Interaction between the Cardiac Rapidly (I_{Kr}) and Slowly (I_{Ks}) Activating Delayed Rectifier Potassium Channels Revealed by Low K+ -induced hERG Endocytic Degradation*

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The delayed rectifier potassium current, I_{Kr}, plays an important role in the repolarization of cardiac action potentials (1).

Cardiac repolarization is controlled by the rapidly (I_{Kr}) and slowly (I_{Ks}) activating delayed rectifier potassium channels. The human ether-a-go-go-related gene (hERG) encodes I_{Kr}, whereas KCNQ1 and KCNE1 together encode I_{Ks}. Decreases in I_{Kr} or I_{Ks} cause long QT syndrome (LQTS), a cardiac disorder with a high risk of sudden death. A reduction in extracellular K+ concentration ([K+]_o) induces LQTS and selectively causes endocytic degradation of mature hERG channels from the plasma membrane. In the present study, we investigated whether I_{Ks} compensates for the reduced I_{Kr} under low K+ conditions. Our data show that when hERG and KCNQ1 were expressed separately in human embryonic kidney (HEK) cells, exposure to 0 mM K+ for 6 h completely eliminated the mature hERG channel expression but had no effect on KCNQ1. When hERG and KCNQ1 were co-expressed, KCNQ1 significantly delayed 0 mM K+ -induced hERG reduction. Also, hERG degradation led to a significant reduction in KCNQ1 in 0 mM K+ conditions. An interaction between hERG and KCNQ1 was identified in hERG + KCNQ1 -expressing HEK cells. Furthermore, KCNQ1 preferentially co-immunoprecipitated with mature hERG channels that are localized in the plasma membrane. Biophysical and pharmacological analyses indicate that although hERG and KCNQ1 closely interact with each other, they form distinct hERG and KCNQ1 channels. These data extend our understanding of delayed rectifier potassium channel trafficking and regulation, as well as the pathology of LQTS.

Whereas I_{K} was originally considered to be mediated by a single type of channel (1, 2), it is now clear that I_{K} is mediated by two distinct types of channels, the rapidly (I_{Kr}) and the slowly activating delayed rectifier potassium channels (I_{Ks}) (3–6). I_{Kr} is encoded by the human ether-a-go-go-related gene (hERG, also known as KCNH2) (3, 4). I_{Ks} is encoded by both KCNQ1 and KCNE1. KCNQ1 (also known as KvLQT1) encodes the pore-forming α subunit, and KCNE1 (also known as minK) encodes the regulatory β subunit of I_{Ks} (5, 6). Both I_{Kr} and I_{Ks} are critical for cardiac repolarization. Naturally occurring mutations in KCNQ1 cause type 1 long QT syndrome (LQT1). Similarly, mutations in hERG cause LQT2 and mutations in KCN1 cause LQT5. These mutations impair the function of either I_{Ks} or I_{Kr} and account for the majority (>90%) of inherited long QT syndromes (7). Furthermore, a number of medications can interfere with proper hERG function, which results in acquired long QT syndrome (8).

A reduction in extracellular K+ concentration ([K+]_o), clinically known as hypokalemia, also causes long QT syndrome (9). We previously demonstrated that a reduction in [K+]_o prolongs rabbit QT intervals on the electrocardiogram (ECG) and decreases cell surface density of both I_{Kr} in rabbit hearts and hERG channels in stable cell lines (10, 11). We further showed that low K+ exposure induces rapid endocytic degradation of mature hERG channels, leading to a decreased hERG channel density at the plasma membrane (10–12). In contrast, low K+ exposure does not affect the expression level of either the EAG or Kv1.5 potassium channels and only moderately decreases the KCNQ1 + KCNE1 current (10).

The repolarization of the cardiac action potential is under joint control of I_{Kr} and I_{Ks} (8). In most physiological systems, when one component declines in function or abundance, another component with similar function compensates by promoting itself to higher activity levels, a phenomenon known as functional compensation (13). It would therefore be expected that a decrease in I_{Kr} may lead to an increase in I_{Ks} under hypokalemic conditions. In the present study, using electrophysiology, Western blot analysis, and immunocytochemistry, we investigated the interactions between I_{Kr} and I_{Ks} in a...
hypokalemia rabbit model and in the HEK 293 cell lines. Our data demonstrated that physical interactions exist between hERG and KCNQ1 proteins at the plasma membrane. Although KCNQ1 delays low K⁺-induced hERG degradation, the endocytotic degradation of hERG channels subsequently promotes KCNQ1 degradation. As a result, low [K⁺], reduces the density of both hERG and KCNQ1 in the plasma membrane.

**EXPERIMENTAL PROCEDURES**

**Hypokalemia Rabbit Model**—New Zealand White rabbits (2.5–3.0 kg) were divided into two groups (nine in each group) and fed a normal or a low K⁺ diet (TestDiet) for 6 weeks. To determine the earliest experimental end point and to ensure manageability of the electrophysiological experiments on isolated cardiac myocytes, the starting time of the rabbit experiments was staggered so that one rabbit was added to each group (control and low K⁺ diet) every week. The compositions of both diets were otherwise identical except for K⁺ content (0.62% versus 0.1%). Blood samples were taken weekly to monitor serum electrolyte levels at Kingston General Hospital Clinical Laboratory (Kingston, Canada). For each rabbit, a 9-min ECG recording was taken once a week on a lightly anesthetized control rabbit in a custom-based medium at room temperature. The cells were harvested from the culture dish by trypsinization method was used. The standard bath solution contained 135 mM NaCl, 5 mM KCl, 1 mm CaCl₂, 1 mm MgCl₂, 10 mM glucose, and 10 mM HEPES, and 0.5% BSA (pH 7.2). The hearts were then perfused with Kreb’s solution plus collagenase II (80 mg/100 ml; 295 units/mg; Worthington). Left ventricular tissues were taken at different time points between 35 and 50 min and minced in Kraft-Brühe solution to obtain optimally isolated myocytes. The Kraft-Brühe solution contained 10 mM KCl, 120 mM potassium glutamate, 10 mM KH₂PO₄, 1.8 mM MgSO₄, 10 mM taurine, 10 mM HEPES, 0.5 mM EGTA, 20 mM glucose, and 10 mM mannitol (pH 7.3). The cells were filtered through cheesecloth and kept in 1% BSA-containing Kraft-Brühe solution at room temperature for electrophysiological studies.

**Molecular Biology**—hERG cDNA was provided by Dr. Gail Robertson (University of Wisconsin-Madison); The G601S hERG mutant was created using site-directed mutagenesis (11). KCNQ1 and KCNE1 cDNAs were provided by Dr. Michael Sanguinetti (University of Utah, Salt Lake City). A hERG-expressing HEK 293 stable cell line (hERG-HEK cells) was obtained from Dr. Craig January (University of Wisconsin-Madison). Stable cell lines expressing KCNQ1+KCNE1 or hERG+KCNQ1+KCNE1 were created. For the KCNQ1+KCNE1 stable cell line, 2 μg each of KCNQ1 and KCNE1 plasmids were co-transfected into HEK 293 cells grown in 60-mm dishes using Lipofectamine 2000. For the hERG+KCNQ1+KCNE1 stable cell line, 1.3 μg each of hERG, KCNQ1, and KCNE1 plasmids were co-transfected into HEK 293 cells grown in 60-mm dishes using Lipofectamine 2000. After transfection, the cells were cultured in 10% FBS-supplemented minimum essential medium (MEM) containing 1 mg/ml G418 for selection of transfected cells. Twenty-four single-cell derived colonies were selected for electrophysiological screening. The colony with characteristic Iₖₛ current (presence of both KCNQ1 and KCNE1) was selected to establish the KCNQ1+KCNE1 stable cell line. Similarly, the colony with both characteristic Iₖₛ current (slowly activating, presence of both KCNQ1 and KCNE1) and hERG current (presence of the hERG-specific tail current) was selected to establish the hERG+KCNQ1+KCNE1 stable cell line. The selected cell clones were amplified, confirmed for the expression of hERG, KCNQ1, and KCNE1 using Western blot analysis, and stored in liquid N₂. To maintain the stable cell lines, the cells were cultured in MEM supplemented with 10% fetal bovine serum and 0.4 mg/ml G418. For the hERG+KCNQ1+KCNE1 stable cell line, all genes were maintained within 40 passages as confirmed by the presence of both hERG and Iₖₛ (KCNQ1+KCNE1) currents. For transient transfection, 2 μg of KCNQ1, 2 μg KCNE1, or 2 μg of KCNQ1 plus 2 μg of KCNQ1 plasmids were transfected into hERG-HEK cells growing in a 60-mm dish at 60–70% confluence using Lipofectamine 2000 (Invitrogen). A GFP plasmid (1 μg, pIRE2-EGFP, Clontech) was co-expressed to identify transfected cells in electrophysiological studies. 24–36 h after transfection, the cells were cultured in a custom made 0 mM K⁺ MEM-based medium or standard (5 mM K⁺) MEM-based medium for various periods. The cells were then harvested for Western blot, immunocytochemistry, and electrophysiological analysis. For electrophysiological studies, the cells were harvested from the culture dish by trypsinization with 0.05% trypsin (Invitrogen) and stored in standard MEM-based medium at room temperature. The cells were studied within 8 h of harvest.

**Electrophysiological Recordings**—The whole cell patch clamp method was used. The standard bath solution contained 135 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES. This standard bath solution was used for recording Iₖrg, IₖCNQ1+KCNE1 in cell lines, and the transient outward K⁺ current (Iₒ), inwardly rectifying K⁺ current (Iₖ₁), resting membrane potentials and action potentials in rabbit ventricular myocytes. The bath solution for recording Iₖₛ in rabbit ventricular myocytes contained (in mm): 140 NMG, 1 MgCl₂, 1 CaCl₂, 10 glucose, 10 HEPES, 0.01 nifedipine and 0.005 E4031. The pipette solution contained (in mm): 135 KCl, 5 EGTA, 1 MgCl₂, and 10 HEPES. The pipette solution contained (in mm): 135 KCl, 5 EGTA, 5 Kₐ ATP, 10 HEPES. The pipette solution for recording Iₖₛ, the transient outward K⁺ current (Iₒ), inwardly rectifying K⁺ current (Iₖ₁), resting membrane potentials and action potentials contained 135 mM KCl, 10 mM EGTA, 1 mM MgCl₂, 5 mM MgATP, and 10 mM HEPES. For Iₖₛ recordings in rabbit ventricular myocytes, Cs⁺-rich solutions were used to isolate Iₖₛ from all other currents (14). The bath
solution contained 135 mM CsCl, 1 mM MgCl₂, 10 mM glucose, 10 mM HEPES, and 0.01 mM nifedipine. The pipette solution contained 135 mM CsCl, 10 mM EGTA, 5 mM MgATP, and 10 mM HEPES. To record the Ba²⁺-mediated L-type Ca²⁺ currents in rabbit ventricular myocytes, the bath solution contained 140 mM TEACl, 5.4 mM BaCl₂, 1 mM MgCl₂, 10 mM glucose, 10 mM HEPES, and the pipette solution contained 135 mM CsCl, 10 mM EGTA, 1 mM MgCl₂, 5 mM MgATP, and 10 mM HEPES. The pH of all bath solutions was adjusted to 7.4, and that of all pipette solutions was adjusted to 7.2 using appropriate hydroxide salts or HCl. Patch clamp experiments were performed at room temperature (22 ± 1°C).

Western Blot Analysis and Co-immunoprecipitation (co-IP)—Whole cell proteins from HEK 293 cells expressing various channels were used for analysis (10–12). Proteins were separated on 8 or 12% SDS-polyacrylamide electrophoresis gels, transferred onto PVDF membrane, and blocked for 1 h with 5% nonfat milk. The blots were incubated with the primary antibody for 1 h at room temperature and then incubated with a horseradish peroxidase-conjugated secondary antibody. Actin expression was used for loading controls. The blots were visualized with Fujifilm using the ECL detection kit (GE Healthcare).

For immunoprecipitation, whole cell protein (0.5 mg) or cell surface protein was incubated with the appropriate primary antibody overnight at 4 °C and then precipitated with protein A/G plus agarose beads (Santa Cruz) for 4 h at 4 °C. The beads were washed three times with ice-cold radioimmune precipitation assay lysis buffer, resuspended in 2x Laemmli sample buffer, and boiled for 5 min. The samples were centrifuged at 20,000 × g for 5 min, and the supernatants were collected and analyzed using Western blot.

Isolation of Cell Surface Protein—A cell surface protein isolation kit (Pierce) was used. The hERG+ KCNQ1+ KCNE1 stably expressing HEK cells were prepared in 100-mm cell culture plates and grown to 90% confluence. The cells were labeled with 10 ml of membrane-impermeant biotinylating reagent, Sulfo-NHS-SS-biotin, for 30 min at 4 °C. The quenching solution (0.5 ml) was then added to quench the reaction. The cells were then lysed with 0.5 ml of lysis buffer containing a protease inhibitor mixture. After centrifugation at 10,000 × g for 2 min at 4 °C, the cell lysate was precipitated with Immobilized NeutrAvidin Gel (agarose beads). The bound proteins were eluted by incubating the resin in a Tris buffer (62.5 mM Tris-HCl, pH 6.8, 1% SDS, 10% glycerol) containing 50 mM DTT. The cell surface protein was then subjected to co-IP analysis to determine hERG-KCNQ1 interactions.

Cleavage of Cell Surface Proteins—To confirm that the 155-kDa hERG protein is localized at the plasma membrane, the hERG expressions between control (treated with the buffer solution, see below) and proteinase K-treated hERG-HEK cells were compared. Live cells were washed with PBS and treated with 200 µg/ml proteinase K (Sigma) in a physiological buffer (10 mM HEPES, 150 mM NaCl, and 2 mM CaCl₂, pH 7.4) at 37 °C for 30 min to cleave cell surface proteins. The reaction was terminated by adding ice-cold PBS containing 6 mM phenylmethylsulfonyl fluoride and 25 mM EDTA. The whole cell proteins were then extracted from the control and proteinase K-treated cells for Western blot analysis.

Immunofluorescence Microscopy—hERG-HEK cells were transfected with an empty vector (control), KCNQ1, KCNE1, or KCNQ1+ KCNE1. Thirty-six hours after transfection, the cell surface hERG channels were labeled by incubating the live cells with a rabbit anti-hERG primary antibody (Sigma). The cells were then exposed to 0 mM K⁺ medium for 4 h and fixed with freshly prepared 4% paraformaldehyde for 15 min. The fixed cells were permeabilized with 0.1% Triton X-100 for 10 min and blocked with 5% BSA for 1 h. The permeabilized cells were treated with a goat anti-KCNQ1 or goat anti-KCNE1 primary antibody (Santa Cruz) and Alexa Fluor 594-conjugated donkey anti-goat secondary antibody to detect KCNQ1 or KCNE1. After washing off excess secondary antibody with PBS (pH 7.4), cell surface hERG channels bound with rabbit anti-hERG (Kv11.1) primary antibody (Sigma) were detected using Alexa Fluor 488 goat anti-rabbit secondary antibody. The nuclei were stained using Hoechst 33342 (0.2 µg/ml; Sigma). Images were acquired using a Leica TCS SP2 Multi Photon confocal microscope (Leica, Germany).

Reagents and Antibodies—MEM, custom 0 mM K⁺ MEM, and FBS were purchased from Invitrogen. Rabbit anti-Kv11.1 (hERG) antibody, electrolytes, MgATP, EGTA, HEPES, glucose, nifedipine, astemizole, and G418 were purchased from Sigma. Goat anti-hERG (C20 and N20), goat anti-KCNQ1, mouse anti-KCNQ1, goat anti-KCNE1, mouse anti-GAPDH, goat anti-actin primary antibodies, protein A/G Plus-agarose, donkey anti-goat IgG, goat anti-mouse IgG, and goat anti-rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Alexa Fluor 594 donkey anti-goat and Alexa Fluor 488 goat anti-rabbit secondary antibodies were purchased from Invitrogen. E4031 was purchased from Calbiochem-EMD4 Biosciences.

All of the data are expressed as the means ± S.E. A one-way analysis of variance was used to test for statistical significance between the control and test groups. A p value of 0.05 or less was considered significant.

RESULTS

Hykopenia Prolongs the QT Interval on ECG and Decreases Both Iₖᵣ and Iₖₛ in Rabbits—We have previously shown that lowering [K⁺]₀ prolongs the QT interval on the ECG and decreases Iₖₛ in a rabbit model (10). However, the effects of low [K⁺]₀ on Iₖₛ were not well defined. Although our previous data showed that Iₖₛ was reduced in rabbits after 4 weeks on a low K⁺ diet, the reduction did not reach statistical significance (10). Furthermore, a compensatory increase in Iₖᵣ was not found. To investigate the role of Iₖₛ and the potential interactions between Iₖᵣ and Iₖₛ under hypokalemic conditions, we studied Iₖₛ in rabbits with hypokalemia induced by a low K⁺ diet for 6 weeks. Nine rabbits in each group were included in the study.

As shown in Fig. 1A, prior to feeding, there was no difference in serum [K⁺] between the two groups of rabbits. Serum [K⁺] remained stable in rabbits fed the control diet during the 6-week experimental period (3.6 ± 0.1 mm at week 6 versus 3.5 ± 0.3 mm at week 0, n = 9, p > 0.05). However, serum [K⁺] in rabbits on the low K⁺ diet decreased significantly after 1
FIGURE 1. Reduction in serum K⁺ concentration prolongs the QT interval on ECG and decreases both I_{Kr} and I_{Ks} in rabbit ventricular myocytes. A, serum K⁺ concentrations of rabbits on control or the low K⁺ diet for 6 weeks. B, QT index of rabbits on control or the low K⁺ diet for 6 weeks. QT index was generated by dividing the observed QT interval on ECG by the expected QT calculated using the formula (QT_{exp} = \frac{86 + 0.22 \times RR}{2}) generated by Bruner et al. (16) in nonanesthesized rabbits. As shown in Fig. 1B, the QT index remained constant during the 6-week experimental period in control rabbits but significantly increased in rabbits fed on low K⁺ diet (n = 8, p < 0.01; Fig. 1B). These data are consistent with the fact that the reduction in serum [K⁺] prolongs QT intervals in our previous study, in which the Bazett’s formula was used to correct QT intervals (10). The heart rate did not change significantly during 6 weeks of experiments and was not different between the control and low K⁺ diet groups (at week 6, 240 ± 4/min, n = 9, in control versus 255 ± 14/min, n = 8, in low K⁺ diet rabbits, p > 0.05). 

Representative ECG recordings from rabbits on low K⁺ diet at weeks 1 and 6 are shown in Fig. 1C. Because sudden death occurs beyond week 6, the experiments on hypokalemic rabbits were terminated at week 6. Ventricular myocytes from rabbits on the control and low K⁺ diet were isolated, and electrophysiological experiments were performed on the isolated cells using the patch clamp method.

After 6 weeks on the normal or low K⁺ diet, the resting membrane potentials of rabbit ventricular myocytes were not different between control and low K⁺ groups (control, 79.7 ± 1.1 mV, n = 7; low K⁺, 77.2 ± 1.3 mV, n = 8, p > 0.05). For recording I_{K1} and I_{to}, cells were held at −40 mV. To analyze I_{K1}, the current at the end of a 1-s hyperpolarizing step to −120 mV was measured. I_{K1} was not different between control and hypokalemic rabbit ventricular myocytes (21.9 ± 3.4 pA/pF in control, n = 6; 22.8 ± 2.3 pA/pF in hypokalemic rabbits, n = 9; p > 0.05). For I_{to} analysis, the peak current upon a 200-ms depolarizing step to 50 mV was measured. I_{to} was 3.7 ± 0.4 pA/pF in control (n = 6) and 3.9 ± 0.7 pA/pF in hypokalemic rabbit ventricular myocytes (n = 8, p > 0.05). To record the Ba²⁺-mediated L-type Ca²⁺ currents, ventricular myocytes were held at −40 mV to inactivate Na⁺ current. The peak Ba²⁺

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current was observed upon a depolarizing step to 0 mV. The densities of the Ba2+-mediated L-type Ca2+ currents were 15.0 ± 2.9 pA/pF in control (n = 7) and 14.6 ± 2.7 pA/pF in low K+ diet rabbit ventricular myocytes (n = 8, p > 0.05). These data are consistent with our previous study (10).

Action potential duration at 90% repolarization (APD90) was significantly prolonged in ventricular myocytes from rabbits on the low K+ diet compared with that from control rabbits (Fig. 1D). Because Ikr and Is are jointly responsible for the repolarization of ventricular myocytes, they were the primary focus in the present study. To isolate Ikr in ventricular myocytes for analysis, we used Cs+ as the charge carrier (14). Cs+ blocks other cardiac K+ channels, such as IK1, Ito, and IK, but uniquely permeates through the native IKr and cloned hERG channels (14). Thus, recording Cs+-carried IKr (IKr-Cs) using symmetrical Cs+ solutions represents an effective way to record pure IKr (14, 18). The IKr-Cs in ventricular myocytes from hypokalemic rabbits was significantly smaller than that from control rabbits (Fig. 1E). This conclusion is consistent with our previous data (10).

IKs was activated by depolarizing steps to voltages between −70 and 50 mV in 10-mV increments for 4 s. IKs was significantly decreased in ventricular myocytes of low K+ diet rabbits compared with that of control rabbits (Fig. 1F). Thus, IKs did not compensate for the decreased IKr but also decreased under hypokalemic conditions.

**Co-expression of KCNQ1 + KCNE1 with hERG Alters the Response of hERG Channels to Reduced [K+]o**—Studies have suggested that hERG interacts with KCNQ1 + KCNE1 (IKs) (16, 19–22). We hypothesize that because of a physical association between IKs and IKr, endocytic degradation of hERG may promote KCNQ1 + KCNE1 degradation and thus simultaneously decrease IKs. To address this possibility, we created a stable cell line that expresses both hERG and KCNQ1 + KCNE1 channels. Fig. 2A shows families of currents recorded from HEK 293 cell lines stably expressing hERG, KCNQ1 + KCNE1, or hERG + KCNQ1 + KCNE1. As shown in the top panel, hERG displayed its unique fast, voltage-dependent recovery from inactivation. This unique hERG property did not exist in the KCNQ1 + KCNE1 stable cell line (middle panel) but did exist in the hERG + KCNQ1 + KCNE1 stable cell line (bottom panel). The hERG + KCNQ1 + KCNE1 stable cell line also displayed the time-dependent, slow activation property of the KCNQ1 + KCNE1 channel, which was not seen in the hERG-HEK cell line. Fig. 2B shows the presence or absence of the unique, fast, voltage-dependent inactivation of hERG channels in each of the stable cell lines. In short, both hERG and KCNQ1 + KCNE1 currents were present in the hERG + KCNQ1 + KCNE1 stable cell line, whereas the individual channels were expressed alone in their respective cell lines.

We then examined the effects of 0 mm K+ exposure on the currents recorded from the hERG, KCNQ1 + KCNE1, or hERG + KCNQ1 + KCNE1 stable cell lines. We previously showed that overnight incubation in 0 mm K+ medium completely eliminated IHERG and reduced IKCNQ1 + IKCN1 by 30.3 ± 8.0% (10). Our subsequent study on the time course of 0 mm K+ exposure-induced reduction in the 155-kDa hERG band showed that the protein decrease by 2 h and essentially disappear by 6 h (11). Thus, a 6-h exposure to 0 mm K+ would be expected to minimally affect the KCNQ1 + KCNE1 current. Thus, in the present study, we exposed cells to 0 mm K+ medium for 6 h to take advantage of the disparity between hERG and KCNQ1 + KCNE1 in response to 0 mm K+ exposure. Indeed, exposure to 0 mm K+ medium for 6 h completely eliminated IHERG (n = 7) and decreased IKCNQ1 + IKCN1 by 19.2 ± 2.3% (n = 11 for 0 mm K+, n = 9 for control; Fig. 3, A and C). Interestingly, in contrast to the hERG or KCNQ1 + KCNE1 separate cell lines, the hERG + KCNQ1 + KCNE1 stable cell line displayed a different response to 6-h 0 mm K+ exposure; IHERG was reduced by 55.5 ± 7.9% (n = 14 for 0 mm K+, n = 17 for control), and IKCNQ1 + IKCN1 was reduced by 76.4 ± 4.9% (n = 11 for 0 mm K+, and n = 15 for control; Fig. 3, B and C).

The effects of 6-h exposure to 0 mm K+ on expression levels of hERG and KCNQ1 were examined using Western blot analysis of HEK 293 cells stably expressing hERG, KCNQ1 + KCNE1, or hERG + KCNQ1 + KCNE1. Exposure to 0 mm K+ for 6 h eliminated the 155-kDa, fully glycosylated, mature form of hERG channels in the hERG stable cell line (Fig. 4, A and E) and had no significant effect on the KCNQ1 protein expression in the KCNQ1 + KCNE1 stable cell line (Fig. 4, B and E). However, the same treatment reduced the hERG 155-kDa form to a lesser extent and KCNQ1 to a greater extent in the hERG + KCNQ1 + KCNE1 stable cell line (Fig. 4, C–E). These results are consistent with the electrophysiological data and indicate that the presence of KCNQ1 + KCNE1 retains some of the hERG channels in the plasma membrane during the 6-h exposure to 0 mm K+. Conversely, internalization of hERG pro-
tein in 0 mM K\(^+\) resulted in the simultaneous internalization of some KCNQ1 proteins.

KCNQ1, but Not KCNE1, Delays Endocytic Degradation of hERG Channels Induced by 0 mM K\(^+\)—Both KCNQ1 and KCNE1 are required to generate the functional IKs current (5, 6). Expression of KCNQ1 alone does not generate IKs but instead produces currents with amplitudes within hundreds of pA and fast activation properties. Similarly, expression of KCNE1 alone does not produce any current (5, 6). When either KCNQ1 or KCNE1 was expressed independently in HEK cells, neither protein’s expression level was affected by a 6-h exposure to 0 mM K\(^+\) medium (Fig. 5A). We have shown that KCNQ1 + KCNE1 can retain hERG in the plasma membrane under low K\(^+\) conditions (Figs. 3 and 4). To study whether either KCNQ1 or KCNE1 alone is sufficient to prevent mature hERG channels from degrading in 0 mM K\(^+\) medium, we transfected hERG-HEK cells with empty vector (pcDNA3, control), KCNQ1, or KCNE1 plasmids. Thirty-six hours after transfection, the cells were exposed to 5 or 0 mM K\(^+\) medium for 6 h. Compared with the control cells, co-expression of KCNQ1, but not KCNE1, effectively retained the 155-kDa hERG band in cells cultured in 0 mM K\(^+\) medium (Fig. 5B).

To investigate whether the retained 155-kDa band indeed represents the mature hERG channels in the plasma membrane, we performed immunocytochemistry to examine the localization of hERG channels in hERG-HEK cells under 0 mM K\(^+\) culture conditions with or without co-expression of KCNQ1 or KCNE1. To this end, KCNQ1, KCNE1, or KCNQ1 + KCNE1 were transiently transfected into hERG-HEK cells. Thirty-six hours after transfection, the cell surface hERG channels were labeled by incubating live cells with an anti-hERG antibody. The cells were then exposed to 0 mM K\(^+\) medium for 4 h, fixed, and permeabilized. KCNQ1 and KCNE1 were labeled using appropriate primary antibodies. The cells were then incubated using appropriate Alexa Fluor-conjugated secondary antibodies to stain either KCNQ1 or KCNE1 and hERG. As shown in Fig. 5C, although every cell expresses hERG...
channels, only a fraction of the cells express KCNQ1 or KCNE1 (the hERG stable cell line being transiently transfected with KCNQ1, KCNE1, or KCNQ1/H11001 KCNE1). Compared with the nontransfected cells, KCNQ1- or KCNQ1/H11001 KCNE1-transfected cells retained hERG expression in the plasma membrane. However, KCNE1 alone failed to retain hERG at the membrane.

We further examined the effects of KCNQ1 or KCNE1 co-expression on hERG response to 0 mM K⁺ exposure by recording IhERG. After 6 h of culture in 0 mM K⁺ medium, hERG-HEK cells transfected with empty pcDNA3 vector, KCNQ1, or KCNE1 were collected, and IhERG was recorded with the 135 mM K⁺-containing pipette solution and the 5 mM K⁺-containing bath solution. Although IhERG in hERG-HEK cells transfected with pcDNA3 (control) or KCNE1 was completely eliminated, 40% of IhERG remained in hERG-HEK cells transfected with KCNQ1 under the same treatment (Fig. 6). Thus, consistent with the Western blots and immunocytochemical data (Fig. 5, B and C), KCNQ1 decreased the sensitivity of hERG channels to 0 mM K⁺ exposure, whereas KCNE1 did not affect hERG sensitivity to 0 mM K⁺ exposure, whereas KCNE1 did not affect hERG expression levels of KCNQ1 or KCNE1 independently expressed in HEK 293 cells (n = 3).

**p < 0.01 versus hERG expression alone (n = 4–5). C, KCNQ1, but not KCNE1, retains hERG membrane expression in cells cultured in 0 mM K⁺ medium for 4 h. Cell surface hERG channels were stained green. KCNQ1 or KCNE1 proteins were stained red. Nuclei were stained blue. Scale bar, 10 μm.

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**FIGURE 5.** KCNQ1, but not KCNE1, interrupts 0 mM K⁺-induced endocytic degradation of mature hERG channels. A, 0 mM K⁺ culture for 6 h does not affect protein expression levels of KCNQ1 or KCNE1 independently expressed in HEK 293 cells (n = 3). B, KCNQ1, but not KCNE1, interrupts 0 mM K⁺-induced reduction of the 155-kDa hERG band intensity. The relative reduction in the 155-kDa band intensity under each condition is summarized underneath the Western blots. **p < 0.01 versus hERG expression alone (n = 4–5). C, KCNQ1, but not KCNE1, retains hERG membrane expression in cells cultured in 0 mM K⁺ medium for 4 h.

**FIGURE 6.** KCNQ1, but not KCNE1, decreases hERG sensitivity to 0 mM K⁺ exposure. A, current traces from hERG-HEK cells co-transfected with empty pcDNA3 vector (control), KCNQ1, or KCNE1 after 6 h of culture in 5 or 0 mM K⁺ medium. The whole cell currents were recorded in the 5 mM K⁺ containing bath solution and the 135 mM K⁺ containing pipette solution. **p < 0.01 versus control hERG-HEK cells. **p < 0.01 versus control hERG-HEK cells.

**FIGURE 7.** Co-expression of KCNQ1 Delays 0 mM K⁺ Exposure-induced Acute Reduction of IhERG—We have previously shown that upon exposure to 0 mM K⁺, the hERG channel enters into a nonconducting state, which triggers channel internalization and degradation. To investigate the role of KCNQ1 in the conductance loss of hERG channels induced by 0 mM K⁺ exposure, we recorded IhERG from control hERG-HEK cells (transfected with the empty pcDNA3 vector) or hERG-HEK cells transfected with KCNQ1. GFP was co-transfected to identify the transfected cells for electrophysiological analysis. Consistent with our previous finding (11), when IhERG from hERG-HEK cells was repetitively evoked by the depolarizing steps (shown above Fig. 7A) at a pulse-interval of 15 s, exposure to 0 mM K⁺ Tyrode solution led to a progressive decline of the current by more than 80% in 16 pulses (n = 6; Fig. 7, A and C). However, when IhERG from hERG+KCNQ1-expressing cells was recorded, the same treatment only decreased the current by 26 ± 6% (n = 9; Fig. 7, B and C). Thus, co-expression of KCNQ1 significantly slowed the acute IhERG reduction upon exposure to 0 mM K⁺ solutions, which may contribute to the effects of KCNQ1 on retaining hERG in the plasma membrane.
Co-expression of KCNQ1 Does Not Change Either the Biophysical Properties or Drug Sensitivity of hERG Channels—To investigate the effects of KCNQ1 expression on the gating kinetics of the hERG current, the current-voltage (I-V) relationships, as well as the activation curves of currents from hERG-HEK cells transfected with empty vector (control) or KCNQ1, were compared. GFP was co-transfected to identify transfected cells for electrophysiological analysis. I-V relationships were constructed by plotting the current amplitudes measured at the end of 4-s depolarization steps (Fig. 8A). Also, the hERG activation curves were obtained by plotting the hERG tail currents at −50 mV against the depolarizing voltages and fitting the data to the Boltzmann equation (Fig. 8B). Co-expression of KCNQ1 did not affect the hERG activation curves. The $V_1/2$ and the slope factor were $-1.7 \pm 0.4$ mV and $9.5 \pm 0.4$, respectively, for hERG, and $-2.9 \pm 0.2$ mV and $9.4 \pm 0.1$, respectively, for hERG + KCNQ1 ($n = 5–7, p > 0.05$). To determine the effects of KCNQ1 co-expression on hERG deactivation, the tail current at −50 mV after full channel activation (4-s depolarizing step to 50 mV) was fitted to double exponential functions. The fast and slow deactivation time constants were $334 \pm 16$ and $1680 \pm 56$ ms, respectively, with a relative amplitude of the slow component of 0.71 for hERG expression alone ($n = 9$). The fast and slow deactivation time constants were $297 \pm 25$ and $1826 \pm 155$ ms, respectively, with a relative amplitude of slow component of 0.68 for hERG+KCNQ1 co-expression ($n = 8, p > 0.05$). Thus, the deactivation rate of the hERG current was not significantly affected by KCNQ1 co-expression.

We also studied the voltage-dependent inactivation of the currents from cells expressing hERG or hERG + KCNQ1. For this analysis, the channel was activated and inactivated by a depolarizing step to 60 mV for 200 ms. Voltage was then changed to −100 ms for 10 ms, a period that is sufficient to allow inactivated hERG channels to recover to the open state but too short for channel deactivation. The cell membrane was then depolarized to various voltages to induce voltage-dependent inactivation (Fig. 8C). The current traces were fitted to a single exponential function to obtain the time constants of current inactivation, which were plotted against voltages (Fig. 8D, $n = 4–6$). Co-expression of KCNQ1 did not affect the voltage-dependent inactivation of the hERG channel (Fig. 8D).

As can be seen from Fig. 8 (A and B), co-expression of KCNQ1 with hERG did not significantly change either the pulse or tail current amplitudes of hERG channels. Compared with the current from cells expressing hERG alone, the current from cells expressing hERG + KCNQ1 also displayed the unique, “bell-shaped” I-V relationship. However, the pulse current amplitudes upon depolarizing voltages between 50 and 70 mV were slightly bigger in hERG + KCNQ1-expressing cells. This may reflect the overlap with the KCNQ1 current, which does not display voltage-dependent inactivation properties. These data, and those showing that KCNQ1 co-expression did not affect the biophysical property of hERG channels, suggest that KCNQ1 and hERG may not form heterologous channels. Instead, they form distinct hERG and KCNQ1 channels. Our pharmacological data described below directly support this notion. The hERG channel, but not KCNQ1, displays uniquely
high sensitivity to a wide spectrum of drugs (8, 23). Astemizole is one of the most potent hERG antagonists with an IC$_{50}$ (half-maximal inhibitory concentration) in the nanomolar range (24). Although 100 nM astemizole completely eliminated the tail current from hERG+KCNQ1-expressing cells, it only slightly inhibited the pulse current (Fig. 8, E–H). When hERG current is specifically blocked in hERG-HEK cells, no endogenous current is apparent (24–26) (also see Fig. 6). Thus, in the presence of astemizole, the current of hERG+KCNQ1-expressing cells represents KCNQ1 current. These data indicate that KCNQ1 and hERG form distinct channels in HEK cells where they are co-expressed.

**KCNQ1 Interacts with hERG at the Cell Surface**—We hypothesized that mature hERG channels physically interact with KCNQ1 and that such an interaction stabilizes hERG channels in the plasma membrane, thus delaying 0 mM K$^+$-induced hERG endocytic degradation. To study the hERG-KCNQ1 association, we performed co-IP experiments using whole cell proteins extracted from the hERG+KCNQ1+KCNE1 stable cell line. When the extracted proteins were precipitated with an anti-hERG antibody, the KCNQ1 protein was detected in the precipitated proteins (Fig. 9A). Inversely, when the whole cell protein was precipitated with an anti-KCNQ1 antibody and detected with anti-hERG antibody, the mature 155-kDa hERG band was detected (Fig. 9B). It has been shown that the 155-kDa hERG is localized at the plasma membrane (18). To confirm the 155-kDa band is localized in the plasma membrane, we applied the membrane-impermeant proteinase K to the culture solution to digest the cell surface proteins in live hERG-HEK cells. The cells were then collected, and the whole cell protein was subject to Western blot analysis. Extracellularly applied proteinase K selectively digests the 155-kDa hERG band. Live hERG-HEK cells were treated with proteinase K (200 µg/ml) in a physiological solution at 37 °C for 30 min to digest cell surface proteins. After washing, the treated cells were collected, and their protein was extracted for Western blot analysis. Extracellularly applied proteinase K selectively digests the 155-kDa hERG band, suggesting that KCNQ1-hERG interaction takes place at the plasma membrane. To confirm this, we isolated cell surface protein using the biotinylation method (18). We then performed KCNQ1-hERG co-IP analyses on cell surface proteins. Our data show that KCNQ1 interacts with the 155-kDa hERG band in proteins isolated from cell surface (Fig. 9D).

**DISCUSSION**

We have recently demonstrated that cell surface hERG channels are sensitive to [K$^+$]o and undergo rapid endocytic degradation upon exposure to a reduced [K$^+$]o. In contrast, KCNQ1+KCNE1 (IKs) channels are relatively insensitive to [K$^+$]o reduction (10). In the present study, using HEK cells expressing each type of channel either separately or in combination, we have demonstrated an interaction between hERG and KCNQ1 and obtained mechanistic insight into this interaction.

The delayed rectifier potassium current, I$_{Kr}$, plays a pivotal role in the repolarization of cardiac action potentials (8). Although I$_{Kr}$ was originally described as a single current (1), it has become evident that I$_{Kr}$ is composed of two distinct currents, I$_{Kr}$a and I$_{Kr}$o, whose channel proteins are encoded by different genes and display currents with distinct biophysical and pharmacological characteristics (3–6). Although they are separate channels, I$_{Kr}$a and I$_{Kr}$o have important functional interactions during cardiac action potential repolarization. Reductions in I$_{Kr}$a prolong the action potential, which consequently activate more I$_{Kr}$o to prevent excess repolarization delay (30, 31). In addition to the functional interactions, a direct physical interaction between I$_{Kr}$a and I$_{Kr}$o was first reported by Ehrlich et al. (19) and confirmed by several recent studies (20–22). Expression of a dominant-negative hERG or KCNQ1 mutant in transgenic rabbits led to the down-regulation of the reciprocal current, indi-
cating that an interaction between $I_{Kr}$ and $I_{KS}$ also occurs in animals in vivo (16).

Despite these observations, the nature of the interaction between $I_{Kr}$ and $I_{KS}$ is not well understood, and the reported results are conflicting. Although it was reported that the presence of KCNQ1 increases hERG current and enhances hERG current deactivation (19), another study showed that KCNQ1 does not affect hERG deactivation gating (21). Moreover, a more recent study reported that overexpression of either KCNQ1 or hERG significantly decreases the reciprocal current (22).

In the present study, we investigated hERG-KCNQ1 interactions using the unique extracellular K$^+$ sensitivity of hERG channels. Because low K$^+$ exposure only triggers hERG internalization but has a much weaker effect on KCNQ1+KCNE1 (10), this approach enables us to study the hERG and KCNQ1 interaction at a robust level. Our data demonstrated that hERG and KCNQ1 physically associate at the plasma membrane (Fig. 5). As a result, KCNQ1 stabilizes hERG membrane localization under low K$^+$ conditions. On the other hand, low K$^+$-induced hERG endocytic degradation drags KCNQ1, as well as KCNE1, into the cell for degradation.

Neither KCNQ1 nor KCNE1 displayed a significant sensitivity to 0 mM K$^+$ exposure (Figs. 4B and 5A). KCNE1 has been shown to interact with both hERG and KCNQ1 (5, 6, 32). In fact, KCNE1 is required for KCNQ1 to form the functional $I_{KS}$ channel (5, 6). Also the interaction between KCNE1 and hERG is supported by our data. When KCNE1 was expressed alone in HEK cells, it was not affected by 6-h exposure to 0 mM K$^+$ conditions (Fig. 5A). However, when KCNE1 was co-expressed with hERG, it was significantly reduced by the same treatment (Fig. 5B). Although it is possible that KCNE1 serves as a linker between hERG and KCNQ1, our data showed that overexpression of KCNQ1 alone is sufficient to delay the degradation of mature hERG channels under low K$^+$ conditions (Fig. 5). Furthermore, our co-IP data indicate a direct interaction between hERG and KCNQ1 in hERG+KCNQ1-expressing HEK cells (Fig. 9). This conclusion is also in line with the previous reports (19–21).

KCNQ1, but not KCNE1, stabilizes hERG membrane expression under low K$^+$ conditions (Fig. 5). The mechanism for this difference is not known and may be related to the size of the molecule. Although KCNQ1 has six transmembrane segments, KCNE1 is a small molecule with only one transmembrane segment (33, 34). Furthermore, it has been shown that KCNQ1 interacts with the protein kinase A-anchoring protein Yotiao, which may enable KCNQ1 to retain hERG channels in the plasma membrane (35).

Different from previous reports that KCNQ1 either increases or decreases hERG current amplitude (19–22), our data show that under normal (5 mM K$^+$) culture conditions, KCNQ1 did not significantly affect the hERG current amplitude (Fig. 8). On the other hand, under conditions where hERG membrane stability is compromised, such as hypokalemia, KCNQ1 stabilizes hERG in the plasma membrane. Our data also show that KCNQ1 did not affect the biophysical or pharmacological properties of hERG currents. The unchanged hERG biophysical and pharmacological properties in hERG+KCNQ1-expressing cells (Fig. 8) suggest that hERG and KCNQ1 do not coassemble to form heterologous channels. Also, after hERG channel antagonist astemizole was applied, the KCNQ1 current upon depolarizing steps was observed in hERG+KCNQ1 cells. Thus, although hERG and KCNQ1 channels associate with each other, they may only form a macromolecular channel complex consisting of distinct hERG and KCNQ1 channels. Then again, KCNQ1 must have a close interaction with hERG because the 0 mM K$^+$-induced acute hERG conductance loss was significantly weakened by the KCNQ1 co-expression (Fig. 7).

Previously, we have illustrated that [K$^+$]$\text{in}$ is a prerequisite for the function and membrane stability of cell surface hERG channels (10, 11). Upon removal of [K$^+$]$\text{in}$, hERG channels enter into a nonconducting state within minutes, reflecting a conformational change of the hERG channel. This conformational change triggers subsequent internalization and degradation of the channel (10, 11). Thus, whereas the conductance loss induced by acute [K$^+$]$\text{in}$ depletion is reversible, prolonged exposure of hERG-expressing cells to 0 mM K$^+$ medium causes progressive reduction in the expression level of hERG channels (11, 12, 36). Under 0 mM K$^+$ culture conditions, the surface density of the mature hERG protein begins to decrease by 2 h and is essentially eliminated by 6 h (10, 11). In the present study, our data show that co-expression of KCNQ1 slowed the conductance loss of hERG channels, which suggests that KCNQ1 interferes with the hERG protein conformational change upon 0 mM K$^+$ exposure, and this may contribute to the enhanced membrane stability of hERG channels.

Our data show that only the plasma membrane-localized mature form (155-kDa) of hERG co-immunoprecipitated with KCNQ1 (Fig. 9). This observation prompts us to propose that the KCNQ1-hERG interaction occurs at the plasma membrane, and such a physical interaction stabilizes mature hERG channels under low K$^+$ conditions (10–12). Our data on cell surface proteins from hERG+KCNQ1+KCNE1-expressing cells directly support this notion (Fig. 9C).

To investigate the physiological relevance of potential hERG-KCNQ1 interaction, we used a hypokalemia rabbit model. Our data show that both $I_{Kr}$ and $I_{KS}$ are decreased in hypokalemic rabbits. Because a certain concentration of serum K$^+$ is required for rabbits to survive, the extent of reduction of $I_{Kr}$ and $I_{KS}$ in the rabbit model is less than the reduction of hERG and KCNQ1+KCNE1 current seen in cell lines that were exposed to 0 mM K$^+$ culture. Culturing adult cardiac myocytes and studying native $I_{Kr}$ and $I_{KS}$ trafficking are difficult tasks. We therefore cultured hERG and/or KCNQ1-expressing cell lines in 0 mM K$^+$ conditions to enhance the mechanistic investigation of $I_{Kr}$-$I_{KS}$ interactions. Although this extreme condition may never occur in humans or animals in vivo, we have previously demonstrated that a reduction in [K$^+$]$\text{in}$ decreases hERG expression in the plasma membrane in a concentration-dependent manner (10). Thus, our data regarding the hERG-KCNQ1 interaction under low [K$^+$]$\text{in}$ conditions provide an explanation for the reduced $I_{KS}$ in the hypokalemic rabbits. Under hypokalemic conditions with reduced $I_{Kr}$, $I_{KS}$ is critical for cardiac repolarization. Its dysfunction would result in a loss of compensatory potential and increase the risk of long QT syndrome, arrhythmias, and sudden death (30, 31).
REFERENCES

1. Noble, D., and Tsien, R. W. (1969) J. Physiol. 200, 205–231
2. Matsuura, H., Ebara, T., and Imoto, Y. (1987) Pflugers Arch. 410, 596–603
3. Sanguinetti, M. C., Jiang, C., Curran, M. E., and Keating, M. T. (1995) Cell 81, 299–307
4. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) Science 269, 92–95
5. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Specter, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80–83
6. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Gomёy, G. (1996) Nature 384, 78–80
7. Keating, M. T., and Sanguinetti, M. C. (2001) Cell 104, 569–580
8. Sanguinetti, M. C., and Tristani-Firouzi, M. (2006) Nature 440, 463–469
9. Roden, D. M., Wooley, R. L., and Primm, R. K. (1986) Circ. Res. 59, 92–95
10. Guo, J., Massaeli, H., Xu, J., Shallow, H., Yang, T., Xu, J., Li, W., Hanson, C., Wu, J. G., and Zhang, S. (2010) J. Clin. Invest. 119, 1088–1093
11. Sun, T., Guo, J., Shallow, H., Yang, T., Xu, J., Li, W., Hanson, C., Wu, J. G., and Zhang, S. (2011) J. Biol. Chem. 286, 6751–6759
12. Sun, T., Guo, J., Shallow, H., Yang, T., Xu, J., Li, W., Hanson, C., Wu, J. G., and Zhang, S. (2010) J. Clin. Invest. 119, 2745–2757
13. Massaeli, H., Guo, J., Xu, J., and Zhang, S. (2010) Circ. Res. 106, 1072–1082
14. Sun, T., Guo, J., Shallow, H., Yang, T., Xu, J., Li, W., Hanson, C., Wu, J. G., Li, X., Massaeli, H., and Zhang, S. (2011) J. Biol. Chem. 286, 6751–6759
15. Osteen, J. D., Sampson, K. J., and Kass, R. S. (2010) J. Biol. Chem. 285, 27259–27264
16. Brunner, M., Peng, X., Liu, G. X., Ren, X. Q., Ziv, O., Choi, B. R., Mathur, R., Hajarí, M., Odening, K. E., Steinberg, E., Folco, E. J., Pringa, E., Centracchio, J., Macharzina, R. R., Donahay, T., Schofield, L., Rana, N., Kirk, M., Mitchell, G. F., Poppas, A., Zehnder, M., and Koren, G. (2008) J. Clin. Invest. 118, 2246–2259
17. Funck-Brentano, C., and Jaillon, P. (1993) Am. J. Physiol. Heart Circ. Physiol. 265, H2264–H2272
18. Rajamani, S., Anderson, C. L., Anson, B. D., and January, C. T. (2002) Circulation 106, 2830–2835
19. Delisle, B. P., Anderson, C. L., Balijepalli, R. C., Anson, B. D., Kamp, T. J., and January, C. T. (2003) J. Biol. Chem. 278, 35749–35754
20. Varro, A., Baláti, B., Iost, N., Takács, J., Virág, L., Lathrop, D. A., Csaba, L., Tálosi, L., and Papp, J. G. (2000) J. Physiol. 523, 67–81
21. Biliczki, P., Virág, L., Iost, N., Poppas, A., and Varró, Á. (2002) Br. J. Pharmacol. 137, 361–368
22. McDonald, T. V., Yu, Z., Ming, Z., Palma, E., Meyers, M. B., Wang, K. W., Goldstein, S. A., and Fishman, G. I. (1997) Nature 388, 289–292
23. Nakajo, K., Ulbrich, M. H., Kubo, Y., and Isacoff, E. Y. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 18862–18867
24. Osteen, J. D., Sampson, K. J., and Kass, R. S. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 18751–18752
25. Marx, S. O., Utsunomiya, R., Reiken, S., Motoike, H., D’Armiento, J., Marks, A. R., and Kass, R. S. (2002) Science 295, 496–499
26. Nakajo, K., Ulbrich, M. H., Kubo, Y., and Isacoff, E. Y. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 18751–18752
27. Marx, S. O., Utsunomiya, R., Reiken, S., Motoike, H., D’Armiento, J., Marks, A. R., and Kass, R. S. (2002) Science 295, 496–499
28. Rajamani, S., Anderson, C. L., Anson, B. D., and January, C. T. (2002) Circulation 105, 2830–2835
29. Delisle, B. P., Anderson, C. L., Balijepalli, R. C., Anson, B. D., Kamp, T. J., and January, C. T. (2003) J. Biol. Chem. 278, 35749–35754
30. Sun, T., Guo, J., Shallow, H., Yang, T., Xu, J., Li, W., Hanson, C., Wu, J. G., and Zhang, S. (2010) Circ. Res. 107, 18751–18752
31. Kupershmidt, S. (2010) J. Physiol. 289, 45469–45474
32. Moos, M., and Zauzer, M. (2000) J. Biol. Chem. 275, 35075–35079
33. Nakajo, K., Ulbrich, M. H., Kubo, Y., and Isacoff, E. Y. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 18751–18752
34. Osteen, J. D., Sampson, K. J., and Kass, R. S. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 18751–18752
35. Marx, S. O., Utsunomiya, R., Reiken, S., Motoike, H., D’Armiento, J., Marks, A. R., and Kass, R. S. (2002) Science 295, 496–499
36. Massaeli, H., Sun, T., Li, X., Shallow, H., Wu, J., Xu, J., Li, W., Hanson, C., Guo, J., and Zhang, S. (2010) J. Biol. Chem. 285, 27259–27264

Interaction between $I_{Ks}$ and $I_{Kr}$