Subunit Interaction Determines $I_{Ks}$ Participation in Cardiac Repolarization and Repolarization Reserve

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Background—The role of $I_{Ks}$, the slow delayed rectifier $K^+$ current, in cardiac ventricular repolarization has been a subject of debate.

Methods and Results—We develop a detailed Markov model of $I_{Ks}$ and its $\alpha$-subunit KCNQ1 and examine their kinetic properties during the cardiac ventricular action potential at different rates. We observe that interaction between KCNQ1 and KCNE1 (the $\beta$-subunit) confers kinetic properties on $I_{Ks}$ that make it suitable for participation in action potential repolarization and its adaptation to rate changes; in particular, the channel develops an available reserve of closed states near the open state that can open rapidly on demand.

Conclusions—Because of its ability to form an available reserve, $I_{Ks}$ can function as a repolarization reserve when $I_{Ks}$, the rapid delayed rectifier, is reduced by disease or drug and can prevent excessive action potential prolongation and development of arrhythmogenic early afterdepolarizations. (Circulation. 2005;112:1384-1391.)

Key Words: action potentials ■ electrophysiology ■ ion channels

Mutations to the cardiac potassium channel gene KCNQ1 (KvLQT1) have been linked to the long QT syndrome LQT1, which predisposes patients to arrhythmia during exercise and emotional stress, conditions that involve high levels of $\beta$-adrenergic stimulation. KCNQ1 is a 6-transmembrane domain protein that can form functional homomeric potassium channels and can also coassemble with the single transmembrane domain protein KCNE1 (MinK).1 Together these gene products reconstitute the $I_{Ks}$ channel. Mutations to KCNE1 have also been linked to LQT (LQT5).2 In addition, transmural heterogeneity of $I_{Ks}$ expression in ventricular myocardium gives rise to mid-myocardial cells (M cells) with a longer action potential (AP) duration (APD) and greater APD rate adaptation than epicardial or endocardial cells in many species.3 $I_{Ks}$ is also augmented by $\beta$-adrenergic stimulation,4 suggesting its important role in mediating cardiac electrophysiological response.

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These findings suggest that $I_{Ks}$ is important for AP repolarization and APD adaptation to changes in rate, as demonstrated in guinea pig.5–7 However, $I_{Ks}$ density has been reported to be much lower in larger mammals, specifically in canine and human ventricle.8 In canine myocytes, L-type calcium current, an inward depolarizing current, has been shown to mediate APD rate adaptation under control conditions without $\beta$-adrenergic effects.7 However, AP repolarization requires a sufficient outward repolarizing current during phases 2 and 3 of the AP. Such current is carried by $I_{Ks}$ (the rapid delayed rectifier) and $I_{Ks}$, with $I_{Ks}$ playing a primary role in large mammals under normal physiological conditions and in the absence of $\beta$-adrenergic stimulation. Because phases 2 and 3 depend on a delicate balance between inward and outward currents, one cannot rule out a priori an important role for $I_{Ks}$. Moreover, the arrhythmic consequences of LQT1 and LQT5 mutations, the existence of only 2 repolarizing currents ($I_{Ks}$ and $I_{Ks}$) that constitute the delayed rectifier, and incorporation of the $\beta$-adrenergic signaling molecules into the $I_{Ks}$ channel complex strongly suggest an important role for $I_{Ks}$ in human heart electrophysiology under various conditions. It has been hypothesized that in large mammals $I_{Ks}$ constitutes a “repolarization reserve” (RR) that compensates for reductions in other repolarizing currents, in particular $I_{Ks}$, caused by mutations (hereditary LQT2) or drugs (acquired LQT1 syndrome).9,10 There is growing consensus that in the absence of RR, certain drugs such as sotalol (antiarrhythmic), erythromycin (anti-infective), chlorpromazine (antipsychotic), and methadone can trigger a life-threatening arrhythmia.9 The possibility of $I_{Ks}$ generating this reserve and the dependence of $I_{Ks}$ participation on its kinetics remain to be elucidated.

In the present study we examine the hypothesis that $I_{Ks}$ can participate in AP repolarization because of kinetic properties conferred by interaction between its KCNQ1 and KCNE1 subunits. We present detailed, experimentally based Markov...
models of KCNQ1 and $I_{Ks}$ and examine their kinetic behavior during the AP at slow and fast rates. By comparing KCNQ1 behavior with $I_{Ks}$, we isolate the effect of the modulatory KCNE1 subunit on the AP. Results show that because of its kinetic properties, $I_{Ks}$ can create an available reserve (AR) of channels at fast rates that can open and generate a larger repolarizing current. In the presence of $I_{Ks}$ block, this AR prevents excessive AP prolongation and the formation of arrhythmogenic early afterdepolarizations (EADs). Such properties are not present in homomeric KCNQ1 channels and therefore require interaction with KCNE1. The AR concept relates to reserve within a single channel, thereby extending the RR concept that involves compensation for one repolarizing current by another.

Methods
Markov models of KCNQ1 and $I_{Ks}$ are derived from experimental data and published K$^+$ channel models. Koren et al described the K$^+$ delayed rectifier RCK1 with a Markov model of 4 independent voltage sensor transitions and 1 cooperative voltage-independent transition before the open state. Zagotta et al expanded this model to study Shaker K$^+$ channels by assuming that each voltage sensor undergoes 2 conformational changes before channel opening and successfully reproduced delayed activation (sigmoidal activation). A delay of several milliseconds has also been observed for KCNQ1 and $I_{Ks}$ activation, suggesting that at least 2 voltage sensor transitions occur before channel opening. Experiments (J. Cui, PhD, personal communication, November 2004) also suggest a voltage-independent transition immediately before the first $I_{Ks}$ open state, as proposed by Koren et al for RCK1.

The Markov schemes we developed for KCNQ1 and $I_{Ks}$ are shown in Figure 1A and 1B. Two closed-state zones are shown; green represents states in which at least 1 voltage sensor must still make a first transition, and blue represents states in which only faster second transitions are necessary for opening. Model derivation is described in the online-only Data Supplement. Criteria for fitting parameters are described below.

The KCNQ1 model is based on frog oocyte recordings (Figure 2). Simulated activation to various potentials (Figure 2A), inactivation measured by a triple-pulse protocol (Figure 2C), and dependence of deactivation time constant on prepulse duration (Figure 2E) were included in the fitting procedure (details are in the online-only Data Supplement). The reversal potential was $-50$ mV on the basis of the current-voltage relationship that shows no activation below $-10$ mV (Figure 2B). Therefore, instantaneous current (possibly due to leakage) was subtracted. Current tracings were normalized according to the current-voltage relationship curve so that the steady state value for each tracing would match the experimental average. Deactivation at $-50$ mV was fit directly to published data. Rate of deactivation at resting $V_m$ and current accumulation at fast pacing rates were simulated with $I_{Ks}$ inserted in the whole-cell model (see below); comparison with experimental data is shown in Figure 2B. Additionally, mean current increase at fast rates and dynamic conductance (corresponding to APs and $I_{Ks}$ simulated in Figure 4) were compared with recordings from AP clamp (Figure 5). A previously developed formulation of the reversal potential that accounts for sodium conductance through $I_{Ks}$ channels was used (ratio of Na$^+$:K$^+$ permeability of 0.01833).

Human $I_{Ks}$ activation kinetics (Figure 3C) were fit to data from Kupershmidt et al, who recorded expressed human $I_{Ks}$ in HEK cells at $37^\circ$C. [Na$^+$]o was 145 mmol/L, and [K$^+$]o was 4 mmol/L. Expressed currents were fit because they display activation kinetics more clearly than currents recorded from native cells while still displaying activation at $\sim 10$ mV and a delay before slow activation, as in native cells. Deactivation was constrained by time constants measured in human ventricular myocytes at $37^\circ$C for [Na$^+$]o = 144 mmol/L and [K$^+$]o = 4 mmol/L to ensure that open-state accumulation was not overestimated. Reversal potential was calculated as for guinea pig $I_{Ks}$. Comparisons with experimental data are shown in Figure 3D.

Markov models for $I_{Ks}$, $I_{Ko}$ (updated version), and $I_{Ks}$ were inserted in the Luo-Rudy (LRd) model of the guinea pig ventricular cell. AP simulation conditions are discussed in the online-only Data Supplement.

Results
KCNQ1 Model Validation and Current Properties
The KCNQ1 model (Figure 1A) was optimized to reproduce experimental protocols designed to isolate the essential channel features. Sigmoidal activation is reproduced with multiple closed-state transitions before opening. This allows for minimal initial current followed by a steep rise after $\sim 5$ ms, as observed experimentally (Figure 2A). Two time constants of activation (fast followed by slow) are evident, with a slow rise in current observed even at 2-second depolarization, the last time point for which the current-voltage relationship is experimentally measured (Figure 2A, 2B).

Figure 1. KCNQ1 and $I_{Ks}$ Markov models. A, Model of KCNQ1 contains 15 closed (C1 to C15) states to account for 2 transitions of each of the 4 voltage sensors before channel opening. Green closed states represent channels in zone 2 that have not completed the first transition for all 4 channel subunits. Blue closed states represent channels in zone 1 that have completed 4 first voltage sensor transitions. The boxed transition (f) to the open state is voltage independent. There are 5 open states (O1 to O5, red) and an inactivation state (l, purple). B, Model of $I_{Ks}$ contains 2 open states with no inactivation.
Next, the triple-pulse protocol (Figure 2C, 2D), which measures channel inactivation at different potentials, is simulated. A short hyperpolarizing pulse to $-130$ mV allows most channels to recover from inactivation while preventing significant deactivation (Figure 2C). In the model, 5 open states allow channels to occupy open states far from the closed state ($O_4$ and $O_5$, Figure 2C), which delays deactivation during the hyperpolarizing pulse. However, transitions toward the closed state are rapid enough to reproduce the measured time constant of deactivation at $-70$ mV (Figure 2A; simulated $\tau_{\text{deact}} = 377$ ms, measured $\tau_{\text{deact}} = 348$ ms). Peak current and time constant of inactivation during the following (third) pulse are consistent with experimental results\textsuperscript{13} (Figure 2D), demonstrating rapid and voltage-dependent inactivation.

Finally, the dependence of deactivation time constant and relative inactivation (defined in the online-only Data Supplement) on pulse duration are simulated. KCNQ1 deactivation rate varies with pulse duration. As channels enter open states that are farther from the closed states, the rate of entry back into the closed states ($\tau_{\text{deact}}$) is slowed (Figure 2F). $\tau_{\text{deact}}$ is greater than estimated experimentally from the dependence of deactivation on pulse duration (Figure 2E inset). However, a different experimental protocol (voltage dependence of deactivation) in the same study\textsuperscript{13} estimates $\tau_{\text{deact}}$ close to the model-determined value at $-60$ mV (502±27 ms for experiment; 594 ms for model) with the same protocol. The different $\tau_{\text{deact}}$ from the 2 experimental protocols may be explained by the need to compensate for the inactivation hook in the pulse-duration protocol (Figure 2E inset) when fitting an exponential, a procedure that can have a major effect on the estimate of $\tau_{\text{deact}}$. In the model such correction is not necessary because deactivation is measured directly as the decay of occupancy in the open and inactivated states.

In contrast to rapid inactivation during the third pulse (above), a single depolarizing pulse from $-80$ mV to $20$ mV results in slow onset of inactivation ($>200$ ms pulse duration needed to elicit hook in tail current; Figure 2E) because of multiple transitions through open states. These transitions also govern relative inactivation (protocol not included in optimization), which shows an experimentally observed 75-ms delay\textsuperscript{13} (Figure 2F).

$I_{Ks}$ Model Validation and Current Properties
The $I_{Ks}$ model resembles the KCNQ1 model closely, with the number of open-state transitions truncated and inactivation...
removed (Figure 1). Significant differences are introduced by changes in the transition rates. For example, increased KCNE1 mRNA in frog oocytes results in greater delay before activation. This subunit effect is simulated by decreasing the transition rate from the first (resting) voltage sensor position in the heteromeric guinea pig $I_{Ks}$ channel to half the homomeric channel rate, confining more channels to zone 2 (Figure 3A, 3C insets). Once channels transition out of this zone, they activate rapidly. However, some channels remain in zone 2, causing a slow rise in current that continues even after several seconds (Figure 3A).

The first voltage sensor position is more stable for human $I_{Ks}$ than KCNQ1 (C1 occupancy is 9 times greater at -80 mV), resulting in slower activation and a continuous current increase even after a 5-second pulse to 60 mV. Once channels transition into O1, they rapidly enter O2 (at 40 mV, the rate into O2 is 6.6 times faster than C1 to O1). The presence of 2 open states allows for simultaneous reproduction of fast activation, steady state current-voltage relationship, and slow deactivation by facilitating slow deactivation without requiring slow activation to reproduce the steady state current.

$I_{Ks}$ Role in Rate Adaptation of APD
Simulated whole-cell APs computed with the guinea pig $I_{Ks}$ Markov model are shown at fast and slow pacing rates (Figure 4A). At the fast rate, peak $I_{Ks}$ increases moderately compared with the slow rate (Figure 4B); however, the more rapid rise of $I_{Ks}$ at the fast rate leads to greater repolarizing...
Dynamic Conductance

Figure 5. Dynamic guinea pig $I_{Ks}$ conductance during AP clamp. Bottom, Experimentally measured $I_{Ks}$ conductance (Chromanol 293B-sensitive current, $g_{293B}$) at fast (CL=250 ms) and slow rates (CL=1000 ms) (thick and thin lines are mean and confidence limits, respectively). Arrows indicate open-state occupancy at AP outset. Reprinted from reference 16 with permission from Blackwell Publishing. Top, Simulation also shows little open-state accumulation (arrow) even at fast rates; instead, in both simulation and experiment an increase in $I_{Ks}$ activation rate during fast pacing is responsible for greater $I_{Ks}$ during the AP.

current earlier during the AP. This earlier $I_{Ks}$ rise causes a 19.7% increase in mean $I_{Ks}$ during the AP, from 1.27 μA/μF at cycle length (CL)=1000 ms to 1.52 μA/μF at CL=250 ms. This is a conservative value compared with AP clamp experiments that measured a 28% increase at these rates.16 The $I_{Ks}$ increase during the AP accelerates repolarization to cause APD shortening at fast rates (adaptation).

$I_{Ks}$ mediation of adaptation is a result of its closed-state transitions rather than its open-state accumulation. Figure 4C and 4D show total occupancy in zone 2 (green), zone 1 (blue), and the open states $O_1$ and $O_2$ (red) (Figure 1). During slow pacing (Figure 4C), most $I_{Ks}$ channels reside in zone 2 before AP depolarization and must make a slow transition into zone 1 before opening. In contrast, at fast rates (Figure 4D) channels accumulate in zone 1 between APs, allowing $I_{Ks}$ to activate rapidly via the second transition. There is not sufficient time between beats for the channels to transition back to zone 2 before the next AP, as at slow rates. Although there is some open-state accumulation due to slow deactivation, its effect on the current is opposed by the lower driving force resulting from a lower peak membrane potential at fast rates. Consequently, the primary mechanism for adaptation is the accumulation of closed states in zone 1, where a fast transition to the open states generates a rapid $I_{Ks}$ rise during phase 1. Thus, at fast rates an AR is built ready to open very rapidly on demand. The overall low probability of channels residing in open states at slow and fast rates (Figure 4C, 4D) indicates that many channels are closed during the AP, creating a large $I_{Ks}$ reserve.

AP clamp experiments have measured $I_{Ks}$ conductance ($g_{Ks}$) during the AP at fast and slow rates, showing that open-state accumulation is minimal (Figure 5, bottom panel, arrows). Rather, $I_{Ks}$ activates more rapidly at a fast rate than at a slow rate, as also observed in our simulations (Figure 5, top panel). The faster activation at fast pacing in experiments can be explained by the accumulation in closed states near to the open state that are readily available to open at the fast rate, as simulated by the model (Figure 4). Thus, the experiments support the concept of AR as a mechanism for $I_{Ks}$ participation in repolarization and its dependence on rate.

Human $I_{Ks}$ activates more slowly than guinea pig $I_{Ks}$, and deactivates more rapidly (Figure 3C; compare with Figure 3A). The guinea pig cell provides a natural model for testing $I_{Ks}$ in an environment in which $I_{Ks}$ plays only a secondary role in repolarization. To evaluate the capability of human $I_{Ks}$ to serve as a RR and to compensate for reduced $I_{Ks}$, we inserted a model of human $I_{Ks}$ in the guinea pig LRd model (Figure 6). As can be seen in Figure 6F (solid line), with increased maximum conductance, human $I_{Ks}$ can generate APD adaptation ($g_{Ks}$ ~4 times greater than guinea pig to obtain 200 ms APD). In contrast, when homomeric KCNQ1 channels are inserted, there is less APD adaptation at fast rates (Figure 6F, dashed line). This attenuation of APD adaptation is surprising because the deactivation kinetics of KCNQ1 are slower than human $I_{Ks}$, allowing for open-state accumulation (Figure 6D).

However, $I_{Ks}$ channels accumulate in zone 1 (Figure 6E, black), which is composed of closed states that only require rapid transitions to open, allowing for rapid activation that leads to large current during the repolarization phase of the AP (Figure 6C, thin line). This large $I_{Ks}$ current during repolarization proves more important for shortening APD than the instantaneous current generated by KCNQ1. As shown earlier (Figure 4), zone 1 accumulation rather than open-state accumulation underlies the increase of $I_{Ks}$ at fast rates. Such AR closed-state accumulation in zone 1 does not occur in KCNQ1 channels (Figure 6E, gray). Instead, a large percentage of channels activate even at slow rates (open-state occupancy at CL=10,000 ms is 30% for KCNQ1 versus 7% for $I_{Ks}$), preventing an increase at fast rates.

$I_{Ks}$ and KCNQ1 During $I_{Kr}$ Block

Figure 7A shows mean $I_{Ks}$ (black) compared with KCNQ1 current (gray) during the first and 40th APs at CL=500 ms. $I_{Ks}$ increases ~50% from the first to the 40th AP, whereas KCNQ1 remains almost the same over 40 paced beats (inset shows current tracings for the first 9 beats). When $I_{Kr}$ is blocked, greater $I_{Ks}$ increase is observed over 40 paced beats (relative to control conditions), whereas only a limited increase is noted for KCNQ1. The difference between $I_{Ks}$ and KCNQ1 behavior in the presence of $I_{Kr}$ block is due to the nature of current accumulation. $I_{Ks}$ block prolongs the AP plateau and elevates its potential, thus enhancing transitions toward the open states. KCNQ1 accumulation is mostly in the
open state, which is at the expense of populating closed states that constitute the AR. Consequently, most channels open on every beat, allowing for only limited accumulation between beats. In contrast, \(I_{Ks}\) shows minimal open-state accumulation, and \(I_{Kr}\) block facilitates transitions into closed states that constitute the AR, allowing for greater \(I_{Ks}\) accumulation over many beats.

When a pause is simulated after 40 beats in the presence of \(I_{Kr}\) block (Figure 7B), the postpause AP with KCNQ1 develops an EAD as a consequence of insufficient KCNQ1 current late during the AP. In contrast, the AP with \(I_{Ks}\) repolarizes normally because of the superior ability of \(I_{Ks}\) to activate during phase 3 of a prolonged AP.

Discussion

We show that interaction of KCNQ1 with the \(\beta\)-subunit KCNE1 to form \(I_{Ks}\) alters kinetics so that an AR is created at fast pacing rates. In contrast, \(I_{Ks}\) shows minimal open-state accumulation, and \(I_{Ks}\) block facilitates transitions into closed states that constitute the AR, allowing for greater \(I_{Ks}\) accumulation over many beats.

A gapped double-pulse protocol with variation in the gap width could be used to characterize the AR in both guinea pig and human ventricular myocytes. In this protocol, a depolarizing pulse to 40 mV for 2 seconds would activate the channel. This step would then be followed by a repolarizing step of variable duration (from 10 ms to 1 second) to 80 mV so that the channel could partially deactivate. A second depolarizing pulse to 40 mV would be used to measure the rate of activation, providing a measure of the AR.

Our simulations also predict that \(I_{Ks}\) will increase more at fast rates when \(I_{Kr}\) is blocked (Figure 7), a manifestation of AR. This prediction could be experimentally tested by blocking \(I_{Kr}\) and measuring the increase in \(I_{Ks}\) during an AP clamp at slow and fast rates. Another protocol to test interaction between \(I_{Ks}\) and \(I_{Kr}\) would be to reduce \(I_{Ks}\) and measure the change in APD from control during pacing, and then to apply \(I_{Kr}\) reduction to control and block \(I_{Ks}\) to the same degree as in the previous protocol. Our simulations predict that the same \(I_{Ks}\) reduction will have a much greater effect on APD when \(I_{Kr}\) is also reduced.

At fast rates, the ability of guinea pig \(I_{Ks}\) to accumulate between APs allows it to participate in APD shortening. However, in human myocytes \(I_{Ks}\) deactivates rapidly, bringing...
ing into question its ability to accumulate and mediate APD adaptation. Our simulations show that open-state accumulation is not necessary for $I_{Ks}$ to increase at fast rates. Because of the complex nature of potassium channel activation, channels can accumulate in closed states near the open states (zone 1) to form an AR that opens rapidly and generates large $I_{Ks}$ current during AP repolarization. This effect is confirmed by AP clamp experiments that show little accumulation in the open state but rapid activation at fast rates (Figure 5) that leads to greater $I_{Ks}$ during the AP. In canine myocytes, $I_{Ks}$ accumulation is also observed in the presence of $\beta$-agonist. Here the mechanism of $I_{Ks}$ accumulation is identical for both human and guinea pig $I_{Ks}$, indicating a common mechanism for $I_{Ks}$ increase across species.

This novel mechanism of adaptation contrasts with the behavior of homomeric KCNQ1 channels that accumulate in the open states because of slow deactivation but show no significant AR. Consequently, the KCNQ1 AP does not adapt as much as the $I_{Ks}$ AP (Figure 6). The fundamental difference between $I_{Ks}$ and KCNQ1 channels is the degree of participation of the first voltage sensor transition in channel activation. Because of the stabilization of the voltage sensor first position by KCNE1, only a small fraction of $I_{Ks}$ channels activate at slow rates, creating a reserve of channels that can activate at fast rates to accelerate repolarization. In contrast, a large percentage of KCNQ1 channels activate even at slow rates, preventing buildup of such a reserve. As shown in Figure 6C, the initial jump (arrow) of $I_{Ks}$ current that reflects open-state accumulation at fast rates is small and is followed by a steady increase to a peak of 3.4 $\mu$A/$\mu$F late during the AP. At this phase of the AP, $I_{Ks}$ has a maximal effect on repolarization and APD. In contrast (Figure 6D), KCNQ1 current displays a large initial jump (arrow) due to large open-state accumulation but does not increase during the AP in the absence of an AR; its magnitude stays at $\approx$2 $\mu$A/$\mu$F and lacks the late peak that is important for repolarization. Thus, interaction with the KCNE1 subunit strongly influences the $I_{Ks}$ profile during the AP; it augments AP shortening and is essential to normal $I_{Ks}$ function and its participation in APD adaptation. Other differences between KCNQ1 and $I_{Ks}$, such as open-state behavior and flickery block, are discussed in the online-only Data Supplement.

As stated earlier in this report, $I_{Ks}$ is not likely to participate in rate adaptation in large mammals under control conditions. However, when $I_{Ks}$ is reduced, $I_{Ks}$ is the only remaining major repolarizing current. The guinea pig myocyte provides a natural electrophysiological environment in which $I_{Ks}$ is reduced. Under these conditions, we show that human $I_{Ks}$ can mediate rate adaptation when the maximum conductance of the current is increased (Figure 6). $\beta$-Agonists, which are present in the normal physiological environment even at basal heart rates, can readily confer such an increase in $I_{Ks}$ conductance.

In the case of pathologically reduced $I_{Ks}$ (by a mutation or a drug), outward currents carried by other channels (RR) can prevent excessive APD prolongation, EADs, and triggered activity. It is hypothesized that $I_{Ks}$ reduction in conjunction with a compromised RR is a precursor to arrhythmia, especially after a pause. To test the ability of $I_{Ks}$ to participate in the RR, we compared $I_{Ks}$ with KCNQ1 accumulation under control conditions and with $I_{Ks}$ block. During pacing, $I_{Ks}$ displays greater accumulation than KCNQ1 and increases further when $I_{Ks}$ is reduced (Figure 7A). When the pause protocol is simulated with blocked $I_{Ks}$, increased $I_{Ks}$ results in normal repolarization, whereas the postpause AP with KCNQ1 develops an EAD (Figure 7B). These simulations indicate that the ability of $I_{Ks}$ to form an AR, which leads to accumulation at fast rates, makes it an ideal candidate to play a critical role in RR. The accepted RR concept involves compensation for one repolarizing current by another. This study demonstrates the existence of a reserve (AR) within a single channel ($I_{Ks}$), thereby extending the RR concept to include single-current reserve.

We describe $I_{Ks}$ with 2 transitions, a slow transition (to zone 1) followed by a fast transition (to open), which implies
that a 2-closed-state model (rather than 15 states) representing lumped voltage sensor transitions could adequately describe $I_{\text{Ks}}$ activation. However, this model would not reproduce the experimentally observed delay before activation, which has important consequences during the AP. To reproduce these kinetics, a semi-Markov 2-closed-state model could be used that would introduce a memory property to the channel via time-dependent transition rates. Such models have been proposed but introduce another level of complexity with the addition of memory. The present model was chosen because of its correlation to the tetrameric symmetry of $K^+$ channels and the simplicity of 4 transitions without memory that describe $I_{\text{Ks}}$ activation. This detailed description of activation, in particular the delay before activation, is a channel feature that has not previously been incorporated into an $I_{\text{Ks}}$ model.

Model parameters were determined with the use of nonlinear optimization. Although we incorporated a large set of experiments, there may be different parameters that also reproduce channel properties. Our conclusions depend on the participation of numerous closed states in $I_{\text{Ks}}$ activation. The necessity of these states for generating a delay before activation has been rigorously documented. Thus, any model of this topology (Figure 1) that fits $I_{\text{Ks}}$ activation, regardless of the parameter set, would generate an AR and cause more adaptation at fast rates (see online-only Data Supplement).

Not all data were obtained from native myocytes. For KCNQ1, we used frog oocyte experiments conducted at room temperature, in which transition rates differ from body temperature. Still, KCNQ1 activation in mammalian cells (37°C) and in oocytes (25°C) is quite similar, with 2 time constants of activation and similar current-voltage relationships.

Future work should incorporate β-adrenergic modulation of $I_{\text{Ks}}$ and study its effects on whole-cell electrophysiological function. To accomplish this, a β-adrenergic model that simulates its effects on many cellular processes is necessary; these processes include sarcoplasmic reticulum calcium handling, $I_{\text{Ca,L}}$, the transient outward Cl− current ($I_{\text{to}}$), $I_{\text{NaCa}}$, $I_{\text{Ks}}$, and $I_{\text{K1}}$. To date there have been attempts to create such a model, but a sufficiently complete model that allows accurate study of AP dynamics in the context of β-adrenergic stimulation awaits development.

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References

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Appendix

Derivation of KCNQ1 and \(I_{Ks}\) Models

Activation

This section describes the rationale for the model structure; it follows model development for the Shaker \(K^+\) channel by Zagotta, Hoshi and Aldrich.\(^1\)

The transition between two energetic states of a channel can be modeled as following:

\[
R \xrightarrow{\alpha} A
\]

Assuming that the rates are exponentially dependent on voltage, they are described by these equations.

\[
\alpha = \alpha_0 \cdot \exp\left(z_\alpha \cdot \frac{V_m \cdot F}{R \cdot T}\right)
\]

\[
\beta = \beta_0 \cdot \exp\left(-z_\beta \cdot \frac{V_m \cdot F}{R \cdot T}\right)
\]

\(\alpha_0\) and \(\beta_0\) (ms) are the transition rates when \(V_m = 0\) mV.

\(z_\alpha\) and \(z_\beta\) (C) are the equivalent charge movements during the state transition.

\[
F = 96485 \text{ C mol}^{-1} \quad \text{(Faraday Constant)}
\]

\[
R = 8314 \text{ J kmol}^{-1} \cdot \text{K} \quad \text{(Gas Constant)}
\]

\[
T = 310 \text{ K} \quad \text{(Body Temperature)}
\]

The model describes two transitions for each of four voltage sensors. If a single transition for a single voltage sensor is \(R \xrightarrow{\alpha} A\), then the following describes two transitions for a single voltage sensor. \(R_1\) and \(R_2\) are resting positions; \(A\) is the activated position.

\[
R_1 \xrightarrow{\alpha} R_2 \xrightarrow{\gamma} A
\]
This presentation can be extended to four voltage sensors as in Zagotta et al (1). The channel is open when all four voltage sensors are in the activated position.

\[
R_1 \xrightarrow{a} R_2 \xrightarrow{\gamma \delta} A \quad \text{Open}
\]

Since each voltage sensor is assumed to move independently, the open probability is the occupancy in A to the fourth power (A^4). These transitions can also be described by a fifteen closed-state Markov model (for each permutation of voltage sensor positions described below), which should be used when there are transitions that depend on open state occupancy. The presence of transitions that depend on open-state occupancy makes the activation transitions dependent on each other, not allowing the simplification of A^4 that applies only to independent transitions. The Markov representation is used in the models of IKs and KCNQ1 and accounts for C_1 thru C_{15} as shown in Figure 1 of the paper.

Each closed state represents a permutation of voltage sensor positions. For example, C_1 is the energetic state where all four sensors are in R_1. The transition rate from C_1 to C_2, where one sensor is in R_2 and three sensors are in R_1, is 4·α since movement of any one of four sensors from R_1 to R_2 can change the channel state from C_1 to C_2. The transition from C_2 to C_6 has the rate γ because only one sensor can transition from R_2 to A. When all four sensors are in the activated position C_{15}, the channel is able to transition to the open state.

The large number of transitions before opening generates a delay before activation, while the symmetry of the model (four identical voltage sensors) requires only four transition rates to describe the process.

Koren et al 2 have described a voltage-independent transition before the open state for RCK1, as revealed by pulses to highly negative potentials that do not increase the rate of deactivation. Unpublished data (J. Cui) suggest that such a voltage-independent transition exists also for IKs and KCNQ1. The final model of activation is shown below. Experiments were best fit with a voltage-independent transition into the open state and a voltage-dependent transition in the reverse direction, from the open to closed state.

\[
R_1 \xrightarrow{a} R_2 \xrightarrow{\gamma \delta} A \quad \text{Open}
\]
Open State Transitions

KCNQ1 channels show a delay before inactivation as well as varying rates of deactivation that depend on pulse duration (Figure 2E and 2F in paper). Experiments show that the minimal number of open states is two, based on the delay and varying rates of deactivation \(^3,4\). However, to account quantitatively for deactivation rate as well as delay, five open states were necessary. These states could possibly account for four independent transitions of the channel subunits after the channel is open, as shown below. The transition rates are analogous to the activation transition rates.

\[
\begin{align*}
\text{Early open states} & \quad \{ O_a \xrightleftharpoons{\psi}{\omega} O_b \} \\
\text{Late open states} & \quad \{ O_a \xrightleftharpoons{\psi}{\omega} O_b \}
\end{align*}
\]

\[O_1 \xrightarrow{\frac{4\psi}{\omega}} O_2 \xrightarrow{\frac{3\psi}{2\omega}} O_3 \xrightarrow{\frac{2\psi}{3\omega}} O_4 \xrightarrow{\psi}{\omega} O_5\]

Even with the additional open states a sufficient delay was not achieved. To increase the delay, negative cooperativity was introduced in the model. This was accomplished, as in Zagotta, Hoshi and Aldrich \(^1\), by

\[
\begin{align*}
\psi & = \exp(z_\psi \cdot (z_\psi + z_\omega) \cdot \nu \cdot \frac{F}{RT}) \\
\omega & = \exp(z_\omega \cdot (z_\psi + z_\omega) \cdot \nu \cdot \frac{F}{RT})
\end{align*}
\]

\[O_1 \xrightarrow{\frac{4\psi \cdot \psi}{\omega \cdot \omega}} O_2 \xrightarrow{\frac{3\psi \cdot \psi}{2\omega \cdot \omega}} O_3 \xrightarrow{\frac{2\psi \cdot \psi}{3\omega \cdot \omega}} O_4 \xrightarrow{\frac{\psi \cdot \psi}{4\omega \cdot \omega}} O_5\]

In Figure 1 of the paper, these transitions are abbreviated as:

\[
\psi_n = \psi \cdot x_\psi^{n-1}, \quad \omega_n = \omega \cdot x_\omega^{n-1}
\]

The \(I_{Ks}\) model closely resembles the KCNQ1 model with fifteen closed states that are derived from the theory described above. However, \(I_{Ks}\) has fewer open state transitions (only two open states) and no inactivation. Experimental evidence, described below, provides some indication of the difference between KCNQ1 and \(I_{Ks}\) open state transitions and the nature of inactivation.

KCNQ1 open states show differential block depending on pulse duration when probed by sodium \(^5\), indicating earlier and later open states. This
differential block is not observed in heteromeric \( I_{Ks} \) channels, suggesting that multiple open states are not present. However, probing \( I_{Ks} \) channels with rubidium results in sigmoidal deactivation \(^6\), which indicates at least two open states. One way to reconcile these results is to assume that the transition from the first open state to the second open state is relatively rapid. Under these conditions, the first open state would not be detected by sodium block, because the transition would be too rapid to be seen experimentally; however, sigmoidal deactivation would still be possible. Our \( I_{Ks} \) model is derived from macroscopic data that indicate a faster transition into the open state and slower deactivation, and requires two open states to reproduce steady-state current. The transition rate between \( O_1 \) and \( O_2 \) in our model is relatively rapid \((\tau<<200 \text{ ms})\) and would not have been detected by the sodium block experiments. Thus the model is in agreement with the hypothesis put forward by Pusch et al \(^5\) to reconcile rubidium and sodium block experiments.

Pusch et al \(^6\) have also observed that a flickery block is responsible for KCNQ1 inactivation. This flickery block affects all channel states, but is facilitated when the channel enters a later open state (farther from the closed state; \(^7\). During a depolarizing pulse channels enter the later open state, allowing more inactivation \(^6,7\). Our model only allows inactivation from a single open state. Flickery block from other channel states is accounted for by introducing a lower macroscopic conductance in our simulations.
Fitting Model Parameters

Simulated KCNQ1 activation was compared to biexponential fits (equation below) to experimental current traces at different voltages provided by M. Sanguinetti.

\[ I_{\text{Activation}} = A_1 \cdot \exp\left(\frac{t}{\tau_1}\right) + A_2 \cdot \exp\left(\frac{t}{\tau_2}\right) \]

Where \( A_1 \) and \( A_2 \) are the amplitudes of the exponentials and \( \tau_1 \) and \( \tau_2 \) are the time constants for activation.

Channels were assumed to be closed at -80 mV; therefore instantaneous (leak) current was subtracted so that there was no current at time zero. Deactivation (\( I_{\text{Deactivation}} \)) at -70 mV was fit to data reconstructed from published mean values of steady-state inactivation, recovery from inactivation, activation and time constant of deactivation according to the following equation:

\[ I_{\text{Deactivation}} = \text{Activation}(V_m) \cdot \exp\left(-\frac{t}{\tau_{\text{Deactivation}}}\right) - \text{Inactivation}(V_m) \cdot \exp\left(-\frac{t}{\tau_{\text{Recovery}}}\right) \]

Where:
- Activation is the percentage of channels open at \( V_m \) (-70 mV).
- \( t \) is time (ms).
- \( \tau_{\text{Deactivation}} \) is the time constant of deactivation.
- Inactivation is the percentage of channels inactivated at \( V_m \) (-70 mV).
- \( \tau_{\text{Recovery}} \) is the time constant for recovery from inactivation.

Simulated time constants of inactivation and deactivation were measured by fitting exponentials with the Nelder-Mead simplex algorithm. Peak current in the triple pulse protocol was normalized to steady-state current at 40 mV.

In KCNQ1 experiments, relative inactivation is used to measure the percentage of activated channels that become inactivated. This measurement is especially useful for KCNQ1 because channels only partially inactivate. Relative inactivation is measured at the beginning of the tail current as 1-x/y, where x is initial tail current and y is extrapolated current (found by fitting a single exponential to tail current). The extrapolated current measures what the tail current would be if no channels were inactivated during the depolarizing pulse.

In our simulations (shown in Figure 2E in the paper), x corresponds to \( \sum_{i=1}^{5} O_i \) (the sum of the open states at the initial tail current); y is \( \sum_{i=1}^{5} O_i + I \) at the same point in time, (I is the inactivated state). Thus, the expression used to find relative
inactivation using the model is \[ 1 - \frac{\sum_{i=1}^{5} O_i}{\sum_{i=1}^{5} O_i + I} \], or one minus the ratio of open state occupancy to sum of open state and inactivated state occupancy.

Oocyte experiments by Tristani-Firouz and Sanguinetti \(^4\) isolate various channel properties, allowing specific transitions to be fit. Activation current traces at many different potentials (Figure 2A, in the paper) constrain the parameters describing the closed-state forward transition rates \(\alpha, \gamma\) and the voltage-independent transition, \(\theta\). The two time constants of activation described in the results section of the paper (fast and slow) each have an associated weight that is determined by the closed-state occupancy at rest, which in turn is governed by the magnitude of the forward rate relative to the magnitude of the reverse rate at the resting potential. Thus, the steady-state occupancy of the closed states at rest constrains \(\beta\) and \(\delta\) at negative potentials. The voltage dependence and magnitude of \(\lambda\) are determined by the time constant of inactivation at different voltages (Figure 2C and 2D, of the paper). Finally, the peak current, which reflects the steady-state inactivation during the depolarizing pulse, constrains the value of \(\mu\) at positive potential, while at negative potential it is constrained by the rate of recovery from inactivation (hook, Figure 2A of the paper).

Similar to KCNQ1, published \(I_{K_\text{s}}\) current traces were fit with biexponentials, leak current was subtracted, and steady-state values were normalized to experimental average. The fitting procedure relies on experiments that display the important channel properties including the time-course of activation (including delay), steady-state current-voltage relationship, rate of deactivation and accumulation during pacing. The forward rate transitions, \(\alpha\) and \(\gamma\), were constrained by activation current traces to different potentials. The closed state occupancy that determines the delay before activation, constrains \(\beta\) and \(\delta\) as in KCNQ1. These transitions also affect \(I_{K_\text{s}}\) accumulation during pacing, which is within the experimental range (see results section in paper). Deactivation was also included in the optimization and constrains the reverse open state transitions, \(\eta\) and \(\omega\). These transitions and the forward state transitions, \(\theta\) and \(\psi\), are also affected by the steady-state I-V relationship. The predictive ability of the model was verified by its ability to reproduce AP morphology, APD rate-dependent adaptation over the entire range of physiological frequencies, and frequency independent APD prolongation with a drug model that uses an experimentally measured off-rate \(^9\).

Parameters for both KCNQ1 and \(I_{K_\text{s}}\) models were estimated using the Nelder-Mead simplex algorithm \(^8\) and Asynchronous Parallel Pattern Search \(^10\). Optimization and simulations were performed on a cluster with fifty-two 2.8 GHz Intel Xeon processors.
Action Potential Simulations

For AP simulations, sets of differential equations describing the $I_{Ks}$, $I_{Kr}$, and $I_{Na}$ Markov models were solved using a 4th order Rosenbrock method with Shampine parameters $^{11}$. All variables were scaled to a maximum probability of 1 and absolute error was set not to exceed $10^{-6}$. The maximum step size was limited to 0.1 ms and was decreased to 0.01 before the stimulus. The Markov model requiring the smallest step determined the time step used to solve all equations. Calcium dynamics, pumps, exchangers, and background currents are from the LRd model $^{12-14}$ available at http://rudylab.wustl.edu.

Extracellular concentrations were set to $[Na^+]_o=136$ mM, $[K^+]_o=5.4$ mM, and $[Ca^{2+}]_o=2$ mM, while intracellular concentrations varied dynamically. Temperature was always set to 37 °C to match experimental conditions in Bosch et al $^{15}$, with the exception of KCNQ1 channels, which were simulated at 23 °C because sufficient body temperature data were not available. These concentrations and temperature were maintained for all simulations to allow for comparison. Cells were kept quiescent for 10 min to achieve steady-state resting conditions before all protocols.

Action potential duration (APD) was measured at 90% repolarization to $V_{rest}$ (recorded immediately before stimulus) from peak $V_m$ with time zero at $dV_m/dt_{max}$. The stimulus applied was -80 $\mu$A/µF for 0.5 ms.
KCNQ1 Rates (ms⁻¹)

\[ I_{KCNQ1} = \bar{G}_{KCNQ1} \cdot O_{KCNQ1} \cdot (V_m - E_{Ks}) \]

Where maximum conductance, \( \bar{G}_{KCNQ1} \), is:

\[ \bar{G}_{KCNQ1} = 0.054 \cdot \left( 1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_i} \right)^{1.4}} \right) \]

Accounts for dependence of conductance on intracellular calcium concentration \([Ca^{2+}]_i\).

and open probability, \( O \):

\[ O_{KCNQ1} = O_1 + O_2 + O_3 + O_4 + O_5 \]

\[ E_{Ks} = \frac{R \cdot T}{F} \cdot \log \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_i}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i} \]

\[ \alpha = 9.57 \cdot 10^{-4} \cdot \exp(1.98 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \beta = 5.00 \cdot 10^{-5} \cdot \exp(-3.33 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \gamma = 3.77 \cdot 10^{-2} \cdot \exp(3.33 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \delta = 4.77 \cdot 10^{-2} \cdot \exp(-4.77 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \theta = 7.98 \cdot 10^{-2} \]

\[ \eta = 2.06 \cdot 10^{-2} \cdot \exp(-1.04 \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \psi = 2.54 \cdot 10^{-2} \cdot \exp(6.46 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \omega = 1.78 \cdot 10^{-2} \cdot \exp(-5.32 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \lambda = 2.32 \cdot 10^{-2} \cdot \exp(1.21 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \mu = 6.19 \cdot 10^{-2} \cdot \exp(-9.46 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \nu = -9.00 \cdot 10^1 \]

\[ x_\psi = \exp(z_\psi \cdot (z_\psi + z_\omega)) \cdot \nu \cdot \frac{F}{RT} \]

\[ x_\omega = \exp(z_\omega \cdot (z_\psi + z_\omega)) \cdot \nu \cdot \frac{F}{RT} \]

\[ \psi_n = \psi \cdot x_\psi^{n-1} \]

\[ \omega_n = \omega \cdot x_\omega^{n-1} \]
Guinea Pig $I_{KS}$ Rates ($\text{ms}^{-1}$)

$$I_{KS} = \overline{G_{KS}} \cdot O_{KS} \cdot (V_m - E_{KS})$$

$$\overline{G_{KS}} = 0.2165 \cdot \left( 1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{\text{Ca}^{2+}} \right)^{1.4}} \right)$$

$$O_{KS} = O_1 + O_2$$

$$E_{KS} = \frac{R \cdot T}{F} \cdot \log \left( \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_i}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i} \right)$$

$$\alpha = 3.72 \cdot 10^{-3} \cdot \exp(2.10 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\beta = 2.35 \cdot 10^{-4} \cdot \exp(-2.42 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\gamma = 7.25 \cdot 10^{-3} \cdot \exp(2.43 \cdot 10^0 \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\delta = 1.53 \cdot 10^{-3} \cdot \exp(-6.26 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\theta = 1.96 \cdot 10^{-3}$$

$$\eta = 1.67 \cdot 10^{-2} \cdot \exp(-1.34 \cdot 10^0 \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\psi = 3.41 \cdot 10^{-4} \cdot \exp(1.24 \cdot 10^0 \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\omega = 2.62 \cdot 10^{-4} \cdot \exp(-8.07 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$
Human $I_{Ks}$ Rates (ms$^{-1}$)

$$I_{Ks} = \frac{G_{Ks}}{O_{Ks}} \cdot (V_m - E_{Ks})$$

$$G_{Ks} = 0.779 \cdot \left( \frac{1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_{i}} \right)^{1.4}}}{1} \right)$$

$$O_{Ks} = O_1 + O_2$$

$$E_{Ks} = \frac{R \cdot T}{F} \cdot \log \left[ \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_i}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i} \right]$$

$$\alpha = 3.98 \cdot 10^{-4} \cdot \exp(3.61 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\beta = 5.74 \cdot 10^{-5} \cdot \exp(-9.23 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\gamma = 3.41 \cdot 10^{-3} \cdot \exp(8.68 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\delta = 1.20 \cdot 10^{-3} \cdot \exp(-3.30 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\theta = 6.47 \cdot 10^{-3}$$

$$\eta = 1.25 \cdot 10^{-2} \cdot \exp(-4.81 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\psi = 6.33 \cdot 10^{-3} \cdot \exp(1.27 \cdot 10^{0} \cdot \frac{V_m \cdot F}{R \cdot T})$$

$$\omega = 4.91 \cdot 10^{-3} \cdot \exp(-6.79 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})$$
Guinea Pig $I_{Kr}$ (Rapid Delayed Rectifier) Model

A previous Markov model of guinea pig $I_{Kr}$\textsuperscript{17} was updated. Simulations generated by the revised model are compared to the current-voltage relationship (Figure A1-A), activation (Figure A1-B), and rectification (Figure A1-C) measured by Sanguinetti and Jurkiewicz\textsuperscript{18}. Current-voltage relationship was measured at the end of a 550 ms pulse to various potentials and activation was the sum of the occupancy in the open and inactivated states at the end of the same pulse. Rectification was reproduced with a 225 ms pulse to various potentials and calculated as the occupancy in the open state over the sum of the occupancy of the open and inactivated states, $O/(O+I)$. Currents simulated for a 550 ms pulse are shown in Figure A1-D. The updated model was also able to reproduce recent experiments by Rocchetti et al\textsuperscript{19} (Figure A1-E and A1-F).

Consistent with AP-clamp experimental observations, peak current during the AP does not increase significantly as rate increase. However, in simulations $I_{Kr}$ is greater at the start and during the plateau of the AP (Figure A1-E and A1-F) than in the experiment. Increasing rectification to decrease early $I_{Kr}$ led to conflicting results with rectification experiments\textsuperscript{18}. A possible explanation to this conflict is an observation by Gurrola et al\textsuperscript{20} that ErgTx, which was used in the AP clamp experiments, is inhibited at higher membrane potentials\textsuperscript{20}, which would cause underestimation at the beginning of the AP. Furthermore, ErgTx does not block inactivated channels, resulting in underestimation of current contribution as channels recover from inactivation during the plateau of the AP.
Figure A1

A. $I_{Kr}$ I-V Relationship

B. $I_{Kr}$ Activation

C. $I_{Kr}$ Rectification

D. Time Dependence of $I_{Kr}$

E. $I_{Kr}$ during the AP at CL = 1000 ms

F. $I_{Kr}$ during the AP at CL = 250 ms
Figure A1: Simulations of $I_{Kr}$. $I_{Kr}$ was fit to experimental data from Sanguinetti and Jurkiewicz. Protocol used to obtain Current-Voltage (I-V) relationship (Panel A) and Activation (Panel B) is shown in inset of panel A. Rectification (Panel C) was measured using a step from -100 mV to various potentials. The current traces generated by the protocol shown in A are shown in Panel D. Panels E and F show $I_{Kr}$ during the action potential at CL= 1000 ms (Panel E) and CL = 250 ms (Panel F). Simulated currents are shown above, and the experimentally observed ErgTx sensitive current is shown below.
**I_{Kr} Markov Model**

\[
\begin{array}{c}
\text{C}_3 \xrightarrow{\alpha} \text{C}_2 \xrightarrow{\alpha_1} \text{C}_1 \xrightarrow{\alpha_2} \text{O} \\
\xrightarrow{\beta} \xrightarrow{\beta_1} \xrightarrow{\beta_2}
\end{array}
\]

**I_{Kr} Equations**

\[I_{Kr} = G_{Kr} \cdot O_{Kr} \cdot (V_m - E_{Kr})\]

\(O_{Kr}\) is the open probability

\[G_{Kr} = 0.0135 \cdot [K^+]_o^{0.59}\]

\[E_{Kr} = \frac{R}{F} \cdot \log \frac{[K^+]_o}{[K^+]_i}\]

\[\alpha_2 = 1.31 \cdot 10^{-2} \cdot \exp(1.48 \cdot 10^0 \cdot \frac{V_m \cdot F}{R \cdot T})\]

\[\beta_2 = 3.30 \cdot 10^{-3} \cdot \exp(-5.77 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})\]

\[\alpha_1 = 2.17\]

\[\beta_1 = 1.08\]

\[\alpha = 3.02 \cdot 10^{-2} \cdot \exp(1.48 \cdot 10^0 \cdot \frac{V_m \cdot F}{R \cdot T})\]

\[\beta = 2.90 \cdot 10^{-3} \cdot \exp(-9.78 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T})\]

\[\alpha_i = 5.45 \cdot 10^{-1} \cdot \exp(-8.17 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \cdot \frac{4.5}{[K^+]_o}\]

\[\beta_i = 8.20 \cdot 10^{-1} \cdot \exp(5.04 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \cdot \left(\frac{4.5}{[K^+]_o}\right)^3\]

\[\mu = \alpha_i \cdot \beta_2 / \beta_i\]
References


