UltraRapid Communication

Autonomic Control of Cardiac Action Potentials
Role of Potassium Channel Kinetics in Response to Sympathetic Stimulation

Cecile Terrenoire,* Colleen E. Clancy,* Joseph W. Cormier,* Kevin J. Sampson, Robert S. Kass

Abstract—$I_{Ks}$, the slowly activating component of the delayed rectifier current, plays a major role in repolarization of the cardiac action potential (AP). Genetic mutations in the α- ($KCNQ1$) and β- ($KCNE1$) subunits of $I_{Ks}$ underlie Long QT Syndrome type 1 and 5 (LQT-1 and LQT-5), respectively, and predispose carriers to the development of polymorphic ventricular arrhythmias and sudden cardiac death. β-adrenergic stimulation increases $I_{Ks}$, and results in rate dependent AP shortening, a control system that can be disrupted by some mutations linked to LQT-1 and LQT-5. The mechanisms by which $I_{Ks}$ regulates action potential duration (APD) during β-adrenergic stimulation at different heart rates are not known, nor are the consequences of mutation induced disruption of this regulation. Here we develop a complementary experimental and theoretical approach to address these questions. We reconstituted $I_{Ks}$ in CHO cells (ie, $KCNQ1$ coexpressed with $KCNE1$ and the adaptor protein Yotiao) and quantitatively examined the effects of β-adrenergic stimulation on channel kinetics. We then developed theoretical models of $I_{Ks}$ in the absence and presence of β-adrenergic stimulation. We simulated the effects of sympathetic stimulation on channel activation (speeding) and deactivation (slowing) kinetics on the whole cell action potential under different pacing conditions. The model suggests these kinetic effects are critically important in rate-dependent control of action potential duration. We also investigate the effects of two LQT-5 mutations that alter kinetics and impair sympathetic stimulation of $I_{Ks}$, and show the likely mechanism by which they lead to tachyarrhythmias and indicate a distinct role of $I_{Ks}$ kinetics in this electrical dysfunction. The full text of this article is available online at http://circres.ahajournals.org. (Circ Res. 2005;96:e25-e34.)

Key Words: $I_{Ks}$ • sympathetic nervous system • electrophysiology • ion channels • Long-QT syndrome

Stimulation of the sympathetic nervous system (SNS) in response to exercise or emotional stress results in a rapid and dramatic increase in heart rate. To ensure adequate diastolic filling time between beats, the ventricular action potential duration (APD) exhibits a concomitant shortening at the cellular level, which results in a reduction in the corresponding QT interval of the ECG. SNS control of cardiac electrical activity is mediated by the activation of β-adrenergic receptors (β-ARs) that regulate the activity of select ion channel proteins via cAMP-dependent protein kinase A (PKA) or by direct binding of cAMP to channel subunits. One key substrate for PKA-dependent phosphorylation in the regulation of the cardiac action potential is the slowly activating $I_{Ks}$ potassium channel.1,2

$I_{Ks}$ is produced by the coassembly of $KCNQ1$ (α-subunit) and $KCNE1$ (β-subunit).3,4 PKA regulation of the channels is coordinated by a macromolecular signaling complex composed of the channel and the targeting protein Yotiao, which recruits PKA and protein phosphatase 1 (PP1) to the carboxy terminal domain of the $KCNQ1$ subunit.4 This complex forms a channel microdomain that permits sensitive temporal control of the channel phosphorylation state in response to SNS stimulation.

Mutations in the genes encoding $I_{Ks}$ subunits cause LQT-1 ($KCNQ1$ mutations) and LQT-5 ($KCNE1$ mutations). Mutation carriers are at increased risk of sudden death in the face of elevated SNS activity.5 Despite the elucidation of the $KCNQ1/KCNE1$ macromolecular signaling complex and these striking clinical data, the precise mechanism by which PKA phosphorylation translates into shortening of APD and how disruption of the signaling underlies elevated arrhythmias risk is unknown.

In this study, we have begun to explore precisely how the SNS regulates $I_{Ks}$ by directly measuring and quantifying the changes in channel gating that arise in response to PKA phosphorylation. We next ask how disruption of the pathway

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may contribute to the genesis of tachyarrhythmias by using a combined experimental and computational approach. First, we measured the effects of CAMP-dependent regulation of hKCNQ1/hKCNE1 channels expressed in Chinese Hamster Ovary (CHO) cells and focused on CAMP-dependent changes in the voltage-dependence and time course of channel gating. Second, we developed a simple computational model of the Ik channel, which is able to reconstitute the experimentally determined CAMP-dependent changes in channel gating to explore the role of modulated Ik in the Luo-Rudy model. To study the effects of adrenergic modulation of Ik, we also incorporated a recently reported computational model of cardiac myocyte β-AR signaling that we modified to include targeted regulation of Ik. We simulated the effects of sympathetic stimulation on channel activation (speeding) and deactivation (slowing) kinetics on the whole-cell action potential under different pacing conditions. The model suggests these kinetic effects are critically important in rate-dependent control of action potential duration. We also investigate the effects of two LQT-5 mutations that alter kinetics and impair sympathetic stimulation of Ik and show the likely mechanism by which they lead to tachyarrhythmias.

Materials and Methods

Cell Culture and Transfection

Chinese hamster ovary (CHO) cells (American Type Cell Culture) were cultured in Ham’s F12 medium and kept at 37°C in an incubator with 5% CO$_2$. The day before transfection, cells were passaged at 30% to 40% confluence in 25-cm$^2$ tissue culture flask. Cells cotransfected with CD8 (0.4 μg) and channel-subunit cDNAs of interest (0.4 μg human KCNQ1, 0.4 μg human KCNE1, 2 μg yotiao) were identified using Dynabeads M-450 anti-CD8 (Dynal, Oslo).

Electrophysiology

Cells culture dishes were placed on the stage of an inverted microscope (Nikon), and currents were recorded at room temperature using the whole-cell configuration of Patch-clamp technique with Axopatch 200B amplifier (Axon Instruments, Inc). Series resistance was 2 to 4 MΩ, and data were acquired using pCLAMP 8.0 software (Axon Instruments, Inc). Voltage-clamp protocols were applied 13 minutes after membrane rupture so to allow stabilization of current rundown. Activation of Ik (ie, human KCNQ1 coexpressed with human KCNE1 and the adaptor protein Yotiao) was assumed using 2-second depolarizing steps ranging from -60 to +80 mV (20-mV increments), triggered every 15 seconds from a holding potential of -80 mV, and followed by a 5-second repolarizing step to -40 mV during which a decreasing tail current was recorded, reflecting progressive deactivation of channels. Activation curves were obtained by plotting normalized peak tail currents as a function of the voltage.

Simulation of Macroscopic Current

Cardiac myocyte β-AR signaling was simulated according to the model of Saucerman et al., which computes PKA-dependent regulation of L-type calcium channel current (I$_{Ca,L}$) and phospholamban (PLB). We added targeted enzymatic modification of KCNQ1 by PKA and PP1 to this model and assumed similar kinetics (kcat and Km) to those presented for I$_{Ca,L}$ and PLB by Saucerman et al. With respect to cellular modeling, we used the Luo-Rudy guinea pig ventricle model. Changes to this model were made in the calculations for I$_{Ca,L}$ and Ik as described in the supplemental data. The L-type calcium current was modeled using a mammalian model fit to experimental data for basal and maximally phosphorylated states (see the online data supplement available at http://circres.ahajournals.org).

We used a Hodgkin-Huxley type model to reconstitute Ik activation characteristics based on Zeng et al. The model consists of three identical independent activation gates that are time and voltage dependent, because activation of the channel in response to depolarization is well fit by a triexponential process. The following expression was used to describe Ik at a given time and voltage:

$$I_{K_i} = g_{K_i} \times x_a \times x_a \times (V_m - E_{K_i}),$$

where $g_{K_i}$ is the maximum membrane conductance, $x_a$ is the activation gating parameter, $V_m$ is membrane potential, and $E_{K_i}$ is the reversal potential of the channel. For the nonphosphorylated state, $g_{K_i}$ was assigned the value from the Luo-Rudy formulation. For phosphorylated channels, $g_{K_i}$ was scaled according to experimental data summarized in Results.

Computational Methods

Simulation of Macroscopic Current

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Total Ik in the cell was modeled as two populations of channels, nonphosphorylated and phosphorylated, denoted by superscript open-circles and asterisks, respectively:

$$I_{K_i} = P_{K_i}(1-n) + P_{K_i}^{*}(n),$$

where $n$ is the fraction of phosphorylated channels. For the D76N and W87R KCNE1 mutations, four populations of channels were considered: wild-type nonphosphorylated, wild-type phosphorylated, mutant nonphosphorylated, and mutant phosphorylated.

$$P_{K_i} = (1-m) \times [P_{K_i}(1-n) + P_{K_i}^{*}(n)] + m \times [P_{K_i}(1-n) + P_{K_i}^{*}(n)].$$

where $m$ is the fraction of mutant channels and the $mut$ notation represents either D76N or W87R. For all simulations, m is zero for wild-type, 0.5 for the heterozygote, and 1 for the homozygote.

The specific kinetic parameters are given in the online data supplement, where the activation parameter $x_a$ is determined by two voltage-dependent transitions, a forward (or opening) transition ($\alpha_{on}$) and a reverse (or closing) transition ($\beta_{on}$), which determine the gate transition time constant ($\tau_{on}$). In all cases, the parameters were fit empirically over the range of voltages to reproduce experimental data resulting in rate constants for nonphosphorylated and rate constants for phosphorylated channels, which are shown explicitly in the online data supplement. We also measured endogenous currents (which resulted in an instantaneous current at some voltages) in
nontransfected cells and subtracted these from experimental $I_{Ks}$ recordings before fits to ensure fits to the kinetics of $I_{Ks}$ alone.

The same paradigm was used in calculating L-type calcium current based on the functional results of Dilly et al. The experimental data, along with the equations, are provided in the online data supplement. All of the single cell simulations were run at 37°C using a Q10=2.57 (from Unsold et al).

The cellular model of the action potential is a Luo-Rudy Phase 2 model. The formulation is based on a numerical reconstruction of the action potential using the following differential equation that describes the rate of change of the membrane potential ($V_m$):

$$\frac{dV_m}{dt} = \frac{1}{C_m} (I_{Kn} + I_s),$$

where $I_{Kn}$ is the sum of all the ionic currents through the membrane and $I_s$ is the stimulus current applied across the membrane. In the model, $I_{Kn}$ includes currents carried through voltage-gated channels, pumps, and exchangers.

Initial conditions were obtained after prolonged pacing at a 1000 ms stimulation rate. These conditions and parameters, as well as new or modified equations are found in the online data supplement.

Figure 1.

**Results**

**PKA Stimulatory Effects on $I_{Ks}$ Activation and Deactivation Kinetics**

The coexpression of the $I_{Ks}$, $\alpha$ (KCNQ1) and $\beta$ (KCNE1) subunits in heterologous systems produces a voltage-dependent potassium current that reconstitutes features of the cardiac-delayed rectifier potassium current $I_{Ks}$. The current activates slowly on depolarization and saturates only after many seconds (Figure 1A). The time course of $I_{Ks}$ activation is voltage dependent and is faster with increasing depolarization (Figure 1B, white columns): time-to-half activation ($t_{1/2}$) = 1050±41.7 ms at +20 mV, 909±44.3 ms at +40 mV, and 770.8±41.1 ms at +60 mV ($n=6$, $P<0.05$ between each voltage).

In cardiac myocytes, $I_{Ks}$ is well known to be stimulated by the SNS and this regulation can be reconstituted in heterologous systems by coexpressing the adapter protein Yotiao with KCNQ1 and KCNE1. In addition to PKA, Yotiao binds protein phosphatase 1 (PP1). We have shown previously that the maximum response of $I_{Ks}$ to PKA is obtained when a protein phosphatase inhibitor (okadaic acid, OA) is combined with the PKA activator cAMP. Hence, we investigated maximal PKA stimulatory effects on $I_{Ks}$ using cAMP (0.2 mmol/L) in addition to OA (0.2 mmol/L).

First, we investigated the effect of maximal PKA stimulation on $I_{Ks}$ activation kinetics. PKA stimulation significantly increased $I_{Ks}$ current measured at the end of the depolarizing pulse (Figure 1A). This effect, observed at all test potentials, is associated with an increase in the rate of channel activation (Figure 1A and 1B) and a 20-mV leftward shift in the $I_{Ks}$ activation curve (Figure 1C), which is consistent with a previous report: $V_{1/2}=27.7±2.9$ mV (control, $n=6$) versus $V_{1/2}=6.0±3.8$ mV (cAMP/OA, $n=4$, $P<0.05$).

We next tested the effect of PKA phosphorylation on $I_{Ks}$ deactivation kinetics (Figure 2). Deactivation reflects the movement of open channels into nonconducting closed states and is voltage dependent. Deactivation is hastened with increasing hyperpolarization (top traces of Figure 2A and 2C): $\tau_{-60 mV}=584.5±46.2$ ms versus $\tau_{-100 mV}=802.8±89.9$ ms ($n=6$, $P<0.05$). In the presence of cAMP/OA, the rate of $I_{Ks}$ deactivation is also voltage dependent (bottom traces of Figure 2A and 2C): $\tau_{-60 mV}=986±232.8$ ms versus $\tau_{-100 mV}=1213.9±235.3$ ms ($n=4$, $P<0.05$). However, PKA stimulation slows the $I_{Ks}$ deactivation rate at all potentials tested compared with rates measured under control conditions. For instance, at −20 mV, a potential close to the level of AP plateau, $\tau_{-20 mV}=807±46.5$ ms ($n=6$) versus $\tau_{-20 mV}=1085.8±127.6$ ms ($n=4$, $P<0.05$) (Figure 2B and 2C).

**Model Fits to Data: PKA Effects on Kinetics and Whole Cell Currents**

Figure 3 illustrates the predictions of a Hodgkin-Huxley type model of $I_{Ks}$ that we developed from the experimental data and focuses on the effects of PKA stimulation on the kinetics of channel activation. Shown are comparisons of experiments (red lines) and simulations (black lines) in the absence
(Figure 3A) and presence of maximal PKA stimulation (cAMP/OA) (Figure 3B) at three different voltages: +20, +40, and +60 mV. Traces were normalized to the maximum current at the end of the depolarizing pulse to emphasize the changes in \( I_{ks} \) kinetics. The model, consisting of three identical and independent activation gates (see Materials and Methods), enabled us to reconstitute the control voltage-dependent acceleration of \( I_{ks} \) activation kinetics at all tested voltages with good fits to experimental recordings (Figure 3A). PKA induced acceleration in \( I_{ks} \) activation kinetics observed experimentally was also reproduced well by the model at all tested voltages (Figure 3B).

Figure 4 illustrates the results of simulating the effects of PKA stimulation on \( I_{ks} \) channel deactivation. \( I_{ks} \) deactivation is seen as a decay of tail current on membrane repolarization and reflects channels moving from open to closed states. We investigated the effects of PKA stimulation on \( I_{ks} \) deactivation rates at different voltages and optimized model parameters to fit the experimentally obtained data. \( I_{ks} \) model fits (black) to experiment recordings (red) of kinetics of \( I_{ks} \) activation in the absence (left) and presence (right) of cAMP and okadaic acid (OA). Cells were depolarized from –80 mV to test potential for 2 seconds and then repolarized to –40 mV for 5 seconds. Note overlap of experimental and simulated traces.

Figure 2. \( I_{ks} \) deactivation kinetics are slowed by PKA stimulation. A, Normalized averaged \( I_{ks} \) (4 to 6 cells) recorded on 2-second depolarizing steps to +80 mV (holding potential was –80 mV) followed by 5-second repolarizing steps to voltages ranging from –120 to –20 mV (20-mV increments). Pulses were applied at 0.083 Hz in control conditions (top traces) or in presence of 0.2 mmol/L cAMP with 0.2 \( \mu \)mol/L okadaic acid in pipette solution (bottom traces). For clarity, tail currents (normalized to maximum current at +80 mV and plotted as arbitrary units) are emphasized. Dashed lines indicate zero current level. B, Normalized tail currents measured at –20 mV after a pre-pulse potential to +80 mV (averaged traces from 4 to 6 cells). Deactivation rate of \( I_{ks} \) was slowed in the presence of cAMP with OA in the pipette (thick lines) compared with control conditions (thin lines). C, PKA stimulation significantly slowed \( I_{ks} \) deactivation rate (\( \tau \)) at all voltages measured (4 to 6 cells; \( P < 0.05 \)). Tau (\( \tau \)) values were obtained by fitting the tail current with a single exponential.

Mechanisms of APD Shortening: \( \beta \)-Adrenergic Regulation of \( I_{ks} \)

We next integrated our \( I_{ks} \) model in the Luo-Rudy dynamic model of the ventricular cell where we incorporated a computational model of cardiac myocyte \( \beta \)-AR signaling modified to include targeted regulation of \( I_{ks} \). This combined model allowed us to model the cellular impact of \( \beta \)-AR receptor-mediated modification of \( I_{ks} \) concomitant with receptor-stimulated regulation of L-type calcium channel current \( I_{ca} \) and phospholamban (PLB) as described by
Saucerman et al.8 Modeling targeted regulation of I_{Ks} allowed us to explore the consequences of disruption of I_{Ks} but not I_{CaL} or PLB regulation in response to /H9252-AR stimulation. Effects of PKA stimulation on rate-dependent changes in I_{Ks} activity is revealed by Figure 6A and 6B. Shown in Figure 6 are two action potentials (100th beat in a train of action potentials as in Materials and Methods) top traces and corresponding I_{Ks} (below) for cells paced at the indicated cycle lengths in the absence (/H11002) and presence (/H11001) of isoproterenol. In both cases, increases in I_{Ks} are caused both by effects on time-independent g_{Ks} and on PKA-modified gating kinetics. Comparing the relative changes in channel activity at the two stimulation rates provides insights into the role of I_{Ks} kinetics into control of APD. At slow rates (Figure 6A, 1000 ms), I_{Ks} is larger in part during /H9252-adrenergic stimulation because of the changes in channel activation rate (ie, leftward shift in activation curve) (Figure 1C). The effect on activation kinetics leads to an enhanced activation of channels during depolarization, therefore producing a larger repolarizing current, which contributes to APD shortening. At the beginning of each action potential, there is little instantaneous I_{Ks} (arrow). This is because of the fact that, at slow pacing, there is sufficient time for most I_{Ks} channels to deactivate between beats. In contrast, during rapid pacing (Figure 6B; 500 ms) of the simulated cell, there is an increase in the instantaneous I_{Ks} (indicated by the arrow) that is due to incomplete deactivation of I_{Ks} between beats, which is apparent in control simulations, but markedly enhanced by PKA modulation due to the PKA-induced slowing of I_{Ks} deactivation. This effect, which becomes much larger at faster rates, is in addition to the PKA induced increase in activation rate and the PKA increase in maximal conductance (g_{KS}) and results in further AP shortening compared with slow heart rates. These simulations suggest a kinetic mechanism whereby initial AP shortening occurs, in part, because of faster activation of the channels in response to β-AR activation. This results in a faster rate and buildup of channels in the open state (slow deactivation) and more resultant shortening. Figure 6C, action potential adaptation curves simulated with (/H11001) and without (/H11002) /H9252-AR stimulation, confirms that the predicted effects of isoproterenol on APD_{90} are more pronounced at longer rather than shorter cycle lengths.

We next asked how selective disruption of I_{Ks} regulation via the β-AR signaling cascade might affect action potential waveforms. Figure 7 illustrates mechanisms by which cellular action potentials are able to successfully adapt to changes in pacing rate and the consequences of selective disruption of I_{Ks} phosphorylation that can occur in variants of LQT-S. As indicated in the figure, this disruption can have marked consequences, particularly during rapid stimulation. Figure
7A presents summary data reporting fidelity of action potential adaptation over a range of paced cycle lengths for three different simulated cellular conditions: pacing in the presence of isoproterenol (bottom row); pacing under isoproterenol-free (control) conditions (middle row); and pacing in the presence of isoproterenol but with \( I_{Ks} \) uncoupled from \( \beta \)-AR regulation (top row). The keys to the bar graph are illustrated by the computed action potentials in the subsequent panels. The white bars in Figure 7A represent cellular action potentials that adapt in a physiologically correct manner to increased heart rate (Figure 7B). Dysfunctional action potential adaptation that leads to action potential alternans (Figure 7C) and adaptation failure that leads to depolarization block of impulses (Figure 7D) is plotted as the black bars. The arrows indicate the current levels at time of action potential upstroke, the instantaneous current. C, Action potential adaptation curves in the absence (solid line) and presence (dashed line) of \( \beta \)-AR stimulation.

Interestingly, in the model, action potential alternans occur only when \( I_{Ks} \) is selectively disrupted (data not shown). Alternans are widely thought to act as an arrhythmogenic substrate that can promote the initiation of phase 2 reentry.

**Two LQT-5 Mutations: Effects on \( I_{Ks} \) and AP Adaptation and Duration**

We next investigated the effects of two previously described KCNE1 mutations (W87R and D76N) that are linked to LQT-5 and predispose carriers to tachyarrhythmias. The W87R mutation results in a reduction in overall current (50%) and a speeding of deactivation kinetics (3-fold faster in the mutant compared with WT) and consequently a positive shift in the voltage-dependence of activation, but not a disruption of PKA-mediated regulation. The D76N mutation also reduces the current and speeds channel deactivation but, in addition, renders the \( I_{Ks} \) channel insensitive to \( \beta \)-adrenergic–mediated PKA phosphorylation. Thus comparison of the effects of these two mutations on action potential morphology allows dissection of the role of altered \( I_{Ks} \) kinetics in the response to sympathetic stimulation. We studied the effects of these mutations in the modified LRd model by incorporating 50% mutant and 50% WT channels, to mimic heterozygous patients, and 100% mutant channels to simulate homozygous cases. We also made use of our ability to simulate targeted \( I_{Ks} \) regulation by PKA. Hence, in the simulations that follow, neither PKA-dependent regulation of \( I_{Cal} \) nor PLB is disrupted.
Figure 8 illustrates action potentials and action potential adaptation curves for WT as well as heterozygous and homozygous W87R mutant cells. Action potentials (top traces) and concomitant $I_{Ks}$ activity (bottom traces) are illustrated under basal conditions (solid curves, /\text{H11002}/) and simulated isoproterenol stimulation of /\text{H9252}-ARs (dashed curves, /\text{H11001}/). Basal cellular activity was computed at a 1-second cycle length, and isoproterenol-stimulated activity was computed at a 500-ms cycle length to simulate /\text{H9252}-AR mediated changes in heart rate. In the case of WT $I_{Ks}$ channels, there is a marked shortening of action potential duration, which is due, in part, to the enhanced $I_{Ks}$ activity that is revealed in the lower traces. This activity is reduced, almost in a gene dosage manner, in heterozygous W87R cells (middle panel) and homozygous W87R cells (right panel), and consequently, there is a graded impact on control of action potential duration (top row). Figure 8B, which plots computed APD$_{90}$ versus cycle length (APD adaptation curve) for each condition, reveals a similar graded effect on action potential adaptation over broad range of cycle lengths. At cycle lengths shorter than 250 ms, the computations predict that homozygous W87R cells fail to adapt resulting in depolarization block of impulses (see Figure 7D). However, our computations did not predict the development of alternans (a beat-to-beat alternation of APD in a long-short pattern) (see Figure 7C) for the W87R mutation regardless of cycle length. The simulations suggest that disrupting PKA-induced altered channel kinetics alone is not sufficient to induce alternans.

The effect of the D76N mutation on action potential duration is actually prolonged in the face of sympathetic stimulation (Figure 9A, right column). This is because of the marked reduction in $I_{Ks}$ that occurs due to mutation-induced changes in channel kinetics as well as the uncoupling of the response of the $I_{Ks}$ channels from PKA-mediated regulation. This occurs while PKA-mediated increases in $I_{CaL}$ are maintained. As was the case for the W87R mutation, at faster pacing rates, action potentials fail to shorten sufficiently (ie, disruption of AP adaptation). But in the case of the D76N mutation, this failure occurs for both heterozygous (at 250 ms) and homozygous (at 350 ms) cells. Importantly, in the case of homozygous D76N cells, reducing the basic cycle length to 317 ms, results in the development of alternans, a dynamic pattern widely associated with electrical instabilities that is a common precursor to the development of lethal reentrant arrhythmias Figure 7C.

**Discussion**

$I_{Ks}$, the slowly activating component of the delayed rectifier current, plays a major role in repolarization of cardiac action potential duration is actually prolonged in the face of sympathetic stimulation (Figure 9A, right column). This is because of the marked reduction in $I_{Ks}$ that occurs due to mutation-induced changes in channel kinetics as well as the uncoupling of the response of the $I_{Ks}$ channels from PKA-mediated regulation. This occurs while PKA-mediated increases in $I_{CaL}$ are maintained. As was the case for the W87R mutation, at faster pacing rates, action potentials fail to shorten sufficiently (ie, disruption of AP adaptation). But in the case of the D76N mutation, this failure occurs for both heterozygous (at 250 ms) and homozygous (at 350 ms) cells. Importantly, in the case of homozygous D76N cells, reducing the basic cycle length to 317 ms, results in the development of alternans, a dynamic pattern widely associated with electrical instabilities that is a common precursor to the development of lethal reentrant arrhythmias Figure 7C.
Mechanisms by which these changes alter APD have been experimental and theoretical approaches by reconstituting faster rates (500 ms) under conditions of heart rate. Both of these effects were shown to alter modulation of APD at different heart rates during (APD). Cardiac APs are shortened by sympathetic stimulation as a result of a PKA-dependent effect and an increase in the rate of activation of the channel, which is observed in the faster time-course of activation during depolarization and also as a leftward shift of the activation curve. The other major kinetic effect of PKA phosphorylation on Iks is a reduction in the channel deactivation rate, which reflects the speed that open channels move to nonconducting closed states. The kinetics of Iks deactivation is a particularly relevant parameter in that it can be responsible for beat-to-beat accumulation in Iks current. In guinea-pig ventricular myocytes, in the absence of PKA stimulation, fast pacing was suggested to produce an increase in Iks as a result of incomplete deactivation of the channels between beats. Confirming the results obtained in other studies, we have shown with our model that such a mechanism could indeed occur. At slower heart rates, there is no buildup of open channels because diastole is sufficiently long to allow for complete deactivation between heartbeats. As heart rate hastens and APDs and diastolic intervals become shorter, there is incomplete deactivation of Iks between beats. This results in an accumulation of channels in the open state during diastole. Although there is no current flowing during diastole due to a lack of driving force, the open channels conduct instantly during the next action potential upstroke. Channels build-up in the open state, and Iks becomes larger with successive increases in heart rate, which causes APD shortening. This shortening is expected to be more pronounced when Iks is PKA phosphorylated because its deactivation rate is slowed: open channels take longer to close under β-adrenergic stimulation; there is a net increase in the channel open probability and a resultant stronger increase in current. Accumulation of Iks as a result of β-adrenergic stimulation has been suggested in canine ventricular myocytes but this phenomenon was not considered as significant at very fast heart rates. We have shown with our model that Iks accumulation caused by β-adrenergic stimulation is physiologically relevant at fast heart rates.

In congenital Long-QT (LQT) syndrome, sympathetic discharge as experienced during exercise or in response to

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**Figure 9.** Cellular effect of LQT-5 mutation D76N. A, Steady-state (100th) action potentials (top row) and corresponding Iks traces (bottom row) for WT (left), heterozygous (middle), and homozygous (right) D76N mutants. Cells were paced at slow rates (1000 ms) without stimulating β-ARs (−, solid lines), and at faster rates (500 ms) under conditions of β-AR stimulation (+, dashed lines). B, Mutations cause a dose-dependent change in the action potential adaptation curve (WT indicated by filled circles and solid line; heterozygous D76N, open squares and dashed line; homozygous D76N, open circles and dotted line). Heterozygous D76N cells are unable to adapt to the 200-ms stimulation rate, and homozygous D76N cells do not properly adapt at rates below 350 ms. Only rates where normal AP adaptation is observed are presented.

Potential (AP) and therefore in determining AP duration (APD). Cardiac APs are shortened by sympathetic stimulation as a result of a PKA-dependent effect and an increase in heart rate. Both of these effects were shown to alter Iks kinetics in separate studies. However, exactly how PKA phosphorylation affects the kinetics of Iks and the mechanisms by which these changes alter APD have been incompletely understood.

In the present study, we investigated the contribution of Iks to modulation of APD at different heart rates during β-adrenergic stimulation. We developed and used a combined experimental and theoretical approach by reconstituting Iks in CHO cells and using these data to develop a simple computer model.

The model is based on the Hodgkin-Huxley formalism and was fit to experimental recordings that were then adjusted to body temperature for use in cellular simulations. In previous models established from recordings in guinea-pig ventricular myocytes, Iks was described by two activation gates that reproduced sigmoidal activation of the channel. Although reasonable fits of our recordings were obtained with two exponentials, Iks activation was better fit with three exponentials as suggested by the initial study of Iks in CHO cells. Hence, we incorporated three identical and independent activation gates in the channel model. As in previous models, the model did not include an inactivation gate because the Iks tail current lacks the “hook” characteristic of an inactivation process. Rather, the channels transition to nonconducting closed states via a deactivation transition. At all voltages throughout the physiological range, our model of Iks showed good agreement compared with experimental recordings in CHO cells.

We also developed a model of Iks kinetics in the presence of PKA stimulation, which resulted in two significant and discrete effects on channel kinetics as well as effects on maximal conductance. We incorporated this model of Iks regulation into a model cell in which IcaL and PLB were PKA-regulated according to the model of Saucerman et al. The model provided insight into key roles of Iks kinetics in PKA-dependent changes in rate-dependent control of APD. PKA phosphorylation of Iks resulted in an increase in the rate of activation of the channel, which is observed in the faster time-course of activation during depolarization and also as a leftward shift of the activation curve. The other major kinetic effect of PKA phosphorylation on Iks is a reduction in the channel deactivation rate, which reflects the speed that open channels move to nonconducting closed states. The kinetics of Iks deactivation is a particularly relevant parameter in that it can be responsible for beat-to-beat accumulation in Iks current. In guinea-pig ventricular myocytes, in the absence of PKA stimulation, fast pacing was suggested to produce an increase in Iks as a result of incomplete deactivation of the channels between beats. Confirming the results obtained in other studies, we have shown with our model that such a mechanism could indeed occur. At slower heart rates, there is no buildup of open channels because diastole is sufficiently long to allow for complete deactivation between heartbeats. As heart rate hastens and APDs and diastolic intervals become shorter, there is incomplete deactivation of Iks between beats. This results in an accumulation of channels in the open state during diastole. Although there is no current flowing during diastole due to a lack of driving force, the open channels conduct instantly during the next action potential upstroke. Channels build-up in the open state, and Iks becomes larger with successive increases in heart rate, which causes APD shortening. This shortening is expected to be more pronounced when Iks is PKA phosphorylated because its deactivation rate is slowed: open channels take longer to close under β-adrenergic stimulation; there is a net increase in the channel open probability and a resultant stronger increase in current. Accumulation of Iks as a result of β-adrenergic stimulation has been suggested in canine ventricular myocytes but this phenomenon was not considered as significant at very fast heart rates. We have shown with our model that Iks accumulation caused by β-adrenergic stimulation is physiologically relevant at fast heart rates.

In congenital Long-QT (LQT) syndrome, sympathetic discharge as experienced during exercise or in response to
auditory input increases the susceptibility of mutation carriers to tachyarrhythmias that precede sudden cardiac death.\textsuperscript{27–29} The mutated genes have been identified and were found to code for KCNQ1 (LQT-1) and KCNE1 (LQT-5), which constitute $I_{\text{Ks}}$.\textsuperscript{3,4} Sympathetic regulation of $I_{\text{Ks}}$ is disrupted in some of these LQT mutants.\textsuperscript{5,6} We investigated the consequences of such a disruption in action potential model simulations. The model showed that, as might have been expected, when $I_{\text{Ks}}$ PKA-modulation is intact, the range of cycle lengths over which action potentials adapt properly to altered heart rate is extended (Figure 7A). At faster rates, $I_{\text{Ks}}$ deactivates rapidly in the “nonresponsive $I_{\text{Ks}}$” cell compared with the “wild-type” cell, preventing buildup of channels in the open state between beats. This results in a reduced APD adaptation to fast heart rate: action potentials fail to repolarize fully between beats and a window of cycle lengths for which APD alternans is predicted (Figure 7A).

Finally, we investigated the effects of specific mutations (W87R and D76N in KCNE1) on action potential adaptation. The W87R mutation results in a reduction in overall current and a speeding of deactivation kinetics. The D76N mutation also reduces current, speeds channel deactivation, but also renders the $I_{\text{Ks}}$ channel functionally insensitive to $\beta$-adrenergic–mediated PKA phosphorylation. In both cases, our computations revealed that the mutations result in reduced adaptation to increases in pacing rate. The D76N mutation is more severe, as simulated cells heterozygous for the mutation are extremely slow to adapt. At slow pacing rates, the mutations result in APD prolongation, the extent of prolongation is largest with the D76N mutation; and in the case of homozygous D76N cells, APD alternans is predicted. Because the parameters of the Luo-Rudy cellular model are based on data for guinea pig ventricle, it is worth noting that both heterozygous and homozygous D76N mutations are predicted to be proarrhythmic within the physiological parameters of guinea pig heart as they fail to adapt to cycle lengths less than 206 and 317 ms, respectively, whereas $\beta$-adrenergic stimulated guinea pig hearts have cycle lengths averaging 170 ms.\textsuperscript{30}

In this study, we have investigated the role of $\beta$-adrenergic-mediated PKA phosphorylation of the $I_{\text{Ks}}$ channel in the context of mutations that underlie LQT-5. One of the benefits of computational modeling is that we can separate complicated physiological processes to investigate the effects of single interventions, in this case, PKA effects on $I_{\text{Ks}}$. In our simulations of the mutations, we find that the model suggests an increased propensity to mutation carriers at fast rates and prolongation of the QT interval at all heart rates, as observed clinically. Interestingly, the cellular phenotype and cellular action potential adaptation result from complex nonlinear interactions between multiple ionic components, which we can identify because the model is a simplification of the actual processes involved. This is also the weakness of this approach, and leads us to interpret the model results with some degree of caution and also to suggest experimental follow-ups for verification of our computed findings.

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Supplemental Figure: Computational fit of experimental data

Current-voltage curves for L-type calcium channels in either the presence (filled circles) or absence (open squares) of cAMP and okadaic acid (OA). Simulated curves are overlayed. Computed curves were fit to reproduce the shift in the curve peak, as well as the increase in peak amplitude. All currents are normalized to the peak of the non-stimulated experimental curve.
Equations and Constants

1. $I_{Ks}$ Regulation Module

$$I_{Ks, PHOSPH} = \frac{k_{cat, PKA-iks} \cdot [PKAC] \cdot I_{Ks, np}}{K_{m, PKA-iks} + I_{Ks, np}}$$

$$I_{Ks, DEPHOSPH} = \frac{k_{cat, PP1-iks} \cdot [PP1] \cdot I_{Ks, p}}{K_{m, PP1-iks, p} + I_{Ks, p}}$$

$$\frac{dl_{Ks, np}}{dt} = I_{Ks, DEPHOSPH} - I_{Ks, PHOSPH}$$

$$\frac{dl_{Ks, p}}{dt} = I_{Ks, PHOSPH} - I_{Ks, DEPHOSPH}$$

$$\text{Frac}_{IKs,p} = \frac{l_{Ks, p}}{l_{Ks, p} + l_{Ks, np}}$$

2. $I_{Ks}$ Current

1. Non-phosphorylated Current

$$g_{ks} = 0.433 \cdot (1 + \frac{0.68}{1 + (0.000038 / [Ca]_t)^{1.4}})$$

$$E_{Ks} = \frac{RT}{F} \cdot \log \left( \frac{[K]_0 + 0.01833 \cdot [Na]_i}{[K]_f + 0.01833 \cdot [Na]_i} \right)$$

$$\alpha_x = 2.6 \cdot \frac{7.19 \times 10^{-5} \cdot (V_m + 60)}{1 - e^{-0.148 \cdot (V_m + 60)}}$$

$$\beta_x = 1.5 \cdot \frac{1.3 \times 10^{-4} \cdot (V_m + 30)}{e^{0.0487 \cdot (V_m + 30) - 1}}$$

$$x_{a_{\infty}} = \frac{\alpha_x}{\alpha_x + \beta_x}$$

$$\tau_{xa} = \frac{12}{\alpha_x + \beta_x}$$

$$\frac{dx_{a}}{dt} = Q_{10,xa} \cdot \frac{(x_{a_{\infty}} - xa)}{\tau_{xa}} \quad Q_{10,xa} = 2.57$$

$$I_{Ks,mut} = g_{Ks,mut} \cdot xa_{\text{mut}}^3 \cdot (V_m - E_{Ks})$$

2. Phosphorylated Current

$$g_{ks}^* = g_{ks} \cdot 1.3$$

$$\alpha_x^* = 5.2 \cdot \frac{6.5 \times 10^{-5} \cdot (V_m + 60)}{1 - e^{-0.096 \cdot (V_m + 60)}}$$

$$\beta_x^* = 1.75 \cdot \frac{1.2 \times 10^{-4} \cdot (V_m + 30)}{e^{0.0487 \cdot (V_m + 30) - 1}}$$

$$x_{a_{\infty}} = \frac{\alpha_x}{\alpha_x^* + \beta_x}$$

$$\tau_{xa} = \frac{12}{\alpha_x^* + \beta_x}$$

$$\frac{dx_{a}}{dt} = Q_{10,xa} \cdot \frac{(x_{a_{\infty}} - xa)}{\tau_{xa}} \quad Q_{10,xa} = 2.57$$

$$I_{Ks} = g_{Ks} \cdot x_{a}^3 \cdot (V_m - E_{Ks})$$

3. Mutant Non-phosphorylated Current

$$g_{ks,mut} = g_{ks} \cdot 0.5$$

$$\beta_{x,mut} = \beta_x \cdot 3$$

$$I_{Ks,mut} = g_{Ks,mut} \cdot x_{a_{\text{mut}}}^3 \cdot (V_m - E_{Ks})$$

4. Mutant Phosphorylated Current

$$g_{ks}^* = g_{ks,mut} \cdot 1.3$$

$$\beta_{x,mut}^* = \beta_x^* \cdot 3$$

$$I_{Ks,mut}^* = g_{Ks,mut}^* \cdot x_{a_{\text{mut}}}^3 \cdot (V_m - E_{Ks})$$

5. Fraction Phosphorylated Current

$$n = \text{Frac}_{IKs,p}$$

$$m = 0$$

When calculating W87R Mutant:

$$n_{mut} = \text{Frac}_{IKs,p}$$

$$m = \text{Frac}_{W87R}$$

When calculating D76N Mutant:

$$n_{mut} = 0$$

$$m = \text{Frac}_{D76N}$$

6. General Formula for $I_{Ks}$

$$I_{Ks} = (1 - m) \cdot (I_{Ks}^0 (1 - n) + I_{Ks}^* (n)) + m \cdot (I_{Ks,mut}^0 (1 - n_{mut}) + I_{Ks,mut}^* (n_{mut}))$$
III. $I_{Ca,L}$ Current

1. Non-phosphorylated Current

$$d_{\infty,v} = -9$$
$$d_{\infty} = \frac{1}{1 + e^{(d_{\infty,v} - V_m) / 5.8}}$$
$$\tau_d = 2 + 2.7 \cdot e^{-((V_m + 35) / 30)^2}$$
$$f_{\infty} = \frac{1}{1 + e^{(V_m + 27.4) / 7.1}} + \frac{0.4}{1 + e^{(50 - V_m) / 20}}$$
$$\tau_f = 10 + 161 \cdot e^{-((V_m + 40) / 14.2)^2}$$
$$\tau_{f2} = 62.6 + 1332.3 \cdot e^{-((V_m + 40) / 14.2)^2}$$
$$f_{Ca} = \frac{[Ca]_d}{[Ca]_d + k_{Ca}}$$

$$\frac{dd}{dt} = \frac{(d_{\infty} - d)}{\tau_d}$$
$$\frac{df}{dt} = \frac{(f_{\infty} - f)}{\tau_f}$$
$$\frac{df2}{dt} = \frac{(f_{\infty} - f2)}{\tau_{f2}}$$

$$E_{Ca} = \frac{RT}{2F} \cdot \log \left( \frac{[Ca]_o}{[Ca]_i} \right)$$

$$g_{Ca} = 0.759 \cdot (1 + 1.429 \cdot \text{Frac}_{Ca,L,\alpha \rho})$$

$$I_{Ca,L}^0 = g_{Ca} \cdot d \cdot (f_{Ca} \cdot f + ((1 - f_{Ca}) \cdot f2)) \cdot (V_m - E_{Ca})$$

2. Phosphorylated Current

$$d_{\infty,v}^* = -19$$
$$d_{\infty}^* = \frac{1}{1 + e^{(d_{\infty,v}^* - V_m) / 5.8}}$$
$$\tau_d^* = 2 + 2.7 \cdot e^{-((V_m + 35) / 30)^2}$$
$$f_{\infty}^* = \frac{1}{(V_m + 27.4) / 7.1 + 0.4} \cdot \frac{(50 - V_m) / 20}{1 + e^{(V_m + 40) / 14.2)^2}$$

$$\tau_{f2}^* = 62.6 + 1332.3 \cdot e^{-((V_m + 40) / 14.2)^2}$$

$$f_{Ca}^* = \frac{[Ca]_d}{[Ca]_d + k_{Ca}}$$

$$\frac{dd^*}{dt} = \frac{(d_{\infty}^* - d^*)}{\tau_d^*}$$
$$\frac{df^*}{dt} = \frac{(f_{\infty}^* - f^*)}{\tau_f^*}$$
$$\frac{df2^*}{dt} = \frac{(f_{\infty}^* - f2^*)}{\tau_{f2}^*}$$

$$I_{Ca,L}^* = g_{Ca} \cdot d^* \cdot (f_{Ca}^* \cdot f^* + ((1 - f_{Ca}^*) \cdot f2^*)) \cdot (V_m - E_{Ca})$$

3. General Formula for $I_{Ca,L}$

$$n = \text{Frac}_{Ca,L,\alpha \rho}$$

$$I_{Ca,L} = I_{Ca,L}^0 (1-n) + I_{Ca,L}^* (n)$$

IV. Constant Definitions

$$k_{cat, \text{PKA-IKs}} = 54 \times 10^{-3} \text{ ms}^{-1}$$
$$K_m, \text{PKA-IKs} = 21 \ \mu\text{M}$$
$$k_{cat, \text{PP1-IKs}} = 8.52 \times 10^{-3} \text{ ms}^{-1}$$
$$K_m, \text{PP1-IKs,\beta} = 7 \ \mu\text{M}$$
$$k_{Ca} = 0.25 \ \text{mM}$$
$$[Ca]_d = 7.2495 \times 10^{-5} \text{ mM}$$
$$R = 8314 \text{ J} \cdot \text{kmol}^{-1} \cdot \text{K}^{-1}$$
$$T = 310 \text{ K}$$
$$F = 96485 \text{ C} \cdot \text{mol}^{-1}$$
V. Variable Descriptions

\( I_{Ks, \text{PHOSPH}} \)  
Phosphorylation of \( I_{Ks} \) for a given time step (\( \mu M \cdot ms^{-1} \))

\( I_{Ks, \text{DEPHOSPH}} \)  
Dephosphorylation of \( I_{Ks} \) for a given time step (\( \mu M \cdot ms^{-1} \))

\( k_{\text{cat}, \text{PKA-IKs}} \)  
Rate of catalysis for PKA phosphorylation of \( I_{Ks} \) (\( ms^{-1} \))

\( k_{\text{cat}, \text{PP1-IKs}} \)  
Rate of catalysis for PP1 dephosphorylation of \( I_{Ks} \) (\( ms^{-1} \))

\( K_{m, \text{PKA-IKs}} \)  
Binding constant for PKA to non-phosphorylated \( I_{Ks} \) (\( \mu M \))

\( K_{m, \text{PP1-IKs,p}} \)  
Binding constant for PP1 to phosphorylated \( I_{Ks} \) (\( \mu M \))

\([\text{PKAC}]\)  
Concentration of available, active PKA catalytic subunits (\( \mu M \))

\([\text{PP1}]\)  
Concentration of available, active PP1 (\( \mu M \))

\( I_{Ks,p} \)  
Concentration of phosphorylated \( I_{Ks} \) channels (\( \mu M \))

\( I_{Ks,np} \)  
Concentration of non-phosphorylated \( I_{Ks} \) channels (\( \mu M \))

\( I_{Ks,p,\text{tot}} \)  
Total fraction of \( I_{Ks} \) channels which show phosphorylation-dependent changes (\( \mu M \))

\( g_{ks} \)  
Conductance of the \( I_{Ks} \) channel (\( mS \cdot \mu F^{-1} \))

\( \text{Frac}_{I_{Ks,p}} \)  
Fraction of \( I_{Ks} \) channels that are phosphorylated

\( \text{Frac}_{D76N} \)  
Fraction of \( I_{Ks} \) channels that contain the KCNE1 D76N mutation [0, 0.5, 1]

\( \text{Frac}_{W87R} \)  
Fraction of \( I_{Ks} \) channels that contain the KCNE1 W87R mutation [0, 0.5, 1]

\( \text{Frac}_{I_{Ca,L,\alpha p}} \)  
Fraction of \( I_{Ca,L} \) \( \alpha \)-subunits that are phosphorylated

\( \text{Frac}_{I_{Ca,L,\beta p}} \)  
Fraction of \( I_{Ca,L} \) \( \beta \)-subunits that are phosphorylated

\( d_{x,v} \)  
Voltage where the \( d_{x} \) curve evaluates to 0.5; the \( V_{1/2} \) of activation for \( I_{Ca,L} \) (mV)

\( g_{cal} \)  
Conductance of the \( I_{CaL} \) channel (\( mS \cdot \mu F^{-1} \))

VI. Initial Starting Conditions

\( I_{Ks,p} \)  
4.8425\times10^{-3} \( \mu M \)

\( I_{Ks,np} \)  
3.24075\times10^{-2} \( \mu M \)

\( x_{a} \)  
0.0250726

\( d \)  
3.09595\times10^{-6}

\( f \)  
0.999683

\( f2 \)  
0.999683