abundance

Ad-GFP Ad-KCNE2

**

B  
KCNE2
α-actin

Fold change of KCNE2 abundance

Ad-GFP Ad-KCNE2

**

C
NRVM  
ARVM

D  
APD (ms)

Ad-GFP Ad-KCNE2

* *

E  
APD (ms)

Ad-GFP Ad-KCNE2

F  
RMP (mV)

Ad-GFP Ad-KCNE2

G  
Over shoot (mV)

Ad-GFP Ad-KCNE2

NRVM ARVM

Fig. 2
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 8

A: Peak current

- Ad-GFP
- Ad-KCNE2

Ctrl-shRNA
KCNE2-shRNA

B: pA/pF vs. Vt (mV)

C: HpTx2 sensitive

- Ad-GFP
- Ad-KCNE2

Ctrl-shRNA
KCNE2-shRNA

D: pA/pF vs. Vt (mV)

E: 4-AP sensitive

- Ad-GFP
- Ad-KCNE2

Ctrl-shRNA
KCNE2-shRNA

F: pA/pF vs. Vt (mV)

G: TEA sensitive

- Ad-GFP
- Ad-KCNE2

Ctrl-shRNA
KCNE2-shRNA

H: pA/pF vs. Vt (mV)

I: Immunoblot

- Kv4.2
- Kv4.3
- Kv1.4
- Kv1.5
- α-actin

J: Target protein/α-actin protein (%Control)

- Ad-GFP
- Ad-KCNE2
- Ctrl-shRNA
- KCNE2-shRNA

Fig. 8
**Fig. 9**

A

-80 mv

0 mv

50 ms

- Ad-GFP
- Ad-KCNE2

B

- Ctrl-shRNA
- KCNE2-shRNA
Table 1  Characteristics of action potential in neonatal mouse ventricular myocytes (NMVMs) with or without KCNE2 overexpression (or KCNE2 knockdown).

<table>
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<tr>
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<th>$V_{rest}$ (mV)</th>
<th>OS (mV)</th>
<th>APD20 (ms)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
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<td>Ad-GFP</td>
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<td>30.43±0.77</td>
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<td>KCNE2-shRNA</td>
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<td>148.61±4.9*</td>
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$V_{rest}$: resting membrane potential; OS: overshoot; APD20: action potential duration (20% repolarization); APD50: action potential duration (50% repolarization); APD90: action potential duration (90% repolarization). *p<0.05 vs Ad-GFP, # p<0.05 vs ctrl-shRNA.
Table 2 Simulated and experimental action potential characteristics in control, KCNE2 overexpression or knockdown adult rat ventricular myocytes (ARVMs).

<table>
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<td>8.8</td>
<td>13.5**</td>
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V_{rest}: resting membrane potential; OS: overshoot; APD_{50}: action potential duration (50% repolarization); APD_{90}: action potential duration (90% repolarization), **# p<0.01 vs ctrl-shRNA.**