Role of the Calcium-Independent Transient Outward Current \(I_{\text{to1}}\) in Shaping Action Potential Morphology and Duration

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Abstract—The Kv4.3-encoded current \(I_{\text{Kv4.3}}\) has been identified as the major component of the voltage-dependent Ca\(^{2+}\)-independent transient outward current \(I_{\text{to1}}\) in human and canine ventricular cells. Experimental evidence supports a correlation between \(I_{\text{to1}}\) density and prominence of the phase 1 notch; however, the role of \(I_{\text{to1}}\) in modulating action potential duration (APD) remains unclear. To help resolve this role, Markov state models of the human and canine Kv4.3- and Kv1.4-encoded currents at 35°C are developed on the basis of experimental measurements. A model of canine \(I_{\text{to1}}\) is formulated as the combination of these Kv4.3 and Kv1.4 currents and is incorporated into an existing canine ventricular myocyte model. Simulations demonstrate strong coupling between L-type Ca\(^{2+}\) current and \(I_{\text{to1}}\) and predict a bimodal relationship between \(I_{\text{Kv4.3}}\) density and APD whereby perturbations in \(I_{\text{Kv4.3}}\) density may produce either prolongation or shortening of APD, depending on baseline \(I_{\text{to1}}\) current level. (Circ Res. 2000;87:1026-1033.)

Key Words: K\(^{+}\) channel ■ transient outward current ■ ventricular action potential ■ action potential duration

The voltage-dependent calcium (Ca\(^{2+}\))-independent transient outward current \(I_{\text{to1}}\) is a key contributor in shaping the early phase of the cardiac ventricular action potential (AP). Recordings obtained from single ventricular myocytes isolated from different depths within the ventricular wall have shown a correlation between \(I_{\text{to1}}\) density and prominence of the phase 1 notch.\(^1\)-\(^5\) \(I_{\text{to1}}\) magnitude is also reduced substantially in ventricular myocytes isolated from failing human and canine hearts.\(^6\),\(^7\) and APs recorded from these cells exhibit a decreased phase 1 notch depth. In addition, blockers of \(I_{\text{to1}}\), such as 4-aminopyridine (4-AP), reduce or eliminate the phase 1 notch.\(^6\)-\(^9\)

Although the evidence linking \(I_{\text{to1}}\) magnitude to characteristics of the phase 1 notch is strong, the role of \(I_{\text{to1}}\) on AP duration (APD) remains unclear. Heart failure–induced reduction of \(I_{\text{to1}}\) density in canine and human myocytes is accompanied by significant prolongation of APD.\(^6\)-\(^7\) However, heart failure is also accompanied by altered expression of genes encoding the inward rectifier potassium (K\(^{+}\)) current \(I_{\text{K1}}\),\(^6\) the sarcoplasmic reticulum (SR)-Ca\(^{2+}\) ATPase,\(^10\),\(^11\) and the sodium–calcium exchanger.\(^10\),\(^12\) Recently, a model of the failing canine ventricular myocyte was developed and used to investigate mechanisms influencing APD in heart failure.\(^13\) Model predictions are that reduction of both \(I_{\text{to1}}\) and \(I_{\text{K1}}\) magnitude, on the basis of the average decrease in current densities measured in terminal heart failure,\(^7\) have only modest effects on APD and that AP prolongation occurs mainly because of altered expression of intracellular Ca\(^{2+}\)-handling proteins and the accompanying reduction of both SR Ca\(^{2+}\) concentration and Ca\(^{2+}\)-mediated inactivation of the L-type Ca\(^{2+}\) current \(I_{\text{CaL}}\).\(^13\)

Experiments designed to reveal the role of \(I_{\text{to1}}\) on APD have yielded conflicting results. In the absence of Ca\(^{2+}\) buffers, low concentrations of 4-AP (1 mmol/L) shorten APD in isolated canine midmyocardial\(^9\) and epicardial\(^8\) ventricular cells. Higher doses of 4-AP (3 to 5 mmol/L) prolong the AP in Ca\(^{2+}\)-buffered canine\(^7\) and human\(^7\) ventricular midmyocardial cells. Interpretation of these findings is complicated by the lack of specificity of 4-AP for \(I_{\text{to1}}\) and the use of Ca\(^{2+}\) buffers. AP prolongation may result from modest block of delayed rectifier K\(^{+}\) currents in response to higher concentrations of 4-AP. In guinea pig myocytes, the introduction of \(I_{\text{to1}}\) by cell fusion techniques produces a reduction of APD that is correlated with increasing \(I_{\text{to1}}\) density.\(^14\) However, the presence of sustained inward currents may have influenced APD in these studies. Functional knockout of a major component of \(I_{\text{to1}}\) (\(I_{\text{to1}}\)) in mouse also prolongs APD.\(^15\),\(^16\) However, \(I_{\text{to1}}\) density is much larger in mouse than in canine or human myocytes and APD is significantly shorter.\(^2\),\(^7\),\(^15\)

These data highlight the uncertainty of the role of \(I_{\text{to1}}\) in controlling APD. To help clarify this role, we have functionally expressed and characterized the human Kv4.3-encoded current (long splice variant, denoted as hKv4.3.3-2) at 35°C; developed a Markov state model of the hKv4.3.2-encoded
current on the basis of these data; developed a Markov state model of the human Kv1.4-encoded current on the basis of data of Po et al.\textsuperscript{17,18} and combined the hKv1.4 and hKv4.3-2 models to form a model of canine $I_{\text{tot}}$; incorporated the $I_{\text{tot}}$ model into a computational model of a canine midmyocardial ventricular cell\textsuperscript{16}; and determined the mechanisms by which $I_{\text{tot}}$ influences AP shape and duration.

Materials and Methods

Composition of $I_{\text{tot}}$

Canine and human $I_{\text{tot}}$ is likely a combination of Kv4.3-2- and Kv1.4-encoded currents ($I_{\text{Kv4.3-2}}$ and $I_{\text{Kv1.4}}$, respectively). Each component has different kinetics of recovery from inactivation.\textsuperscript{2,5,19,20} The Kv4.3-encoded current\textsuperscript{21–23} has kinetics and pharmacological sensitivity similar to the $I_{\text{tot}}$ component with fast recovery.\textsuperscript{2} Reduction in Kv4.3 mRNA transcript level is also correlated with reduction in $I_{\text{tot}}$ density in human and canine heart failure.\textsuperscript{7,24} The Kv4.3 current has kinetics similar to the slowly recovering component of $I_{\text{tot}}$.\textsuperscript{2,17} In addition, Kv4.3 mRNA transcripts have been detected in canine\textsuperscript{23} and human\textsuperscript{24} myocytes at levels 16% and 72% as abundant, respectively, as those of Kv4.3.

On the basis of these data, the model of $I_{\text{tot}}$ is constructed as a combination of $I_{\text{Kv4.3-2}}$ and $I_{\text{Kv1.4}}$. The balance of $I_{\text{Kv4.3-2}}$ and $I_{\text{Kv1.4}}$ (77% and 23%, respectively) is based on the relative magnitudes of fast versus slow recovery time constants measured by Kääb et al.\textsuperscript{2} (estimated from their Figure 7D) in canine midmyocardial cells. The strategy for modeling $I_{\text{Kv4.3-2}}$ and $I_{\text{Kv1.4}}$ is described below.

Characterization of $I_{\text{Kv4.3-2}}$

The hKv4.3 gene has two splice variants with quantitatively similar biophysical properties in the basal state. In this study, only the long splice variant, denoted hKv4.3-2, is expressed. Full-length cDNA encoding hKv4.3-2\textsuperscript{21} was subcloned into the pIREs-GFP vector for bicistronic expression of the hKv4.3-2 channel and green fluorescence protein in mouse Ltk fibroblasts. Transient transfection was performed using the lipofectamine method (GIBCO-BRL), as previously described.\textsuperscript{21} Cells were transfected to the stage of an inverted microscope (Nikon Diaphot) and selected by epifluorescence for bicistronic expression of the hKv4.3-2 channel and green fluorescence protein.

Characterization of $I_{\text{Kv1.4}}$

The hKv1.4-encoded current\textsuperscript{21–23} has kinetics and pharmacological sensitivity similar to the slowly recovering component of $I_{\text{Kv4.3}}$; the recovery time constant of inactivation of $I_{\text{Kv1.4}}$ is slightly to ensure behavior consistent with native currents\textsuperscript{1–3} (see online data supplement).

Modeling $I_{\text{tot}}$ Effects on the AP

The canine $I_{\text{tot}}$ is incorporated into the Winslow-Rice-Jafri (WRJ)\textsuperscript{13} canine ventricular cell model to investigate its interaction with other membrane currents. Additional myocyte models are implemented to test robustness of simulation results (see Discussion). APs are simulated at 1 and 2 Hz periodic pacing to steady state. For brevity, only those at 1 Hz are shown.

An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

Functional Expression of hKv4.3 and $I_{\text{Kv4.3}}$

Representative normalized whole-cell currents elicited by a family of depolarizing voltage steps from 0 to 60 mV in 20-mV increments are shown in Figure 2A (solid lines) with corresponding model-simulated currents (dashed lines). Experimental currents are normalized by the peak current measured at 60 mV (2118 pA). Successive current traces are displaced vertically by 0.1 normalized units (212 pA) for clarity. The current activates and inactivates rapidly, decaying within 100 ms. Time to peak decreases monotonically from $\approx$6.5 ms at $-10$ mV to $\approx$2 ms at 60 mV (Figure 2B).

The time constant of inactivation becomes nearly voltage-independent, with a time constant of $\approx$13.5 ms at potentials $>10$ mV (Figure 2C). The current activates in the range of $-40$ to $-30$ mV, and peak current increases nearly linearly over more positive potentials (Figure 2D). In all cases, experimental data (symbols) are well fit by the model (lines).

The steady-state availability curve is shown in Figure 2E. Boltzmann function fits to experimental data (dashed line), fit to and the model (Δ, data; fit not shown) both yield half-maximal current at $V_{1/2} = -51.1 \pm 0.7$ mV, with slope factor (k) of $5.6 \pm 0.4$ mV ($\pm$SE values and n=6 for experiments), in agreement with previous measurements.\textsuperscript{14,21,22}
current inactivates fully at potentials more positive than $-10$ mV. Recovery kinetics were determined at $-100$ and $-80$ mV (Figure 2F). The currents recover monoexponentially with time constants of $20.23 \pm 1.72$ ms ($\bullet$, experiment; dashed line, model) and $37.69 \pm 1.76$ ms ($\triangle$, experiment; simulated current; solid line, fit) for the model at $-100$ and $-80$ mV, respectively. These data demonstrate the ability of the model to reproduce properties measured experimentally.

**Model Validation of $I_{Kv1.4}$ and Canine $I_{to1}$**

Figure 3 shows features of the $I_{Kv1.4}$ model at $22^\circ$C. Figure 3A shows peak $I_{Kv1.4}$ model current (normalized to the $+100$-mV peak current) with the corresponding model current traces in the inset. The current activates at potentials greater than $-50$ mV, and the current-voltage relation shows slight outward rectification in agreement with experimental data. The time constant of inactivation is nearly voltage-independent at potentials $>-10$ mV, ranging from $52.4$ ms at $-10$ mV to $49.1$ ms at $+100$ mV. Time to peak is 10 to 17 ms, depending on clamp potential (not shown). Steady-state availability curve (Figure 3B; symbols, model; line, fit) exhibits $V_{1/2}$ of $-66.3$ mV and $k$ of $4$ mV, in close agreement with experiments.

Peak $I_{to1}$ current in response to a family of depolarizing voltage steps is shown in Figure 4A, with corresponding current traces shown in the inset. Currents are normalized by peak $I_{to1}$ magnitude at $60$ mV. Model $I_{to1}$ activates at $\approx -40$ mV, and the peak current-voltage relation increases monotonically. The steady-state availability curve (Figure 4B; symbols, model; line, fit) exhibits a $V_{1/2}$ of $-55.5$ mV, with $k$ of $6.8$ mV. The features of Figures 4A and 4B agree well with native $I_{to1}$ measured in both canine$^1,7$ and human$^3,27,28$ myocytes. Currents in these experiments activate in the range of $-20$ to $-10$ mV$^{1,3,7,27,28}$ and have $V_{1/2}$ in the range of $-23$ to $-37$ mV$^{1,3,7,28}$ The $\approx 20$-mV difference in both the voltage where $I_{to1}$ first activates and in the half-inactivation voltage is accounted for by the presence of extracellular divalent cations (usually 0.1 to 0.3 mmol/L Cd$^{2+}$), which produce a 15 to 25 mV difference in both the voltage where $I_{to1}$ first activates and in the half-inactivation voltage.
positive shift in both the peak current-voltage relation and the steady-state inactivation curve of native $I_{\text{to}}$.\textsuperscript{28,29} and expressed Kv4.3 currents.\textsuperscript{30}

The time constant of inactivation for model $I_{\text{to}}$ is nearly voltage-independent at potentials greater than $-10$ mV, ranging from 10.8 ms at $-10$ mV to 8.4 ms at $+60$ mV, and the current reaches its peak value in 2 to 5 ms, depending on test potential. The inactivation kinetics agree with values obtained at 35°C for human subendocardial and subepicardial myocytes (7 and 7.9 ms, respectively)\textsuperscript{27} and for canine midmyocardial cells (9.4 ms, estimated from Figure 8 of Liu et al\textsuperscript{1}). Figure 4C shows the time course of recovery from inactivation at $-80$ mV. Normalized peak currents ($C$) in response to a 2-pulse protocol with 200-ms steps to $+40$ mV are fit to the biexponential recovery function $1-\alpha_1 e^{-\tau_1}-\alpha_2 e^{-\tau_2}$ (solid line). These data are replotted on a log scale to illustrate the clearly biexponential nature of the model recovery process (Figure 4C, inset). The fit yields values of 37 ms and 583.6 ms for $\tau_1$ and $\tau_2$, respectively, where the relative amplitude of $\tau_1$ (ie, $a_1/[a_1+a_2]$) is 0.779. These time constants and their relative weights have the values expected on the basis of the individual recovery properties and the combination ratio of the component currents $I_{\text{Kv4.3}}$ and $I_{\text{Kv1.4}}$ and are in agreement with those measured experimentally at or near 35°C in both canine\textsuperscript{1,7} and human\textsuperscript{3,27} ventricular midmyocardial cells.

**Effect of $I_{\text{to}}$ Density on Canine AP Shape and Duration**

Downregulation of Kv4.3, without an associated reduction in Kv1.4 level, is believed to be the basis for the reduction of $I_{\text{to}}$ observed in failing canine myocytes.\textsuperscript{24} Therefore, the $I_{\text{to}}$ model was incorporated into the WRJ canine ventricular cell model to study the impact of Kv4.3 downregulation on AP properties. The effect of varying the density of the Kv4.3 component of $I_{\text{to}}$ on model AP shape (Figure 5A) and duration (Figure 5B, •) is multifaceted. With complete elimination of $I_{\text{Kv4.3}}$, APD at 90% repolarization (APD\textsubscript{90}) is approximately 250 ms. As $I_{\text{Kv4.3}}$ current density is increased, phase 1 repolarization becomes more prominent, resulting in an AP with a spike and dome configuration. Hyperpolarization of phase 1 membrane potential attributable to an early repolarizing current is commonly observed in experiments; however, the model reveals an additional effect of $I_{\text{Kv4.3}}$ on APD. At relatively low densities of $I_{\text{Kv4.3}}$, incremental changes in current density produce progressive prolongation of APD. For example, an $I_{\text{Kv4.3}}$ with maximal conductance ($G_{\text{Kv4.3}}$) of 0.07 nS/pF (corresponds to 4.6 pA/pF peak current in response to a depolarization to $+20$ mV from $-80$ mV) results in an APD of 263 ms. Increasing $G_{\text{Kv4.3}}$ to 0.10 and 0.12 nS/pF produces APs with durations of 275 and 300 ms, respectively. Additional increases in the density of $I_{\text{Kv4.3}}$ reveal the presence of a threshold phenomenon, whereby the AP configuration switches from the spike and dome morphology with relatively long duration to a short triangular AP that lacks a plateau phase. The short APs resemble those measured in species normally expressing high levels of $I_{\text{to}}$, such as mouse and rat.\textsuperscript{15} At these relatively high current densities, any additional increase in maximal conductance leads to shortening of APD. The same simulations were repeated with a 50% reduction in density of the fast inward sodium current $I_{\text{Na}}$ (Figure 5B, □). This decrease in $I_{\text{Na}}$ shifts the $G_{\text{Kv4.3}}$ versus APD relationship only slightly toward lower conductance values. The effects of $G_{\text{Kv4.3}}$ on APD are seen to depend on the baseline value of $G_{\text{Kv4.3}}$ against which perturbations in current density are made. At lower levels of $I_{\text{Kv4.3}}$ expression, increasing $G_{\text{Kv4.3}}$ prolongs APD, whereas at higher levels of expres-
tion, increasing $G_{Kv4.3}$ shortens APD. Qualitatively similar results were obtained at both 1 Hz (Figure 5) and 2 Hz (not shown) pacing rates.

**Mechanism of $I_{Kv4.3}$ Influence on AP Shape**

To understand mechanisms underlying the influence of $I_{Kv4.3}$ on AP shape, the effect of varying $G_{Kv4.3}$ on individual membrane currents and state variables was examined. $I_{cat}$ shape and magnitude is closely coupled to the density of $I_{Kv4.3}$. Figure 6A shows 3 simulated canine APs. In case 1, $I_{Kv4.3}$ is underexpressed by 70% (dashed line, $G_{Kv4.3}=0.0358$ nS/pF); in case 2, $I_{Kv4.3}$ is expressed at normal levels (solid line, $G_{Kv4.3}=0.1194$ nS/pF); and in case 3, $I_{Kv4.3}$ is overexpressed by 20% (dotted line, $G_{Kv4.3}=0.1432$ nS/pF). The normal current level of case 2 is set such that total $I_{to1}$ density ($\approx 9.5$ pA/pF for peak current in response to a step to +20 mV) agrees with that measured in control canine left ventricular midmyocardial cells (5 to 11 pA/pF)7 and consists of 77% $I_{Kv4.3}$.7 Figures 6B and 6C (inset) show corresponding $L$-type Ca$^{2+}$ channel open probability ($P_{open-ICaL}$), $I_{CaL}$, and $I_{to1}$ for each of the 3 cases. An increase in the expression level of $I_{Kv4.3}$ leads to an increase in the phase 1 repolarization rate, which results in stronger hyperpolarization of the notch potential (Figure 5A). This decrease in phase 1 notch potential has 2 effects on $I_{cat}$. The rate of decline of $P_{open-ICaL}$ during phase 1 increases for case 2 versus case 1 due to a partial deactivation of the $L$-type channel. There is a concurrent increase in occupation probability of the closed states immediately preceding the open state of the $L$-type channel (not shown), brought about by activation of $I_{Kv4.3}$. In addition, the driving force for the $L$-type current is increased for case 2 versus case 1, increasing peak $I_{cat}$ by $\approx 70\%$ (Figure 6C). Indeed, $I_{cat}$ for case 2 remains greater than that for case 1 throughout phase 1 even though $P_{open-ICaL}$ is decreased (Figures 6B and 6C). This increased inward current prevents the relatively large $I_{Kv4.3}$ from truncating the AP and allows for the subsequent return to activation of the $L$-type channel after inactivation of $I_{Kv4.3}$ and progression to phase 2 of the AP. The delayed secondary activation of the $L$-type channel is shown by the increase in $P_{open-ICaL}$ and $I_{CaL}$, which occurs between 30 and 50 ms after the upstroke of the AP for case 2 (Figure 6B, arrow). Subsequent to this secondary activation, $P_{open-ICaL}$ and $I_{CaL}$ for cases 1 and 2 are time-shifted versions of each other (Figure 6B, inset). Thus, the difference in APD in case 1 versus case 2 can be attributed to the difference in duration of phase 1. The case 3
AP exhibits an even more rapid early phase 1 rate of hyperpolarization compared with case 2. This results in a more complete deactivation of the L-type channel, thus eliminating the ability of \(I_{\text{CaL}}\) to overcome \(I_{\text{Kv4.3}}\) regardless of the increase in L-type channel driving force. The resulting AP repolarizes rapidly and is therefore lacking a plateau phase.

**Discussion**

Recent experimental findings suggest that reductions in magnitude of \(I_{\text{tot}}\), as a consequence of reduced Kv4.3 expression, may be important in modulating APD in normal versus failing canine and human cardiac myocytes.\(^5\) To investigate the role of \(I_{\text{tot}}\) in AP profile and duration, a new model of canine \(I_{\text{tot}}\), built on descriptions of \(I_{\text{Kv4.3}}\) and \(I_{\text{Kv1.4}}\), has been developed in this study. Incorporation of this \(I_{\text{tot}}\) model into the WRJ canine ventricular cell model\(^1\) reveals a complex interaction between \(I_{\text{Kv4.3}}\) density and \(I_{\text{tot}}\) magnitude, which in turn modulates APD. At relatively low levels of \(I_{\text{Kv4.3}}\), increasing \(I_{\text{Kv4.3}}\) augments the driving force for \(I_{\text{CaL}}\) and produces a delay in activation of the late phase of \(I_{\text{CaL}}\). Both effects contribute to the modest prolongation of APD. Additionally increasing \(I_{\text{Kv4.3}}\) density reveals a threshold phenomenon, whereby the early outward current overcomes \(I_{\text{CaL}}\), thus eliminating phase 2 producing a short AP with triangular shape. Loss of the AP dome resulting from imbalance of membrane currents during phase 1 has been observed previously both experimentally\(^4\) and in simulations.\(^31\) As a consequence of this bimodal phenomenon, increasing \(I_{\text{Kv4.3}}\) density shortens APD at high baseline densities, whereas at lower levels, increasing \(I_{\text{Kv4.3}}\) density produces modest prolongation of APD. Thus, the effect of perturbing \(I_{\text{Kv4.3}}\) density is dependent on the underlying current level against which the changes are imposed (Figure 5).

This study indicates that the relationship between APD and \(I_{\text{tot}}\) or \(I_{\text{Kv4.3}}\) density is not a simple monotonic correlation. Rather, this relationship exhibits a bifurcation separating 2 distinct modes of behavior. In mouse ventricular myocytes, a 57% reduction in mean peak outward current density induced by overexpression of dominant-negative Kv4 \(\alpha\) subunits leads to prolongation of APD.\(^15\) Because \(I_{\text{tot}}\) density is significantly greater in mouse than in human or canine cells,\(^2,7,15\) model predictions for the APD versus \(I_{\text{tot}}\) relationship at high baseline levels of \(I_{\text{tot}}\) are consistent with these data. However, the complex nature of the relationship between APD and \(I_{\text{tot}}\) over a wider range of \(I_{\text{tot}}\) density suggests that extrapolation of the consequences of altering expression levels of \(I_{\text{tot}}\) in mouse to other species may not be valid. In fact, the WRJ canine model predicts that reduction of \(I_{\text{tot}}\) from normal levels will lead to modest shortening of APD (Figure 6A). Via this mechanism, a rate-dependent decrease in the availability of \(I_{\text{tot}}\) is expected to contribute to both a shallower phase 2 notch potential and a shorter APD. Such rate-dependent changes in AP morphology have been observed in canine epicardial\(^1\)-4,8 and midmyocardial\(^9\) cells and human subepicardial cells\(^2,3\) and have led to the suggestion that the main impact of \(I_{\text{tot}}\) on APD is secondary to its effects on \(I_{\text{CaL}}\),\(^8,9\) consistent with the mechanism described in this study.

![Figure 7. Effect of \(I_{\text{Kv4.3}}\) current density on simulated guinea pig APs](image)

To show that the \(I_{\text{Kv4.3}}\) versus APD behavior is not unique to the WRJ canine cell model, the hKv4.3-2 model was incorporated into the Luo-Rudy Phase II (LRII)\(^2\) and the Jafari-Rice-Winslow (JRW)\(^3\) guinea pig ventricular cell models. Descriptions of Ca\(^{2+}\) handling and \(I_{\text{CaL}}\) differ greatly in the WRJ/JRW versus the LRII models. Simulations using the guinea pig models (Figures 7A and 7B) produced qualitatively similar results to those of the WRJ canine model (Figure 5). The similar effects of \(I_{\text{Kv4.3}}\) density on AP morphology in all 3 models demonstrate that this behavior is not likely to be attributed to any particular mathematical formulation of Ca\(^{2+}\) cycling or detailed representation of ionic currents. Rather, this behavior emerges as a general consequence of interactions between a rapidly activating and inactivating outward current and a rapidly activating and partially inactivating inward current.

The finding that introduction of \(I_{\text{Kv4.3}}\) to guinea pig ventricular cell models produces APD prolongation (at low \(I_{\text{Kv4.3}}\) density) contrasts with experimental findings.\(^14\) In these experiments, expressed rat Kv4.3 current was introduced into isolated guinea pig myocytes via cell fusion techniques. Kv4.3 current density had a strong influence on AP plateau potential and was inversely correlated with APD over the entire range of \(I_{\text{Kv4.3}}\) densities studied (see Figure 5 of Hoppe...
et al.\(^4\)). The presence of a maintained outward current complicates interpretation of these results. The \(I_{\text{to}}\)-related maintained current magnitude (measured as the difference between the fully activated current and the current following an inactivating prepulse to 0 mV) was not correlated with Kv4.3 current density, consistent with complete inactivation of the Kv4.3 current. However, the possibility remains that a time-independent maintained outward current was correlated with APD. JRW model simulations show that concurrent increases in both \(I_{\text{KChIP}}\) and an instantaneous leak current (\(I_{\text{leak}}\) with reversal potential \(E_{\text{leak}}\)) produce monotonic decreases in both APD and plateau potential qualitatively similar to those observed experimentally\(^4\) (Figure 7C). For each AP, conductance of \(I_{\text{to}}\) is equal to 10% of \(G_{\text{Kv4.3}}\), corresponding to a background current that is \(\approx 16\%\) of peak \(I_{\text{Kv4.3}}\), which is in the lower range of those observed experimentally.\(^4\) Clearly, the \(G_{\text{Kv4.3}}\) versus APD relationship described in Figures 7A and 7B may be obscured in the presence of background currents.

The properties of \(I_{\text{to}}\) and its relationship to AP shape may be modulated by additional factors. Recently, members of the KChIP family of proteins were found to modulate Kv4.3 currents in oocytes at room temperature.\(^3\) Extrapolation of the lower range of those observed experimentally.\(^4\) Clearly, the \(G_{\text{Kv4.3}}\) versus APD relationship described in Figures 7A and 7B may be obscured in the presence of background currents.

The properties of \(I_{\text{to}}\) and its relationship to AP shape may be modulated by additional factors. Recently, members of the KChIP family of proteins were found to modulate Kv4.3 currents in oocytes at room temperature.\(^3\) Extrapolation of these data to myocytes at 35°C is difficult. At this time, there is insufficient data to explore this issue quantitatively. Moreover, the close similarity in kinetic properties of expressed Kv4.3 and Kv1.4 currents to the 2 major components of native \(I_{\text{to}}\) in human and canine myocytes suggests that the role of accessory proteins may be subtle. Since \(I_{\text{calc}}\) (Figure 6) and, to a lesser extent, \(I_{\text{leak}}\) (Figure 5B) interact with \(I_{\text{to}}\), any factors that modulate these currents, in addition to direct modulators of \(I_{\text{to}}\), are likely to influence the impact of \(I_{\text{to}}\) on AP shape.

The influence of \(I_{\text{to}}\) on the trajectory of \(I_{\text{calc}}\), and on the profile and duration of the AP demonstrates the complex interaction of currents that are active during phase 1. A reduction of \(I_{\text{to}}\) from normal levels tends to produce modest shortening of APD, contrary to the belief that loss of \(I_{\text{to}}\) may be responsible for the extreme APD prolongation observed in heart failure. Implicit in this finding is that \(I_{\text{to}}\) may not play a critical role in APD prolongation-induced arrhythmias, such as early afterdepolarizations.

Interactive Model Available on the Internet

An interactive version of the WRJ canine myocyte model as described in this article is available at the National Simulation Resource (NSR) via the Internet at http://nsr.bioeng.washington.edu/Software/DEMO/CANINE-AP. Please see the online data supplement at http://www.circresaha.org for more details.

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References


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Joseph L. Greenstein, Richard Wu, Sunny Po, Gordon F. Tomaselli, and Raimond L. Winslow

Interactive Model Available on the Internet

We would like to encourage our readers to take the opportunity to interact with our computational myocyte model. An interactive version of the Winslow-Rice-Jafri (WRJ)$^1$ canine ventricular cell model including the new Kv4.3/Kv1.4-based model of $I_{to1}$ as described in this paper is available via the internet at http://nsr.bioeng.washington.edu, the National Simulation Resource (NSR) website at the University of Washington. Users may access the WRJ canine action potential model, an accompanying model description and software user manual directly at: http://nsr.bioeng.washington.edu/Software/DEMO/CANINE-AP. The NSR simulation software, XSIM, is an X-Windows program and therefore requires your local machine to be running an X-server. See the website for details. The available tools allow users to set parameters, run multiple simulations, and visualize output graphically. We would like to thank James Bassingthwaighte and Zheng Li for implementing our model in the NSR, and making it available to the scientific community. We are grateful to Eduardo Marbán and the editors of Circulation Research for embracing web-based interactive modeling technologies as a valued component of its online publication content.
Expanded Methods

Markov State Model of the hKv4.3 Channel:

The hKv4.3-2 channel Markov model is derived based on the assumption that members of the Kv4 family of K⁺ channels are homotetrameric. Each subunit contains a voltage sensor for activation in a similar manner to Kv1 channels.²,³ Therefore, a model consisting of four closed and four closed-inactivated states, one open state, and one open-inactivated state, as shown in Online Figure 1, is employed. Each transition from left to right within the upper row of states represents progression toward the open state via activation of a single subunit’s voltage sensor. The channel conducts once all four of the subunits have been activated. The scaling of forward and reverse activation rates is based on the assumption that the four subunits activate in a manner that is identical and independent. The forward and reverse rates for the activation process, αᵢ and βᵢ respectively, are exponential functions of voltage in accordance with Eyring rate theory. For each state in the upper row, there is a corresponding inactivated state in the lower row. In a manner similar to activation, transition rates into and out of the inactivated states, βᵢ and αᵢ respectively, are expressed as exponential functions of voltage. The voltage dependent transition rates (in ms⁻¹) are calculated as follows:

\[ a_a(V) = a_{a0}e^{a_aV} \]  (1)
\[ b_a(V) = b_{a0}e^{-b_aV} \]  (2)
\[ a_i(V) = a_{i0}e^{-a_iV} \]  (3)
\[ b_i(V) = b_{i0}e^{b_iV} \]  (4)

where \( V \) is membrane potential in mV. The forward and reverse rates between non-inactivated and inactivated states (i.e. transitions between upper and lower rows of Online Figure 1) are assigned the scaling factors \( f_1 \) - \( f_4 \) and \( b_1 \) - \( b_4 \) respectively. The scaling factors allow these
transition rates to differ as a function of the state of activation of the channel. This assumption of coupling of inactivation to activation provides the necessary additional degrees of freedom to enable the model to accurately fit all aspects of measured macroscopic current behavior. Inactivation is not constrained to be voltage-independent, as it is generally the case for Kv1 channels.\textsuperscript{4,5} Microscopic reversibility\textsuperscript{6} is satisfied by choosing appropriate scaling rates for transitions between inactivated states (in the lower row).

The hKv4.3-2 ionic current is computed as

\[ I_{\text{Kv4.3}}(V,t) = G_{\text{Kv4.3}} \times P_{\text{open}}(V,t) \times \{V(t) - E_K\} \]  \hspace{1cm} (5)

where \( I_{\text{Kv4.3}} \) is the hKv4.3-2 current, \( G_{\text{Kv4.3}} \) is the maximal channel conductance, \( P_{\text{open}}(V,t) \) is the probability the channel is in the open state, \( V(t) \) is membrane potential, and \( E_K \) is the potassium reversal potential as determined by the Nernst equation.

\[ \]

**Online Figure 1.** State diagram of the hKv4.3-2 encoded K\textsuperscript{+} channel Markov model. The model structure consists of four closed states (C\textsubscript{0} – C\textsubscript{3}), four closed-inactivated states (CI\textsubscript{0} – CI\textsubscript{3}), one open state (O), and one open-inactivated state (OI). The transition rates \( \alpha_a, \beta_a, \alpha_i, \) and \( \beta_i \) are voltage dependent, and the scaling factors, \( f_1 – f_4 \) and \( b_1 – b_4 \), allow for coupling of inactivation to activation.
Markov State Model of the hKv1.4 Channel:

The model of $I_{Kv1.4}$ is based on the model structure used for $I_{Kv4.3}$ (Online Figure 1) with the additional constraint that inactivation is assumed to be voltage independent$^4,5$ (i.e. $a_i = b_i = 0$ in Eqs. 1-4). Permeation of $K^+$ through the Kv1.4 channel exhibits outward rectification, which is described using the Goldman-Hodgkin-Katz current equation.$^7$ The Kv1.4 $K^+$ current is therefore computed as

$$I_{Kv1.4,K} = \frac{P_{Kv1.4}}{C_{sc}} \times \frac{F^2V}{RT} \times P_{open}(V,t) \times \frac{[K^+]_i - [K^+]_o e^{-VF/RT}}{1 - e^{-VF/RT}} \quad (6)$$

where $P_{Kv1.4}$ is the maximum channel permeability to $K^+$, $C_{sc}$ is the specific membrane capacity ($10^4 \text{ pF/mm}^2$), $F$ is the Faraday constant, $R$ is the gas constant, $T$ is absolute temperature, $[K^+]_i$ and $[K^+]_o$ are intracellular and extracellular $K^+$ concentrations respectively, and $P_{open}$ is the probability the channel is in the open state. The Kv1.4 channel exhibits a small permeability to $Na^+$ ions.$^7$ The $Na^+$ current through the Kv1.4 channel and the total Kv1.4 current are described by Eqs. 7 and 8 respectively

$$I_{Kv1.4,Na} = \frac{\alpha P_{Kv1.4}}{C_{sc}} \times \frac{F^2V}{RT} \times P_{open}(V,t) \times \frac{[Na^+]_i - [Na^+]_o e^{-VF/RT}}{1 - e^{-VF/RT}} \quad (7)$$

$$I_{Kv1.4} = I_{Kv1.4,K} + I_{Kv1.4,Na} \quad (8)$$

where $[Na^+]_i$ and $[Na^+]_o$ are intracellular and extracellular $Na^+$ concentrations respectively and $\alpha$ is the ratio of $Na^+$ to $K^+$ permeability with a value of 0.02.$^7$
Model Fitting Process:

The time evolution of the state occupation probabilities for any finite state Markov process is described by the Kolmogorov forward equations. In matrix notation, these equations are

$$\dot{\mathbf{P}}(t) = \mathbf{A}\mathbf{P}(t) \quad (9)$$

where $\mathbf{P}(t)$ is expressed as a column vector of probabilities for occupying each state, and $\mathbf{A}$ is the state transition matrix. Since the rate constants are functions of voltage, $\mathbf{A}$ is generally a voltage-dependent matrix. Under applied voltage clamp protocols, however, this system is a piece-wise linear time invariant system. In this case the form of the analytic solution for an $n$ state Markov process is

$$\mathbf{P}(t) = \sum_{i=1}^{n} c_i \mathbf{e}_i \exp(\lambda_i t) \quad (10)$$

where $\lambda_i$ is the $i^{th}$ eigenvalue of the matrix $\mathbf{A}$, $\mathbf{e}_i$ is its corresponding eigenvector, and $c_i$ is a constant of integration which depends on the initial value, $\mathbf{P}(t=0)$.

The free parameters of each model are determined using a Nelder-Mead simplex search algorithm implemented by MATLAB (The Mathworks Inc.). In order to compare model behavior to experimental data, Eq. 10 is used to calculate model response to the identical voltage clamp protocols used for experimental characterization of the hKv4.3-2 and hKv1.4 expressed currents. For hKv4.3-2, parameter sets were determined at 35°C based on the data obtained in this study. For hKv1.4, parameters are fit at room temperature (21-23°C) based on data reported by Po et al. The quality of fit for any set of parameters is measured by a weighted cost function. The cost function accounts for differences between experiment and simulation by incorporating the mean squared error in time-to-peak (over the range $-10$ mV to $+60$ mV for
Kv4.3, at +100 mV for Kv1.4), normalized peak current (over the range −40 mV to +60 mV), and time constant of inactivation (over the range −20 mV to +60 mV for Kv4.3, −10 mV to +40 mV for Kv1.4). The cost function accounts for differences in steady state availability by including the squared error of the half maximal availability \( (V_{1/2}) \) and slope factor \( (k) \), in Boltzmann function fits (i.e. \( [1 + e^{-(V-V_{1/2})/k}]^{-1} \)) to model currents vs. experimental currents. An error term (mean steady state availability over the range 0 mV to 55 mV) is included to insure that the current is fully inactivating. The cost function accounts for the time course of recovery from inactivation by including the squared error in time constants from fits to monoexponential recovery curves for model simulations vs. experimental data. Recovery curves are obtained by depolarizing cells to 50 mV for 500 ms, then holding at −100 mV or −80 mV for a variable duration, then depolarizing to 50 mV for 500 ms. The currents during the second pulse are normalized by the current measured on the first pulse. The optimal set of parameters for the hKv4.3-2 channel expressed in mouse Ltk\(^{-}\) cells at 35°C based on the data obtained in this study are given in the Online Table.

**Model of Canine I\(_{\text{to1}}\):**

Canine I\(_{\text{to1}}\) is assumed to consist of two distinct currents, I\(_{\text{Kv4.3}}\) and I\(_{\text{Kv1.4}}\). Minor adjustments are made to each of these components to insure agreement of the I\(_{\text{to1}}\) model with native canine I\(_{\text{to1}}\) characteristics. The time constant of inactivation of Kv4.3 is reduced based on fast inactivation time constants measured for native I\(_{\text{to1}}\) in both human and canine ventricular cells.\(^{11-13}\) The inactivation time constant was reduced to \( \approx9 \) ms at depolarized potentials (from \( \approx13.5 \) ms in expressed hKv4.3-2). All other aspects of Kv4.3 behavior were left unchanged.

The Kv1.4 time constant of recovery from inactivation was reduced from that measured by Po et al\(^{10}\) in order to match data from human and canine myocytes. For behavior at 22°C,
this time constant was set to 3500 ms and 2000 ms at recovery potentials of –80 mV and –100 mV respectively. These values are in the midrange of time constants for the slow component of recovery from inactivation measured by Näbauer et al in human left ventricular subendocardial cells (3109±155 ms at –80 mV)\textsuperscript{12} and failing human ventricular midmyocardial cells (4425 ms at –80 mV, 2331 ms at –100 mV),\textsuperscript{14} and by Kääb et al\textsuperscript{15} in canine ventricular midmyocardial cells (1593±323 ms at –100 mV). The parameters of the Kv1.4 model are scaled to 35ºC using a Q\textsubscript{10} of 3.6 based on measurements of inactivation kinetics made in human native I\textsubscript{to1}.\textsuperscript{12} In order to maintain steady state behavior, all rates in the model are scaled by the same Q\textsubscript{10}. This yields a model time constant of recovery from inactivation of 580 ms at 35ºC for a recovery potential of –80 mV, a value which is in the midrange of time constants measured by Liu et al\textsuperscript{11} in canine midmyocardial cells at 37ºC (456±212 ms), by Näbauer et al\textsuperscript{12} in human left ventricular subendocardial cells at 35ºC (839±38 ms), and by Li et al\textsuperscript{13} in human right ventricular endocardial cells at 36ºC (490±31 ms).

The canine I\textsubscript{to1} current is formed by the combination of the canine I\textsubscript{Kv4.3} and I\textsubscript{Kv1.4} currents in a 77:23 ratio (based on peak currents elicited by a voltage step from –100 mV to 40 mV). This ratio is based on the relative magnitudes of fast vs. slow recovery time constants measured by Kääb et al (estimated based on data of their Figure 7D)\textsuperscript{15} in canine midmyocardial cells and corresponds to values of 0.1194 nS/pF and 1.709x10\textsuperscript{-6} mm/s for G\textsubscript{Kv4.3} and P\textsubscript{Kv1.4} respectively. Optimized parameter values for the Kv4.3 and Kv1.4 components of the canine I\textsubscript{to1} model at 35ºC are given in the Online Table.

**Ventricular Myocyte Models:**

The canine I\textsubscript{to1} is incorporated into the Winslow-Rice-Jafri (WRJ)\textsuperscript{1} canine ventricular cell model. The dynamical equations are solved using the Merson modified Runge-Kutta fourth-
order adaptive step algorithm, with a maximum step size of 100 µsec and a maximum normalized truncation error tolerance of $10^{-6}$. APs were simulated at 1 Hz and 2 Hz periodic pacing to steady state. APs are initiated using a 100 pA/pF current injection with 500 µs duration.

**Online Table.** Optimized Markov model parameter values at 35°C.

<table>
<thead>
<tr>
<th></th>
<th>hKv4.3-2 expressed current</th>
<th>Kv4.3 component of canine $I_{o1}$</th>
<th>Kv1.4 component of canine $I_{o1}$</th>
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<tr>
<td>$\alpha_{d0}$ (ms$^{-1}$)</td>
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References


