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Characterization of Recombinant Human Cardiac *KCNQ1/KCNE1* Channels (I_{Ks}) Stably Expressed in HEK 293 Cells

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Abstract. The present study was designed to characterize pharmacological, biophysical and electrophysiological properties of the recombinant human cardiac I_{Ks} (*KCNQ1*/*KCNE1*) channels at physiological temperature. Human cardiac *KCNQ1* and *KCNE1* genes were cotransfected into HEK 293 cells, and a cell clone stably expressing both genes was selected. Membrane currents were recorded using a perforated patch-clamp technique. The typical I_{Ks} was slowly activated upon depolarization voltages in HEK 293 cells stably expressing human cardiac *KCNQ1* and *KCNE1* genes, and the current was inhibited by I_{Ks} blockers HMR 1556 and chromanol 293B, with 50% inhibitory concentrations (IC_{50}s) of 83.8 nM and 9.2 μM, respectively. I_{Ks} showed a significant temperature-dependent increase in its magnitude upon elevating bath temperature to 36°C from room temperature (21°C). The current was upregulated by the β-adrenoceptor agonist isoproterenol, and the effect was reversed by H89. In addition, I_{Ks} was inhibited by Ba^{2+} in a concentration-dependent manner (IC_{50} = 1.4 mM). Action potential clamp revealed a "bell-shaped" time course of I_{Ks} during the action potential, and maximal peak current was seen at the plateau of the action potential. A significant use- and frequency-dependent increase of I_{Ks} was observed during a train of action potential clamp. These results indicate that the recombinant human cardiac I_{Ks} stably expressed in HEK 293 cells is similar to native I_{Ks} in drug sensitivity and regulated by Ba^{2+} and β-adrenoceptor via the cyclic adenosine monophosphate/protein kinase A pathway. Importantly, the current exhibits significant temperature dependence, a bell-shaped time course during action potential and prominent use- or frequency-dependent accumulation during a train of action potentials.

Key words: Electrophysiology — Ion channel — Slowly activating delayed rectifier potassium current — Recombinant human cardiac *KCNQ1*/*KCNE1* — HEK 293 cell — Perforated recording

Introduction

The slowly activating delayed rectifier potassium current I_{Ks} plays an important role in the repolarization of mammalian cardiac action potential in different species, including humans (Sanguinetti & Jurkiewicz, 1990; Li et al., 1996; Lu et al., 2001). It has been demonstrated that I_{Ks} is a heteromultimeric complex composed of four pore-forming α subunits and several accessory β subunits encoded, respectively, by the *KCNQ1* and *KCNE1* genes (Sanguinetti et al., 1996; Barhanin et al., 1996; Chen et al., 2003). Dysfunction of I_{Ks} due to genetic mutations in the *KCNQ1* or *KCNE1* gene is linked to congenital long QT syndrome (LQT1 or LQT5). Electrical remodeling in diseased hearts is often associated with a reduction of I_{Ks}, e.g., myocardial infarction (Jiang et al., 2004), chronic heart failure (Li et al., 2002, 2004) and cardiac hypertrophy (Nabauer & Kaab, 1998; Xu et al., 2001). All these conditions increase the risk for life-threatening ventricular arrhythmia.

Although some properties of the recombinant I_{Ks} were studied in different transiently expressing systems, detailed information of pharmacological and biophysical properties has not been well documented at physiological temperature. Here, we established a HEK 293 cell line stably expressing recombinant human cardiac I_{Ks} by cotransfecting the human *KCNQ1* and *KCNE1* genes and characterized the

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channel's pharmacological and biophysical properties at physiological temperature (36–37°C). In addition, we studied the time course and frequency dependence of the I_{Ks} current during cardiac action potentials at physiological temperature.

Methods

Cell Culture and Gene Transfection

HEK 293 cells were purchased from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum in 5% CO_2 and 95% air at 37°C.

Human *KCNQ1* and *KCNE1* cDNAs were generous gifts from Dr. Mike Sanguinetti (University of Utah, Salt Lake City, UT) and Dr. Richard Swanson (Merck & Co. Inc, West Point, PA), respectively. These cDNAs were subcloned into the HindIII/BamHI site of the pCEP4 vector (Invitrogen) and the EcoRI/NotI site of the pALTER-Max vector (Promega, Madison, WI), respectively. The vectors of *hKCNQ1* (0.4 μg) and *hKCNE1* (4.0 μg) were cotransfected into HEK 293 cells using Lipofectamine 2000^{TM} (Invitrogen) according to the manufacturer's instructions. The cDNA molar ratio of *hKCNE1:hKCNQ1* was 20:1, ensuring that cells expressing *hKCNQ1* also had *hKCNE1*. After selection in 200 μg/ml hygromycin (Sigma-Aldrich, St. Louis, MO) for 2 weeks, colonies were picked with cloning cylinders and examined for channel expression by whole-cell current recordings. One colony of cells with stable expression of I_{Ks} (*hKCNQ1*/*hKCNE1*) was obtained and maintained in DMEM containing 100 μg/ml hygromycin.

Solutions and Drugs

Tyrode solution contained (mM) NaCl 140, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.33, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 5.0 and glucose 10 (pH adjusted to 7.3 with NaOH). For open-patch whole-cell recordings, the pipette solution contained (mM) KCl 20, K-aspartate 110, $MgCl_2$ 1.0, HEPES 10, ethyleneglycoltetraacetic acid (EGTA) 5, guanosine triphosphate (GTP) 0.1, Na_2-phosphocreatine 5 and Mg_2-adenosine triphosphate (ATP) 5 (pH adjusted to 7.2 with KOH). For perforated-patch recordings, EGTA, GTP and ATP were omitted while amphotericin B (Sigma-Aldrich) was added to the pipette solution (200 μg/ml).

HMR 1556 and chromanol 293B were gifts from Aventis (Frankfurt, Germany). The stock solutions were made as 1 and 100 mM in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and kept at 4°C; H89 (Sigma-Aldrich) and forskolin (Alomone Labs, Jerusalem, Israel) were prepared as 10 mM stock solutions in DMSO and distilled water, respectively, then divided into aliquots and kept at −20°C. Isoproterenol (Sigma-Aldrich) was freshly prepared as 10 mM stock solution in 1 mM ascorbic acid and kept in the dark at 4°C. Propranolol (Sigma-Aldrich) was prepared as 10 mM stock. DMSO had no measurable effect on I_{Ks} currents at the maximal final concentration (0.1%).

Data Acquisition and Analysis

Cells on a coverslip were transferred to an open cell chamber mounted on the stage of an inverted microscope and superfused with Tyrode solution at ~2 ml/min. Experiments were performed at room temperature (21–22°C) and/or 36–37°C, maintained with a temperature controller (Warner Instrument, Hamden, CT) as specified.

Borosilicate glass electrodes (1.2 mm optical density, OD) were pulled with a Brown-Flaming puller (P-97; Sutter Instrument, Novato, CA) and had tip resistances of 1–2 MΩ when filled with the pipette solution. The currents were measured using an EPC-10 amplifier and Pulse software (Heka Elektronik, Lambrecht, Germany). A 3 M KCl–agar bridge was used as the reference electrode. Tip potential was zeroed before the patch pipette touched the cell. In most experiments, membrane currents were recorded with perforated patch configuration by including 200 μg/ml amphotericin B in pipette solution, and stable access resistance (8–12 MΩ) formed within 30 min. Current and voltage signals were low pass-filtered at 5 kHz and stored in an IBM-compatible personal computer for off-line analysis.

Nonlinear curve fitting was performed using Pulsefit (Heka Elektronik) or Sigmaplot (SPSS, Chicago, IL). Comparison was made using a paired or unpaired Student's *t*-test to evaluate the statistical significance between two group means. Analysis of variance was used for multiple groups. Data were expressed as mean ± standard error (SE). Differences were considered significant at $P < 0.05$.

Results

Cell Line Stably Expressing I_{Ks}

Figure 1A illustrates the membrane currents recorded using the whole-cell patch technique at 21°C, with the voltage protocol shown in the inset in HEK 293 cells with or without transfecting *hKCNQ1* and *hKCNE1* genes. A small endogenous membrane current was observed in the cell without any gene transfection, while a slowly activating current was elicited by the depolarization voltages also with a significant tail current at −40 mV in HEK 293 cells stably expressing *hKCNQ1*/*hKCNE1* and showed a characterization typical of I_{Ks}. We therefore referred to it as I_{Ks}.

Rundown is a well-known problem for studies of I_{Ks} with whole-cell dialysis patch-clamp configuration. We found that the I_{Ks} stably expressed in HEK 239 cells also showed a significant rundown after cell membrane rupture; however, the perforated patch configuration with 200 μg/ml amphotericin B in the pipette solution showed a stable recording. Figure 1B illustrates time courses of normalized I_{Ks} recorded with a 3-s voltage step to +40 mV from −80 mV, then back to −40 mV in two typical experiments using conventional whole-cell patch recording and perforated patch recording, respectively. In the whole-cell patch configuration, I_{Ks} showed continuous rundown (55% at 30 min) after membrane rupture, while in the perforated patch recording, I_{Ks} showed no significant rundown after stable access formation. Stable I_{Ks} recording was maintained for 30–50 min with perforated patch recording; therefore, perforated recording was employed in the following experiments.

The I_{Ks}-selective blockers HMR 1556 and chromanol 293B were verified in the HEK 293 cell line stably expressing human cardiac I_{Ks} with perforated patch recording. Both HMR 1556 and 293B inhibited I_{Ks} (at +40 mV) in a concentration-

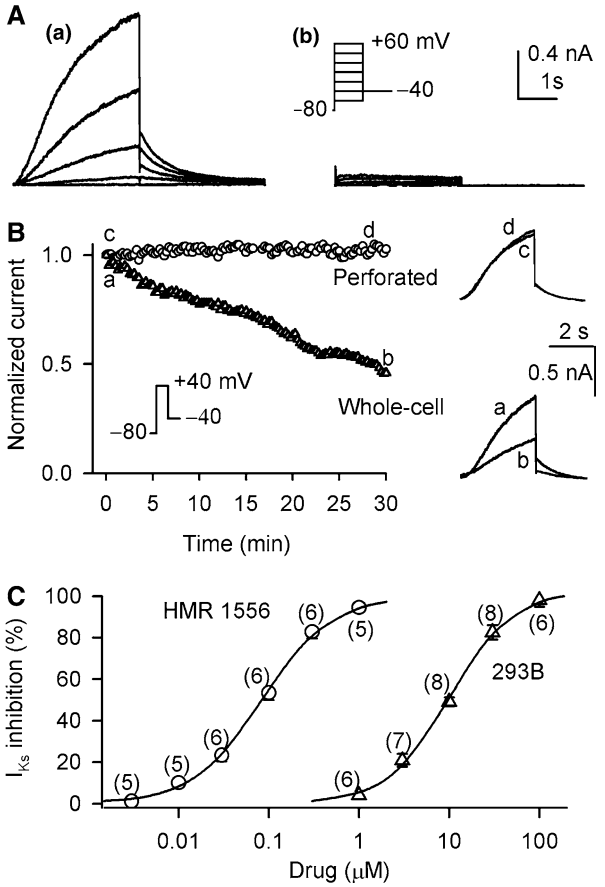

Fig. 1. Membrane currents in HEK 293 cells. (*A*) Membrane currents were recorded at 21°C using the voltage protocol shown in the *inset* in HEK 293 cells with (*a*) and without (*b*) transfecting the *hKCNQ1*/*KCNE1* genes. A slowly activating current was activated in the cell stably expressing *hKCNQ1*/*hKCNE1* upon 3-s voltage steps to between −60 and +40 (20-mV increment) from −80 mV with significant tail current at −40 mV, typical of I_{Ks}. A small endogenous current was observed in the cell without gene transfection. (*B*) Time courses of normalized I_{Ks} tail current recorded at −40 mV after a 3-s voltage step to +40 from −80 mV in whole-cell patch configuration (*open triangles*) and perforated patch recording with 200 μg/ml amphotericin B in pipette solution (*open circles*) in different cells expressing I_{Ks} channels. Continuous rundown of I_{Ks} was observed after membrane rupture in whole-cell patch configuration, while stable I_{Ks} was maintained for 30 min. Original I_{Ks} traces at corresponding time points are shown at the right of the panel. (*C*) Concentration-response relationships of I_{Ks} inhibition by HMR 1556 (open circles) and chromanol 293B (open triangles). The numbers in parentheses are experimental numbers.

dependent manner (Fig. 1C), and the concentration-response relationship curves for the inhibition of I_{Ks} by HMR 1556 in five cells completed all concentrations (0.003–1 μM) and by 293B in six cells completing all concentrations (1–100 μM) were fitted to the Hill equation: $E = E_{max}/[1 + (IC_{50}/C)^b]$, where E is the effect at concentration C, E_{max} is the maximal effect, IC_{50} is the concentration for half-maximal effect and b is the Hill coefficient. The IC_{50} for HMR 1556 was 83.8 ± 3.4 nM, with a b value of 1.13 (E_{max} = 95%), while the IC_{50} for 293B was 9.2 ± 0.7 μM, with a b value of 1.23 (E_{max} = 98%).

Temperature Dependence Of I_{Ks}

To investigate the temperature dependence of I_{Ks}, the current was recorded at room temperature (21–22°C) and then at 36–37°C. Each cell was tested at the two temperatures to avoid issues of current variations among cells. Figure 2A shows I_{Ks} traces recorded in a representative cell with a similar voltage protocol shown in the inset of Figure 1A at 21°C and 36°C. Magnitude and kinetics for activation and deactivation of I_{Ks} clearly increased as bath temperature was elevated to 36°C from 21°C. Figure 2B displays the current-voltage (I-V) relationships of I_{Ks} tail current at 21°C and 36°C in eight cells. The increase of bath temperature resulted in a substantial augmentation of current magnitude. I_{Ks} tail current at +40 mV was 0.12 ± 0.01 nA at 21°C and 0.44 ± 0.05 nA at 36°C (increased by 2.7-fold, $P < 0.01$).

The voltage dependence of I_{Ks} activation was determined by normalizing I_{Ks} tail current (measured at −40 mV from the peak tail to the completed deactivation level). Figure 2C displays the activation curves fit to the Boltzmann function: $f = 1/\{1 + \exp[(V_{0.5}-V_t)/S]\}$, where V_t is the test potential, $V_{0.5}$ is the midpoint of activation voltage and S is the slope factor. $V_{0.5}$ was 21.4 ± 1.4 mV at 21°C and 10.2 ± 1.1 mV at 36°C (n = 7, $P < 0.01$), while S was not significantly affected (13.3 ± 2.0 for 21°C, 14.8 ± 2.1 for 36°C), suggesting that activation conductance of I_{Ks} is increased by elevating bath temperature.

Figure 2D shows raw data for activation (+40 mV) and deactivation (−40 mV) of I_{Ks} fitted by single exponential functions with time constants at 21°C and 36°C. The activation of I_{Ks} was fitted from the significant activation of the current to the end of the voltage steps, while the deactivation of I_{Ks} was fitted from the peak tail to the complete deactivation level of the tail current. Both activation and deactivation time constants of I_{Ks} were decreased by elevating bath temperature from 21°C to 36°C. Figure 2E illustrates the mean values for the activation time constant of I_{Ks} at different voltages. The activation time constant of I_{Ks} was significantly reduced at 36°C at test potentials of −20 to +40 mV ($P < 0.01$). Figure 2F displays the deactivation time constant of I_{Ks} determined at potentials of −90 to −30 mV after a 3-s voltage step to +40 mV (inset) measured at 21°C and then at 36°C. The mean values of the deactivation time constant of I_{Ks} at all test potentials were shorter at 36°C than those at 21°C (n = 8, $P < 0.01$). Therefore, I_{Ks} activation and deactivation kinetics are markedly accelerated at physiological temperature relative to those at room temperature. This

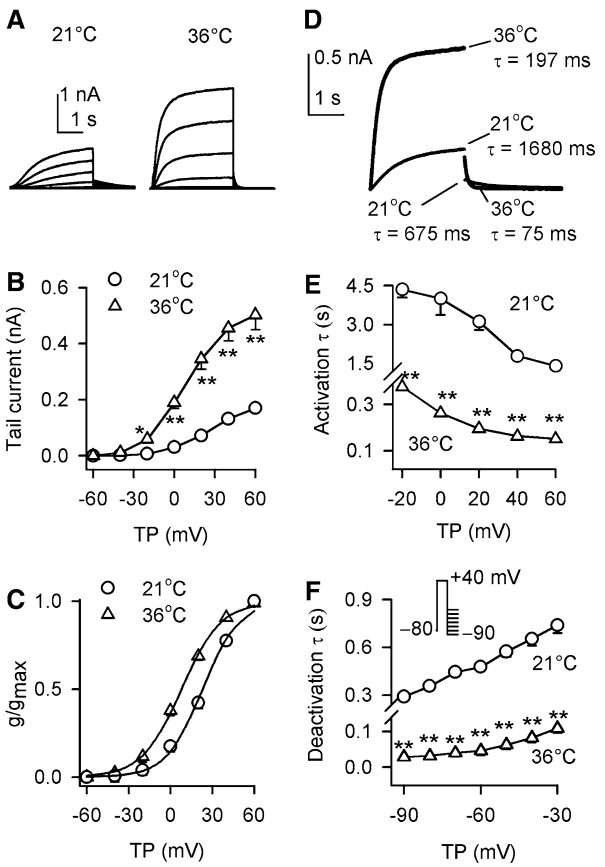

Fig. 2. Temperature dependence of I_{Ks}. (*A*) I_{Ks} traces recorded in a representative cell with similar voltage protocol shown in the *inset* of Figure 1A (3-s steps to between −60 and +60 from −80 mV with 20-mV increment, then to −40 mV) at 21°C, then at 36°C. (*B*) I-V relationships of I_{Ks} tail current measured at −40 mV from peak tail current to the complete deactivation level at 21°C (*open circles*) and 36°C (*open triangles*) in the same group of cells (each cell tested at the two temperatures). (*C*) Steady-state activation of I_{Ks} determined by normalizing I_{Ks} tail current at 21°C (*open circles*) and at 36°C (*open triangles*). The curve was fit to the Boltzmann function. (*D*) I_{Ks} traces recorded from a representative cell with a 3-s voltage step to +40 from −80 mV, then to −40 mV at 21°C, then at 36°C. Raw data (*points*) of I_{Ks} and corresponding I_{Ks} tail current at −40 mV were fit to a monoexponential function (*solid line,* superimposed with raw data) with activation and deactivation time constants shown. (*E*) Activation time constant was greater at 21°C than at 36°C in the entire voltage range (**$P < 0.01$, $n = 8$). (*F*) Deactivation time constant of I_{Ks} determined with the voltage protocol shown in the *inset*. The time constant was shorter at 36°C (*open circles*) than at 21°C (*open triangles*) over the whole voltage range examined(**$P < 0.01$, $n = 8$).

temperature dependence is important when considering the contribution of I_{Ks} to action potential repolarization at body temperatures.

Q_{10} is the characteristic temperature coefficient, which is used to assess temperature dependence of ion channel currents (Noble & Tsien, 1968; Kiyosue et al., 1993; Li et al., 1998). We determined Q_{10} values for the amplitude and kinetics of the recombinant human cardiac I_{Ks} expressed in HEK 293 cells. The Q_{10} for I_{Ks} magnitude was calculated by the equation $Q_{10} = 1 + 10\,(A_2 - A_1)/[A_1\,(T_2 - T_1)]$, where A_1 and A_2 are I_{Ks} magnitude at different temperatures T_1 and T_2, respectively; the Q_{10} for I_{Ks} activation and deactivation time constants was calculated with the equation $Q_{10} = \exp\{[10/(T_2 - T_1)]\ \ln(\tau_1/\tau_2)\}$, where τ_1 and τ_2 are the time constants obtained at T_1 and T_2, respectively. Q_{10} values were 1.8 ± 0.1 for the magnitude of I_{Ks} at +40 mV, 4.9 ± 0.2 for the time constant of I_{Ks} activation at +40 mV and 3.9 ± 0.2 for the time constant of deactivation of I_{Ks} at −40 mV. No voltage dependence of Q_{10} was observed for the magnitude, activation and deactivation time constants of I_{Ks}.

Modulation of I_{Ks} by β-Adrenoceptor via the Cyclic Adenosine Monophosphate/Protein Kinase A Pathway

To study whether the recombinant I_{Ks} expressed in HEK 293 cells could be modulated by β-adrenoceptor via the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway as in native cardiac myocytes, we tested the effects of a β-adrenoceptor agonist (isoproterenol), an adenylate cyclase activator (forskolin) and a PKA inhibitor (H89) on I_{Ks} at 36°C.

Figure 3 illustrates the effect of isoproterenol (100 nM) on voltage dependence and kinetics of I_{Ks}. The magnitude of I_{Ks} was augmented by application of 100 nM isoproterenol for 3 min (Fig. 3A). Mean values of I_{Ks} tail current were increased by isoproterenol at voltage steps from −20 to +60 mV (Fig. 3B, $n = 7$, $P < 0.05$ or $P < 0.01$ *vs.* control). Figure 3C shows the steady-state activation of I_{Ks} determined by normalizing the tail current in the absence and presence of 100 nM isoproterenol. The $V_{0.5}$ of I_{Ks} activation was shifted toward hyperpolarization by 4 mV with 100 nM isoproterenol ($V_{0.5}$ 10.9 ± 1.0 mV for control, 6.9 ± 0.8 mV for isoproterenol; $P < 0.05$, $n = 7$), while the slope factor was not affected (13.8 ± 1.2 for control, 14.5 ± 1.3 for isoproterenol).

Figure 3D and E display the activation and deactivation time constants of I_{Ks} in the absence and presence of 100 nM isoproterenol. The activation time constant of I_{Ks} was reduced by isoproterenol at −20 to +40 mV ($n = 7$, $P < 0.01$ *vs.* control); however, the deactivation time constant was increased by isoproterenol at potentials of −90 to −20 mV ($n = 7$, $P < 0.01$ *vs.* control). These results indicate that isoproterenol accelerates the activation and slows down the deactivation of I_{Ks}.

Pretreatment with 1 μM propranolol fully prevented the augmentation of I_{Ks} by 100 nM isoproterenol ($n = 6$); however, forskolin at 1 μM significantly increased I_{Ks} (by 28.5 ± 7.4%, $n = 7$, $P < 0.01$) in the presence of propranolol. The

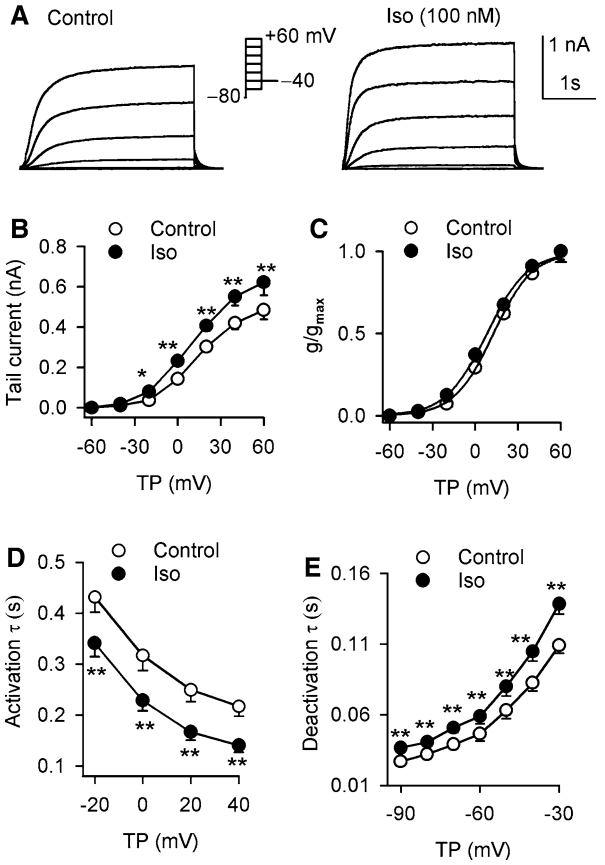

Fig. 3. Effects of the β-adrenoceptor agonist isoproterenol (Iso) on I_{Ks} in HEK 293 cells. (*A*) I_{Ks} traces recorded in a representative cell with the voltage protocol as shown in the *inset* in the absence and presence of isoproterenol. Isoproterenol at 100 nM substantially increased I_{Ks}. (*B*) I-V relationships of I_{Ks} tail current in the absence (*open circles*) and presence of 100 nM isoproterenol (*filled circles*, $^*P < 0.05$; $^{**}P < 0.01$ *vs.* control). (*C*) Steady-state activation curves were determined by normalizing I_{Ks} tail current measured at −40 mV and fit to the Boltzmann distribution in the absence and presence of 100 nM isoproterenol. (*D*) Time constant of I_{Ks} activation in the absence and presence of 100 nM isoproterenol ($n = 7$, $^{**}P < 0.01$ *vs.* control). (*E*) Voltage-dependent time constant of I_{Ks} deactivation determined by the voltage protocol as shown in the *inset* of Figure 2F in the absence and presence of 100 nM isoproterenol. The deactivation time constant of I_{Ks} was increased by application of 100 nM isoproterenol($n = 7$, $^{**}P < 0.01$ *vs.* control).

stimulatory effect of I_{Ks} by isoproterenol or forskolin was completely reversed by the coapplication of 1 μM H89 ($n = 7$). These results support the notion that isoproterenol increased I_{Ks} by activating endogenous β-adrenoceptor in HEK cells (Freeman et al., 2002; Werry et al., 2002), and the effect was mediated by the cAMP/PKA pathway.

Inhibition of I_{Ks} by Ba^{2+}

It has been reported that Ba^{2+} inhibits several K^+ channel currents (Lesage et al., 1997; Weerapura et al., 2000; Li et al., 2000). Here, we determined whether human cardiac I_{Ks} would be affected by application of Ba^{2+}. Figure 4A shows I_{Ks} traces recorded in a representative cell with a similar voltage protocol as shown in the inset of Figure 3A in the absence and presence of $BaCl_2$. Ba^{2+} at 3 mM substantially inhibited voltage-dependent I_{Ks}, and the effect was reversed by washout. Figure 4B displays the I-V relationships of mean values of I_{Ks} step current during control, in the presence of 3 mM Ba^{2+} and after drug washout for 5 min. Ba^{2+} significantly suppressed I_{Ks} at −20 to +60 mV ($n = 6$, $P < 0.05$ or $P < 0.01$ *vs.* control). Figure 4C illustrates the concentration-response relation for the inhibition of I_{Ks} (+40 mV) by Ba^{2+}. The IC_{50} of Ba^{2+} for inhibiting I_{Ks} was 1.4 mM, the Hill coefficient was 1.0 and E_{max} was 60%.

Figure 4D illustrates the steady-state activation curves of I_{Ks} determined by normalizing the tail current in the absence and presence of Ba^{2+}. Ba^{2+} at 3 mM shifted the $V_{0.5}$ of I_{Ks} activation toward depolarization by 6 mV (10.1 ± 1.1 mV in control, 16.1 ± 0.9 mV for Ba^{2+}; $n = 6$, $P < 0.01$). Figure 4E displays the normalized current traces recorded in a representative cell by a 3-s voltage step to +40 from −80 mV in the absence and presence of Ba^{2+}. The raw data (points) were fit to monoexponential functions (solid lines) with time constants shown during control and after 3 mM Ba^{2+}. Figure 4F shows the mean values of voltage-dependent activation time constants in the absence and presence of Ba^{2+}. Ba^{2+} at 3 mM significantly increased the activation time constant of I_{Ks} at −20 and +40 mV ($n = 6$, $P < 0.01$), indicating a slowed activation of the current. However, Ba^{2+} did not affect the deactivation time constant (*data not shown*).

Time Course of I_{Ks} during Human Cardiac Action Potential

The distribution of I_{Ks} during cardiac action potential was studied using an action potential waveform, as described previously (Li et al., 1999). Figure 5A illustrates the cardiac action potential obtained from human ventricular myocytes (Li et al., 1998) as a voltage waveform to clamp cells and the current elicited by the action potential in a HEK 293 cell expressing I_{Ks} channels in the absence and presence of HMR 1556. HMR 1556 at 1 μM almost abolished the outward current activated by the action potential. Figure 5B shows the HMR 1556-sensitive I_{Ks} obtained by digital subtraction of the currents after application of HMR 1556 from those before drug application. The normalized HMR 1556-sensitive I_{Ks} exhibited a "bell-shaped" distribution during the cardiac action potential, which activated slowly after depolarization, reached a maximum level at plateau phase of the action potential and declined with repolarization of the action potential. Figure 5C shows instantaneous

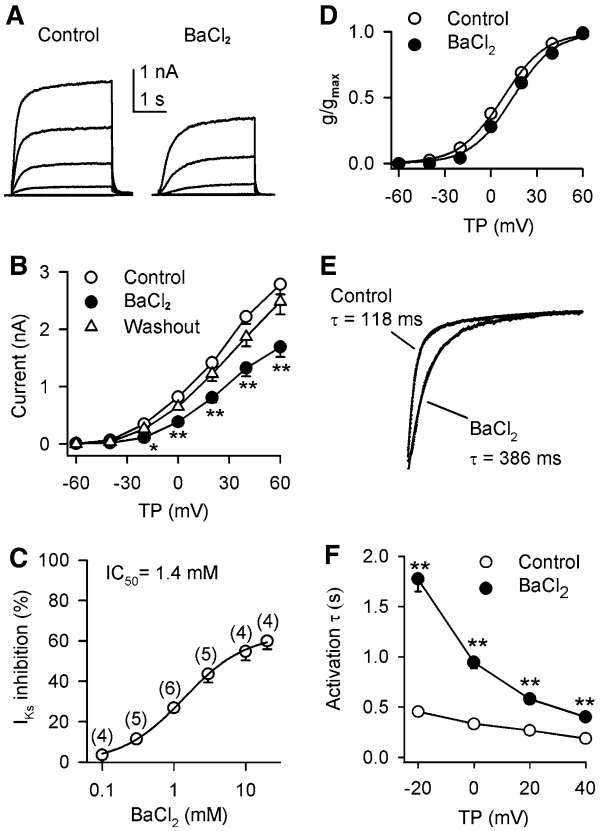

Fig. 4. Effects of Ba^{2+} on I_{Ks}. (*A*) Voltage-dependent I_{Ks} recorded in a typical experiment with the voltage protocol shown in the *inset* of Figure 3A in the absence and presence of $BaCl_2$. Ba^{2+} at 3 mM reversibly suppressed I_{Ks}. (*B*) I-V relationships of I_{Ks} during control (*open circles*), in the presence of 3 mM $BaCl_2$ (*filled circles*) and after washout of Ba^{2+} for 5 min (*open triangles*, $n = 6$, $^*P < 0.05$; $^{**}P < 0.01$ *vs.* control). (*C*) Concentration-response relationship of I_{Ks} inhibition by Ba^{2+}. IC_{50} was 1.4 mM, Hill coefficient was 1.0 and E_{max} was 60%. (*D*) Steady-state activation curves of I_{Ks} were determined by normalizing I_{Ks} tail current in the absence and presence of Ba^{2+} and fit to the Boltzmann distribution. (*E*) I_{Ks} traces recorded with 3-s voltage step to +40 mV in a representative cell in the absence and presence of 3 mM Ba^{2+}. Raw data were fit to a monoexponential function with time constants shown. Ba^{2+} remarkably increased the activation time constant of I_{Ks}. (*F*) Voltage-dependent time constants of I_{Ks} activation during control and after application of 3 mM Ba^{2+} ($n = 6$, $^{**}P < 0.01$ *vs.* control).

I-V relationship obtained by plotting the action potential clamp voltage against normalized HMR 1556-sensitive I_{Ks} current in Figure 5B, indicating that the I-V relationship peaked at a plateau of +30 mV during the action potential.

Rate-dependent Increase of I_{Ks}

The rate-dependent increase or accumulation of I_{Ks} was described in native cardiomyocytes of guinea pig heart at physiologically relevant rates (Jurkiewicz & Sanguinetti, 1993), and a similar finding observation was made in canine ventricular myocytes with application of the β-adrenoceptor agonist isoproterenol (Stengl et al., 2003). To study whether the frequency dependence was present in the recombinant human cardiac I_{Ks} expressed in HEK 293 cells, the action potential waveform was employed to record I_{Ks} at 0.5–2 Hz. Figure 6 illustrates use and frequency dependence of I_{Ks} with action potential clamp. I_{Ks} was recorded at 0.5, 1.0, 1.5 and 2.0 Hz with a train of 20 action potential waveforms (60-s interval between trains). Figure 6A displays the action potential waveform and representative current traces from the first, second and twentieth waveforms at 2 Hz in a typical experiment. The magnitude of I_{Ks} was clearly larger during the second and twentieth waveforms than during the first waveform, and the time of peak current at plateau of the action potential shifted to 120 ms during the twentieth waveform from 185 ms during the first waveform. Similar results were obtained in a total of six cells.

Figure 6B shows changes in normalized I_{Ks} during each waveform expressed as a function of the first waveform at each frequency. Statistically significant use dependence was noted at all frequencies from 0.5 to 2.0 Hz ($n = 8$, $P \leq 0.05$ or $P < 0.01$). The use-dependent increase in I_{Ks} resulted in frequency dependence of the current, as shown in Figure 6C, which illustrates the relation between pulse frequency and steady-state peak I_{Ks} at the twentieth waveform. The normalized I_{Ks} was greater at 1, 1.5 and 2.0 Hz than at 0.5 Hz ($P < 0.01$).

The effects of the β-adrenoceptor agonist isoproterenol on time course and use and frequency dependence of I_{Ks} were studied using the waveform of human cardiac action potential in another set of experiments. Figure 7A shows representative I_{Ks} traces recorded at 0.5 Hz before (control) and after application of isoproterenol in a typical experiment. The magnitude of I_{Ks} was increased and the time of peak current at plateau of the action potential was slightly shifted to 184 ms from 196 ms during the action potential waveform by application of 100 nM isoproterenol. Figure 7B displays changes in normalized I_{Ks} during each waveform expressed as a function of the first waveform at each frequency during control and after 100 nM isoproterenol. Isoproterenol increased the degree of use-dependent accumulation at 1 and 2 Hz and at 0.5 and 1.5 Hz (*data not shown*). The frequency-dependent accumulation of I_{Ks} peak current (twentieth waveform) during the action potential was increased by 5.5%, 4.3%, 4.1% and 4.3% with 100 nM isoproterenol ($n = 6$, $P < 0.05$ *vs.* before isoproterenol).

Discussion

In the present study, we established a HEK 293 cell line stably expressing high levels of recombinant human cardiac I_{Ks} channels and characterized in

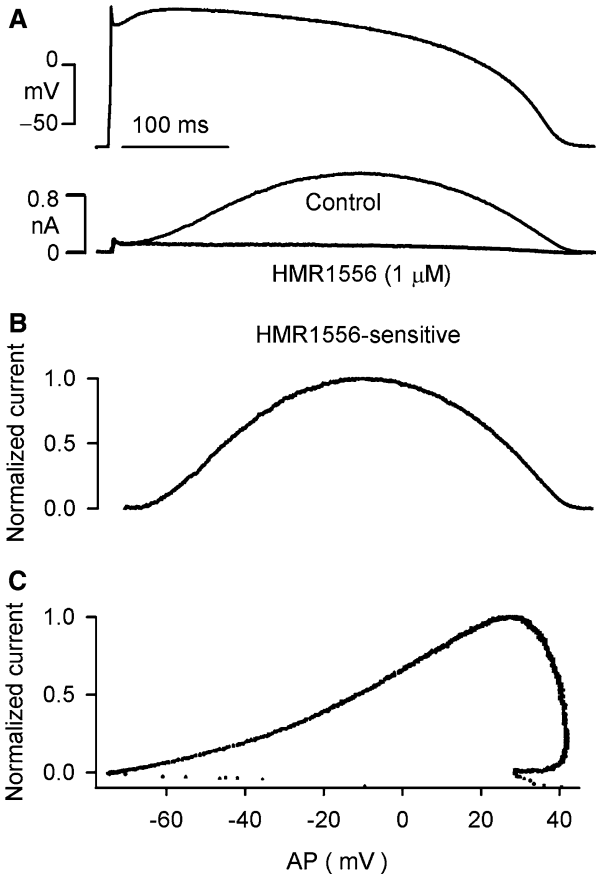

Fig. 5. Time courses of I_{Ks} during human cardiac action potential (*AP*). (*A*) Action potential obtained from a human ventricular myocyte was used to clamp the HEK 293 cell expressing I_{Ks} channels, and currents were elicited by the action potential during control and after application of 1 μM HMR 1556. (*B*) HMR 1556-sensitive current, obtained by subtracting membrane currents before and after application of HMR 1556 and then normalized by the maximum current, showing a "bell-shaped" distribution during the action potential. (*C*) Instantaneous I–V relationship of HMR 1556-sensitive I_{Ks} during the action potential against the action potential voltage, peaked at about +30 mV.

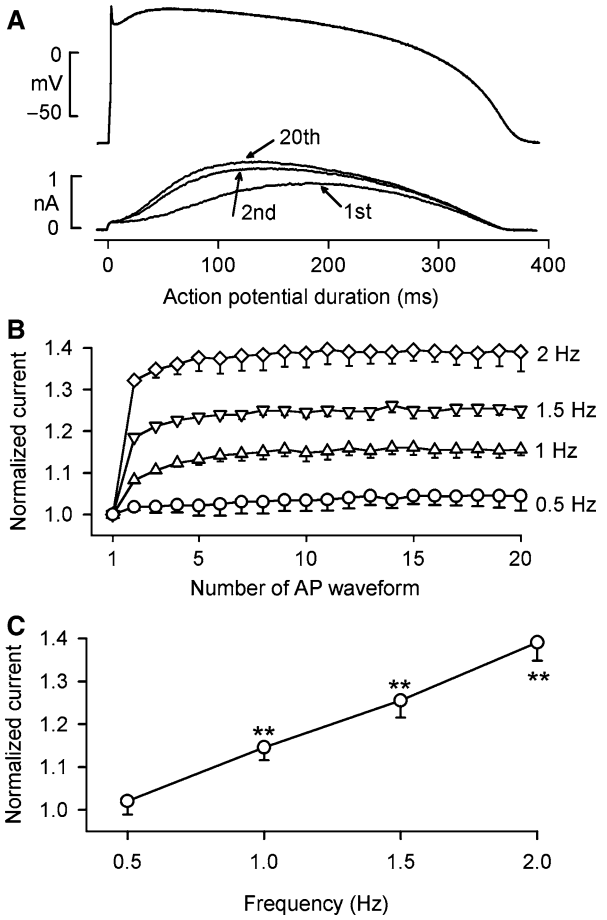

Fig. 6. Use or frequency dependence of I_{Ks}. (*A*) The same human cardiac action potential (*AP*) as in Figure 5A used to clamp a HEK 293 cell expressing I_{Ks} channels and representative current traces from first, second and twentieth action potentials at 2 Hz. (*B*) Results (means ± SE) are normalized to current elicited by the first action potential of a train after resting for 60 s. The action potential clamp frequencies were 0.5, 1, 1.5 and 2 Hz ($n = 8$). (*C*) Steady-state frequency (increase) of I_{Ks}. Results (mean ± SE) for each cell during twentieth action potential at each frequency ($n = 8$, $^{**}P < 0.01$ *vs.* 0.5 Hz).

detail the pharmacological and biophysical properties of the current at physiological temperature with a perforated patch technique.

HEK 293 cells were originally derived from human embryonic kidney and subsequently demonstrated to be a useful cell type to produce adenovirus, other viral vectors and human recombinant proteins. This type of cell has been used to stably (or transiently) express cloned cardiac ion channels, including hKv1.5 (Choi et al., 2005), hERG (Zhou et al., 1998), Kv4.2 and Kv4.3 (Guo et al., 2002), Kv2.1 (Leung et al., 2005) and HCN (Ludwig et al., 1999) channels. The present study established a HEK 293 cell line stably expressing human cardiac I_{Ks} channels by cotransfecting the *hKCNQ1* and *hKCNE1* genes, and the current exhibited a characterization typical of I_{Ks}, consistent with that of I_{Ks} transiently expressed in HEK cells reported by Zhang and colleagues (2001). However, it is different from that described by Kupershmidt and coworkers (2002), who reported in a comparative study that I_{Ks} was generated in HEK 293 cells only when a domain-containing protein (fhl2) was coexpressed with KCNQ1 and hKCNE1. The results from Zhang et al. (2001) and the present observations suggest that fhl2 is not necessary for expressing functional I_{Ks} channels in HEK 293 cells. The variation is likely related to different vectors applied.

A rundown of I_{Ks} was observed in whole-cell patch configuration due to alteration of the intracellular milieu. Therefore, perforated patch recording with amphotericin B was used (Fig. 1B). The selective I_{Ks} blockers HMR 1556 and chromanol 293B blocked I_{Ks} channels and had IC_{50}s of 83.8 nM and

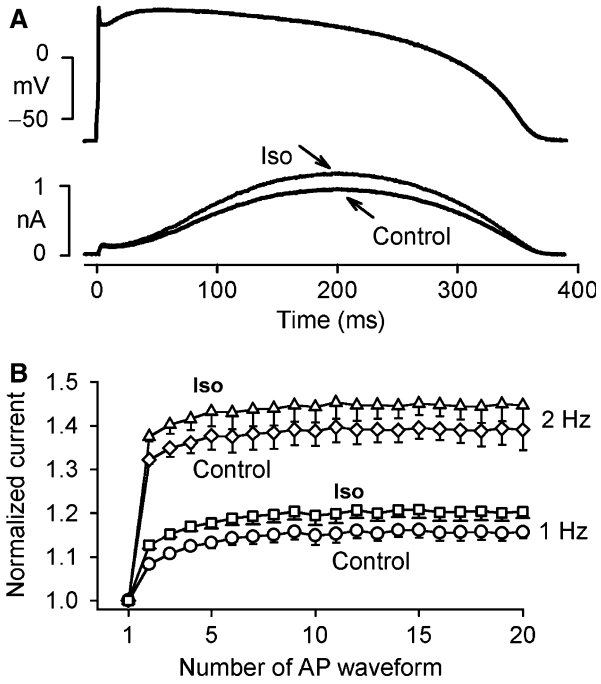

Fig. 7. Effects of isoproterenol on time courses of I_{Ks} during human cardiac action potential (*AP*) and use dependence of the current. (*A*) The same human cardiac action potential as in Figure 6A used to clamp a representative HEK 293 cell expressing I_{Ks} channels, and current traces were recorded at 0.5 Hz before (control) and after application of 100 nM isoproterenol. (*B*) Results are normalized to current elicited by the first action potential of a train after resting for 60 s. The action potential clamp frequencies were 1 and 2 Hz ($n = 6$, $P < 0.05$ *vs.* before isoproterenol).

9.2 μM, respectively, for the inhibition of human cardiac I_{Ks}. The IC_{50} values are close to those (65 nM for HMR 1556, 8 μM for 293B) observed in canine ventricular myocytes (Volders et al., 2003), suggesting the cell line stably expressing human cardiac I_{Ks} would be a good model for pharmacological evaluation. In addition, cells stably expressing human cardiac I_{Ks} are much more convenient than those of transiently transfected I_{Ks} channels. Especially when the perforated patch configuration is employed to limit the current rundown, a highly successful recording may easily be obtained with the stable cell line. Although perforated patch configuration had high access resistance, the voltage error would not significantly affect the major conclusion of this slowly activating current (Sanguinetti & Jurkiewicz, 1990).

The temperature dependence was observed in several ion channel currents of cardiac myocytes (Li et al., 1998; Tiwari & Sikdar, 1999) and related to the variation of cardiac action potential duration at different temperatures (Kiyosue et al., 1993). The Q_{10} value of 1.8 we observed here in human cardiac I_{Ks} stably expressed in HEK 293 cells was close to those (1.5–1.8) of other K^+ channels (Li et al., 1998; Tiwari & Sikdar, 1999) and that (1.7) in human cardiac I_{Ks} expressed in Chinese hamster ovary cells (Seebohm et al., 2001) but smaller than those (3.5–4.4) in native cardiac I_{Ks} in guinea pig ventricular myocytes (Kiyosue et al., 1993) and that (5.1) in cloned *hKCNQ1*/*hKCNE1* genes expressed in *Xenopus* oocytes (Seebohm et al., 2001). Whether the different Q_{10}s of I_{Ks} are related to different cell types and/or species remains to be studied.

It is interesting that the $V_{0.5}$ (+10.2 mV, Fig. 2) of I_{Ks} activation obtained in HEK cells stably expressing recombinant human cardiac I_{Ks} is close to that (+9.4 mV) observed in human right ventricular myocytes from explanted heart (Li et al., 1996), to those (+7.0–15 mV) observed in rabbit ventricular myocytes (Salata et al., 1998; Xu et al., 2001) and to that (+15.7 mV) in guinea pig cardiac myocytes (Sanguinetti & Jurkiewicz, 1990). The time constant (110 ms at −30 mV) of deactivation of recombinant I_{Ks} is close to that (122 ms) observed in undiseased human ventricular myocytes (Virag et al., 2001) but different from that (220 ms) in relatively normal human right ventricular myocytes (Li et al., 2004). The time constant (162 ms at +40 mV) of I_{Ks} activation in HEK cells is, however, smaller than that (903 ms) observed in undiseased human ventricular myocytes (Virag et al., 2001). These different values are likely related at least in part to the difficulty of separating I_{Ks} current in native cells from other currents.

The β-adrenoceptor agonist isoproterenol, as in native mammalian cardiac cells (Salata et al., 1998; Han, et al., 2001), upregulated the recombinant human cardiac I_{Ks} expressed in HEK cells, shifted the conductance-voltage relationship to more negative potentials and speeded up the activation kinetics and slowed down the deactivation rate (Fig. 3). The observation of β-adrenoceptors in HEK 293 cells is supported by a previous report (Friedman, et al., 2002). In addition, the antagonism of the effects of isoproterenol and forskolin by the PKA inhibitor H89 suggested an intact β-adrenoceptor cAMP/PKA system in HEK 293 cells.

It was reported that Ba^{2+} inhibited several K^+ channel currents (Lesage et al., 1997; Weerapura et al., 2000; Li et al., 2000). Ba^{2+} also inhibited the recombinant human cardiac I_{Ks} in a concentration-dependent manner with an IC_{50} of 1.4 mM (E_{max} = 60%), shifted rightward the voltage-dependent activation and slowed activation without affecting the deactivation kinetics of I_{Ks} channels (Fig. 4). The effect suggests that I_{Ks} channels contain a Ba^{2+} binding site. No information is available in the literature regarding the effect of Ba^{2+} on native cardiac I_{Ks} to compare this observation. This effect should be considered when Ba^{2+} is used for blocking other K^+ currents in native cells.

I_{Ks} is an important contributor to the repolarization in cardiac action potential. The present results with the action potential clamp technique demonstrate a bell-shaped distribution of I_{Ks} during the

action potential (Fig. 5), similar to the prediction of the mathematical model (Zeng et al., 1995) and the observation in guinea pig ventricular myocytes (Rocchetti et al., 2001). The current slowly activated and reached a maximum level at plateau of the action potential (about +30 mV), then gradually inactivated to zero at termination of the action potential.

A significant use- and frequency-dependent increase of I_{Ks} was observed at physiologically relevant rates of 1–2 Hz with the action potential clamp technique (Fig. 6), consistent with the reports in guinea pig cardiac myocytes (Lu et al., 2001; Jurkiewicz & Sanguinetti, 1993). A previous study described that the accumulation of I_{Ks} at high heart rate was related to the higher density and slow deactivation kinetics of the current in guinea pig ventricular myocytes, while no significant accumulation of I_{Ks} in rabbit ventricular cells was due to the lower density and faster deactivation of the current (Lu et al., 2001). However, the frequency dependence of I_{Ks} observed in this study seems mainly related to the fast activation and large magnitude (or density) of the current since the deactivation time constant of the current is much faster (Fig. 3, 63 ms at −50 mV) than that (151 ms) observed in rabbit ventricular cells (Lu et al., 2001). The notion that the speed of activation and the density of the current dominate the accumulation of I_{Ks} is supported by recent observations in canine ventricular myocytes (Stengl et al., 2003). These authors found that the accumulation of I_{Ks} was significant only when the current was augmented by application of isoproterenol in action potential clamp experiments (Stengl et al., 2003). We also found that isoproterenol increased the degree of accumulation of I_{Ks} (Fig. 7). Our earlier study showed that the small I_{Ks} did not show a significant increase at a higher rate in ventricular cells from explanted human hearts (Li et al., 1999). Although the accumulation of I_{Ks} was observed in HEK 293 cells, whether the accumulation of I_{Ks} is significant in human heart and involved in the rate-dependent abbreviation of human cardiac action potential remains to be studied in undiseased human cardiac myocytes.

In summary, the present study provides detailed information that the recombinant human cardiac I_{Ks} stably expressed in HEK 293 cells is similar to native I_{Ks} in drug sensitivity and modulated by Ba^{2+} and β-adrenoceptor via the cAMP/PKA pathway. Importantly, the current exhibits significant temperature dependence, a bell-shaped time course during action potential and prominent use- or frequency-dependent accumulation of activation during a train of pulses.

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References

Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., Romey, G. 1996. K(v)LQT1 and lsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* **384:**78–80

Chen, H., Kim, L.A., Rajan, S., Xu, S., Goldstein, S.A. 2003. Charybdotoxin binding in the I_{Ks} pore demonstrates two MinK subunits in each channel complex. *Neuron* **40:**15–23

Choi, W.S., Khurana, A., Mathur, R., Viswanathan, V., Steele, D.F., Fedida, D. 2005. Kv1.5 surface expression is modulated by retrograde trafficking of newly endocytosed channels by the dynein motor. *Circ. Res.* **97:**363–371

Freeman, J.L., Gonzalo, P., Pitcher, J.A., Claing, A., Lavergne, J.P., Reboud, J.P., Lefkowitz, R.J. 2002. Beta 2-adrenergic receptor stimulated, G protein-coupled receptor kinase 2 mediated, phosphorylation of ribosomal protein P2. *Biochemistry* **41:**12850–12857

Friedman, J., Babu, B., Clark, R.B. 2002. $Beta_2$-adrenergic receptor lacking the cyclic AMP-dependent protein kinase consensus sites fully activates extracellular signal-regulated kinase 1/2 in human embryonic kidney 293 cells: lack of evidence for G_s/G_i switching. *Mol. Pharmacol.* **62:**1094–1102

Guo, W., Li, H., Aimond, F., Johns, D.C., Rhodes, K.J., Trimmer, J.S., Nerbonne, J.M. 2002. Role of heteromultimers in the generation of myocardial transient outward K^+ currents. *Circ. Res.* **90:**586–593

Han, W., Wang, Z., Nattel, S. 2001. Slow delayed rectifier current and repolarization in canine cardiac Purkinje cells. *Am. J. Physiol.* **280:**H1075–H1080

Jiang, M., Zhang, M., Tang, D.G., Clemo, H.F., Liu, J., Holwitt, D., Kasirajan, V., Pond, A.L., Wettwer, E., Tseng, G.N. 2004. KCNE2 protein is expressed in ventricles of different species, and changes in its expression contribute to electrical remodeling in diseased hearts. *Circulation* **109:**1783–1788

Jurkiewicz, N.K., Sanguinetti, M.C. 1993. Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class III antiarrhythmic agent. Specific block of rapidly activating delayed rectifier K^+ current by dofetilide. *Circ. Res.* **72:**75–83

Kiyosue, T., Arita, M., Muramatsu, H., Spindler, A.J., Noble, D. 1993. Ionic mechanisms of action potential prolongation at low temperature in guinea pig ventricular myocytes. *J. Physiol.* **486:**85–106

Kupershmidt, S., Yang, I.C., Sutherland, M., Wells, K.S., Yang, T., Yang, P., Balser, J.R., Roden, D.M. 2002. Cardiac-enriched LIM domain protein fhl2 is required to generate I_{Ks} in a heterologous system. *Cardiovasc. Res.* **56:**93–103

Lesage, F., Lauritzen, I., Duprat, F., Reyes, R., Fink, M., Heurteaux, C., Lazdunski, M. 1997. The structure, function and distribution of the mouse TWIK-1 K^+ channel. *FEBS Lett.* **402:**28–32

Leung, Y.M., Kang, Y., Xia, F., Sheu, L., Gao, X., Xie, H., Tsushima, R.G., Gaisano, H.Y. 2005. Open form of syntaxin-1A is a more potent inhibitor than wild-type syntaxin-1A of Kv2.1 channels. *Biochem. J.* **387:**195–202

Li, G.R., Feng, J., Yue, L., Carrier, M. 1998. Transmural heterogeneity of action potentials and Ito1 in myocytes isolated from the human right ventricle. *Am. J. Physiol.* **275:**H369–H377

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

Li, G.R., Lau, C.P., Ducharme, A., Tardif, J.C., Nattel, S. 2002. Transmural action potential and ionic current remodeling in ventricles of failing canine hearts. *Am. J. Physiol.* **283:**H1031–H1041

Li, G.R., Lau, C.P., Leung, T.K., Nattel, S. 2004. Ionic current abnormalities associated with prolonged action potentials in cardiomyocytes from diseased human right ventricles. *Heart Rhythm* **1:**460–468

Li, G.R., Yang, B., Feng, J., Bosch, R.F., Carrier, M., Nattel, S 1999. Transmembrane I_{Ca} contributes to rate-dependent changes of action potentials in human ventricular myocytes. *Am. J. Physiol.* **276:**H98–H106

Li, G.R., Yang, B., Sun, H., Baumgarten, C.M. 2000. Existence of a transient outward K^+ current in guinea pig cardiac myocytes. *Am. J. Physiol.* **279:**H130–H138

Lu, Z., Kamiya, K., Opthof, T., Yasui, K., Kodama, I. 2001. Density and kinetics of I_{Kr} and I_{Ks} in guinea pig and rabbit ventricular myocytes explain different efficacy of I_{Ks} blockade at high heart rate in guinea pig and rabbit: implications for arrhythmogenesis in humans. *Circulation* **104:**951–956

Ludwig, A., Zong, X., Stieber, J., Hullin, R., Hofmann, F., Biel, M. 1999. Two pacemaker channels from human heart with profoundly different activation kinetics. *EMBO J.* **18:**2323–2329

Nabauer, M., Kaab, S. 1998. Potassium channel down-regulation in heart failure. *Cardiovasc. Res.* **37:**324–334

Noble, D., Tsien, R.W. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol.* **195:**185–214

Rocchetti, M., Besana, A., Gurrola, G.B., Possani, L.D., Zaza, A. 2001. Rate dependency of delayed rectifier currents during the guinea-pig ventricular action potential. *J. Physiol.* **534:**721–732

Salata, J.J., Jurkiewicz, N.K., Wang, J., Evans, B.E., Orme, H.T., Sanguinetti, M.C. 1998. A novel benzodiazepine that activates cardiac slow delayed rectifier K^+ currents. *Mol. Pharmacol.* **54:**220–230

Sanguinetti, M.C., Curran, M.E., Zou, A., Shen, J., Spector, P.S., Atkinson, D.L., Keating, M.T. 1996. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* **384:**80–83

Sanguinetti, M.C., Jurkiewicz, N.K. 1990. Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents. *J. Gen. Physiol.* **96:**195–215

Seebohm, G., Lerche, C., Busch, A.E., Bachmann, A. 2001. Dependence of I_{Ks} biophysical properties on the expression system. *Pfluegers Arch.* **442:**891–895

Stengl, M., Volders, P.G., Thomsen, M.B., Spatjens, R.L., Sipido, K.R., Vos, M.A. 2003. Accumulation of slowly activating delayed rectifier potassium current (I_{Ks}) in canine ventricular myocytes. *J. Physiol.* **551:**777–786

Tiwari, J.K., Sikdar, S.K. 1999. Temperature-dependent conformational changes in a voltage-gated potassium channel. *Eur. Biophys. J.* **28:**338–345

Virag, L., Iost, N., Opincariu, M., Szolnoky, J., Szecsi, J., Bogats, G., Szenohradszky, P., Varro, A., Papp, J.G. 2001. The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes. *Cardiovasc. Res.* **49:**790–797

Volders, P.G., Stengl, M., van Opstal, J.M., Gerlach, U., Spatjens, R.L., Beekman, J.D., Sipido, K.R., Vos, M.A. 2003. Probing the contribution of I_{Ks} to canine ventricular repolarization: key role for beta-adrenergic receptor stimulation. *Circulation* **107:**2753–2760

Weerapura, M., Nattel, S., Courtemanche, M., Doern, D., Ethier, N., Hebert, T. 2000. State-dependent barium block of wild-type and inactivation-deficient HERG channels in *Xenopus* oocytes. *J. Physiol.* **526:**265–278

Werry, T.D., Christie, M.I., Dainty, I.A., Wilkinson, G.F., Willars, G.B. 2002. Ca^{2+} signalling by recombinant human CXCR2 chemokine receptors is potentiated by P2Y nucleotide receptors in HEK cells. *Br. J. Pharmacol.* **135:**1199–1208

Xu, X., Rials, S.J., Wu, Y., Salata, J.J., Liu, T., Bharucha, D.B., Marinchak, R.A., Kowey, P.R. 2001. Left ventricular hypertrophy decreases slowly but not rapidly activating delayed rectifier potassium currents of epicardial and endocardial myocytes in rabbits. *Circulation* **103:**1585–1590

Zeng, J., Laurita, K.R., Rosenbaum, D.S., Rudy, Y 1995. Two components of the delayed rectifier K^+ current in ventricular myocytes of the guinea pig type. Theoretical formulation and their role in repolarization. *Circ. Res.* **77:**140–152

Zhang, S., Rajamani, S., Chen, Y., Gong, Q., Rong, Y., Zhou, Z., Ruoho, A., January, C.T. 2001. Cocaine blocks HERG, but not KvLQT1 + minK, potassium channels. *Mol. Pharmacol.* **59:**1069–1076

Zhou, Z., Gong, Q., Ye, B., Fan, Z., Makielski, J.C., Robertson, G.A., January, C.T. 1998. Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys. J.* **74:**230–241