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# Mechanisms of Altered Excitation-Contraction Coupling in Canine Tachycardia-Induced Heart Failure, II

## Model Studies

Raimond L. Winslow, Jeremy Rice, Saleet Jafri, Eduardo Marbán, Brian O'Rourke

**Abstract**—Ca<sup>2+</sup> transients measured in failing human ventricular myocytes exhibit reduced amplitude, slowed relaxation, and blunted frequency dependence. In the companion article (O'Rourke B, Kass DA, Tomaselli GF, Kääh S, Tunin R, Marbán E. Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart, I: experimental studies. *Circ Res.* 1999;84:562–570), O'Rourke et al show that Ca<sup>2+</sup> transients recorded in myocytes isolated from canine hearts subjected to the tachycardia pacing protocol exhibit similar responses. Analyses of protein levels in these failing hearts reveal that both SR Ca<sup>2+</sup> ATPase and phospholamban are decreased on average by 28% and that Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) protein is increased on average by 104%. In this article, we present a model of the canine midmyocardial ventricular action potential and Ca<sup>2+</sup> transient. The model is used to estimate the degree of functional upregulation and downregulation of NCX and SR Ca<sup>2+</sup> ATPase in heart failure using data obtained from 2 different experimental protocols. Model estimates of average SR Ca<sup>2+</sup> ATPase functional downregulation obtained using these experimental protocols are 49% and 62%. Model estimates of average NCX functional upregulation range are 38% and 75%. Simulation of voltage-clamp Ca<sup>2+</sup> transients indicates that such changes are sufficient to account for the reduced amplitude, altered shape, and slowed relaxation of Ca<sup>2+</sup> transients in the failing canine heart. Model analyses also suggest that altered expression of Ca<sup>2+</sup> handling proteins plays a significant role in prolongation of action potential duration in failing canine myocytes. (*Circ Res.* 1999;84:571-586.)

**Key Words:** excitation-contraction coupling ■ heart failure ■ midmyocardial ventricular action potential  
■ Ca<sup>2+</sup> transient

Recent studies using the canine tachycardia pacing-induced model of heart failure<sup>1–8</sup> demonstrate that changes in cellular electrophysiological and excitation-contraction (E-C) coupling processes are qualitatively similar to those observed in cells isolated from failing human heart. In human heart failure,  $I_{K1}$  current density measured at hyperpolarized membrane potentials is reduced by  $\approx 50\%$ ,<sup>9,10</sup> and density of the transient outward current  $I_{to1}$  is reduced by  $\approx 75\%$  in subepicardial<sup>11</sup> and  $\approx 40\%$  in midmyocardial ventricular cells<sup>9</sup> and is unchanged in subendocardial ventricular cells.<sup>11</sup> The magnitude of  $I_{K1}$  is reduced by  $\approx 40\%$ , and that of  $I_{to1}$  by  $\approx 70\%$  in failing canine midmyocardial cells.<sup>5</sup> Expression of proteins involved in E-C coupling is also altered in human heart failure. Sarcoplasmic reticulum (SR) Ca<sup>2+</sup> ATPase mRNA level,<sup>12–16</sup> protein level,<sup>12,17,18</sup> and uptake rate<sup>19</sup> are reduced by  $\approx 50\%$  in end-stage heart failure. Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) mRNA levels are increased by  $\approx 55\%$  to 79%,<sup>12,20</sup> and NCX protein levels increase 36% to 160%.<sup>12,20–22</sup>

Less information is available with regard to NCX function in heart failure. However, Reinecke et al<sup>22</sup> reported an 89% increase in sodium-gradient-stimulated <sup>45</sup>Ca<sup>2+</sup> uptake in human heart sarcolemmal vesicles.

As described in the preceding article by O'Rourke et al,<sup>23</sup> alterations of intracellular Ca<sup>2+</sup> handling in failing canine midmyocardial ventricular myocytes parallel those observed in human. In particular, the time constant of Ca<sup>2+</sup> uptake in the absence of Na<sup>+</sup>/Ca<sup>2+</sup> exchange is prolonged in failing cells ( $576 \pm 83$  versus  $282 \pm 30$  ms in controls), suggesting a functional downregulation of the SERCA2a. This observation is consistent with Western blot analyses indicating that SR Ca<sup>2+</sup> ATPase protein levels are reduced in failing heart by 28%. Additionally, in the presence of cyclopiazonic acid (CPA, a blocker of the SR Ca<sup>2+</sup> ATPase pump), the time constant of Ca<sup>2+</sup> extrusion is larger in normal than failing cells ( $813 \pm 269$  versus  $599 \pm 48$  ms). This observation is consistent with Western blot analyses indicating a 104%

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increase in the level of expression of the NCX in failing cells. Taken together, these results suggest that SR  $\text{Ca}^{2+}$  uptake is impaired and that  $\text{Ca}^{2+}$  extrusion via the NCX is enhanced in myocytes isolated from the failing canine heart in a way that is similar qualitatively to that seen in human patients.

In this article, we use the data of O'Rourke et al<sup>23</sup> to develop a computational model of the action potential and of intracellular  $\text{Ca}^{2+}$  handling in normal and failing canine ventricular myocytes using biophysically detailed descriptions of both sarcolemmal currents and key components of E-C coupling. With the limits of individual alterations fixed using experimentally derived values, the model is used to quantify the extent to which each parameter ( $I_{\text{to1}}$ ,  $I_{\text{K1}}$ , SR  $\text{Ca}^{2+}$  ATPase, and NCX) contributes to the overall change in electrical and  $\text{Ca}^{2+}$  dynamics in heart failure. The results support the hypothesis that differences in expression of sarcolemmal ion channels and  $\text{Ca}^{2+}$  handling proteins measured experimentally are sufficient to account for the altered action potential waveform and  $\text{Ca}^{2+}$  transient of the failing canine cardiomyocyte.

## Materials and Methods

### Normal Canine Ventricular Cell Model

Jafri et al<sup>24</sup> have presented a model of  $\text{Ca}^{2+}$  handling in the guinea pig ventricular myocyte that incorporates the following: (1) sarcolemmal ion currents of the Luo-Rudy phase II ventricular cell model,<sup>25</sup> (2) a state model of the L-type  $\text{Ca}^{2+}$  current in which  $\text{Ca}^{2+}$ -mediated inactivation occurs via the mechanism of mode switching,<sup>26</sup> (3) calcium-induced calcium release from SR via ryanodine-sensitive calcium release (RyR) channels using a model adapted from that of Keizer and Levine,<sup>27</sup> and (4) a restricted subspace located between the junctional SR (JSR) and T tubules into which both L-type  $\text{Ca}^{2+}$  and RyR channels empty. The model of the canine midmyocardial ventricular cell used in this study is derived from this guinea pig ventricular cell model. All dynamic equations, parameters, and initial conditions for this new model are given in the Appendix. The following modifications to the model of Jafri et al<sup>24</sup> have been made to better represent properties of canine midmyocardial ventricular cells.

#### $I_{\text{to1}}$

Canine epicardial and midmyocardial ventricular cell action potentials exhibit a prominent notch in phase 1 of the action potential that results from the presence of 2 transient outward currents: a  $\text{Ca}^{2+}$ -independent 4-aminopyridine (4-AP)-sensitive current ( $I_{\text{to1}}$ )<sup>5,28,29</sup> and a  $\text{Ca}^{2+}$ -dependent current ( $I_{\text{to2}}$ ).<sup>29,30</sup> The  $\text{Ca}^{2+}$ -independent component  $I_{\text{to1}}$  is modeled on the basis of the formulation of Campbell et al<sup>31</sup> for ferret ventricular cells. Peak  $I_{\text{to1}}$  conductance ( $G_{\text{to1}}$ ) was adjusted to yield a linear plot of peak current density in response to 500-ms-duration voltage-clamp stimuli from a holding potential of  $-80$  mV, with slope  $0.3$  pA/pF-mV and y-intercept  $4.6$  pA/pF. This agrees well with experimental measurements reported for canine  $I_{\text{to1}}$  at  $37^\circ\text{C}$  by Liu et al<sup>28</sup> (see their Figure 10B: slope,  $0.28$  pA/pF-mV, and y-intercept,  $5$  pA/pF). Activation rate constants were scaled to yield a time to peak of  $\approx 8$  ms at a clamp potential of  $+10$  mV (see Figure 5B of Tseng and Hoffman).<sup>29</sup> Inactivation rate constants were adjusted to yield a decay time constant of  $\approx 20$  ms.<sup>29</sup> The  $\text{Ca}^{2+}$ -dependent chloride ( $\text{Cl}^-$ ) current  $I_{\text{to2}}$  was not incorporated in this model.

#### $I_{\text{Kr}}$

The delayed rectifier current  $I_{\text{K}}$  in both canine and guinea pig ventricular myocytes consists of rapid- and slow-activating components known as  $I_{\text{Kr}}$  and  $I_{\text{Ks}}$ , respectively. Models of  $I_{\text{Kr}}$  and  $I_{\text{Ks}}$  in guinea pig ventricular cells have been developed.<sup>32</sup> These models have been modified to approximate properties of corresponding

currents measured in isolated canine midmyocardial ventricular cells.  $I_{\text{Kr}}$  is described using a closed-open-state model in which forward ( $K_{12}$ ) and backward ( $K_{21}$ ) rate constants are exponential functions of voltage ( $V$ ) with the following form:

$$(1) \quad K_{ij}(V) = e^{a_{ij} + b_{ij}V}.$$

Parameters of this model are fully constrained by knowledge of the time constant  $\tau(V)$ , defined as

$$(2) \quad \tau(V) = \frac{1}{K_{12}(V) + K_{21}(V)},$$

at 2 voltages and by knowledge of the steady-state activation function. Activation was fit using a Boltzmann function determined by Liu and Antzelevitch<sup>33</sup> (see their Figure 11). The time constant of activation at  $+5$  mV was set to  $100$  ms,<sup>33</sup> and the time constant of deactivation at  $-60$  mV was set to  $3000$  ms,<sup>34</sup> thereby constraining the rate constants  $K_{12}(V)$  and  $K_{21}(V)$ . A fixed increment of  $27$  ms was added to Equation 2 to bound the time constant away from 0 at depolarized potentials. The maximum conductance  $\bar{G}_{\text{Kr}}$  was adjusted to yield a tail current density of  $0.2$  pA/pF in response to a voltage-clamp step to  $+25$  mV for  $3.0$  seconds, followed by a step to  $-35$  mV for  $1.0$  seconds, as described by Gintant.<sup>35</sup>

#### $I_{\text{Ks}}$

The slow-activating delayed rectifier current  $I_{\text{Ks}}$  is present in epicardial, midmyocardial, and endocardial canine ventricular cells.  $I_{\text{Ks}}$  is modeled as described in Zeng et al,<sup>32</sup> with the exception that the steady-state activation function is fit using a Boltzmann function determined by Liu and Antzelevitch.<sup>33</sup> The voltage-dependent time constant is also shifted by  $+40$  mV in the depolarizing direction to fit the experimental data of Liu and Antzelevitch<sup>33</sup> (see their Figure 13). Maximum conductance ( $\bar{G}_{\text{Ks}}$ ) is adjusted to yield a tail current density of  $0.4$  pA/pF in response to  $3.0$ -second-duration voltage-clamp steps from the holding potential of  $-35$  to  $+25$  mV, followed by a return to the holding potential<sup>34</sup> (see Figure 5). The  $\text{Ca}^{2+}$  dependence of  $I_{\text{Ks}}$  described in the Luo-Rudy phase II guinea pig model is not included, as there are no experimental data constraining this dependence in canine ventricular cells.

#### $I_{\text{K1}}$

$I_{\text{K1}}$  is fit using data measured at  $22^\circ\text{C}$  in isolated canine midmyocardial ventricular myocytes measured by Kääh et al<sup>5</sup> and scaled to  $37^\circ\text{C}$ . These data indicate that maximum outward  $I_{\text{K1}}$  density is  $\approx 2.5$  pA/pF at  $-60$  mV<sup>5</sup> (see Reference 5, Figure 4B). These data also show that  $I_{\text{K1}}$  density is nonnegligible at voltages within the plateau range of the canine action potential. For example,  $I_{\text{K1}}$  density is  $0.3$  pA/pF at  $0$  mV, a value comparable with the density of  $I_{\text{Kr}}$  during the plateau phase of the action potential. The functional representation of  $I_{\text{K1}}$  in the Luo-Rudy phase II model can therefore not be used, as it approaches 0 at plateau membrane potentials. An alternative formulation better approximating the canine data is presented in the Appendix.

#### $I_{\text{Ca,L}}$

The model of L-type  $\text{Ca}^{2+}$  current used is identical to the mode-switching model presented in Jafri et al,<sup>24</sup> with 3 exceptions. First, the voltage dependence of the activation transition rates  $\alpha(V)$  and  $\beta(V)$  and the inactivation variable  $y(V)$  are shifted by  $+10$  mV in the depolarizing direction to position the peak L-type  $\text{Ca}^{2+}$  current in response to voltage-clamp stimuli at  $+5$  mV, as measured experimentally.<sup>5</sup> Second, the monotonic decreasing steady-state (voltage-dependent) inactivation function  $y_\infty$  is modified to have an asymptotic value of  $0.2$  for large positive membrane potentials  $V$ . This modification reproduces the slow component of  $\text{Ca}^{2+}$  current observed under voltage-clamp stimuli in canine ventricular cells.<sup>5,37</sup> Finally, peak L-type  $\text{Ca}^{2+}$  current density is adjusted to a value of  $2.5$  pA/pF at a clamp voltage of  $+5$  mV.

#### $J_{\text{np}}$

In the model of Jafri et al,<sup>24</sup>  $\text{Ca}^{2+}$  uptake into network SR (NSR) is modeled using a Hill function with coefficient of  $2$ . Reverse pump

rate is assumed to be 0, and  $\text{Ca}^{2+}$  leak from NSR to cytoplasm is assumed to be proportional to the gradient of NSR and cytosolic  $\text{Ca}^{2+}$  concentrations. Recently, Shannon et al<sup>38</sup> have proposed the hypothesis that SR  $\text{Ca}^{2+}$  accumulation at rest is not limited by leak of  $\text{Ca}^{2+}$  from SR but rather is limited by a reverse component of SR  $\text{Ca}^{2+}$  ATPase pump current. They have proposed a new model of the SR  $\text{Ca}^{2+}$  ATPase pump that includes forward- and reverse-current components, each with its own binding constant and peak forward and reverse rates (denoted  $V_{\text{maxf}}$  and  $V_{\text{maxr}}$ , respectively).<sup>39</sup> The forward mode exhibits slight cooperativity, whereas the reverse mode is noncooperative. The relative magnitudes of forward- and reverse-current components determine whether SR load increases, is constant, or decreases during diastole. The model is presented in the Appendix.

### Failing Canine Ventricular Cell Model

Kääb et al<sup>5</sup> have shown that in the canine tachycardia pacing-induced model of heart failure,  $I_{\text{to1}}$  and  $I_{\text{K1}}$  are downregulated on average by 66% and 32%, respectively, in terminal heart failure. Only the number of expressed channels is changed; the kinetic properties of  $I_{\text{to1}}$  and gating behavior of  $I_{\text{K1}}$  are unaltered. On the basis of these data, the effects of terminal heart failure are modeled by reducing the peak conductance of  $I_{\text{to1}}$  and  $I_{\text{K1}}$  by the factors indicated above. Downregulation of the SR  $\text{Ca}^{2+}$  ATPase is modeled by simultaneous scaling of both the forward and reverse maximum pump rates  $V_{\text{maxf}}$  and  $V_{\text{maxr}}$  by a scale factor,  $K_{\text{SR}}$ . Upregulation of the NCX is modeled by increasing a scale factor,  $K_{\text{NaCa}}$ .

### Numerical Methods

The dynamical equations in the Appendix are solved on a Silicon Graphics workstation using the Merson modified Runge-Kutta fourth-order adaptive step algorithm (No. 25, Reference 52), with a maximum step size of 100 microseconds and maximum error tolerance of  $10^{-6}$ . The error from all variables is normalized to ensure that each contributes equally to the calculation of global error, as described in Jafri et al.<sup>24</sup> Initial conditions listed in the Appendix are used in all calculations, unless noted otherwise. These initial conditions were computed in response to a periodic pulse train of frequency 1 Hz and were determined immediately before the 11th pulse. Action potentials are initiated using  $0.1 \mu\text{A}\mu\text{F}^{-1}$  current injection for 500 microseconds.

The canine ventricular cell model is used to derive quantitative estimates of the NCX scale factor  $K_{\text{NaCa}}$  and the SR  $\text{Ca}^{2+}$  ATPase scale factor  $K_{\text{SR}}$  from experimental data by fitting model  $\text{Ca}^{2+}$  transient decay rates to those measured experimentally. To do this, a series of 10 voltage-clamp stimuli ( $-97\text{-mV}$  holding potential,  $3\text{-mV}$  step potential, and  $200\text{-ms}$  duration) are applied at a frequency of 1 Hz.  $\text{Ca}^{2+}$  transient decay rate is estimated from response to the final voltage-clamp stimulus to assure that model SR  $\text{Ca}^{2+}$  concentrations have reached equilibrium values.

## Results

### Action Potentials and $\text{Ca}^{2+}$ Transients: Model Versus Experimental Results

Figure 1 demonstrates the ability of the model to reconstruct action potentials and  $\text{Ca}^{2+}$  transients of both normal and failing canine midmyocardial ventricular myocytes. The solid and dotted lines in Figure 1A show experimental measurements of normal and failing action potentials, respectively. Model action potentials are shown in Figure 1C. In this figure, the solid line shows a normal action potential. The dashed line shows an action potential when  $I_{\text{to1}}$  is reduced by 66% of the normal values and  $I_{\text{K1}}$  by 32% of the normal values (the average percentage reductions observed in terminal heart failure.)<sup>5</sup> The dotted line corresponds to these same reductions of  $I_{\text{to1}}$  and  $I_{\text{K1}}$ , in addition to a 62% reduction of the SR  $\text{Ca}^{2+}$  ATPase pump and a

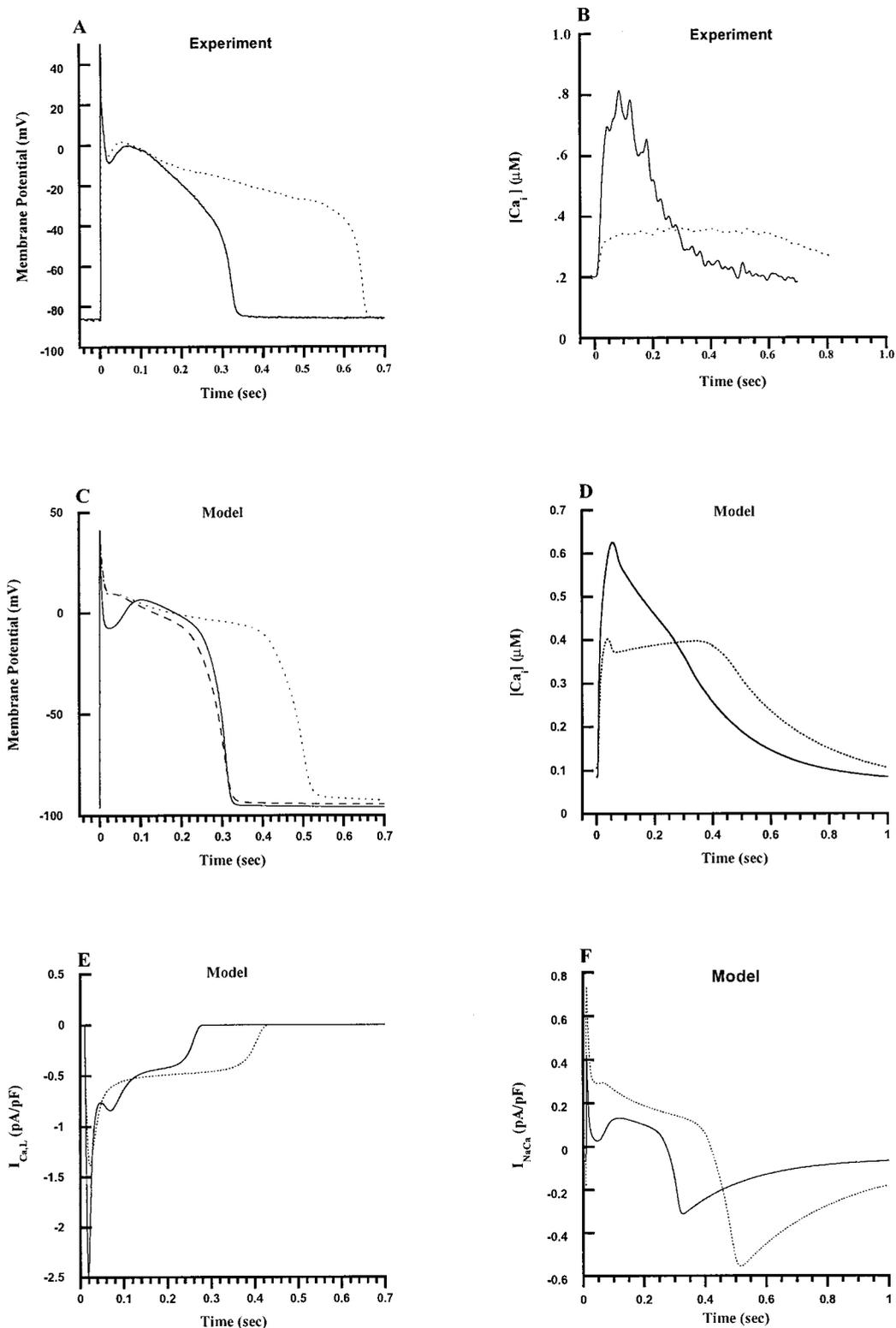
75% increase of the NCX. These values are model-based estimates of the average percentage change in activity of these proteins determined using experimentally derived limits on their function, as described in the following sections.

The model data of Figure 1C show that downregulation of  $I_{\text{to1}}$  and  $I_{\text{K1}}$  reduces the depth of the phase 1 notch. However, notch depth is larger in the experimental measurements from the failing myocyte (Figure 1A, dotted line) than is predicted by the model (Figure 1C, dashed line). This greater notch depth is due to the presence of the  $\text{Ca}^{2+}$ -dependent transient outward current  $I_{\text{to2}}$ , which is not included in the model. The most significant change in model action potential duration (APD) occurs with upregulation of the NCX and downregulation of the SR  $\text{Ca}^{2+}$  ATPase (Figure 1C, dotted line). These 2 changes alone increase APDs at 90% repolarization (APD<sub>90</sub>) by  $\approx 200$  ms.

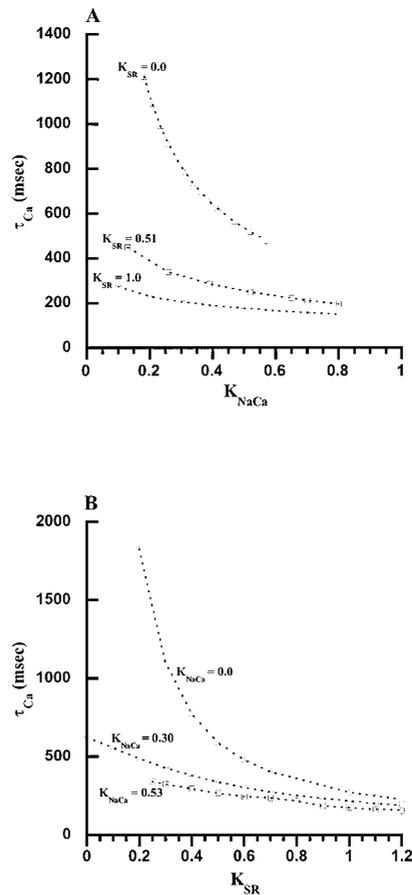
Figure 1D illustrates model normal (solid line) and failing (dotted line)  $\text{Ca}^{2+}$  transients. Amplitude of the  $\text{Ca}^{2+}$  transient is reduced significantly in the heart failure model.  $\text{Ca}^{2+}$  transient shape is flattened, duration is prolonged, and relaxation is slowed. These changes are similar qualitatively to those seen in the experimental data of Figure 1B.

Figures 1E and 1F show L-type  $\text{Ca}^{2+}$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchange currents for normal (solid lines) and failing (dotted lines) model cells. The reduction in peak magnitude of the L-type  $\text{Ca}^{2+}$  current seen in Figure 1E for the failing model cell results from downregulation of  $I_{\text{to1}}$ , which reduces depth of the phase 1 notch and therefore driving force during onset of the L-type  $\text{Ca}^{2+}$  current. Figure 1E also shows that L-type  $\text{Ca}^{2+}$  current is increased during the later plateau phase of the action potential in failing model cells. The mechanism of this increase will be considered in subsequent sections. Figure 1F shows that  $\text{Na}^+/\text{Ca}^{2+}$  exchange operates in reverse mode, generating a net outward current during most of the plateau phase of the action potential. The magnitude of this outward current decreases during the plateau phase, and in the failing cell model the current becomes significantly smaller than the inward L-type  $\text{Ca}^{2+}$  current.

These simulations demonstrate the ability of the model to reproduce both normal and failing canine myocyte action potentials and  $\text{Ca}^{2+}$  transients. The following sections describe application of the model to estimation of the degree of functional change in the NCX and SR  $\text{Ca}^{2+}$  ATPase in control and failing myocytes. The approach is as follows: (1) the time constant of  $\text{Ca}^{2+}$  decay ( $\tau_{\text{Ca}}$ ) measured with SR function blocked using CPA data is used to estimate the model  $\text{Na}^+/\text{Ca}^{2+}$  exchange scale factor  $K_{\text{NaCa}}$ ; (2) with  $K_{\text{NaCa}}$  fixed at this value, the model SR  $\text{Ca}^{2+}$  ATPase scale factor  $K_{\text{SR}}$  required to reproduce the  $\tau_{\text{Ca}}$  measured in physiological solutions is determined; (3) the SR  $\text{Ca}^{2+}$  ATPase reduction in heart failure is cross-checked independently by determining the model SR  $\text{Ca}^{2+}$  ATPase scale factor required to reproduce the  $\tau_{\text{Ca}}$  measured under  $\text{Na}^+$ -free conditions (0-Na data) with the model  $\text{Na}^+/\text{Ca}^{2+}$  exchange set to 0; and (4) the model  $\text{Na}^+/\text{Ca}^{2+}$  exchange scale factor is estimated independently from  $\tau_{\text{Ca}}$  in physiological solutions using the estimate of SR function determined in step 3.



**Figure 1.** Model vs experimental action potentials and  $Ca^{2+}$  transients. Each action potential and  $Ca^{2+}$  transient is in response to a 1-Hz pulse train, with responses measured in the steady state. A, Experimentally measured membrane potential as a function of time in normal (solid line) and failing (dotted line) canine myocytes. B, Experimentally measured cytosolic  $Ca^{2+}$  concentration ( $\mu$ mol/L) as a function of time for normal (solid line) and failing (dotted line) canine ventricular myocytes. C, Membrane potential as a function of time simulated using the normal canine myocyte model (solid line), the myocyte model with  $I_{to1}$  and  $I_{K1}$  downregulation (dashed line; downregulation by 66% and 32%, respectively), and the heart failure model (dotted line; downregulation of  $I_{to1}$  and  $I_{K1}$  as described previously,  $K_{SR}=0.38$  corresponding to 62% downregulation and  $K_{NaCa}=0.53$  corresponding to 75% upregulation). D, Cytosolic  $Ca^{2+}$  concentration ( $\mu$ mol/L) as a function of time simulated using the normal (solid line) and heart failure (dotted line) model, with parameters as described in panel A. E, L-type  $Ca^{2+}$  current as a function of time for the normal (solid line) and failing (dotted line) cell models. F,  $Na^{+}/Ca^{2+}$  exchange current as a function of time for the normal (solid line) and failing (dotted line) cell models.



**Figure 2.** A, Model voltage-clamp Ca<sup>2+</sup> transient relaxation time constant  $\tau_{Ca}$  as a function of NCX scale factor  $K_{NaCa}$  at SR Ca<sup>2+</sup> ATPase pump scale factors of  $K_{SR}=0.0$  (corresponding to CPA block), 0.51 (value estimated in 0-Na experiments for failing myocytes), and 1.0 (normal value).  $\Delta$  indicates  $K_{SR}=0.0$ ;  $\square$ ,  $K_{SR}=0.51$ ; and  $\circ$ ,  $K_{SR}=1.0$ . B, Model Ca<sup>2+</sup> transient relaxation time constant  $\tau_{Ca}$  as a function of SR Ca<sup>2+</sup> ATPase pump scale factor  $K_{SR}$  for  $K_{NaCa}$  values of 0.0 (0-Na conditions), 0.30 (value estimated in normal myocytes in presence of CPA), and 0.53 (value estimated for failing myocytes during CPA block).  $\Delta$  indicates  $K_{NaCa}=0.0$ ;  $\square$ ,  $K_{NaCa}=0.53$ ; and  $\circ$ ,  $K_{NaCa}=0.30$ .

### Estimation of NCX and SR Ca<sup>2+</sup> ATPase Activity in Normal and Failing Myocytes:

#### CPA Experiments

In the preceding article by O'Rourke et al, Ca<sup>2+</sup> transients in response to voltage-clamp stimuli were measured in the presence and absence of CPA, a blocker of the SR Ca<sup>2+</sup> ATPase pump. In the presence of CPA, Ca<sup>2+</sup> transient decay rate ( $\tau_{Ca}$ ) following termination of a depolarizing voltage step reflects the rate of extrusion of Ca<sup>2+</sup> from the cytosol by the NCX (extrusion by the sarcolemmal Ca<sup>2+</sup> ATPase is small). Estimates of the NCX pump current scale factor  $K_{NaCa}$  may therefore be obtained by setting the model value of  $K_{SR}$  to 0 and varying  $K_{NaCa}$  until model Ca<sup>2+</sup> transient decay rates match those measured experimentally in the presence of CPA.  $K_{NaCa}$  may then be fixed at this value and  $K_{SR}$  varied until model Ca<sup>2+</sup> transient decay rate matches that measured experimentally using physiological solutions. This procedure can be applied to data obtained from both normal and failing cells to assess the extent of functional upregulation and

downregulation of the NCX and SR Ca<sup>2+</sup> ATPase in heart failure.

To estimate  $K_{NaCa}$ , model  $K_{SR}$  was set to 0, 10 voltage-clamp steps (holding potential -97 mV, step potential 3 mV, and duration 200 ms) were applied at a frequency of 1 Hz to assure that Ca<sup>2+</sup> levels in each model Ca<sup>2+</sup> pool were equilibrated, and model  $\tau_{Ca}$  was measured by fitting an exponential function to the decay phase of the final Ca<sup>2+</sup> transient. Figure 2A plots model  $\tau_{Ca}$  (ordinate, ms) as a function of  $K_{NaCa}$  (abscissa) with  $K_{SR}=0.0$  (open triangles).  $K_{NaCa}=0.30$  yields a  $\tau_{Ca}$  equal to the average value measured experimentally in normal myocytes in the presence of CPA ( $813 \pm 269$  ms). One SD of experimental variability is accounted for by  $K_{NaCa}$  values in the interval (0.21, 0.48). This same curve shows that  $K_{NaCa}=0.53$  produces a  $\tau_{Ca}$  matching that measured in failing myocytes in the presence of CPA ( $599 \pm 48$  ms). One SD experimental variability is encompassed by  $K_{NaCa}$  values in the interval (0.48, 0.60). Assuming the normal value of  $K_{NaCa}$  to be 0.30, these data suggest a functional upregulation of the NCX in heart failure in the range of 60% to 100%, with average value  $\approx 75\%$ .

Figure 2B plots model  $\tau_{Ca}$  (ordinate, ms) as a function of  $K_{SR}$  (abscissa). The curve marked with open circles plots this dependence when  $K_{NaCa}$  is constant at the normal value estimated above ( $K_{NaCa}=0.30$ ). The experimental value of  $\tau_{Ca}$  measured in normal myocytes using physiological solutions is  $219 \pm 36$  ms. The maximum forward and reverse SR Ca<sup>2+</sup> ATPase pump rates  $V_{maxf}$  and  $V_{maxr}$  given in Table 4 of the Appendix have been selected to yield a similar time constant when  $K_{SR}=1.0$ . Measured variation about this value is accounted for by  $K_{SR}$  values in the interval (0.85, 1.15).

The experimental value of  $\tau_{Ca}$  measured in failing myocytes using physiological solutions is  $292 \pm 23$  ms. Dependence of model  $\tau_{Ca}$  on  $K_{SR}$  when  $K_{NaCa}$  is fixed at the value estimated for failing canine myocytes (0.53) is shown by the curve labeled with open squares in Figure 2B.  $K_{SR}=0.38$  yields a model  $\tau_{Ca}$  equal to that observed experimentally. The experimental deviation of  $\tau_{Ca}$  is accounted for by  $K_{SR}$  values in the interval (0.26, 0.51). Assuming the average value of  $K_{SR}$  in normal cells to be 1.0, these data suggest a functional downregulation of the SR Ca<sup>2+</sup> ATPase pump in heart failure in the range of 49% to 74%, with average value 62%.

### Estimation of NCX and SR Ca<sup>2+</sup> ATPase Activity in Normal and Failing Myocytes:

#### 0-Na Experiments

To provide a second, independent measure of altered Ca<sup>2+</sup> handling protein expression in heart failure, O'Rourke et al<sup>23</sup> have measured  $\tau_{Ca}$  in the presence and absence of Na<sup>+</sup>/Ca<sup>2+</sup> exchange by removing Na<sup>+</sup> ions from both intracellular and extracellular solutions. In the absence of Na<sup>+</sup>/Ca<sup>2+</sup> exchange,  $\tau_{Ca}$  reflects primarily the rate of Ca<sup>2+</sup> uptake from the cytosol by the SR Ca<sup>2+</sup> ATPase pump. Estimates of the SR Ca<sup>2+</sup> ATPase pump rate scale factor  $K_{SR}$  under 0-Na conditions may therefore be obtained by setting the model value of  $K_{NaCa}$  to 0 and varying  $K_{SR}$  until simulated voltage-clamp Ca<sup>2+</sup> transient decay rates match those measured experimentally. Once the model value of  $K_{SR}$  is constrained,  $K_{NaCa}$  can then be determined by changing its value until model Ca<sup>2+</sup> transient

decay rates match those measured experimentally using physiological solutions. This procedure can be applied to data obtained from both normal and failing cells to assess the extent of functional upregulation and downregulation of the NCX and SR  $\text{Ca}^{2+}$  ATPase in heart failure.

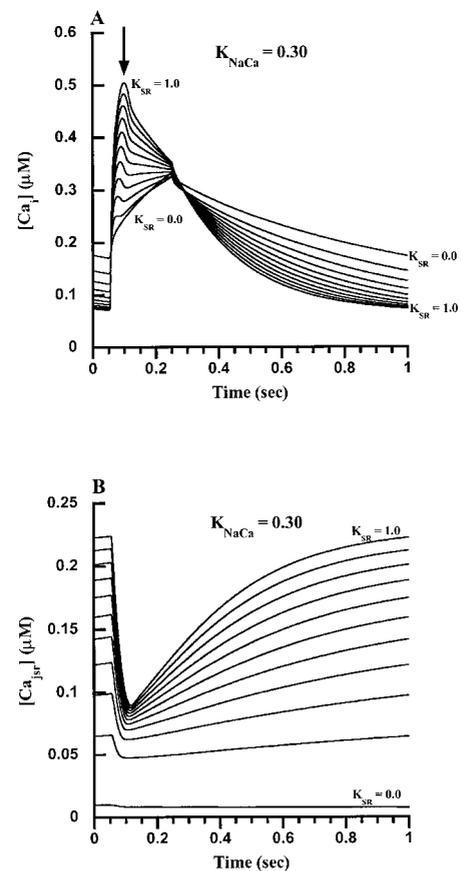
To mimic 0-Na conditions,  $K_{\text{NaCa}}$  was set equal to 0.  $K_{\text{SR}}$  was then varied, and the time constant for  $\text{Ca}^{2+}$  reuptake into SR was computed. Model  $\tau_{\text{Ca}}$  values are plotted as a function of  $K_{\text{SR}}$  in Figure 2B (open triangles). Experimentally measured values of this time constant are  $282 \pm 30$  ms in normal and  $576 \pm 83$  ms in failing canine ventricular cells studied under 0-Na conditions. A  $K_{\text{SR}}$  value of 1.0 accounts for  $\tau_{\text{Ca}}$  measured experimentally in normal cells ( $282 \pm 30$  ms), and values in the interval (0.92, 1.07) account for the observed SD in these measurements. This estimate of the average  $K_{\text{SR}}$  value in normal myocytes based on block of the NCX agrees with that estimated using the CPA data. A  $K_{\text{SR}}$  value of 0.51 accounts for the average  $\tau_{\text{Ca}}$  measured experimentally in failing cells ( $576 \pm 83$  ms), and  $K_{\text{SR}}$  values in the interval (0.46, 0.59) account for the SD. Assuming the normal  $K_{\text{SR}}$  value to be 1.0, these data suggest a functional downregulation of the SR  $\text{Ca}^{2+}$  ATPase pump in failing myocytes in the range of 41% to 54%, with average value 49%. This estimate of SR  $\text{Ca}^{2+}$  ATPase downregulation is qualitatively similar to that obtained using CPA.

Dependence of model  $\tau_{\text{Ca}}$  on  $K_{\text{NaCa}}$  when  $K_{\text{SR}}$  is fixed at the value estimated for normal canine myocytes (1.0) is shown by the curve labeled with open circles in Figure 2A.  $K_{\text{NaCa}} = 0.22$  yields a model  $\tau_{\text{Ca}}$  equal to that observed experimentally using physiological solutions ( $219 \pm 36$  ms). Experimental deviation of  $\tau_{\text{Ca}}$  is accounted for by  $K_{\text{NaCa}}$  values in the interval (0.13, 0.43). Dependence of model  $\tau_{\text{Ca}}$  on  $K_{\text{NaCa}}$  when  $K_{\text{SR}}$  is fixed at the value estimated for failing canine myocytes under 0-Na conditions (0.51) is shown by the curve labeled with open squares in Figure 2A.  $K_{\text{NaCa}} = 0.35$  yields a model  $\tau_{\text{Ca}}$  equal to the average value observed experimentally using physiological solutions ( $292 \pm 23$  ms). Experimental deviation of  $\tau_{\text{Ca}}$  is accounted for by  $K_{\text{NaCa}}$  values in the interval (0.26, 0.46). Assuming the normal value of  $K_{\text{NaCa}}$  to be 0.22, these data suggest a functional upregulation of the NCX in heart failure in the range of 18% to 109%, with average value 38%. This estimate of altered expression of NCX in heart failure has greater variability than that obtained previously using the CPA data but is consistent in that it also indicates increased expression.

### Parametric Dependence of Voltage-Clamp $\text{Ca}^{2+}$ Transients on SR $\text{Ca}^{2+}$ ATPase and NCX Levels

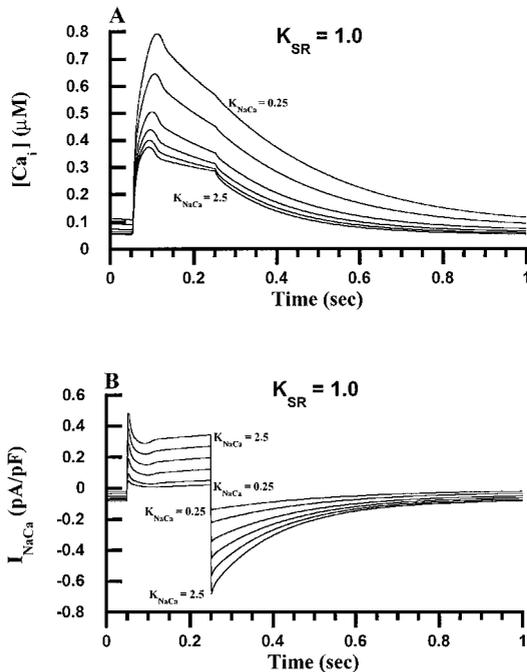
The above analyses provide estimates of  $K_{\text{SR}}$  and  $K_{\text{NaCa}}$  in normal and failing myocytes. Results indicate functional downregulation of the SR  $\text{Ca}^{2+}$  ATPase pump and upregulation of the NCX in heart failure. The parametric dependence of model cytosolic  $\text{Ca}^{2+}$  transients on  $K_{\text{SR}}$  and  $K_{\text{NaCa}}$  is examined next.

Model cytosolic  $\text{Ca}^{2+}$  concentration (ordinate,  $\mu\text{mol/L}$ ) versus time (abscissa, seconds) is shown in Figure 3A as  $K_{\text{SR}}$  is varied. In these simulations,  $K_{\text{NaCa}}$  is constant at the value estimated using CPA data from normal cells ( $K_{\text{NaCa}} = 0.30$ ).  $K_{\text{SR}}$  is varied from 1.0 to 0.0 in steps of 0.1.  $\text{Ca}^{2+}$  transients



**Figure 3.** A, Cytosolic  $\text{Ca}^{2+}$  concentration ( $\mu\text{mol/L}$ ) as a function of time in response to a 10-second-duration periodic sequence of voltage-clamp stimuli with frequency of 1 Hz. Holding potential is  $-97$  mV, and clamp potential is  $+3$  mV, with duration of 200 ms. Only response to the final stimulus is shown. A family of responses is shown in which  $K_{\text{NaCa}}$  is constant at 0.30 (value estimated in CPA experiments with normal canine myocytes), and  $K_{\text{SR}}$  is varied from 1.0 to 0.0 in steps of 0.1. B, Model JSR  $\text{Ca}^{2+}$  concentration in response to the stimuli in panel A.

are in response to a 1-Hz voltage-clamp stimulus (holding potential  $-97$  mV, step potential 3 mV, and duration 200 ms). Response to the final stimulus of 10 stimulus cycles is shown, with the time origin translated to 0 seconds. These data show that reduction of the model SR  $\text{Ca}^{2+}$  ATPase pump, simulating the effects of downregulation of this pump in heart failure, reduces the amplitude of the early peak of the  $\text{Ca}^{2+}$  transient (marked by the arrow). This early peak disappears as  $K_{\text{SR}}$  approaches 0. Figure 3B shows JSR  $\text{Ca}^{2+}$  levels for each of the responses in Figure 3A. Reduction of the early peak in the data of Figure 3A coincides with depletion of JSR  $\text{Ca}^{2+}$  at small values of  $K_{\text{SR}}$ . Thus, the early peak in the model  $\text{Ca}^{2+}$  transient is generated by  $\text{Ca}^{2+}$  release from JSR, and the slow second peak, which is present even when JSR is depleted, results from influx of  $\text{Ca}^{2+}$  through sarcolemmal L-type  $\text{Ca}^{2+}$  channels and reverse-mode  $\text{Na}^{+}/\text{Ca}^{2+}$  exchange. As  $K_{\text{SR}}$  decreases,  $\text{Ca}^{2+}$  levels in JSR decrease, and the  $\text{Ca}^{2+}$  transient becomes reduced in peak amplitude. The  $\text{Ca}^{2+}$  transient exhibits a decrease, no change, or an increase of amplitude during the course of the voltage-clamp stimulus, depending on the value of  $K_{\text{SR}}$ . Decay rate of the  $\text{Ca}^{2+}$  transient decreases



**Figure 4.** A, Cytosolic  $Ca^{2+}$  concentration ( $\mu\text{mol/L}$ ) as a function of time in response to a 10-second-duration periodic sequence of voltage-clamp stimuli with frequency of 1 Hz. Holding potential is  $-97$  mV, and clamp potential is  $+3$  mV, with duration of 200 ms. Only response to the final stimulus is shown. A family of responses is shown in which  $K_{NaCa}$  is varied from 0.5 to 2.5 in steps of 0.5 (response for  $K_{NaCa}=0.25$  is also shown), while  $K_{SR}$  is held constant at the value accounting for the  $Ca^{2+}$  relaxation rate measured in normal myocytes under 0-Na conditions. B, Model NaCa exchange current  $I_{NaCa}$  as a function of time in response to the stimuli described in Figure 3A.

with decreasing  $K_{SR}$  values, as shown in the data of Figure 3A, as well as Figure 2B (open circles).

Figure 4A shows model cytosolic  $Ca^{2+}$  concentration (ordinate,  $\mu\text{mol/L}$ ) versus time (abscissa, seconds) as  $K_{NaCa}$  is varied in steps of 0.5 from 0.5 to 2.5. A plot for  $K_{NaCa}=0.25$  is also shown.  $K_{SR}$  is constant at the value estimated using CPA data from normal myocytes ( $K_{SR}=1.0$ ). Voltage clamp steps from  $-97$  mV to  $+3$  mV with 200 ms duration are applied at a rate of 1.0 Hz. The final  $Ca^{2+}$  transient in a sequence of 10 is displayed, with the time origin translated to 0 seconds. There are 3 effects of increased  $K_{NaCa}$ . These are (1) increased rate of  $Ca^{2+}$  extrusion and lower diastolic  $Ca^{2+}$  at the holding potential, (2) reduction in  $Ca^{2+}$  transient amplitude in response to the  $+3$  mV voltage-clamp step, and (3) "flattening" of the  $Ca^{2+}$  transient during the voltage-clamp step. The increased  $Ca^{2+}$  extrusion at the holding potential is a direct consequence of increased NCX activity when the exchanger is operating in the forward mode at the  $-97$ -mV holding potential, as shown in Figure 4B. This figure also shows that the NCX operates in reverse mode at the  $+3$  mV clamp potential, thus generating  $Ca^{2+}$  influx. The reduction in  $Ca^{2+}$  transient amplitude in response to the voltage step is a consequence of the fact that total  $Ca^{2+}$  extrusion at the holding potential is greater than total  $Ca^{2+}$  influx at the step potential. This produces a smaller  $Ca^{2+}$  transient through reductions in SR  $Ca^{2+}$  loading and therefore a smaller  $Ca^{2+}$

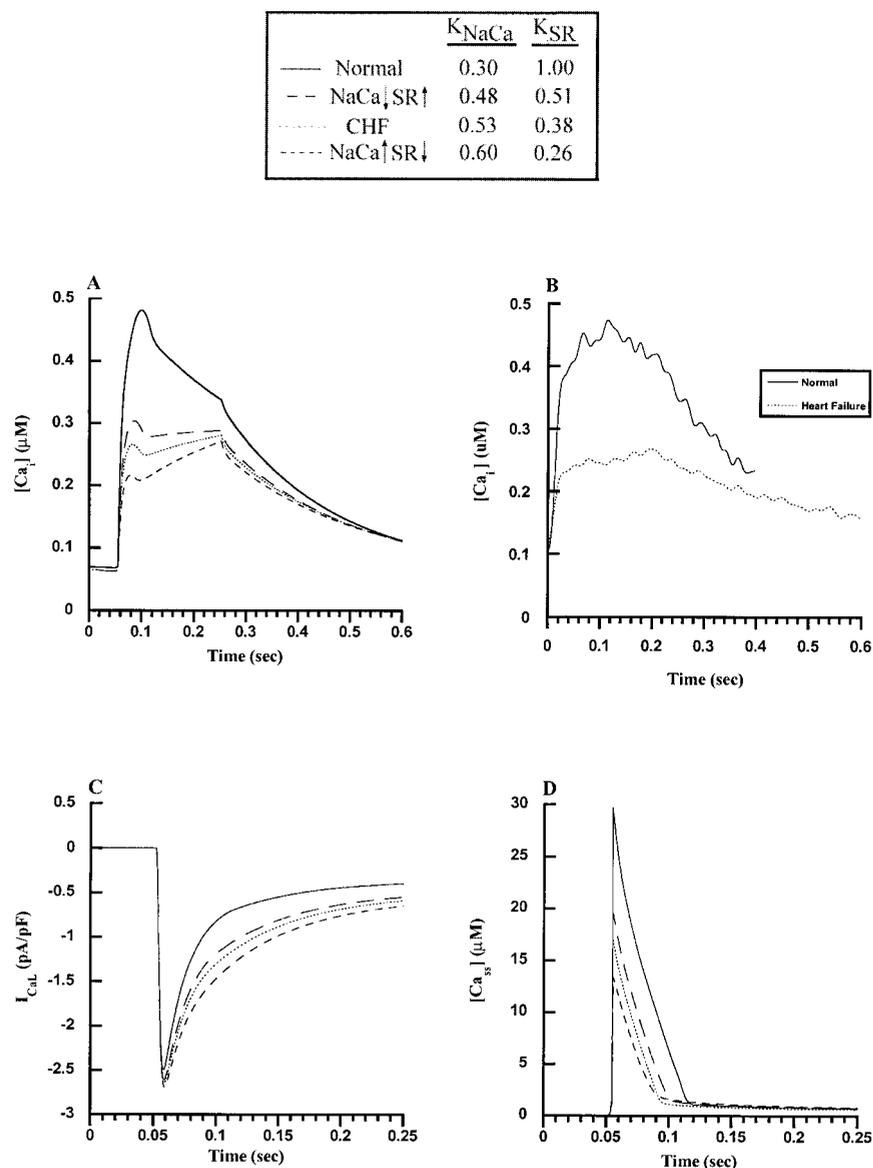
release. The flattening of the  $Ca^{2+}$  transient with increased  $K_{NaCa}$  is a direct consequence of increased  $Ca^{2+}$  influx during the voltage step, as shown in Figure 4B. Decreased  $K_{NaCa}$  values also produce smaller  $Ca^{2+}$  transient decay rates, as seen by the data of Figure 4A, as well as Figure 2A (open circles).

### $Ca^{2+}$ Transients in Response to Voltage-Clamp Stimuli: Model Versus Experimental Results

Figure 5A shows model  $Ca^{2+}$  transients in response to a 1-Hz voltage-clamp pulse train. These transients were computed using  $K_{SR}$  and  $K_{NaCa}$  parameter values determined from the experimental series in the presence and absence of CPA. The solid line is the normal model  $Ca^{2+}$  transient ( $K_{NaCa}=0.30$  and  $K_{SR}=1.0$ ). The peak  $Ca^{2+}$  level (480 nmol/L) agrees well with the value measured experimentally in normal myocytes ( $450 \pm 75$  nmol/L).<sup>23</sup> The dotted line is the model  $Ca^{2+}$  transient computed using the average  $K_{NaCa}$  (0.53) and  $K_{SR}$  (0.38) values for failing myocytes. The remaining 2  $Ca^{2+}$  transients (dashed lines) correspond to  $K_{NaCa}$  and  $K_{SR}$  values selected at  $\pm 1$  SD from the average for failing myocytes. The short dashed line represents parameter choices producing a high degree of SR unloading (large NCX activity,  $K_{NaCa}=0.60$ ; small SR  $Ca^{2+}$  ATPase activity,  $K_{SR}=0.26$ ). The long-dashed line represents parameter choices that minimize SR unloading (small NCX activity,  $K_{NaCa}=0.48$ ; large SR  $Ca^{2+}$  ATPase activity,  $K_{SR}=0.51$ ). These data show that as  $K_{NaCa}$  is increased from a normal value of 0.30 (taking on values of 0.48, 0.53, and 0.60) and  $K_{SR}$  is decreased from the normal value of 1.0 (taking on values 0.51, 0.38, and 0.26),  $Ca^{2+}$  transient peak decreases monotonically from the normal value of 480 nmol/L, taking on values of 300, 266, and 230 nmol/L. These values agree well with the average experimental values measured in failing cells of  $230 \pm 40$  nmol/L.<sup>23</sup>

Figure 5B shows a  $Ca^{2+}$  transient measured experimentally. The amplitude and waveform of the model predictions in Figure 5A are in close agreement with these experimental data.

Figure 5C shows a plot of the L-type  $Ca^{2+}$  current during the  $Ca^{2+}$  transients of Figure 5A. The parameter changes have relatively little effect on peak current, but increases in  $K_{NaCa}$  or decreases in  $K_{SR}$  produce a monotonic increase in the late component of the L-type  $Ca^{2+}$  current. As shown in Figure 5D, these same parameter changes also produce monotonic decreases of the subspace  $Ca^{2+}$  transient peak. Thus, the increase in the late component of L-type  $Ca^{2+}$  current seen in Figure 5C results from a decrease in  $Ca^{2+}$ -mediated inactivation of this current due to reductions in magnitude of the subspace  $Ca^{2+}$  transient, which is in turn a consequence of reduced SR  $Ca^{2+}$  load. As can be appreciated by examining the magnitude of the change in L-type  $Ca^{2+}$  current density with alterations in  $Ca^{2+}$  handling, this late component of the L-type  $Ca^{2+}$  current would be expected to play an important role in determining the action potential plateau. This suggests that in heart failure, alterations in the expression of  $Ca^{2+}$  handling proteins that decrease SR  $Ca^{2+}$  load and reduce the amplitude of the  $Ca^{2+}$  transient may contribute substantially to prolongation of APD by reducing  $Ca^{2+}$ -mediated inactivation of the L-type  $Ca^{2+}$  current.



**Figure 5.** A, Cytosolic  $Ca^{2+}$  concentration ( $\mu\text{mol/L}$ ) as a function of time in response to a 10-second-duration periodic sequence of voltage-clamp stimuli with frequency of 1 Hz. Holding potential is  $-97$  mV, and clamp potential is  $+3$  mV, with duration of 200 ms. Only response to the final stimulus is shown. Results of 4 simulations are shown. Solid line indicates normal model  $Ca^{2+}$  transient ( $K_{NaCa}=0.30$ ;  $K_{SR}=1.0$ ). Dotted line indicates the average failing model  $Ca^{2+}$  transient computed using values of  $K_{SR}$  and  $K_{NaCa}$  estimated in the presence/absence of CPA, as described in the text ( $K_{SR}=0.38$ ;  $K_{NaCa}=0.53$ ). Short-dashed lines show failing model  $Ca^{2+}$  transients computed using  $K_{SR}=0.26$  and  $K_{NaCa}=0.60$ . Long-dashed lines show failing model  $Ca^{2+}$  transients computed using  $K_{SR}=0.51$  and  $K_{NaCa}=0.48$ . B, Experimentally measured cytosolic  $Ca^{2+}$  concentration ( $\mu\text{mol/L}$ ) vs time in response to voltage-clamp stimuli (as described in panel A) in normal (solid line) and failing (dotted line) canine ventricular myocytes. C, L-type  $Ca^{2+}$  current as a function of time for the stimuli and model parameters described in panel A. D, Subspace  $Ca^{2+}$  concentration ( $\mu\text{mol/L}$ ) as a function of time for the stimuli and model parameters described in panel A.

## Discussion

In this article, we present a model of the canine midmyocardial action potential and  $Ca^{2+}$  transient. The model is used to estimate the magnitude of SR  $Ca^{2+}$  ATPase pump rates and NCX current in normal and failing myocytes<sup>23</sup> using 2 methods. In the first method, model SR  $Ca^{2+}$  ATPase current is set to 0, and the NCX current is scaled to yield  $Ca^{2+}$  relaxation time constants in response to voltage-clamp stimuli matching those measured experimentally in normal and failing myocytes in the presence of CPA, a blocker of the SR  $Ca^{2+}$  ATPase. The extent of functional upregulation of the NCX in heart failure estimated using this approach is in the range of 60% to 100%, with average value 75%. Having constrained the model NCX current, model SR  $Ca^{2+}$  ATPase pump current is then estimated by matching the model  $Ca^{2+}$  relaxation rate to experimental data obtained in the absence of CPA. Comparison of model SR  $Ca^{2+}$  ATPase pump currents estimated for normal and failing myocytes suggests a functional downregulation in heart failure in the range of 49% to 74%, with average value 62%.

In the second method, model NCX current is set to 0, and the SR  $Ca^{2+}$  ATPase current is scaled to yield  $Ca^{2+}$  relaxation time constants matching those measured experimentally under 0-Na conditions. Functional downregulation of the SR  $Ca^{2+}$  ATPase current in heart failure estimated using this approach is in the range of 41% to 54%, with average value 49%. Having constrained the model SR  $Ca^{2+}$  ATPase current, NCX current is estimated by matching the model  $Ca^{2+}$  relaxation rate to experimental data obtained in control intracellular and extracellular sodium concentrations. Functional NCX upregulation in heart failure estimated using this approach is in the range of 18% to 109%, with average value 38%.

Analysis of protein levels in canine hearts subjected to the tachycardia pacing protocol reveal that both SR  $Ca^{2+}$  ATPase and phospholamban proteins are reduced on average by 28%<sup>23</sup> and that NCX protein is increased on average by 104%.<sup>23</sup> Both steady-state mRNA and expressed levels of E-C coupling proteins in failing human ventricular cells have

been measured. The majority of reports agree that there is a  $\approx 50\%$  reduction of: (1) mRNA encoding the SR  $\text{Ca}^{2+}$  ATPase pump,<sup>12–16</sup> (2) expressed SR  $\text{Ca}^{2+}$  ATPase protein level,<sup>12,17,18</sup> and (3) direct SR  $\text{Ca}^{2+}$  ATPase uptake rate during heart failure.<sup>19</sup> There is a 55% to 79% increase in Na-Ca exchanger mRNA levels,<sup>12,20</sup> a 36% to 160% increase in expressed protein levels,<sup>12,20–22</sup> and an approximate factor of 2 increase in  $\text{Na}^+/\text{Ca}^{2+}$  exchange activity in human heart failure.<sup>22</sup>

The model-based estimates of functional upregulation and downregulation of the NCX and SR  $\text{Ca}^{2+}$  ATPase pump reported here are consistent with these reports. Model estimates of average SR  $\text{Ca}^{2+}$  ATPase functional downregulation are 49% and 62%, depending on the estimation methods used. These values agree well with estimates of mRNA level, protein level, and SR  $\text{Ca}^{2+}$  ATPase uptake rate measured in human heart failure, but suggest a slightly larger degree of downregulation than indicated by measurements of protein level in canine tachycardia pacing-induced heart failure<sup>23</sup> (28%). Model estimates of average NCX upregulation are 38% and 75%. These estimates agree well with measured increases in mRNA levels in human heart failure and are within the range of variability of measured NCX protein levels in human heart failure. However, the model estimates are slightly lower than is suggested by the increased protein levels measured in the failing canine heart.<sup>23</sup>

$\text{Ca}^{2+}$  transients measured in failing human and canine ventricular myocytes exhibit reduced amplitude and slowed relaxation.<sup>5,40–43</sup> Model simulations of  $\text{Ca}^{2+}$  transients in response to voltage-clamp stimuli reported here demonstrate that the altered expression of the NCX and SR  $\text{Ca}^{2+}$  ATPase pump measured in failing canine myocytes is sufficient to account for these properties. Both changes contribute to reduced SR  $\text{Ca}^{2+}$  load and release and therefore reduced amplitude of the early  $\text{Ca}^{2+}$  transient peak (Figures 3A and 4A). The shape of the  $\text{Ca}^{2+}$  transient is also controlled by both NCX and SR  $\text{Ca}^{2+}$  ATPase levels. As the  $\text{Ca}^{2+}$  ATPase pump is downregulated (Figure 3A), the shape of the plateau portion of the voltage-clamp  $\text{Ca}^{2+}$  transient changes from negative to 0, then to positive slope. This change in slope is produced by a decrease in early  $\text{Ca}^{2+}$  release from JSR, which in turn increases the dependence of  $\text{Ca}^{2+}$  transient shape on  $\text{Ca}^{2+}$  entry through the L-type  $\text{Ca}^{2+}$  channel. Upregulation of NCX also influences  $\text{Ca}^{2+}$  transient shape, tending to flatten the  $\text{Ca}^{2+}$  transient plateau by increasing reverse-mode  $\text{Ca}^{2+}$  entry at depolarized potentials (Figure 4A). The interplay between both of these factors accounts for the flattened  $\text{Ca}^{2+}$  transient shape seen in failing myocytes (Figure 1D, model; Figure 1B, experimental data).

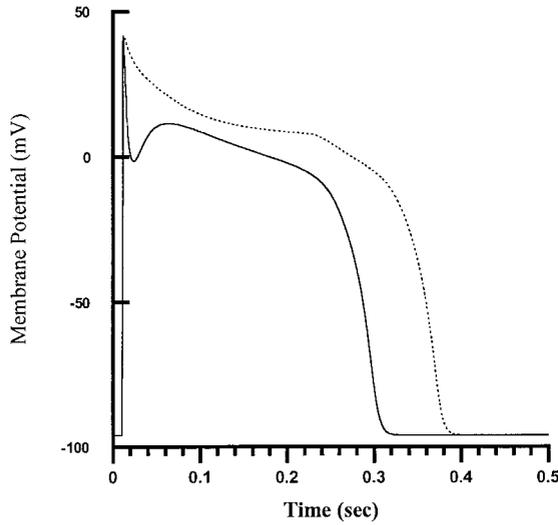
Model  $\text{Ca}^{2+}$  transients in response to voltage-clamp stimuli exhibit a “knob” at the early peak of the transient (see Figure 3A, for example) that does not appear to be present in the experimental data. This knob disappears as the SR  $\text{Ca}^{2+}$  level becomes small (Figure 3A), indicating that the knob is dependent on SR  $\text{Ca}^{2+}$  release. The knob is likely an artifact of model construction. All SR  $\text{Ca}^{2+}$  release in this model occurs from a single functional unit, defined as a set of L-type  $\text{Ca}^{2+}$  channels, RyR channels, and the subspace within which they interact. Stern has referred to such models as common

pool models.<sup>44</sup> The knob reflects a large, single  $\text{Ca}^{2+}$  release event from this single functional unit. In contrast, real cardiac cells have a large number of functional units in which there is local control of calcium-induced calcium release. We have recently implemented a local control model of  $\text{Ca}^{2+}$  release consisting of an ensemble of functional units, in which each functional unit is defined as an L-type  $\text{Ca}^{2+}$  channel interacting with a small set of RyR channels through a diadic space. Both L-type  $\text{Ca}^{2+}$  channels and RyR channels are modeled stochastically using the channel models presented in Jafri et al.<sup>24</sup> In such a model, the stochastic nature of RyR channel openings produces a variable latency of  $\text{Ca}^{2+}$  release in each functional unit. The  $\text{Ca}^{2+}$  transients computed using this model exhibit the property of gradedness and do not exhibit the knob seen in Figure 3A due to temporal smearing of  $\text{Ca}^{2+}$  release times.

A recent study has put forth the hypothesis that coupling between L-type  $\text{Ca}^{2+}$  channels and RyR channels may be altered in heart failure and that this altered coupling leads to a reduction in amplitude of the  $\text{Ca}^{2+}$  transient.<sup>45</sup> The results presented here cannot refute this hypothesis. Indeed, structurally detailed models of RyR channel and L-type  $\text{Ca}^{2+}$  channel interactions in the diadic space predict a strong dependence of these interactions on geometric factors.<sup>46–48</sup> However, the results reported here indicate that such an assumption is not necessary to account for reduced amplitude of  $\text{Ca}^{2+}$  transients in failing myocytes. Rather, these simulations indicate that the altered expression of  $\text{Ca}^{2+}$  handling proteins reported by several different groups in both failing human and canine myocytes could account for changes in  $\text{Ca}^{2+}$  transient amplitude and shape.

The data of Figure 1 demonstrate that downregulation of the outward repolarizing currents  $I_{K1}$  and  $I_{to1}$ , together with altered expression of the NCX and SR  $\text{Ca}^{2+}$  ATPase pump, can account for differences in both action potential and  $\text{Ca}^{2+}$  transient shape in heart failure. However, the data of Figure 1C also indicate that downregulation of  $I_{K1}$  and  $I_{to1}$ , at least to the extent measured on average in failing cells, has a small effect on APD. Instead, altered expression of  $\text{Ca}^{2+}$  handling proteins plays a significant role in APD prolongation.

It is not surprising that downregulation of model  $I_{K1}$  has only a modest impact on APD, as  $I_{K1}$  is primarily responsible for the terminal phase of repolarization. However, the finding that reduction of model  $I_{to1}$  has only a small effect on APD differs from the experimental results of Kääh et al<sup>5</sup> in dog myocytes and of Beuckelmann et al<sup>9</sup> in human cells. These experiments were performed using EGTA as an intracellular  $\text{Ca}^{2+}$  buffer. This buffering minimizes the modulatory effects of  $\text{Ca}^{2+}$  and thus enhances the relative influence of outward  $K$  currents on action potential characteristics. When effects of EGTA buffering are simulated in the model described in this article, block of  $I_{to1}$  has a greater influence on APD. An example is shown in Figure 6. The  $\text{Ca}^{2+}$  buffering effects of EGTA were modeled using the fast buffering approximation developed by Wagner and Keizer,<sup>49</sup> with  $\text{EGTA} = 10 \text{ mmol/L}$  and the dissociation constant  $K_m = 0.15 \text{ } \mu\text{mol/L}$ . Block of  $I_{to1}$  by 95% increases  $\text{APD}_{90}$  by 73 ms, or  $\approx 25\%$  of the control value. These results again emphasize the important modula-



**Figure 6.** Membrane potential as a function of time for the normal model (solid line) and for the model with a 95% reduction in magnitude of  $I_{to1}$  (dotted line). Simulations are done in the presence of EGTA (10 mmol/L;  $K_m=0.15 \mu\text{mol/L}$ ), using the fast buffering approximation of Wagner and Keizer.<sup>49</sup> Response to fifth stimulus at a cycle length of 1200 ms is shown.

tory role of  $\text{Ca}^{2+}$  on action potential characteristics in the canine myocyte.

It is also possible that 4-AP block of  $K$  currents other than  $I_{to1}$  occurred in the Kääh et al<sup>5</sup> experiments, but that such effects were not resolvable. Steady-state current-voltage relations were measured in the presence and absence of 4-AP to assess whether or not this was the case. Data are shown in Figure 10C of Kääh et al<sup>5</sup> and indicate that experimental variability in steady-state current at 0 mV (a potential near that of the action potential plateau) is roughly  $\pm 1.0 \text{ pA/pF}$ . The sum of model outward currents  $I_{to1}$ ,  $I_{K1}$ ,  $I_{Kr}$ , and  $I_{Ks}$  during the plateau is comparable with the magnitude of this variability in the experimental measurements ( $\approx 1.0 \text{ pA/pF}$ ). Genetic approaches for selective suppression of  $I_{to1}$ <sup>50,51</sup> may turn out to be more useful than pharmacological approaches in determining the influence of this current on APD.

The model predicts that one important mechanism of APD prolongation in heart failure is that shown in Figures 1 and 5. Under conditions of reduced SR  $\text{Ca}^{2+}$  release, there is less  $\text{Ca}^{2+}$ -mediated inactivation of the L-type  $\text{Ca}^{2+}$  current. The resulting increase of inward current, as shown for voltage-clamp stimuli in Figure 5C and for action potentials in Figure 1E, helps to maintain and prolong the plateau phase of the action potential. Investigation into the relative contribution of the various  $\text{Ca}^{2+}$ -regulatory mechanisms and  $\text{Ca}^{2+}$ -dependent membrane currents in determining the action potential shape and duration is an important area for future experimental and modeling studies.

## Appendix

Standard Units (Unless Otherwise Noted) Used in the Following Set of Equations

Quantity	Units
Membrane potential	mV
Membrane current	$\mu\text{A}\mu\text{F}^{-1}$
Membrane conductance	$\text{mS}\mu\text{F}^{-1}$
Ionic flux	$\text{mMms}^{-1}$
Concentration	mM
Time constant	ms
Rate constant	$\text{ms}^{-1}$

## Membrane Currents

### $\text{Na}^+$ Current $I_{\text{Na}}$

$$(A.1) \quad I_{\text{Na}} = \bar{G}_{\text{Na}} m^3 h j (V - E_{\text{Na}})$$

$$(A.2) \quad E_{\text{Na}} = \frac{RT}{F} \ln \left( \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right)$$

$$(A.3) \quad \frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m$$

$$(A.4) \quad \frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h$$

$$(A.5) \quad \frac{dj}{dt} = \alpha_j (1 - j) - \beta_j j$$

$$(A.6) \quad \alpha_m = 0.32 \frac{V + 47.13}{1 - e^{-0.1(V + 47.13)}}$$

$$(A.7) \quad \beta_m = 0.08 e^{-\frac{V}{11}}$$

For  $V \geq -40 \text{ mV}$ ,

$$(A.8) \quad \alpha_h = 0.0$$

$$(A.9) \quad \beta_h = \frac{1}{0.13(1 + e^{(V + 10.66)/-11.1})}$$

$$(A.10) \quad \alpha_j = 0.0$$

$$(A.11) \quad \beta_j = 0.3 \frac{e^{-2.535 \times 10^{-7} V}}{1 + e^{-0.1(V + 32)}}$$

For  $V < -40 \text{ mV}$ ,

$$(A.12) \quad \alpha_h = 0.135 e^{(80 + V)/-6.8}$$

$$(A.13) \quad \beta_h = 3.56 e^{0.079 V} + 3.1 \times 10^5 e^{0.35 V}$$

$$(A.14) \quad \alpha_j = (-127140 e^{0.2444 V} - 3.474 \times 10^{-5} e^{-0.04391 V}) \frac{V + 37.78}{1 + e^{0.311(V + 79.23)}}$$

$$(A.15) \quad \beta_j = 0.1212 \frac{e^{-0.01052 V}}{1 + e^{-0.1378(V + 40.14)}}$$

### Rapid-Activating Delayed Rectifier $K^+$ Current $I_{\text{Kr}}$

$$(A.16) \quad I_{\text{Kr}} = \bar{G}_{\text{Kr}} f([\text{K}^+]_o) R(V) X_{\text{Kr}}(V - E_{\text{K}})$$

(A.17) 
$$E_K = \frac{RT}{F} \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)$$

(A.18) 
$$R(V) = \frac{1}{1 + 1.4945e^{0.0446V}}$$

(A.19) 
$$f([K^+]_o) = \sqrt{[K^+]_o/4}$$

(A.20) 
$$\frac{dX_{Kr}}{dt} = K_{12}(1 - X_{Kr}) - K_{21}X_{Kr}$$

(A.21) 
$$K_{12} = e^{-5.495 + 0.1691V}$$

(A.22) 
$$K_{21} = e^{-7.677 - 0.0128V}$$

**Slow-Activating Delayed Rectifier K<sup>+</sup> Current I<sub>Ks</sub>**

(A.23) 
$$I_{Ks} = \bar{G}_{Ks} X_{Ks}^2 (V - E_{Ks})$$

(A.24) 
$$E_{Ks} = \frac{RT}{F} \ln \left( \frac{[K^+]_o + 0.01833[Na^+]_o}{[K^+]_i + 0.01833[Na^+]_i} \right)$$

(A.25) 
$$\frac{dX_{Ks}}{dt} = (X_{Ks}^\infty - X_{Ks}) / \tau_{X_{Ks}}$$

(A.26) 
$$X_{Ks}^\infty = \frac{1}{1 + e^{-(V-24.7)/13.6}}$$

(A.27) 
$$\tau_{X_{Ks}} = \frac{1}{\frac{0.0000719(V-10)}{1 - e^{-0.148(V-10)}} + \frac{0.000131(V-10)}{e^{0.0687(V-10)} - 1}}$$

**Transient Outward K<sup>+</sup> Current I<sub>to1</sub>**

(A.28) 
$$I_{to1} = \bar{G}_{to1} X_{to1} Y_{to1} (V - E_K)$$

(A.29) 
$$\frac{dX_{to1}}{dt} = \alpha_{X_{to1}}(1 - X_{to1}) - \beta_{X_{to1}} X_{to1}$$

(A.30) 
$$\frac{dY_{to1}}{dt} = \alpha_{Y_{to1}}(1 - Y_{to1}) - \beta_{Y_{to1}} Y_{to1}$$

(A.31) 
$$\alpha_{X_{to1}} = 0.04516e^{0.03577V}$$

(A.32) 
$$\beta_{X_{to1}} = 0.0989e^{-0.06237V}$$

(A.33) 
$$\alpha_{Y_{to1}} = \frac{0.005415e^{-(V+33.5)/5}}{1 + 0.051335e^{-(V+33.5)/5}}$$

(A.34) 
$$\beta_{Y_{to1}} = \frac{0.005415e^{(V+33.5)/5}}{1 + 0.051335e^{(V+33.5)/5}}$$

**Time-Independent K<sup>+</sup> Current I<sub>K1</sub>**

(A.35) 
$$I_{K1} = \bar{G}_{K1} K_1^\infty(V) \left( \frac{[K^+]_o}{[K^+]_o + K_{mK1}} \right) (V - E_K)$$

(A.36) 
$$K_1^\infty(V) = \frac{1}{2 + e^{1.5 \frac{F}{RT}(V - E_K)}}$$

**Plateau K<sup>+</sup> Current I<sub>Kp</sub>**

(A.37) 
$$I_{Kp} = \bar{G}_{Kp} K_p(V) (V - E_K)$$

(A.38) 
$$K_p(V) = \frac{1}{1 + e^{(7.488 - V)/5.98}}$$

**NCX Current I<sub>NaCa</sub>**

(A.39)

$$I_{NaCa} = k_{NaCa} \frac{5000}{K_{m,Na}^3 + [Na^+]_o^3} \frac{1}{K_{m,Ca} + [Ca^{2+}]_o} \frac{1}{1 + k_{sat} e^{(\eta-1)VF/RT}} (e^{\eta VF/RT} \times [Na^+]_i^3 [Ca^{2+}]_o - e^{(\eta-1)VF/RT} [Na^+]_o^3 [Ca^{2+}]_i)$$

**Na<sup>+</sup>-K<sup>+</sup> Pump Current I<sub>NaK</sub>**

(A.40) 
$$I_{NaK} = \bar{f}_{NaK} f_{NaK} \frac{1}{1 + \left( \frac{K_{m,Na}}{[Na^+]_i} \right)^{1.5}} \frac{[K^+]_o}{[K^+]_o + K_{m,Ko}}$$

(A.41) 
$$f_{NaK} = \frac{1}{1 + 0.1245e^{-0.1VF/RT} + 0.0365\sigma e^{-VF/RT}}$$

(A.42) 
$$\sigma = \frac{1}{7} (e^{\frac{[Na^+]_o}{67.5}} - 1)$$

**Sarcolemmal Ca<sup>2+</sup> Pump Current I<sub>p(Ca)</sub>**

(A.43) 
$$I_{p(Ca)} = \bar{I}_{p(Ca)} \frac{[Ca^{2+}]_i}{K_{m,p(Ca)} + [Ca^{2+}]_i}$$

**Ca<sup>2+</sup> Background Current I<sub>Ca,b</sub>**

(A.44) 
$$I_{Ca,b} = \bar{G}_{Ca,b} (V - E_{Ca})$$

(A.45) 
$$E_{Ca} = \frac{RT}{2F} \ln \left( \frac{[Ca^{2+}]_o}{[Ca^{2+}]_i} \right)$$

**Na<sup>+</sup> Background Current I<sub>Na,b</sub>**

(A.46) 
$$I_{Na,b} = \bar{G}_{Na,b} (V - E_{Na})$$

**Membrane Potential**

(A.47) 
$$\frac{dV}{dt} = -(I_{Na} + I_{Ca} + I_{Ca,K} + I_{Kr} + I_{Ks} + I_{to1} + I_{K1} + I_{Kp} + I_{NaCa} + I_{NaK} + I_{p(Ca)} + I_{Ca,b} + I_{Na,b})$$

**Ca<sup>2+</sup> Handling Mechanisms**

**L-Type Ca<sup>2+</sup> Current I<sub>Ca</sub>**

(A.48) 
$$\alpha = 0.4e^{(V+2)/10}$$

(A.49) 
$$\beta = 0.05e^{-(V+2)/13}$$

(A.50) 
$$\alpha' = \alpha a$$

(A.51) 
$$\beta' = \frac{\beta}{b}$$

(A.52) 
$$\gamma = 0.10375[Ca^{2+}]_{ss}$$

(A.53) 
$$\frac{dC_0}{dt} = \beta C_1 + \omega C_{Ca0} - (4\alpha + \gamma)C_0$$

(A.54) 
$$\frac{dC_1}{dt} = 4\alpha C_0 + 2\beta C_2 + \frac{\omega}{b} C_{Ca1} - (\beta + 3\alpha + \gamma a)C_1$$

(A.55) 
$$\frac{dC_2}{dt} = 3\alpha C_1 + 3\beta C_3 + \frac{\omega}{b^2} C_{Ca2} - (2\beta + 2\alpha + \gamma a^2)C_2$$

$$(A.56) \quad \frac{dC_3}{dt} = 2\alpha C_2 + 4\beta C_4 + \frac{\omega}{b^3} C_{Ca3} - (3\beta + \alpha + \gamma a^3) C_3$$

$$(A.57) \quad \frac{dC_4}{dt} = \alpha C_3 + gO + \frac{\omega}{b^4} C_{Ca4} - (4\beta + f + \gamma a^4) C_4$$

$$(A.58) \quad \frac{dO}{dt} = fC_4 - gO$$

$$(A.59) \quad \frac{dC_{Ca0}}{dt} = \beta' C_{Ca1} + \gamma C_0 - (4\alpha' + \omega) C_{Ca0}$$

$$(A.60) \quad \frac{dC_{Ca1}}{dt} = 4\alpha' C_{Ca0} + 2\beta' C_{Ca2} + \gamma a C_1 - \left( \beta' + 3\alpha' + \frac{\omega}{b} \right) C_{Ca1}$$

$$(A.61) \quad \frac{dC_{Ca2}}{dt} = 3\alpha' C_{Ca1} + 3\beta' C_{Ca3} + \gamma a^2 C_2 - \left( 2\beta' + 2\alpha' + \frac{\omega}{b^2} \right) C_{Ca2}$$

$$(A.62) \quad \frac{dC_{Ca3}}{dt} = 2\alpha' C_{Ca2} + 4\beta' C_{Ca4} + \gamma a^3 C_3 - \left( 3\beta' + \alpha' + \frac{\omega}{b^3} \right) C_{Ca3}$$

$$(A.63) \quad \frac{dC_{Ca4}}{dt} = \alpha' C_{Ca3} + g' O_{Ca} + \gamma a^4 C_4 - \left( 4\beta' + f' + \frac{\omega}{b^4} \right) C_{Ca4}$$

$$(A.64) \quad \frac{dO_{Ca}}{dt} = f' C_{Ca4} - g' O_{Ca}$$

$$(A.65) \quad \bar{I}_{Ca} = \frac{\bar{P}_{Ca}}{C_{sc}} \frac{4VF^2}{RT} \frac{0.001 e^{2VF/RT} - 0.341 [Ca^{2+}]_o}{e^{2VF/RT} - 1}$$

$$(A.66) \quad I_{Ca} = \bar{I}_{Ca} y \{O + O_{Ca}\}$$

$$(A.67) \quad I_{Ca,K} = \frac{P'_K}{C_{sc}} y \{O + O_{Ca}\}$$

$$\frac{VF^2}{RT} \frac{[K^+]_i e^{VF/RT} - [K^+]_o}{e^{VF/RT} - 1}$$

$$(A.68) \quad P'_K = \frac{\bar{P}_K}{1 + \frac{\bar{I}_{Ca}}{I_{Ca, half}}}$$

$$(A.69) \quad \frac{dy}{dt} = \frac{y_\infty - y}{\tau_y}$$

$$(A.70) \quad y_\infty = \frac{0.8}{1 + e^{(V+12.5)/5}} + 0.2$$

$$(A.71) \quad \tau_y = 20 + \frac{600}{1 + e^{(V+20)/9.5}}$$

**RyR Channel (Keizer and Levine)<sup>27</sup>**

$$(A.72) \quad \frac{dP_{C1}}{dt} = -k_a^+ [Ca^{2+}]_{ss}^n P_{C1} + k_a^- P_{O1}$$

$$(A.73) \quad \frac{dP_{O1}}{dt} = k_a^+ [Ca^{2+}]_{ss}^n P_{C1} - k_a^- P_{O1} - k_b^+ [Ca^{2+}]_{ss}^m P_{O1} + k_b^- P_{O2} - k_c^+ P_{O1} + k_c^- P_{C2}$$

$$(A.74) \quad \frac{dP_{O2}}{dt} = k_b^+ [Ca^{2+}]_{ss}^m P_{O1} - k_b^- P_{O2}$$

$$(A.75) \quad \frac{dP_{C2}}{dt} = k_c^+ P_{O1} - k_c^- P_{C2}$$

$$(A.76) \quad J_{rel} = v_1 (P_{O1} + P_{O2}) ([Ca^{2+}]_{JSR} - [Ca^{2+}]_{ss})$$

**SERCA2a Pump (Shannon et al)<sup>38</sup>**

$$(A.77) \quad f_b = ([Ca^{2+}]_i / K_{fb})^{N_{fb}}$$

$$(A.78) \quad r_b = ([Ca^{2+}]_{NSR} / K_{rb})^{N_{rb}}$$

$$(A.79) \quad J_{up} = K_{SR} \frac{v_{maxf} f_b - v_{maxr} r_b}{1 + f_b + r_b}$$

**Intracellular Ca<sup>2+</sup> Fluxes**

$$(A.80) \quad J_{tr} = \frac{[Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR}}{\tau_{tr}}$$

$$(A.81) \quad J_{xfer} = \frac{[Ca^{2+}]_{ss} - [Ca^{2+}]_i}{\tau_{xfer}}$$

$$(A.82) \quad J_{urpn} = \frac{d[HTRPNCa]}{dt} + \frac{d[LTRPNCa]}{dt}$$

$$(A.83) \quad \frac{d[HTRPNCa]}{dt} = k_{htrpnl}^+ [Ca^{2+}]_i ([HTRPN]_{tot} - [HTRPNCa]) - k_{htrpnl}^- [HTRPNCa]$$

$$(A.84) \quad \frac{d[LTRPNCa]}{dt} = k_{ltrpnl}^+ [Ca^{2+}]_i ([LTRPN]_{tot} - [LTRPNCa]) - k_{ltrpnl}^- [LTRPNCa]$$

**Intracellular Ion Concentrations**

$$(A.85) \quad \frac{d[Na^+]_i}{dt} = -(I_{Na} + I_{Na,b} + 3I_{NaCa} + 3I_{NaK}) \frac{A_{cap} C_{sc}}{V_{myo} F}$$

$$(A.86) \quad \frac{d[K^+]_i}{dt} = -(I_{Kr} + I_{Ks} + I_{to1} + I_{K1} + I_{Kp} + I_{Ca,K} - 2I_{NaK}) \frac{A_{cap} C_{sc}}{V_{myo} F}$$

$$(A.87) \quad \frac{d[Ca^{2+}]_i}{dt} = \beta_i \left\{ J_{xfer} - J_{up} - J_{urpn} - (I_{Ca,b} - 2I_{NaCa} + I_{P(Ca)}) \frac{A_{cap} C_{sc}}{2V_{myo} F} \right\}$$

$$(A.88) \quad \beta_i = \left\{ 1 + \frac{[CMDN]_{tot} K_m^{CMDN}}{(K_m^{CMDN} + [Ca^{2+}]_i)^2} \right\}^{-1}$$

$$(A.89) \quad \beta_{ss} = \left\{ 1 + \frac{[CMDN]_{tot} K_m^{CMDN}}{(K_m^{CMDN} + [Ca^{2+}]_{ss})^2} \right\}^{-1}$$

$$(A.90) \quad \beta_{JSR} = \left\{ 1 + \frac{[CSQN]_{tot} K_m^{CSQN}}{(K_m^{CSQN} + [Ca^{2+}]_{JSR})^2} \right\}^{-1}$$

$$(A.91) \quad \frac{d[Ca^{2+}]_{ss}}{dt} = \beta_{ss} \left\{ J_{rel} \frac{V_{JSR}}{V_{ss}} - J_{xfer} \frac{V_{myo}}{V_{ss}} - (I_{Ca}) \frac{A_{cap} C_{sc}}{2V_{ss} F} \right\}$$

$$(A.92) \quad \frac{d[Ca^{2+}]_{JSR}}{dt} = \beta_{JSR} \{ J_{ir} - J_{rel} \}$$

$$(A.93) \quad \frac{d[Ca^{2+}]_{NSR}}{dt} = J_{up} \frac{V_{myo}}{V_{NSR}} - J_{u} \frac{V_{JSR}}{V_{NSR}}$$

**Tables**

**TABLE 1. Cell Geometry Parameters**

Parameter	Definition	Value
$C_{sc}$	Specific membrane capacity	1.00 $\mu F \text{ cm}^{-2}$
$A_{cap}$	Capacitive membrane area	$1.534 \times 10^{-4} \text{ cm}^2$
$V_{myo}$	Myoplasmic volume	$25.84 \times 10^{-6} \mu L$
$V_{JSR}$	JSR volume	$0.16 \times 10^{-6} \mu L$
$V_{NSR}$	NSR volume	$2.10 \times 10^{-6} \mu L$
$V_{SS}$	Subspace volume	$1.2 \times 10^{-9} \mu L$

**TABLE 2. Standard Ionic Concentrations**

Parameter	Definition	Value
$[K^+]_o$	Extracellular $K^+$ concentration	4.0 mmol/L
$[Na^+]_o$	Extracellular $Na^+$ concentration	138.0 mmol/L
$[Ca^{2+}]_o$	Extracellular $Ca^{2+}$ concentration	2.0 mmol/L

**TABLE 3. Membrane Current Parameters**

Parameter	Definition	Value
$F$	Faraday constant	96.5 coulomb $\text{mmol}^{-1}$
$T$	Absolute temperature	310 K
$R$	Ideal gas constant	$8.314 \text{ Jmol}^{-1} \text{ K}^{-1}$
$\bar{G}_{kr}$	Peak $I_{kr}$ conductance	$0.0034 \text{ mS}\mu F^{-1}$
$\bar{G}_{ks}$	Peak $I_{ks}$ conductance	$0.00271 \text{ mS}\mu F^{-1}$
$\bar{G}_{to1}$	Peak $I_{to1}$ conductance	$0.23815 \text{ mS}\mu F^{-1}$
$\bar{G}_{k1}$	Peak $I_{k1}$ conductance	$2.8 \text{ mS}\mu F^{-1}$
$\bar{G}_{kp}$	Peak $I_{kp}$ conductance	$0.002216 \text{ mS}\mu F^{-1}$
$\bar{G}_{na}$	Peak $I_{na}$ conductance	$12.8 \text{ mS}\mu F^{-1}$
$K_{NaCa}$	Scaling factor of $Na^+$ - $Ca^{2+}$ exchange	$0.30 \mu A\mu F^{-1}$
$K_{mNa}$	$Na^+$ half-saturation constant for $Na^+$ - $Ca^{2+}$ exchange	87.5 mmol/L
$K_{mCa}$	$Ca^{2+}$ half-saturation constant for $Na^+$ - $Ca^{2+}$ exchange	1.38 mmol/L
$K_{mK1}$	$Ca^{2+}$ half-saturation constant for $I_{k1}$	13.0 mmol/L
$K_{sat}$	$Na^+$ - $Ca^{2+}$ exchange sat. factor at negative potentials	0.2
$\eta$	Controls voltage dependence of $Na^+$ - $Ca^{2+}$ exchange	0.35
$\bar{I}_{NaK}$	Maximum $Na^+$ - $K^+$ pump current	$0.693 \mu A\mu F^{-1}$
$K_{m,Na}$	$Na^+$ half-saturation constant for $Na^+$ - $K^+$ pump	10.0 mmol/L
$K_{m,Ko}$	$K^+$ half-saturation constant for $Na^+$ - $K^+$ pump	1.5 mmol/L
$\bar{I}_{p(Ca)}$	Maximum sarcolemmal $Ca^{2+}$ pump current	$0.05 \mu A\mu F^{-1}$
$K_{m,p(Ca)}$	Half-saturation constant for sarcolemmal $Ca^{2+}$ pump	0.00005 mmol/L
$\bar{G}_{Ca,b}$	Maximum background $Ca^{2+}$ current conductance	$0.0003842 \text{ mS}\mu F^{-1}$
$\bar{G}_{Na,b}$	Maximum background $Na^+$ current conductance	$0.0031 \text{ mS}\mu F^{-1}$

**TABLE 4. SR Parameters**

Parameter	Definition	Value
$v_1$	Maximum RyR channel $\text{Ca}^{2+}$ flux	$1.8 \text{ ms}^{-1}$
$K_{fb}$	Forward half-saturation constant for $\text{Ca}^{2+}$ ATPase	$0.168 \times 10^{-3} \text{ mmol/L}$
$K_{rb}$	Backward half-saturation constant for $\text{Ca}^{2+}$ ATPase	$3.29 \text{ mmol/L}$
$K_{SR}$	Scaling factor for $\text{Ca}^{2+}$ ATPase	1.0
$N_{fb}$	Forward cooperativity constant for $\text{Ca}^{2+}$ ATPase	1.2
$N_{rb}$	Reverse cooperativity constant for $\text{Ca}^{2+}$ ATPase	1.0
$v_{maxf}$	$\text{Ca}^{2+}$ ATPase forward rate parameter	$0.813 \times 10^{-4} \text{ mmol/L ms}^{-1}$
$v_{maxr}$	$\text{Ca}^{2+}$ ATPase reverse rate parameter	$0.318 \times 10^{-3} \text{ mmol/L ms}^{-1}$
$\tau_{tr}$	Time constant for transfer from NSR to JSR	0.5747 ms
$\tau_{xfer}$	Time constant from subspace to myoplasm	26.7 ms
$k_a^+$	RyR $\text{P}_{C_1} - \text{P}_{O_1}$ rate constant	$12.15 \times 10^9 \text{ mmol/L}^{-4} \text{ ms}^{-1}$
$k_a^-$	RyR $\text{P}_{O_1} - \text{P}_{C_1}$ rate constant	$0.576 \text{ ms}^{-1}$
$k_b^+$	RyR $\text{P}_{O_1} - \text{P}_{O_2}$ rate constant	$4.05 \times 10^6 \text{ mmol/L}^{-3} \text{ ms}^{-1}$
$k_b^-$	RyR $\text{P}_{O_2} - \text{P}_{O_1}$ rate constant	$1.930 \text{ ms}^{-1}$
$k_c^+$	RyR $\text{P}_{O_1} - \text{P}_{C_2}$ rate constant	$0.100 \text{ ms}^{-1}$
$k_c^-$	RyR $\text{P}_{C_2} - \text{P}_{O_1}$ rate constant	$0.0008 \text{ ms}^{-1}$
$n$	RyR $\text{Ca}^{2+}$ cooperativity parameter $\text{P}_{C_1} - \text{P}_{O_1}$	4
$m$	RyR $\text{Ca}^{2+}$ cooperativity parameter $\text{P}_{O_1} - \text{P}_{O_2}$	3

**TABLE 5. L-Type  $\text{Ca}^{2+}$  Channel Parameters**

Parameter	Definition	Value
$f$	Transition rate into open state	$0.3 \text{ ms}^{-1}$
$g$	Transition rate out of open state	$2.0 \text{ ms}^{-1}$
$f'$	Transition rate into open state for mode Ca	$0.005 \text{ ms}^{-1}$
$g'$	Transition rate out of open state for mode Ca	$7.0 \text{ ms}^{-1}$
$b$	Mode transition parameter	2.0
$a$	Mode transition parameter	2.0
$\omega$	Mode transition parameter	$0.01 \text{ ms}^{-1}$
$\bar{P}_{Ca}$	L-type $\text{Ca}^{2+}$ channel permeability to $\text{Ca}^{2+}$	$3.125 \times 10^{-4} \text{ cm s}^{-1}$
$\bar{P}_K$	L-type $\text{Ca}^{2+}$ channel permeability to $\text{K}^+$	$5.79 \times 10^{-7} \text{ cm s}^{-1}$
$I_{Ca, half}$	$\bar{I}_{Ca}$ level that reduces $P'_K$ by half	$-0.265 \mu\text{M}\mu\text{F}^{-1}$

**TABLE 6. Buffering Parameters**

Parameter	Definition	Value
$[LTRPN]_{tot}$	Total troponin low-affinity site concentration	$70.0 \times 10^{-3} \text{ mmol/L}$
$[HTRPN]_{tot}$	Total troponin high-affinity site concentration	$140.0 \times 10^{-3} \text{ mmol/L}$
$k_{trpn}^+$	$\text{Ca}^{2+}$ on rate for troponin high-affinity sites	$20.0 \text{ mmol/L}^{-1} \text{ ms}^{-1}$
$k_{trpn}^-$	$\text{Ca}^{2+}$ off rate for troponin high-affinity sites	$66.0 \times 10^{-6} \text{ ms}^{-1}$
$k_{trpn}^{+}$	$\text{Ca}^{2+}$ on rate for troponin low-affinity sites	$40.0 \text{ mmol/L}^{-1} \text{ ms}^{-1}$
$k_{trpn}^{-}$	$\text{Ca}^{2+}$ on rate for troponin low-affinity sites	$0.040 \text{ ms}^{-1}$
$[CMDN]_{tot}$	Total myoplasmic calmodulin concentration	$50.0 \times 10^{-3} \text{ mmol/L}$
$[CSQN]_{tot}$	Total NSR calsequestrin concentration	$15.0 \text{ mmol/L}$
$K_m^{CMDN}$	$\text{Ca}^{2+}$ half-saturation constant for calmodulin	$2.38 \times 10^{-3} \text{ mmol/L}$
$K_m^{CSQN}$	$\text{Ca}^{2+}$ half-saturation constant for calsequestrin	$0.8 \text{ mmol/L}$

TABLE 7. State Variable Initial Conditions

Variable	Definition	Initial Value
$t$	Time	0.00 ms
$V$	Membrane potential	-95.87 mV
$m$	$I_{Na}$ activation gate	$2.4676 \times 10^{-4}$
$h$	$I_{Na}$ inactivation gate	0.99869
$j$	$I_{Na}$ slow inactivation gate	0.99887
$X_{Kr}$	$I_{Kr}$ activation gate	0.6935
$X_{Ks}$	$I_{Ks}$ activation gate	$1.4589 \times 10^{-4}$
$X_{to1}$	$I_{to1}$ activation gate	$3.742 \times 10^{-5}$
$Y_{to1}$	$I_{to1}$ inactivation gate	1.00
$[Na^+]_i$	Intracellular $Na^+$ concentration	10.00 mmol/L
$[K^+]_i$	Intracellular $K^+$ concentration	159.48 mmol/L
$[Ca^{2+}]_i$	Myoplasmic $Ca^{2+}$ concentration	$8.464 \times 10^{-5}$ mmol/L
$[Ca^{2+}]_{NSR}$	NSR $Ca^{2+}$ concentration	0.2620 mmol/L
$[Ca^{2+}]_{ss}$	Subspace SR $Ca^{2+}$ concentration	$1.315 \times 10^{-4}$ mmol/L
$[Ca^{2+}]_{JSR}$	JSR $Ca^{2+}$ concentration	0.2616 mmol/L
$P_{C_1}$	Fraction of channels in state $P_{C_1}$	0.4929
$P_{O_1}$	Fraction of channels in state $P_{O_1}$	$6.027 \times 10^{-4}$
$P_{O_2}$	Fraction of channels in state $P_{O_2}$	$2.882 \times 10^{-9}$
$P_{C_2}$	Fraction of channels in state $P_{C_2}$	0.5065
$C_0$	L-type $Ca^{2+}$ channel closed: mode normal	0.99802
$C_1$	L-type $Ca^{2+}$ channel closed: mode normal	$1.9544 \times 10^{-6}$
$C_2$	L-type $Ca^{2+}$ channel closed: mode normal	0.00
$C_3$	L-type $Ca^{2+}$ channel closed: mode normal	0.00
$C_4$	L-type $Ca^{2+}$ channel closed: mode normal	0.00
$O$	L-type $Ca^{2+}$ channel open: mode normal	0.00
$C_{Ca0}$	L-type $Ca^{2+}$ channel closed: mode Ca	$1.9734 \times 10^{-3}$
$C_{Ca1}$	L-type $Ca^{2+}$ channel closed: mode Ca	0.00
$C_{Ca2}$	L-type $Ca^{2+}$ channel closed: mode Ca	0.00
$C_{Ca3}$	L-type $Ca^{2+}$ channel closed: mode Ca	0.00
$C_{Ca4}$	L-type $Ca^{2+}$ channel closed: mode Ca	0.00
$O_{Ca}$	L-type $Ca^{2+}$ channel open: mode Ca	0.00
$y$	$I_{Ca}$ inactivation gate	0.7959
[LTRPNCA]	Concentration of $Ca^{2+}$ -bound low-affinity troponin sites	$5.5443 \times 10^{-3}$ mmol/L
[HTRPNCA]	Concentration of $Ca^{2+}$ -bound high-affinity troponin sites	$136.64 \times 10^{-3}$ mmol/L

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