Proarrhythmic Consequences of a KCNQ1 AKAP-Binding Domain Mutation

Computational Models of Whole Cells and Heterogeneous Tissue

Jeffrey J. Saucerman, Sarah N. Healy, Mary E. Belik, Jose L. Puglisi, Andrew D. McCulloch

Abstract—The KCNQ1-G589D gene mutation, associated with a long-QT syndrome, has been shown to disrupt yotiao-mediated targeting of protein kinase A and protein phosphatase-1 to the IKs channel. To investigate how this defect may lead to ventricular arrhythmia during sympathetic stimulation, we use integrative computational models of β-adrenergic signaling, myocyte excitation-contraction coupling, and action potential propagation in a rabbit ventricular wedge. Paradoxically, we find that the KCNQ1-G589D mutation alone does not prolong the QT interval. But when coupled with β-adrenergic stimulation in a whole-cell model, the KCNQ1-G589D mutation induced QT prolongation and transient afterdepolarizations, known cellular mechanisms for arrhythmogenesis. These cellular mechanisms amplified tissue heterogeneities in a three-dimensional rabbit ventricular wedge model, elevating transmural dispersion of repolarization and creating other T-wave abnormalities on simulated electrocardiograms. Increasing heart rate protected both single myocyte and the coupled myocardium models from arrhythmic consequences. These findings suggest that the KCNQ1-G589D mutation disrupts a critical link between β-adrenergic signaling and myocyte electrophysiology, creating both triggers of cardiac arrhythmia and a myocardial substrate vulnerable to such electrical disturbances. (Circ Res. 2004;95:1216-1224.)

Key Words: β-adrenergic signaling ▪ arrhythmia ▪ long-QT syndrome ▪ computational model

Long QT syndrome (LQTS) is a cardiac disorder in which the QT interval on the electrocardiogram (ECG) is prolonged. Patients with mutations in KCNQ1, which encodes the α subunit of IKs, develop an LQTS (LQT1) particularly susceptible to sudden cardiac death during sympathetic stimulation. Whereas healthy individuals have shortened or unchanged QT intervals with exercise or stress, QT intervals in LQT1 patients prolong further, suggesting a problem at the interface of the sympathetic nervous system and electrophysiology.

Motivated to examine the molecular mechanisms at this interface, Kass and colleagues discovered a signaling complex of KCNQ1, protein kinase A (PKA), and protein phosphatase-1 (PP1) mediated by the A-kinase anchoring protein (AKAP) yotiao. LQT1-associated mutation KCNQ1-G589D disrupted the signaling complex, preventing β-adrenergic regulation of IKs. This suggests the possibility that the KCNQ1-G589D mutation, present in 508 of 939 established Finnish LQTS patients and associated with exercise-induced arrhythmias, may cause an unusual LQTS in which the primary defect occurs in autonomic regulation rather than channel gating per se.

These findings raise a number of integrative questions for which whole-cell and multicellular models are not yet available. What are the cellular mechanisms by which the G589D mutation alters sympathetic response of the myocyte action potential? Does the mutation prolong the baseline action potential duration (APD) or prevent appropriate rate-dependent APD shortening? Could this mutation induce trigger events for arrhythmia (eg, afterdepolarizations), and if so, by what mechanisms are these triggers generated? How do these proarrhythmic cellular mechanisms interact with fiber architecture and cellular heterogeneity to affect action potential propagation and repolarization in the myocardium?

Recent examples have shown a promise of computational models for investigating the impact of gene mutations, tissue heterogeneities, and dynamical instabilities on arrhythmic mechanisms. Here, we use an integrative computational model of β-adrenergic signaling, excitation-contraction coupling, and action potential propagation to investigate the whole-cell and myocardial consequences of KCNQ1/KCNE1 signaling complex disruption. Model analysis indicates that although the KCNQ1-G589D mutation did not necessarily prolong the QT interval at rest, sympathetic-stimulated increases in ICa, left uncompensated by increased IKr, resulted in QT prolongation. This impaired response to sympathetic stimulation precipitated calcium-mediated afterdepolarization.

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tions and transmural dispersion of repolarization in ventricular tissue, significant factors related to arrhythmic risk. However, moderate increases in heart rate protected against these proarrhythmic mechanisms.

**Materials and Methods**

**Signal Transduction Model**

A mechanistic mathematical model of β-adrenergic signaling in rat was adapted for the rabbit ventricular myocyte by making the following changes motivated by experimental data: adjusting protein expression levels for β-adrenergic receptors, PDE4, PKA, and phospholamban; inclusion of the PDE3 isoform; and the addition of a KCNQ1/KCNE1 signaling complex containing KCNQ1, KCNE1, yotiao, PKA, and PP1. Methods for modeling these signaling networks have been described in detail previously. The new signaling model included a total of five PKA phosphorylation targets: the L-type calcium channel (ICaL), phospholamban (PLB), the ryanodine receptor (RyR), troponin I (TnI), and IKs. Based on single-channel recordings of IKs conductance and whole-cell IKs conductance in rabbit ventricular myocytes, KCNQ1 was assumed to be expressed at 25 nmol/L cytosol, with quasi-equilibrium binding (KC50=0.1 mmol/L) to an equivalently expressed yotiao. Yotiao was assumed necessary for interaction between KCNQ1 and PKA or PP1, binding one PP1 and one PKA holoenzyme per anchoring protein. The KCNQ1-G589D defect associated with LQT1 syndrome was modeled as a 10-fold increase in the dissociation constant for KCNQ1 and yotiao (Figure 1A).

**Excitation-Contraction Coupling Model**

We used a previously published model of excitation-contraction coupling in the adult rabbit ventricular myocyte, modified with a simple model of calcium-induced calcium release and a reversible CaSR-ATPase pump. Details of modeling the consequences of ICaL, PLB, RyR, and TnI phosphorylation have been described previously and are summarized in Table S1 in the online data supplement available at http://circres.ahajournals.org. Phosphorylated IKs channels were modeled as being 3.6-fold more likely to be actively gating, with a 35.6 mV leftward shift in the activation curve for the IKs gating variable, consistent with whole-cell patch clamp recordings with cAMP and isoproterenol. An IC50 of 0.43 μmol/L was used for nifedipine block of the L-type calcium channel. The signaling and excitation-contraction coupling mechanisms included in the cellular model have been described in detail previously.
Rabbit Ventricular Wedge Model

To simulate β-adrenergic regulation of electrophysiology at the tissue level, we used a 2-element wedge (dimensions ~0.9 x 0.8 x 0.5 cm) taken from the original 36-element anatomic model of the rabbit ventricular geometry and fiber architecture described by Vetter and McCulloch. This wedge was refined in each direction to yield a 1024-element wedge, which was sufficient to obtain solutions for conduction velocity and action potential duration that were both converged to within 2% (data not shown). Transmural electrical heterogeneity in the wedge was incorporated using endocardial, midmyocardial, and epicardial layers with relative thicknesses of 3:3:2. Relative current densities in each region (see online data supplement for parameters) were estimated from rabbit (endocardial/epicardial data for I_{Na},27 I_{Kr},27 and I_{to}28; endocardial/midmyocardial/epicardial I_{Ks} estimated from upstroke velocity) or when necessary canine (I_{Ks},29 midmyocardial I_{Ks}, Ik, and I_{to}28). Simulations of a larger heart were performed by decreasing the diffusion coefficient of 50%, which increases transmural activation time by 50% as well. Initial conditions for finite element simulations were calculated with the integrated signaling/excitation-contraction coupling cell model. We approximated phosphorylation levels as constant over the interval of tissue simulation, ~2 seconds. Simulations were performed using a collocation-Galerkin finite element method.10

Model Validation

The cellular model was validated with independent experimental data from the literature at a variety of functional levels, obtained from isolated rabbit ventricular myocytes whenever possible. The signaling portion of the model exhibited appropriate concentration response of cAMP and PKA activity to isoproterenol (EC_{50} of 10 nmol/L and 11 nmol/L versus 12 nmol/L and 9 nmol/L), basal particulate cAMP (3.2 versus 3.7 pmol/mg), desensitization magnitude (35% versus 37%), cAMP rise, and PKA activation time (1.4 versus 1 minute), cAMP decline and PKA deactivation time (t_{50} of 5 minutes and 5 minutes versus 4 minutes and 7 minutes), and downstream phosphorylation levels (PLB E_{50} 20 versus 7.1 nmol/L; TnI EC_{50} 21 versus 3.2 nmol/L) (see Figure S1 in the online data supplement). The functional consequences of β-adrenergic signaling in the rabbit ventricular myocyte were validated as well, confirming consistent changes in calcium channel current (1.8- versus 2.2-fold increase), calcium inotropy (1.9- versus 1.8-fold increase in Δ[Ca^{2+}], and decreased action potential duration (14% versus 10%). β-Adrenergic regulation of I_{Ks} caused an increase in maximum conductance (2.7 versus 2.4-fold), a leftward shift in the conductance-voltage relation (13 versus 9 mV), and no change in the activation/inactivation time constant (see Figure S2 in the online data supplement).

In the ventricular wedge model, the diffusion coefficient was chosen to yield a transmural conduction velocity of 27 cm/s at 3 Hz, agreeing with results from Sung et al.,7 who measured transverse velocities of 23±6 cm/s in an isolated Langendorff-perfused rabbit heart. All measurements of electrophysiological function were consistent with the experimental results performed in arterially perfused rabbit wedge preparations of similar size at 1 Hz: endocardial APD_{90} (217 versus 212 ms), epicardial APD_{90} (166 versus 191 ms), QT interval (245 versus 251 ms), and transmural dispersion of repolarization (TDR) (36 versus 43 ms) (see Figure 6C).

Results

Effect of KCNQ1-G589D Mutation on Sympathetic Regulation of I_{Ks}

We incorporated known protein interactions in the KCNQ1/KCNE1 signaling complex into a mathematical model of β-adrenergic signaling and excitation-contraction coupling in the rabbit ventricular myocyte. We found that in wild-type (WT) myocyte models, this signaling complex allows a large dynamic range of KCNQ1 phosphorylation level on isoproterenol stimulation, which is abolished with disruption of the KCNQ1/yotiao interaction caused by the G589D mutation in KCNQ1 (Figure 2A). Incorporating β-adrenergic regulation of I_{Ks} into the model, we predicted dynamic changes in WT but not G589D mutant I_{Ks} with isoproterenol (Figure 2B). Thus, our mathematical model predicts the known subcellular consequences of the KCNQ1-G589D gene defect consistent with experimental patch-clamp data from expression systems.3

KCNQ1-G589D Mutation Prolongs Cardiac Myocyte APD in Response to Isoproterenol

By incorporating the signaling interactions of the KCNQ1/KCNE1 complex into a whole-cell model of signaling and excitation-contraction coupling, we assessed the cellular-level impact of the KCNQ1-G589D mutation. At 1 Hz, WT myocyte models stimulated with isoproterenol exhibited shortened action potentials (decreased APD_{90} 12%) because of the influence of phosphorylated KCNQ1/KCNE channels (Figure 3A). In contrast, G589D myocyte models treated with isoproterenol exhibited 15% larger APD_{90} than untreated myocytes (Figure 3A). The rate dependence of APD is important for normal function of the heart as well; so we examined whether the G589D mutation altered this relationship either in untreated or isoproterenol-treated conditions. Untreated WT and G589D myocyte models have extremely similar action potentials. Isoproterenol-stimulated G589D mutants exhibit longer APDs than WT for all cycle lengths (CLs) such that the general rate-dependent APD shortening is preserved (Figure 3B).
Sympathetic Stimulation May Induce Calcium-Mediated Afterdepolarizations in G589D Mutant Myocytes

Cardiac myocytes with prolonged action potentials are susceptible to early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs), which may serve as cellular-level triggers for cardiac arrhythmias and sudden cardiac death. We found both EADs and DADs in isoproterenol-stimulated G589D myocyte models at long cycle lengths, but not in WT. Figure 4A demonstrates the pattern of observed afterdepolarizations with 1 Hz pacing, exhibiting the first EAD at 8 seconds after isoproterenol exposure as PKA-regulated channels undergo significant transitions. Afterdepolarizations subsequently disappear as the signaling state

Figure 3. G589D mutation prolongs APD in isoproterenol-treated myocyte models. (A) Simulated myocyte action potentials at 1 Hz, showing decreased APD_{90} in isoproterenol-treated WT (1 μmol/L; solid line) and prolonged APD_{90} in isoproterenol-treated G589D mutants (dashed line) compared with untreated WT (dotted line) and G589D mutants (dot-dashed line). (B) Increasing pacing rates shorten both WT (untreated, open circles; 1 μmol/L isoproterenol, filled circles) and G589D (untreated, open squares; isoproterenol, filled squares) APD_{90}s. Isoproterenol-treated G589D myocyte models exhibit increased APD_{90} at all rates compared with WT.

Figure 4. Calcium-mediated afterdepolarizations develop in a KCNQ1-G589D myocyte model with isoproterenol. A, Action potential record of a G589D mutant myocyte model with 0.1 μmol/L isoproterenol added at time 0, starting at steady-state. Several EADs occur; yet the cell returns to a stable state. B, Inset from box in A, showing action potential, [Ca], and I_{Ca} during the first EAD following isoproterenol exposure in G589D (dashed lines), compared with WT (solid lines) myocyte models. As the action potential prolongs in G589D mutants, I_{Ca} reactivates and initiates an EAD.
G589D Mutation Prolongs QT and Elevates TDR in a Rabbit Ventricular Wedge Model

To investigate potential consequences of the observed proarhythmic cellular mechanisms, we developed a three-dimensional model of action potential propagation in a rabbit ventricular wedge. APD rate-dependence of WT endocardial, midmyocardial, and epicardial cell models were validated with published experimental data. Only high concentrations of isoproterenol elicited afterdepolarizations. On the other hand, EAD and DAD incidence in 1 μmol/L isoproterenol-stimulated G589D myocyte models decreases with increasing pacing rate (Figure 5B), presumably because of the shortened APDs (see Figure 4B). Single EADs were also observed following a 1.5 second pause in isoproterenol-stimulated G589D myocyte models nominally at 2 and 3 Hz.

EAD-like responses in the endocardial and midmyocardial regions, but the large EADs seen in single cell simulations appear blunted because of electrotonic coupling. Sympathetic stimulation increases APD in the G589D mutant ventricular wedge heterogeneously (Figure 7B), elevating transmural dispersion of repolarization (TDR) at large cycle lengths (Figure 7C). TDR is often used as an indicator of arrhythmic risk. TDR increased with cycle length in both WT and mutant tissue models, suggesting a protective role for higher heart rates (Figure 7C). Increased TDR (from 19 to 39 ms) was also observed in G89D wedge models following a 1.5-second pause from 500- and 333-ms basic cycle lengths.

The likelihood for the G589D proarhythmic cellular mechanisms to manifest ECG abnormalities appears further increased in simulations of a larger heart. A very large, broad T-wave was observed (Figure 7D, second beat), because of strong EADs elicited by the endocardium. On the subsequent beat, small EADs isolated to a region of midmyocardium cause a bifurcated T-wave with an inverted T2 component. Electrotonic coupling appears lower in the larger heart, allowing more irregular repolarization patterns with G589D mutation.

Discussion

This study investigated potential cell and tissue-level mechanisms that may bridge the KCNQ1-G589D gene mutation to aspects of the LQT1 clinical phenotype. Incorporating the molecular consequences of the KCNQ1-G589D defect into a mathematical model of the rabbit ventricular myocyte, we found increased APD and a susceptibility to afterdepolarizations only with sympathetic stimulation. Extending spatially to model a rabbit ventricular wedge preparation, we examined the role of interactions between these cellular mechanisms, cell-type heterogeneities, and fiber angle distributions on action potential propagation and simulated ECGs. These analyses suggest a mechanistic link from the KCNQ1-G589D gene defect to TDR and possible T-wave abnormalities in the ventricle, clinical indicators of arrhythmic risk in LQT syndrome.

Enhanced calcium channel currents with isoproterenol tend to increase APD, which was counterbalanced by enhanced IKs, current in WT but not G589D mutant models.
These findings are consistent with experimental data showing variable APD changes in isoproterenol-stimulated WT myocytes, but significantly longer APDs when $I_{Ks}$ or $I_{Kr}$ channels are blocked pharmacologically. Isoproterenol shortened WT APD at low but not high rates, qualitatively matching experimental studies showing that high rates either reduce or eliminate isoproterenol-dependent shortening of WT APD. Although the rate dependence of APD has been hypothesized to be altered with KCNQ1/KCNE1 signaling complex disruption, we found that the slow reactivation of $I_{Ks}$ maintained rate-dependent shortening of G589D mutant APDs qualitatively similar to WT. The prolonged APD predicted in isoproterenol-stimulated G589D myocyte models increased susceptibility to afterdepolarizations. EADs and DADs, hypothesized as triggers for arrhythmia, have been studied in both mathematical and experimental models of drug-induced LQTS. The calcium-mediated mechanisms for EAD and DAD initiation, namely $I_{Ca}$ reactivation and spontaneous SR calcium release, were confirmed by afterdepolarization suppression with the calcium channel blocker nifedipine. Experimentally, isoproterenol may trigger occasional afterdepolarizations in WT myocytes, potentially because of stochastic $I_{Ca}$ or RyR gating. Our deterministic WT models appeared somewhat more stable, which should have made the model more resistant to the observed EADs and DADs with G589D mutation.

We found that during sympathetic stimulation, the predicted cellular consequences of the G589D mutation may amplify existing tissue heterogeneities as evidenced by changes in the ECG and underlying action potentials from the ventricular model. Isoproterenol increased QT interval and TDR in the G589D ventricular wedge model, consistent with pharmacological models of LQT1 in canine. Whereas strong electrotonic coupling appeared to blunt EADs in our original rabbit wedge model, a larger G589D mutant wedge allowed pronounced ECG abnormalities including sudden large, broad T-waves and T-wave inversion under otherwise unaltered conditions. Similar EADs, TDR, and ECG abnormalities in acquired LQT models have been reported as precursors to torsade de pointes (TdP), a polymorphic ventricular tachycardia. These mechanistic simulations support the clinical observation that patients with the KCNQ1-G589D mutation are particularly vulnerable to exercise-induced arrhythmias.

Consistent with previous cellular simulations and wedge experiments, QT prolongation, EAD susceptibility, and TDR decreased in models of G589D myocytes and myocardium at increased rates. Whereas sympathetic stimulation increases rate in the intact heart, a vulnerable window to arrhythmia may develop during transitions in sympathetic stimulation or following an excitation pause. Pauses may trigger EADs in LQTS and have been seen preceding 74% of TdP in congenital LQTS patients. We found that pauses allowed EADs in G589D cell models and increased TDR in G589D wedge models at higher pacing rates. This may explain why, as in previous experimental studies, some proarrhythmic mechanisms were seen at rates lower than expected of the normal rabbit heart. Taken together, our models of myocytes and heterogeneous tissue support the hypothesis that sympathetic stimulation of the ventricle may...
allow whole-cell and tissue-level arrhythmic mechanisms in G589D mutants, whereas increased heart rate may be protective.

Because we investigated the direct consequences of the G589D mutation, we did not include secondary changes in protein expression. KCNQ1 mutants in cell expression systems suggest a potential for IKs downregulation, although clinical data are not yet available. Conceivably, the KCNQ1-G589D mutation could work together with IKs downregulation to manifest arrhythmic risk. In preliminary simulation studies, we found that a 50% decrease in IKs in conjunction with G589D mutation would produce a resting long QT phenotype not seen with G589D mutation alone, without qualitatively affecting the cellular and tissue-level responses to sympathetic stimulation shown above. Perhaps prolonged resting QT, the traditional clinical indicator of LQT1, may be a secondary consequence in patients with the G589D mutation. This hypothesis is consistent with experimental models of acquired LQTS that suggest TDR, rather than QT prolongation itself, may allow cellular triggers to propagate into TdP. This hypothesis is also consistent with a clinical study that showed LQT1 genotype to correlate better with epinephrine-stimulated QT prolongation than resting QT.

A mechanistic β-adrenergic signaling model has allowed us to characterize the graded, dynamic regulation of E-C coupling and mechanistically model the G589D mutation. Thus, we predicted rather than imposed the regulatory consequences of G589D mutation and demonstrated robustness to isoproterenol concentration in the predicted phenotypes. With large changes in phosphorylation levels during simulations, the detailed model was vital for describing a transient vulnerable period for EADs and calculating appropriate initial conditions for ventricular wedge simulations. Finally, the detailed model creates a framework for investigating potential therapeutics such as β-blockers, PDE inhibitors, and PLB inhibitors in the context of this congenital LQTS.

A number of limitations in this study must be considered when interpreting the results. At the level of signaling networks, many pathway and cross-talk interactions were not modeled. Sympathetic stimulation also activates the β2-AR and α1-AR pathways, although they are not as prominent as β1-AR signaling in the overall inotropic and electrophysiological response under normal conditions. Calmodulin and CaMKII pathways affect the frequency dependence of several aspects of excitation-contraction coupling; incorporating these regulatory interactions may increase APD and afterdepolarization incidence at higher pacing rates. The controversial β-adrenergic actions on IKr have not been included, as they are thought to act indirectly via unknown mechanisms including PKC rather than direct PKA-mediated phosphorylation networks, many pathway and cross-talk interactions were not modeled. Sympathetic stimulation also activates the β2-AR and α1-AR pathways, although they are not as prominent as β1-AR signaling in the overall inotropic and electrophysiological response under normal conditions. Calmodulin and CaMKII pathways affect the frequency dependence of several aspects of excitation-contraction coupling; incorporating these regulatory interactions may increase APD and afterdepolarization incidence at higher pacing rates. The controversial β-adrenergic actions on IKr have not been included, as they are thought to act indirectly via unknown mechanisms including PKC rather than direct PKA-mediated phosphorylation.
lation. Although we have included the functional roles of some A-kinase anchoring proteins, many mechanisms for signaling compartmentation are still unclear. Our model of cardiac handling uses a simplified representation of calcium-induced calcium release, which is unable to predict stochastic behavior such as calcium sparks or the role of calcium in the junctional subspace. However, our model is sufficient to predict graded calcium release, cytosolic calcium transients, and generation of EADs and DADs.

Although heterogeneities in the myocardium can greatly affect action potential propagation, we have only accounted for the most prominent differences. We have modeled endocardial, midmyocardial, and epicardial myocytes arranged in transmural layers, although these cells may not be arranged in distinct layers in the real heart. We have modeled fiber angle distributions but not the discontinuous architecture of laminar sheets. Although we did not model Purkinje fibers, our endocardial pacing as in experimental wedge preparations is unlikely to be greatly affected. The experimental perfused-wedge preparation has been used in numerous studies of ventricular heterogeneity and acquired LQTS. However, the ventricular wedge used here and in previous experimental work lacks many anatomic and physiologic features of the intact heart. We did not observe TdP, perhaps because of the size of our ventricular wedge. Although we have modeled sympathetic stimulation as uniform within the ventricular wedge (as in experimental wedge preparations), heterogeneity of cardiac innervation and expression of signaling proteins may contribute to arrhythmia. Despite these limitations, our computational models successfully predicted many known aspects of cardiac excitation-contraction coupling and its regulation by β-adrenergic signaling in rabbit.

In conclusion, the KCNQ1-G589D gene defect appears to provide evidence for a strong founder effect in Finland. Toivonen L, Kontula K. Four potassium channel mutations account for 56% of the genetic spectrum underlying long QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland. Ann Med. 2004; 36(suppl 1):53–63.


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Data Supplement

Table

Table S1

Modeled consequences of complete PKA-mediated phosphorylation of targets involved in excitation-contraction coupling. The model predicts graded and dynamic phosphorylation levels, with complete phosphorylation rarely being reached in model simulations with β-adrenergic stimulus alone.

<table>
<thead>
<tr>
<th>PKA Target</th>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLB</td>
<td>Km_up</td>
<td>4.0x ↓</td>
<td>Releases PLB-mediated inhibition of Ca binding to the SR Ca-ATPase (SERCA).</td>
<td>(1)</td>
</tr>
<tr>
<td>I_{CaL}</td>
<td>f_Po</td>
<td>2.0x ↑</td>
<td>Increases channel open probability (α_{iC} subunit) and increases channel availability (β_2 subunit).</td>
<td>(2, 3)</td>
</tr>
<tr>
<td></td>
<td>f_LCCavail</td>
<td>2.3x ↑</td>
<td>Increases channel availability and shifts current-voltage relationship leftward.</td>
<td>(7)</td>
</tr>
<tr>
<td>RyR</td>
<td>Krel</td>
<td>3.0x ↓</td>
<td>Increases Ca sensitivity of RyR.</td>
<td>(4, 5)</td>
</tr>
<tr>
<td>TnI</td>
<td>KmTnC</td>
<td>1.45x ↑</td>
<td>Decreases affinity of Ca for TnC</td>
<td>(6)</td>
</tr>
<tr>
<td>I_{Ks} (KCNQ1)</td>
<td>f_Ksavil</td>
<td>3.6x ↑</td>
<td>Increases channel availability and shifts current-voltage relationship leftward.</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>Xs05</td>
<td>-35.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure Legends

Figure S1

Validation of updated β_1-adrenergic signaling model with whole-cell experimental data from rabbit ventricular myocytes (8, 9). Gradual increase and partial adaptation of (A) total cAMP and (B) total PKA activity to exposure of 1 µmol/L isoproterenol (lines- model; circles- experiment from (8)). (C) Concentration response of cAMP bound to PKA upon isoproterenol exposure (line- model; circles- experiment from (9)). (D) Concentration response of type II PKA
regulatory and catalytic subunit dissociation with isoproterenol (line- model; circles- experiment from (9)). cAMP stimulates PKA subunit dissociation, with type II PKA regulatory subunits generally found in particulate cell fractions.

Figure S2
Validation of $I_{Ks}$ regulation by β-adrenergic signaling with experimental data from (7). The model reproduces experimentally observed (A) increases in maximum conductance, (B) a leftward shift in the conductance-voltage relationship, and (C) a lack of change in activation/inactivation time constant with isoproterenol. Panels A and B were obtained using repeated 2 second pulses to the desired test potential (-60 to 90 mV) from a holding potential of -60 mV. All simulation and experimental data are shown at the steady-state (60 seconds for simulations) reached with 1 µmol/L isoproterenol (Iso).

References


Figure S1

A) Cyclic AMP (pmol/mg protein)

B) PKA activity (normalized)

C) PKA-bound cAMP (% of control)

D) Particulate PKA (% of control)
Figure S2
Model equations and parameters

Default units

Concentration (signaling molecules):  \( \mu \text{M} \)
Concentration (ions):  \( \text{mM} \)
Time:  \( \text{s} \)
Potential:  \( \text{mV} \)
Length:  \( \text{cm} \)

Note: A time-scale conversion factor of 10^3 ms/s has been used in the ODEs for excitation-contraction coupling, so that parameters for these ODEs retain units of milliseconds.

Beta-adrenergic signaling modules

Beta1-adrenergic receptor module

\[
L = L_{tot} - (L_R + L_{RG})
\]

\[
L_R = \frac{L \times \beta_{1\_AR}}{K_L}
\]

\[
L_{RG} = \frac{L \times \beta_{1\_AR} \times G_S}{K_L \times K_R}
\]

\[
R_G = \frac{\beta_{1\_AR} \times G_S}{K_C}
\]

\[
G_S = G_{tot} - (R_G + L_{RG} + G_S_{\_beta\_gamma})
\]

\[
\beta_{1\_AR} = \beta_{1\_AR_{\_act}} - (L_R + L_{RG} + R_G)
\]

\[
\frac{\text{d}(\beta_{1\_AR_{\_act}})}{\text{d}(\text{time})} = \frac{(k_{\_beta\_ARK\_minus} \times \beta_{1\_AR\_S464} - k_{\_beta\_ARK\_plus} \times (L_R + L_{RG}))}{+ (k_{\_PKA\_minus} \times \beta_{1\_AR\_S301} - k_{\_PKA\_plus} \times PKACI \times \beta_{1\_AR_{\_act}})}
\]

\[
\frac{\text{d}(\beta_{1\_AR\_S464})}{\text{d}(\text{time})} = k_{\_beta\_ARK\_plus} \times (L_R + L_{RG}) - k_{\_beta\_ARK\_minus} \times \beta_{1\_AR\_S464}
\]

\[
\frac{\text{d}(\beta_{1\_AR\_S301})}{\text{d}(\text{time})} = k_{\_PKA\_plus} \times PKACI \times \beta_{1\_AR_{\_act}} - k_{\_PKA\_minus} \times \beta_{1\_AR\_S301}
\]
Parameter | Value | Units
---|---|---
$L_{tot}$ | 0.1 | μM
$beta1\_AR_{tot}$ | 0.028 | μM
$G_{stot}$ | 3.83 | μM
$KL$ | 0.285 | μM
$KR$ | 0.062 | μM
$KC$ | 33 | μM
$k_{beta\_ARK\_plus}$ | $1.1\times10^{-3}$ | s$^{-1}$
$k_{beta\_ARK\_minus}$ | $2.2\times10^{-3}$ | s$^{-1}$
$k_{PKA\_plus}$ | $3.6\times10^{-3}$ | s$^{-1}$μM$^{-1}$
$k_{PKA\_minus}$ | $2.2\times10^{-3}$ | s$^{-1}$
$k_{gact}$ | 16 | s$^{-1}$
$k_{hyd}$ | 0.8 | s$^{-1}$
$k_{reassoc}$ | $1.2\times10^{3}$ | s$^{-1}$μM$^{-1}$

**Gs activation module**

$$\frac{d(Gs\_alpha\_GTP_{tot})}{d(time)} = (k_{gact} \times (RG + LRG) - k_{hyd} \times Gs\_alpha\_GTP_{tot})$$

$$\frac{d(Gs\_beta\_gamma)}{d(time)} = (k_{gact} \times (RG + LRG) - k_{reassoc} \times Gs\_alpha\_GDP \times Gs\_beta\_gamma)$$

$$\frac{d(Gs\_alpha\_GDP)}{d(time)} = (k_{hyd} \times Gs\_alpha\_GTP_{tot} - k_{reassoc} \times Gs\_alpha\_GDP \times Gs\_beta\_gamma)$$

**cyclic AMP metabolism module**

$$Gs\_alpha\_GTP = (Gs\_alpha\_GTP_{tot} - Gs\_alpha\_GTP\_AC)$$

$$AC = (AC_{tot} - Gs\_alpha\_GTP\_AC)$$

$$PDE = (PDE_{tot} - PDE_{inhib})$$

$$IBMX = (IBMX_{tot} - PDE_{inhib})$$

$$Gs\_alpha\_GTP\_AC = \frac{Gs\_alpha\_GTP \times AC}{K_{Gs\_alpha}}$$

$$PDE_{inhib} = \frac{PDE \times IBMX}{K_{IBMX}}$$
\[
\frac{d(cAMP_{tot})}{d(time)} = \frac{k_{AC \, basal} \ast AC \ast ATP}{Km_{basal} \ast ATP} + \frac{k_{AC \, Gs \, alpha} \ast Gs \, alpha \ast GTP \ast AC \ast ATP}{Km_{Gs \, alpha} \ast GTP \ast ATP} - \frac{k_{PDE} \ast PDE \ast cAMP}{Km_{PDE} \ast cAMP} 
\]

Parameter | Value | Units
--- | --- | ---
\(AC_{tot}\) | 0.047 | \(\mu M\)
\(ATP\) | \(5 \times 10^3\) | \(\mu M\)
\(PDE_{3\, tot}\) | 0.06 | \(\mu M\)
\(PDE_{4\, tot}\) | 0.036 | \(\mu M\)
\(IBMX_{tot}\) | \(0.103\) | \(\mu M\)
\(K_{Gs \, alpha}\) | 0.4 | \(\mu M\)
\(K_{IBMX}\) | 30 | \(\mu M\)
\(k_{AC \, basal}\) | 0.2 | \(s^{-1}\)
\(Km_{basal}\) | \(1.03 \times 10^3\) | \(\mu M\)
\(k_{AC \, Gs \, alpha}\) | 8.5 | \(s^{-1}\)

PKA activation module

\[
cAMP = (cAMP_{tot} - ((ARCI + 2.0 \ast A2RCI + 2.0 \ast A2RI) + (ARCII + 2.0 \ast A2RCII + 2.0 \ast A2RII)))
\]

\[
PKACI = (2.0 \ast PKAI_{tot} - (RCI + ARCI + A2RCI + PKACI_PKI))
\]

\[
PKACII = (2.0 \ast PKAI_{tot} - (RCII + ARCII + A2RCII + PKACII_PKI))
\]

\[
RCI = \frac{KA \ast KB \ast (cAMP)^{2.0}}{cAMP} \ast \frac{PKACI}{KD} \ast (PKACI + PKACI_PKI)
\]

\[
ARCI = \frac{KA}{cAMP} \ast \frac{PKACI}{KD} \ast (PKACI + PKACI_PKI)
\]

\[
A2RCI = \frac{PKACI}{KD} \ast (PKACI + PKACI_PKI)
\]

\[
A2RI = (PKACI + PKACI_PKI)
\]

\[
RCII = \frac{KA \ast KB \ast (cAMP)^{2.0}}{cAMP} \ast \frac{PKACII}{KD} \ast (PKACII + PKACII_PKI)
\]

\[
ARCII = \frac{KA}{cAMP} \ast \frac{PKACII}{KD} \ast (PKACII + PKACII_PKI)
\]
\[ A2RCII = \frac{PKACII}{KD} \times (PKACII + PKACII_{PI}) \]

\[ A2RII = (PKACII + PKACII_{PI}) \]

\[ PKI = \frac{KPKI \times PKI_{tot}}{(KPKI + PKACI + PKACII)} \]

\[ PKACI_{PI} = \frac{PKACI \times PKI_{tot}}{(KPKI + PKACI + PKACII)} \]

\[ PKACII_{PI} = \frac{PKACI \times PKI_{tot}}{(KPKI + PKACI + PKACII)} \]

<p>|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKAItot</td>
<td>0.46</td>
<td>μM</td>
</tr>
<tr>
<td>PKAILtot</td>
<td>0.084</td>
<td>μM</td>
</tr>
<tr>
<td>PKI_{tot}</td>
<td>0.18</td>
<td>μM</td>
</tr>
<tr>
<td>KA</td>
<td>9.14</td>
<td>μM</td>
</tr>
<tr>
<td>KB</td>
<td>1.64</td>
<td>μM</td>
</tr>
<tr>
<td>KD</td>
<td>4.375</td>
<td>μM</td>
</tr>
<tr>
<td>KPKI</td>
<td>2 \times 10^{-4}</td>
<td>μM</td>
</tr>
</tbody>
</table>

**Phospholamban regulation module**

\[ Inhib1_p = Inhib1_{ptot} - \frac{PP1 \times Inhib1_p}{KInhib1} \]

\[ PP1 = \epsilon \times PP1_{tot} - \frac{PP1 \times Inhib1_p}{KInhib1} \]

\[ \frac{d(Inhib1_{ptot})}{d(time)} = \frac{kPKA_{Inhib1} \times PKACI \times Inhib1}{KmPKA_{Inhib1} + Inhib1} - \frac{V_{maxPP2A_{Inhib1}} \times Inhib1_{ptot}}{KmPP2A_{Inhib1} + Inhib1_{ptot}} \]

\[ Inhib1 = Inhib1_{tot} - Inhib1_{ptot} \]

\[ \frac{d(PLB_p)}{d(time)} = \frac{kPKA_{PLB} \times PKACI \times PLB}{KmPKA_{PLB} + PLB} - \frac{kPP1_{PLB} \times PP1 \times PLB_p}{KmPP1_{PLB} + PLB_p} \]

\[ PLB = PLB_{tot} - PLB_p \]

\[ F_{PLB} = PLB/PLB_{tot} \]

\[ Km_{up} = (3/4 \times F_{PLB}/F_{PLB0} + 1/4) \times Km_{up0} \]
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$PLB_{tot}$</td>
<td>38</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$PP1_{tot}$</td>
<td>0.89</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$Inhib1_{tot}$</td>
<td>0.3</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>10</td>
<td>none</td>
</tr>
<tr>
<td>$k_{PKA_{PLB}}$</td>
<td>54</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$Km_{PKA_{PLB}}$</td>
<td>21</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$k_{PP1_{PLB}}$</td>
<td>8.5</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$Km_{PP1_{PLB}}$</td>
<td>7.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$k_{PKA_{Inhib1}}$</td>
<td>60</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$Km_{PKA_{Inhib1}}$</td>
<td>1</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$V_{max_{PP2A_{Inhib1}}}$</td>
<td>14</td>
<td>$\mu$M*s$^{-1}$</td>
</tr>
<tr>
<td>$K_{Inhib1}$</td>
<td>1*10$^{-3}$</td>
<td>$\mu$M</td>
</tr>
</tbody>
</table>

**L-type calcium channel regulation module**

\[
PKA_{II_{LCC}} = \frac{PKA_{II_{tot}}}{PKA_{II_{tot}}} * PKA_{II}
\]

\[
\frac{d(LCC_{alpha1Cp})}{d(time)} = \frac{\epsilon * k_{PKA_{LCC}} * PKA_{II} * LCC_{alpha1C}}{Km_{PKA_{LCC}} + \epsilon * LCC_{alpha1C}} - \frac{\epsilon * k_{PP2A_{LCC}} * PP2A_{LCC} * LCC_{alpha1C}}{Km_{PP2A_{LCC}} + \epsilon * LCC_{alpha1C}}
\]

\[
LCC_{alpha1C} = LCC_{tot} - LCC_{alpha1Cp}
\]

\[
F_{LCC_{alpha1Cp}} = LCC_{alpha1Cp}/LCC_{tot}
\]

\[
f_{Po} = 0.03 * F_{LCC_{alpha1Cp}}/F_{LCC_{alpha1Cp0}} + 0.97
\]

\[
\frac{d(LCC_{beta2p})}{d(time)} = \frac{\epsilon * k_{PKA_{LCC}} * PKA_{II} * LCC_{beta2}}{Km_{PKA_{LCC}} + \epsilon * LCC_{beta2}} - \frac{\epsilon * k_{PP1_{LCC}} * PP1_{LCC} * LCC_{beta2}}{Km_{PP1_{LCC}} + \epsilon * LCC_{beta2}}
\]

\[
LCC_{beta2p} = LCC_{tot} - LCC_{beta2p}
\]

\[
F_{LCC_{beta2p}} = LCC_{beta2p}/LCC_{tot}
\]

\[
f_{LCCavail} = 0.05 * F_{LCC_{beta2p}}/F_{LCC_{beta2p0}} + 0.95
\]
Parameter | Value | Units
--- | --- | ---
$LCC_{tot}$ | 0.025 | μM
$PKA_{II tot \_ LCC}$ | 0.025 | μM
$PP1_{\_ LCC}$ | 0.025 | μM
$PP2A_{\_ LCC}$ | 0.025 | μM
$kPKA_{\_ LCC}$ | 54 | s$^{-1}$
$KmPKA_{\_ LCC}$ | 21 | μM
$kPP1_{\_ LCC}$ | 8.52 | s$^{-1}$
$KmPP1_{\_ LCC}$ | 3.0 | μM
$kPP2A_{\_ LCC}$ | 10.1 | s$^{-1}$
$KmPP2A_{\_ LCC}$ | 3.0 | μM

**Ryanodine receptor regulation module**

$$PKACII_{\_ RyR} = \frac{PKAII_{\_ RyRtot}}{PKAII_{tot}} \times PKACII - \frac{\epsilon_{\_ RyR} \times PKACII_{\_ RyR}}{Ks_{\_ RyR \_ PKAC}}$$

$$PP1_{\_ RyR} = PP1_{\_ RyRtot} - \frac{\epsilon_{\_ RyRp} \times PP1_{\_ RyR}}{Ks_{\_ RyR \_ PP1}}$$

$$PP2A_{\_ RyR} = PP2A_{\_ RyRtot} - \frac{\epsilon_{\_ RyRp} \times PP2A_{\_ RyR}}{Ks_{\_ RyR \_ PP2A}}$$

$$RyR = RyR_{total} - \frac{\epsilon_{\_ RyR} \times PKACII_{\_ RyR}}{Ks_{\_ RyR \_ PKAC}}$$

$$RyRp = RyR_{total} - \frac{\epsilon_{\_ RyRp} \times PP1_{\_ RyR}}{Ks_{\_ RyR \_ PP1}} - \frac{\epsilon_{\_ RyRp} \times PP2A_{\_ RyR}}{Ks_{\_ RyR \_ PP2A}}$$

$$\frac{d(RyR_{total})}{d(time)} = kPKA_{\_ RyR} \times \frac{\epsilon_{\_ RyR} \times RyR \times PKACII_{\_ RyR}}{Ks_{\_ RyR \_ PKAC}} - kPP1_{\_ RyR} \times \frac{\epsilon_{\_ RyRp} \times PP1_{\_ RyR}}{Ks_{\_ RyR \_ PP1}}$$

$$- kPP2A_{\_ RyR} \times \frac{\epsilon_{\_ RyRp} \times PP2A_{\_ RyR}}{Ks_{\_ RyR \_ PP2A}}$$

$$F_{\_ RyRp} = \frac{RyR_{total}}{RyR_{sum}}$$

$$Krel = \left[ \frac{2}{3} \times \left( \frac{1 - F_{RyRp}}{1 - F_{RyR_{0}}} \right) + \frac{1}{3} \right] \times Krel0$$
Saucerman et al., 092064/R1

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Parameter Value Units
RyRtot 0.135 μM
PKAIItot_RyR 0.034 μM
PP1_RyR 0.034 μM
PP2A_RyR 0.034 μM
kPKA_RyR 54 s⁻¹
KmPKA_RyR 21 μM
kPP1_RyR 8.52 s⁻¹
KmPP1_RyR 7.0 μM
kPP2A_RyR 10.1 s⁻¹
KmPP2A_RyR 4.1 μM

Troponin I regulation module

\[
\frac{d(Tnlp)}{d(time)} = \frac{kPKA_{TnI} \times PKAC \times TnI}{KmPKA_{TnI} + Tnlp} - \frac{kPP2A_{TnI} \times PP2A_{TnI} \times Tnlp}{KmPP2A_{TnI} + Tnlp}
\]

\[TnI = TnItot - Tnlp\]

\[F_{TnI} = \frac{TnI}{TnI_{tot}}\]

\[KmTnC = (1.45 - 0.45 \times F_{TnI}/F_{TnI_0}) \times KmTnC_0\]

Parameter Value Units
TnItot 70 μM
PP2A_TnI 0.67 μM
kPKA_TnI 54 s⁻¹
KmPKA_TnI 21 μM
kPP2A_TnI 10.1 s⁻¹
KmPP2A_TnI 4.1 μM

I_Ks regulation module

\[KCNQ1 = KCNQ1_{tot} - \frac{KCNQ1 \times Yot}{Kyotiao}\]

\[Yot = Yottot - \frac{KCNQ1 \times Yot}{Kyotiao}\]

\[PKACII_{KCNQ1} = \frac{KCNQ1 \times Yot}{KCNQ1_{tot} \times Kyotiao} \times \frac{PKAIItot \times Yot}{PKAIItot \times PKACII}\]

\[PP1_{KCNQ1} = \frac{KCNQ1 \times Yot}{KCNQ1_{tot} \times Kyotiao} \times PP1_{tot \_Yot}\]
\[
\frac{d(KCNQ1p)}{d(time)} = \frac{\epsilon * kPKA_{KCNQ1} * PKACII_{KCNQ1} * KCNQ1}{KmPKA_{KCNQ1} + \epsilon * KCNQ1}
\]
\[
- \frac{\epsilon * kPP1_{KCNQ1} * PP1_{KCNQ1} * KCNQ1p}{KmPP1_{KCNQ1} + \epsilon * KCNQ1p}
\]

\[F_{KCNQ1p} = KCNQ1p/KCNQ1tot\]

\[
f_{Ksavail} = (0.2 * F_{KCNQ1p}/F_{KCNQ1p0} + 0.8)
\]

\[Xs_{05} = 1.5 * (2.0 - F_{KCNQ1p}/F_{KCNQ1p0})\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1tot</td>
<td>0.025</td>
<td>μM</td>
</tr>
<tr>
<td>Yot</td>
<td>0.025</td>
<td>μM</td>
</tr>
<tr>
<td>PKAIItot_Yot</td>
<td>0.025</td>
<td>μM</td>
</tr>
<tr>
<td>PP1tot_Yot</td>
<td>0.025</td>
<td>μM</td>
</tr>
<tr>
<td>Kyotiao(WTorG589D)</td>
<td>10^{-4} or 10^2</td>
<td>μM</td>
</tr>
<tr>
<td>kPKA_{KCNQ1}</td>
<td>54</td>
<td>s^{-1}</td>
</tr>
<tr>
<td>KmPKA_{KCNQ1}</td>
<td>21</td>
<td>μM</td>
</tr>
<tr>
<td>kPP1_{KCNQ1}</td>
<td>8.52</td>
<td>s^{-1}</td>
</tr>
<tr>
<td>KmPP1_{KCNQ1}</td>
<td>7.0</td>
<td>μM</td>
</tr>
<tr>
<td>epsilon</td>
<td>10</td>
<td>none</td>
</tr>
</tbody>
</table>

**Excitation-contraction coupling modules**

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<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_{myo})</td>
<td>25.84*10^{-6}</td>
<td>μL</td>
</tr>
<tr>
<td>(V_{NSR})</td>
<td>2.098*10^{-6}</td>
<td>μL</td>
</tr>
<tr>
<td>(V_{JSR})</td>
<td>0.182*10^{-6}</td>
<td>μL</td>
</tr>
<tr>
<td>(A_{cap})</td>
<td>1.534*10^{-4}</td>
<td>cm²</td>
</tr>
<tr>
<td>(T)</td>
<td>310</td>
<td>K</td>
</tr>
<tr>
<td>(R)</td>
<td>8314</td>
<td>J/kmol*K</td>
</tr>
<tr>
<td>(F)</td>
<td>96485</td>
<td>C/mol</td>
</tr>
</tbody>
</table>

**I\_Na: Sodium current**

\[
i_{Na} = g_{Na} * (m)^{3.0} * h * j * (V - E_{Na})
\]

\[
E_{Na} = \frac{R * T}{F} * \ln \frac{Nao}{Nai}
\]
\[ alpha_m = \frac{0.32 \times (V + 47.13)}{(1.0 - e^{-0.1 \times (V+47.13)})} \]

\[ beta_m = 0.08 \times e^{\frac{V}{11.1}} \]

\[ \frac{d(m)}{d(time)} = 10^3 \times (alpha_m \times (1.0 - m) - beta_m \times m) \]

\[ alpha_h = \begin{cases} 
0.135 \times e^{\frac{(80.0+V)}{0.8}}; & \text{if } V < -40.0, \\
0.0 & \text{otherwise.} 
\end{cases} \]

\[ beta_h = \begin{cases} 
(3.56 \times e^{0.079 \times V} + 310000 \times e^{0.35 \times V}) / (1.0 \times e^{\frac{-[(V+10.66)]}{11.1}}); & \text{if } V < -40.0, \\
0.13 \times e^{\frac{1}{1.0+e^{\frac{(V+79.23)}{11.1}}}} & \text{otherwise.} 
\end{cases} \]

\[ \frac{d(h)}{d(time)} = 10^3 \times (alpha_h \times (1.0 - h) - beta_h \times h) \]

\[ alpha_j = \begin{cases} 
(-127140.0 \times e^{0.2444 \times V} - 0.00003474 \times e^{-0.04391 \times V}) \times \frac{(F+37.78)}{(1.0+e^{0.311 \times (V+79.23)})}; & \text{if } V < -40.0, \\
0.0 & \text{otherwise.} 
\end{cases} \]

\[ beta_j = \begin{cases} 
\frac{0.1212 \times e^{-0.0052 \times V}}{(1.0+e^{0.1378 \times (V+40.14)})}; & \text{if } V < -40.0, \\
0.3 \times e^{-0.000002535 \times V} / (1.0+e^{0.1 \times (V+32.0)}) & \text{otherwise.} 
\end{cases} \]

\[ \frac{d(j)}{d(time)} = 10^3 \times (alpha_j \times (1.0 - j) - beta_j \times j) \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_{Na} )</td>
<td>8.0</td>
<td>mS/( \mu )F</td>
</tr>
<tr>
<td>( P_{Ca} )</td>
<td>5.4e-4</td>
<td>cm/s</td>
</tr>
<tr>
<td>( P_{Na} )</td>
<td>6.75e-7</td>
<td>cm/s</td>
</tr>
<tr>
<td>( P_K )</td>
<td>1.93e-7</td>
<td>cm/s</td>
</tr>
<tr>
<td>( Km_{Ca} )</td>
<td>0.6\times10^{-3}</td>
<td>mM</td>
</tr>
<tr>
<td>( Nif )</td>
<td>0 or 0.2</td>
<td>( \mu )M</td>
</tr>
<tr>
<td>( IC_{50nif} )</td>
<td>0.43</td>
<td>( \mu )M</td>
</tr>
</tbody>
</table>

**I_CaL: L-type Ca channel**

\[ i_{CaCa} = d \times f \times f_{Ca} \times f_{Po} \times f_{LCCavail} \times \frac{IC_{50nif}}{IC_{50nif} + Nif} \times I_{CaCa} \]

\[ i_{CaNa} = d \times f \times f_{Ca} \times f_{Po} \times f_{LCCavail} \times \frac{IC_{50nif}}{IC_{50nif} + Nif} \times I_{CaNa} \]
\[ i_{CaK} = d \cdot f \cdot f_{Ca} \cdot f_{Po} \cdot f_{LCCav} \cdot \frac{IC \cdot 50nif}{IC \cdot 50nif + Nif} \cdot I_{CaK} \]

\[ I_{CaCa} = P_{Ca} \cdot (2.0)^{2.0} \cdot \frac{V \cdot (F)^{2.0}}{R \cdot T} \cdot \left( \gamma_{Cai} \cdot Cai \cdot e^{\frac{2.0+V}{R \cdot T}} - \gamma_{CaO} \cdot CaO \right) \left( e^{\frac{2.0+V}{R \cdot T}} - 1.0 \right) \]

\[ I_{CaNa} = P_{Na} \cdot (1.0)^{2.0} \cdot \frac{V \cdot (F)^{2.0}}{R \cdot T} \cdot \left( \gamma_{Nai} \cdot Nai \cdot e^{\frac{1.0+V}{R \cdot T}} - \gamma_{Nao} \cdot Nao \right) \left( e^{\frac{1.0+V}{R \cdot T}} - 1.0 \right) \]

\[ I_{CaK} = P_{K} \cdot (1.0)^{2.0} \cdot \frac{V \cdot (F)^{2.0}}{R \cdot T} \cdot \left( \gamma_{Ki} \cdot Ki \cdot e^{\frac{1.0+V}{R \cdot T}} - \gamma_{Ko} \cdot Ko \right) \left( e^{\frac{1.0+V}{R \cdot T}} - 1.0 \right) \]

\[ \alpha_{d} = \frac{d_{\text{infinity}}}{\tau_{d}} \]

\[ d_{\text{infinity}} = \frac{1.0}{1.0 + e^{\frac{(V+10.0)}{0.24}}} \]

\[ \tau_{d} = d_{\text{infinity}} \cdot \left( \frac{1.0 - e^{\frac{(V+10.0)}{0.24}}}{0.035 \cdot (V + 10.0)} \right) \]

\[ \beta_{d} = \frac{(1.0 - d_{\text{infinity}})}{\tau_{d}} \]

\[ \frac{d(d)}{d(\text{time})} = 10^{3} \cdot (\alpha_{d} \cdot (1.0 - d) - \beta_{d} \cdot d) \]

\[ \alpha_{f} = \frac{f_{\text{infinity}}}{\tau_{f}} \]

\[ f_{\text{infinity}} = \left( \frac{1.0}{1.0 + e^{\frac{(V+35.06)}{8.6}}} \right) + \left( \frac{0.6}{1.0 + e^{\frac{(20.0-V)}{20.8}}} \right) \]

\[ \tau_{f} = \frac{1.0}{0.0197 \cdot e^{-(0.0337 \cdot (V+10.0))^{2/3}} + 0.02} \]

\[ \beta_{f} = \frac{(1.0 - f_{\text{infinity}})}{\tau_{f}} \]

\[ \frac{d(f)}{d(\text{time})} = 10^{3} \cdot (\alpha_{f} \cdot (1.0 - f) - \beta_{f} \cdot f) \]

\[ f_{Ca} = \left( \frac{1.0}{1.0 + \left( \frac{Ca}{Km_{Ca}} \right)^{2.0}} \right) \]

**I_Kr: Rapidly activating potassium channel**
\[
g_{Kr} = g_{Kr0} \times \sqrt{\frac{Ko}{5.4}}
\]
\[
E_{Kr} = \frac{R \times T}{F} \times \ln \frac{Ko}{K_i}
\]
\[
i_{Kr} = g_{Kr} \times X_r \times R_r \times (V - E_{Kr})
\]
\[
\frac{d(X_r)}{d(time)} = 10^3 \times \frac{(X_r\_infinity - X_r)}{tau_{X_r}}
\]
\[
X_r\_infinity = \frac{1.0}{1.0 + e^{-\frac{(V-50.0)}{7.5}}}
\]
\[
tau_{X_r} = \frac{1.0}{\left(\frac{0.00138(V+7.0)}{1.0-e^{-0.123(V+7.0)} + e^{0.145(V+10.0)+0.145-1.0}}\right) + \left(\frac{0.00061(V+10.0)}{1.0+e^{-0.057(V+50.0)}}\right)}
\]
\[
R_r = \frac{1.0}{1.0 + e^{-\left(\frac{V+33.0}{22.4}\right)}}
\]

Parameter | Value | Units
---|---|---
g\_Kr0 | 0.035 | mS/μF
\(g_{Ks0}\) | 0.5 | mS/μF
\(g_{to}\) | 0.06 | mS/μF
\(g_{K10}\) | 0.54 | mS/μF
\(g_{Kp}\) | 0.008 | mS/μF

**I\_Ks: Slowly activating potassium channel**

\[
g_{Ks} = g_{Ks0} \times f_{Ksavail} \times \frac{(0.057 + 0.19)}{1.0 + e^{\frac{(V-50.0)}{0.6}}}
\]
\[
p_{Ca} = -\left(\log(Cai)\right) + 3.0
\]
\[
E_{Ks} = \frac{R \times T}{F} \times \ln \left(\frac{Ko + P_{NaK} \times Nao}{K_i + P_{NaK} \times Nai}\right)
\]
\[
i_{Ks} = g_{Ks} \times (Xs)^{2.0} \times (V - E_{Ks})
\]
\[
\frac{d(Xs)}{d(time)} = 10^3 \times \frac{(Xs\_infinity - Xs)}{tau_{Xs}}
\]
\[
Xs\_infinity = \frac{1.0}{1.0 + e^{-\frac{(V-50.0)}{16.2}}}
\]
\[
tau_{Xs} = \frac{1.0}{\left(\frac{0.0000719(V+30.0)}{1.0-e^{-0.148(V+30.0)}} + \frac{0.000131(V+30.0)}{e^{0.0667(V+30.0)-1.0}}\right)}
\]
**I_to: Transient outward potassium current**

\[ i_{to} = g_{to} \times X_{to} \times Y_{to} \times (V - E_K) \]

\[ \alpha_{X_{to}} = 0.04561 \times e^{0.03577 \times V} \]

\[ \beta_{X_{to}} = 0.0989 \times e^{-0.06237 \times V} \]

\[
\frac{d(X_{to})}{d(time)} = 10^3 \times (\alpha_{X_{to}} \times (1.0 - X_{to}) - \beta_{X_{to}} \times X_{to})
\]

\[ \alpha_{Y_{to}} = 0.005415 \times e^{\left(\frac{(V+33.5)}{5.0}\right)} \]

\[ \beta_{Y_{to}} = 0.005415 \times e^{\left(\frac{(V+33.5)}{5.0}\right)} \]

\[
\frac{d(Y_{to})}{d(time)} = 10^3 \times (\alpha_{Y_{to}} \times (1.0 - Y_{to}) - \beta_{Y_{to}} \times Y_{to})
\]

**I_K1: Time-independent potassium current**

\[ g_{K1} = g_{K10} \times \sqrt{\frac{Ko}{5.4}} \]

\[ E_{K1} = \frac{R \times T}{F} \times \ln \frac{Ko}{K_1} \]

\[ i_{K1} = g_{K1} \times K1_{infinity} \times (V - E_{K1}) \]

\[ \alpha_{K1} = \frac{1.02}{(1.0 + e^{0.2385 \times ((V - E_{K1}) - 59.215)})} \]

\[ \beta_{K1} = \frac{0.49124 \times e^{\left(\frac{(V+5.476-E_{K1})}{12.45}\right)} + e^{\left(\frac{(V-(E_{K1}+594.31))}{16.2}\right)}}{(1.0 + e^{-0.5143 \times ((V-(E_{K1}+4.753))})} \]

\[ K1_{infinity} = \frac{\alpha_{K1}}{\alpha_{K1} + \beta_{K1}} \]

**I_Kp: Plateau potassium current**

\[ E_{Kp} = E_{K1} \]

\[ Kp = \frac{1.0}{{(1.0 + e^{\left(\frac{17.488 - V}{5.98}\right)})}} \]

\[ i_{Kp} = g_{Kp} \times Kp \times (V - E_{Kp}) \]

**I_NaCa: Na/Ca exchanger**
\[ i_{NaCa} = K_{NaCa} \times \frac{1.0}{(K_{mNa})^{3.0} + (Nao)^{3.0}} \times \frac{1.0}{(K_{mCa} + Cao)} \times \frac{1.0}{(1.0 + K_{sat} \times e^{(eta-1.0) \times \frac{V}{F \times R \times T}})} \times \left( e^{\eta \times \frac{V}{F \times R \times T}} \times Cao - e^{(\eta-1.0) \times \frac{V}{F \times R \times T}} \times (Nao)^{3.0} \times Cai \right) \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{NaCa})</td>
<td>2.6 \times 10^3</td>
<td>(\mu A/\mu F)</td>
</tr>
<tr>
<td>(K_{mNa})</td>
<td>87.5</td>
<td>mM</td>
</tr>
<tr>
<td>(K_{mCa})</td>
<td>1.38</td>
<td>mM</td>
</tr>
<tr>
<td>(K_{sat})</td>
<td>0.1</td>
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<tr>
<td>(eta)</td>
<td>0.35</td>
<td>none</td>
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<tr>
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<th>Value</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
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<td>(\mu A/\mu F)</td>
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<tr>
<td>(K_{mpCa})</td>
<td>0.5 \times 10^{-3}</td>
<td>mM</td>
</tr>
<tr>
<td>(g_{Nab})</td>
<td>9.8 \times 10^{-5}</td>
<td>(mS/\mu F)</td>
</tr>
<tr>
<td>(g_{Cab})</td>
<td>3.0 \times 10^{-3}</td>
<td>(mS/\mu F)</td>
</tr>
<tr>
<td>(Pns)</td>
<td>1.75 \times 10^{-7}</td>
<td>cm/s</td>
</tr>
<tr>
<td>(Km_{ns})</td>
<td>1.2 \times 10^{-3}</td>
<td>mM</td>
</tr>
</tbody>
</table>

**I_{pCa}: Sarcolemmal calcium pump**

\[ i_{pCa} = \frac{Cai}{(K_{mpCa} + Cai)} \]

**I_{Nab}: Sodium background current**

\[ E_{NaN} = E_{Na} \]

\[ i_{Na_b} = g_{Nab} \times (V - E_{NaN}) \]

**I_{Cab}: Calcium background current**

\[ E_{CaN} = \frac{R \times T}{2.0 \times F} \times \ln \frac{Cao}{Cai} \]

\[ i_{Ca_b} = g_{Cab} \times (V - E_{CaN}) \]

**I_{NaK}: Sodium/potassium pump**

\[ f_{NaK} = \frac{1.0}{\left(1.0 + 0.1245 \times e^{-0.1 \times \frac{V}{F \times R \times T}}\right) + 0.0365 \times \sigma \times e^{-\left(\frac{V}{F \times R \times T}\right)}} \]

\[ \sigma = \frac{1.0}{7.0} \times \left(e^{\frac{\eta}{6.3}} - 1.0\right) \]

\[ i_{NaK} = I_{NaK} \times f_{NaK} \times \frac{1.0}{\left(1.0 + \sqrt{\left(K_{mNai} \times Nai\right)^{3.0}}\right)} \times \frac{Ko}{(Ko + K_{mKo})} \]

**I_{ns}: Nonspecific Ca-activated currents**

\[ I_{nsNa} = Pns \times (1.0)^{2.0} \times \frac{V \times (F)^{2.0}}{R \times F} \times \frac{\text{gamma}_{Nai} \times Nai \times e^{-0.1 \times \frac{V}{F \times R \times T}} - \text{gamma}_{Nao} \times Nao}{\left(e^{-0.1 \times \frac{V}{F \times R \times T}} - 1.0\right)} \]
\[ i_{\text{nsNa}} = \frac{I_{\text{nsNa}}}{1 + \left(\frac{K_{m_{\text{ns}}}}{C_{ai}}\right)^3} \]

\[ I_{\text{nsK}} = P_{\text{ns}} \times (1.0)^{2.0} \times \frac{V \times (F)^{2.0}}{R \times T} \times \left(\frac{\gamma_{K_i} \times K_i \times e^{-\frac{V-E_{\text{Cl}}}{K_m_{\text{CaCl}}}} - \gamma_{K_o} \times K_o}{e^{-\frac{V-E_{\text{Cl}}}{K_m_{\text{CaCl}}}} - 1.0}\right) \]

\[ i_{\text{nsK}} = \frac{I_{\text{nsK}}}{1 + \left(\frac{K_{m_{\text{ns}}}}{C_{ai}}\right)^3} \]

**I\_Cl(Ca): Calcium-activated chloride current**

\[ i_{\text{Cl Ca}} = g_{\text{Cl}} \times \left(\frac{V - E_{\text{Cl}}}{1.0 + \frac{K_m_{\text{CaCl}}}{C_{ai}}}\right) \]

**Cytosolic calcium buffers**

\[ TRPN\_\text{buff} = TRPN\_\text{max} \times \frac{C_{ai}}{(C_{ai} + K_{m_{\text{TnC}}})} \]

\[ B_{\text{trpn}} = TRPN\_\text{max} \times \frac{K_m_{\text{TnC}}}{(C_{ai} + K_{m_{\text{TnC}}})^2} \]

\[ CMDN\_\text{buff} = CaM\_\text{max} \times \frac{C_{ai}}{(C_{ai} + K_{m_{\text{CaM}}})} \]

\[ B_{\text{cam}} = CaM\_\text{max} \times \frac{K_m_{\text{CaM}}}{(C_{ai} + K_{m_{\text{CaM}}})^2} \]

\[ B_{\text{MYO}} = \frac{1}{1 + B_{\text{trpn}} + B_{\text{cam}}} \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPN_\text{max}</td>
<td>0.07</td>
<td>mM</td>
</tr>
<tr>
<td>K_{m_{\text{TnC}}}</td>
<td>0.5128*10^{-3}</td>
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</tr>
<tr>
<td>CaM_\text{max}</td>
<td>0.05</td>
<td>mM</td>
</tr>
<tr>
<td>K_{m_{\text{CaM}}}</td>
<td>2.38*10^{-3}</td>
<td>mM</td>
</tr>
</tbody>
</table>

**Sarcoplasmic reticulum fluxes**

\[ RyR\_\text{open} = \frac{1.0}{1.0 + e^{-\frac{-((\text{time})+4.0)}{0.5}}} \]

\[ RyR\_\text{close} = \left(1.0 - \frac{1.0}{1.0 + e^{-\frac{-((\text{time})+4.0)}{0.5}}}\right) \]
\[ G_{rel} = \begin{cases} 
\frac{20}{1.0+e^{-\frac{i_{Ca_{L}, Ca_{b}, p_{Ca}, Ca_{T}}+i_{NaCa}K_{rel}}}{0.9}} & \text{if calcium\_overload = 0.0,} \\
\frac{90+RyR\_open\_RyR\_close}{1.0+e^{-\frac{i_{Ca_{L}, Ca_{b}, p_{Ca}, Ca_{T}}+i_{NaCa}K_{rel}}}{0.9}} & \text{otherwise.} 
\end{cases} \]

\[ i_{rel} = G_{rel} \ast (Ca_{JSR} - Cai) \]

\[ i_{up} = i_{upmax} \ast \frac{(Cai/K_{m_{up}})^2 - (Ca_{NSR}/3.0)^2}{1 + (Cai/K_{m_{up}})^2 + (Ca_{NSR}/3.0)^2} \]

\[ i_{leak} = K_{leak} \ast Ca_{NSR} \]

\[ K_{leak} = \frac{i_{up}}{Ca_{NSR\_max}} \]

\[ i_{tr} = \frac{Ca_{NSR} - Ca_{JSR}}{\tau_{tr}} \]

\[ CSQN\_buff = CSQN\_max \ast \frac{Ca_{JSR}}{(Ca_{JSR} + K_{mCSQN})} \]

\[ \frac{d(Ca_{JSR})}{d(time)} = 10^3 \ast (i_{tr} \ast \frac{V_{NSR}}{V_{JSR}} - i_{rel}) \]

\[ \frac{d(Ca_{NSR})}{d(time)} = 10^3 \ast [i_{up} - (i_{leak} + i_{tr})] \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krel0</td>
<td>5.0</td>
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<tr>
<td>i_{upmax}</td>
<td>4*10^{-3}</td>
<td>mM/ms</td>
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<tr>
<td>Km_{up}0</td>
<td>3*10^{-4}</td>
<td>mM</td>
</tr>
<tr>
<td>Ca_{NSR_max}</td>
<td>15</td>
<td>mM</td>
</tr>
<tr>
<td>tau_{tr}</td>
<td>6.0</td>
<td>ms</td>
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<tr>
<td>CSQN_max</td>
<td>10</td>
<td>mM</td>
</tr>
<tr>
<td>CSQN_th</td>
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</tr>
<tr>
<td>K_{mCSQN}</td>
<td>0.8333</td>
<td>mM</td>
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</tbody>
</table>

**Cytosolic ions**

\[ \frac{d(Nai)}{d(time)} = -10^3 \ast ((i_{Na} + i_{CaNa} + i_{Na\_b} + i_{NaCa} \ast 3.0 + i_{NaK} \ast 3.0)) \ast \frac{A_{cap}}{V_{myo} \ast F} \]

\[ \frac{d(Cai)}{d(time)} = -10^3 \ast B_{MYO} \ast \left[ (i_{CaCa} + i_{p\_Ca} + i_{Ca\_b} - 2.0 \ast i_{NaCa}) \ast \frac{A_{cap}}{2.0 \ast V_{myo} \ast F} + i_{rel} \ast \frac{V_{JSR}}{V_{myo}} + (i_{leak} - i_{up}) \ast \frac{V_{NSR}}{V_{myo}} \right] \]
\[
\frac{d(K_i)}{d(t)} = -10^3 \times (i_{CaK} + i_{Kr} + i_{Ks} + i_{K1} + i_{Kp} + i_{to} - 2.0 \times i_{NaK}) \times \frac{A_{cap}}{V_{myo} \times F}
\]

**Membrane potential**

\[
\frac{d(V)}{d(t)} = 10^3 \times \left[ I_{stim} - \left( i_{Na} + i_{Ca_L} + i_{Kr} + i_{Ks} + i_{NaCa} + i_{K1} + i_{Kp} + i_{p_Ca} + i_{Na_b} + i_{Ca_b} + i_{NaK} + i_{to} + i_{Cl_Ca} \right) \right]
\]

**Tissue simulations**

For tissue simulations, the above ODE for membrane potential was replaced by the following monodomain PDE:

\[
\frac{\partial(V)}{\partial(t)} = \nabla \cdot D \nabla V - \sum I_{ion}
\]

with boundary conditions \(n \cdot (D \nabla V) = 0\), where \(\sum I_{ion}\) represents the sum of all membrane currents at a given point, as in the above ODE for membrane potential. Units for tissue simulations are \(V\ [mV]\), time \([ms]\), and \(D\ [cm^2/ms]\), and \(I\ [\mu A/\mu F]\).

Transmural heterogeneities in current densities used for tissue simulations:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Endo</th>
<th>Mid</th>
<th>Epi</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g_{Na})</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>mS/\mu F</td>
</tr>
<tr>
<td>(g_{to})</td>
<td>0.025</td>
<td>0.06</td>
<td>0.06</td>
<td>mS/\mu F</td>
</tr>
<tr>
<td>(g_{Kp})</td>
<td>0.004</td>
<td>0.004</td>
<td>0.008</td>
<td>mS/\mu F</td>
</tr>
<tr>
<td>(g_{Kr})</td>
<td>0.03</td>
<td>0.015</td>
<td>0.04</td>
<td>mS/\mu F</td>
</tr>
<tr>
<td>(g_{Ks})</td>
<td>0.3</td>
<td>0.25</td>
<td>0.75</td>
<td>mS/\mu F</td>
</tr>
</tbody>
</table>

**Additional notes**