Genetic manipulation of calcium-handling proteins in cardiac myocytes. II. Mathematical modeling studies

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Coutu, Pierre, and Joseph M. Metzger. Genetic manipulation of calcium-handling proteins in cardiac myocytes. II. Mathematical modeling studies. Am J Physiol Heart Circ Physiol 288: H613–H631, 2005. First published August 26, 2004; doi:10.1152/ajpheart.00425.2004.—We developed a mathematical model specific to rat ventricular myocytes that includes electrophysiological representation, ionic homeostasis, force production, and sarcomere movement. We used this model to interpret, analyze, and compare two genetic manipulations that have been shown to increase myocyte relaxation rates, parvalbumin (Parv) de novo expression, and sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA2a) overexpression. The model was used to seek mechanistic insights into 1) the relative contribution of two mechanisms by which SERCA2a overexpression modifies Ca$^{2+}$ sequestration, i.e., more pumps and an increase in the SERCA2a-to- phospholamban ratio, 2) the mechanisms behind postrest potentiation and how Parv and SERCA2a influence this response, and 3) why Parv myocytes retain their fast kinetics when endogenous SERCA2a is partially impaired by thapsigargin (a condition used to mimic diastolic dysfunction). The model was also utilized to predict whether Parv metal-binding characteristics might be modified to improve diastolic and systolic functions and whether Parv or SERCA2a might affect diastolic Ca$^{2+}$ levels and myocyte energetics. One outcome of the model was to demonstrate a higher peak and total ATP consumption in SERCA2a myocytes and more even distribution of ATP throughout the cardiac cycle in Parv myocytes. This may have implications for failing hearts that are energetically compromised.

mathematical model; rat; parvalbumin; sarco(endo)plasmic reticulum calcium-adenosinetriphosphatase 2a

HEART FAILURE affects more than 5,000,000 people in the United States (25). It has been estimated that 40% of heart failure patients exhibit pure diastolic dysfunction (20), a condition characterized by slowing of Ca$^{2+}$ uptake at the cellular level, increase in stiffness during relaxation at the tissue level, and impaired ventricular filling at the organ level. Alterations in Ca$^{2+}$-handling proteins play an important role in diastolic dysfunction (23). Several genetic manipulations have been proposed to restore myocyte Ca$^{2+}$-handling function in failing hearts (12), including modification of expression or functionality of sarco(endo)plasmic Ca$^{2+}$-ATPase (SERCA2a), phospholamban (PLB), Na$^+$/Ca$^{2+}$ exchanger (NCX), and parvalbumin (Parv), a specialized Ca$^{2+}$ buffer (8).

In our experimental study (10), we needed a model that was specific to rat ventricular myocytes at physiological temperature that could reproduce sarcomere-shortening and Ca$^{2+}$ fluorescence measurements. To our knowledge, only one model specific to rat ventricular myocytes has been published with a focus on electrophysiological parameters at room temperature, and it does not include sarcomere shortening (27). We therefore created an integrated rat ventricle cardiac myocyte model that contains a detailed mathematical representation of sarcolemmal ionic channels, Ca$^{2+}$ handling (including effects of genetic manipulations), force production (including cooperativity in the force-pCa relation), and unloaded shortening. The model was adapted and integrated in part from component models that have been published previously (24, 29, 37).

With use of this rat integrated myocyte model, it was possible to elucidate the mechanisms underlying some of the results obtained in our experimental study (10). In particular, the model provided insights into 1) how Parv-transduced myocytes retain their already rapid kinetics when endogenous SERCA2a pumps are partially inhibited by TG, 2) whether the effects of SERCA2a overexpression are the same as removal of its natural inhibition by PLB (21), 3) how PRP works in control myocytes, and 4) how Parv and SERCA2a affect PRP. In addition, the model allowed a theoretical investigation of tractions, both methods resulted in similar increases in myocyte relaxation rate. However, we established that the response of SERCA2a-expressing myocytes to other conditions, such as β-adrenergic stimulation [with isoproterenol (Iso)], diastolic dysfunction [chemically induced with thapsigargin (TG)], a SERCA2a inhibitor, or changes in stimulation pacing [during postrest potentiation (PRP) tests], was different from that of Parv-expressing myocytes. The exact mechanisms underlying these differences are unclear. Moreover, other important issues regarding the use of SERCA2a overexpression or Parv expression have not been addressed experimentally. Indeed, issues such as the impact of these two genetic manipulations on energy consumption and Ca$^{2+}$ diastolic level are of critical importance, because energy deficiency has been postulated to contribute to progression of heart failure (13, 39), and elevated diastolic Ca$^{2+}$ levels have been associated with a calmodulin-dependent signaling pathway leading to maladaptive hypertrophy (7, 28).

To lend insight into the mechanisms underlying the SERCA2a overexpression or Parv expression and to address some of the issues mentioned above, we used mathematical modeling. To effectively use data presented in our experimental study (10), we needed a model that was specific to rat ventricular myocytes and more even distribution of ATP throughout the cardiac cycle in Parv myocytes. This may have implications for failing hearts that are energetically compromised.

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possible genetic modification of Parv’s metal-binding site characteristics, such that its accelerating effect on cardiac myocyte relaxation is optimized without compromising the myocyte’s systolic function. The model simulations also showed that SERCA2a overexpression might have the negative consequence of increasing energy use compared with control or Parv-expressing myocytes. Finally, the simulation results suggest that wild-type Parv expression might create a small increase in diastolic Ca\(^{2+}\) levels because of its slow Ca\(^{2+}\) release during the late part of the contraction cycle.

**METHODS**

**Overview**

The model was constructed by integrating and adapting previously reported models: 1) ionic channel formulation and Ca\(^{2+}\) handling (based on Ref. 37), 2) force development (based on Ref. 29), and 3) sarcomere movement (based on Ref. 24). Each of these components is described in detail. However, first, it seems appropriate to give an overview of how the model is structured. Figure 1 provides an overview of the model. The electrophysiological component of the model is shown in Fig. 1, **top**. It includes the ionic currents and fluxes that control the membrane potential as well as homeostasis for Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) including sarcoplasmic reticulum (SR) Ca\(^{2+}\) handling. Among the features of this first section of the model is the binding of Ca\(^{2+}\) to the low-affinity sites of troponin. The second section of the model represents active force development by the cross bridges (Fig. 1, **bottom**). Finally, the interaction between sarcomere movement and force production (Fig. 1, **bottom**) is based on the myofilament sliding theory (18). This section of the model is fully detailed in the original model (24) and is briefly described below.

The main modifications of the original component models are presented below and are followed by a description of the algorithm used to solve the equations. Detailed equations and parameter values are given in **APPENDIX A**.

**Ionic Channel Formulation**

The ionic channel formulation is based on the model of Winslow et al. (37), which was used to represent experiments conducted in canine myocytes. To make the model rat specific, the voltage-activated K\(^{+}\) channel representation of Winslow et al. was replaced by a formulation representative of rat cardiac myocytes (16). This novel representation, as well as other minor changes on other currents, is discussed in detail in Tables 1–6.

**Ca\(^{2+}\)-Handling Parameters**

The major difference in Ca\(^{2+}\) handling and removal between large (e.g., canine) and smaller (e.g., rat) mammalian myocytes occurs mostly in the relative contribution of each protein, rather than in fundamental differences in their basic functionality. In particular, in

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**Electrophysiology and Ionic Homeostasis**

![Diagram of electrophysiology and ionic homeostasis](image)

**Active Force**

**Total Force**

**Movement**

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**Fig. 1.** Block diagram overview of the model. **Top:** electrophysiological and ionic homeostasis section of the model, consisting of 5 compartments: myoplasm (MYO), subspace (SS), junctional (JSR) and network (NSR) sarcoplasmic reticulum, and extracellular space. Different ionic currents (arrows) and Ca\(^{2+}\) fluxes (arrowheads) are directly linked to equations in **APPENDIX A**. Arrow direction indicates a positive flux (J) or current (I), J\(_{\text{Trp}}\), low-affinity site of troponin. **Bottom left:** 4-state model for active force production by cross bridges. Cross-bridge attachment is permissive (p) or nonpermissive (n) and force-producing (1) or non-force-producing (0). Transitions between nonpermissive and permissive states are controlled by the rate \(k_{\text{on}}\), which is a function of sarcomere length (L) and the fraction of troponin sites occupied by Ca\(^{2+}\) (TCa). Arrows labeled \(V_{\text{Ca}}\) indicate effect of sarcomere shortening velocity on breaking cross bridges and releasing Ca\(^{2+}\) from troponin. **Bottom middle:** overall force production model, which is the active force-generating component (**bottom left**) in parallel with a serial passive elastic component. Total force is the sum of forces produced by the 2 components. **Bottom right:** mechanisms by which sarcomere movement is handled by the model.
Table 1. Cell geometry parameters, universal constants, and ionic concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Csc</td>
<td>Specific membrane capacitance</td>
<td>1.00 μF/cm²</td>
</tr>
<tr>
<td>Aap</td>
<td>Capacitive membrane area</td>
<td>1.53 × 10⁻⁴ cm²</td>
</tr>
<tr>
<td>Vmyo</td>
<td>Myoplasmic volume</td>
<td>2.584 × 10⁻³ μl</td>
</tr>
<tr>
<td>Vsr</td>
<td>Junctional SR volume</td>
<td>2.815 × 10⁻³ μl</td>
</tr>
<tr>
<td>Vnsr</td>
<td>Network SR volume</td>
<td>2.10 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>Vsub</td>
<td>Subspace volume</td>
<td>1.20 × 10⁻⁹ μl</td>
</tr>
<tr>
<td>F</td>
<td>Faraday constant</td>
<td>96,500 mC/mmol</td>
</tr>
<tr>
<td>R</td>
<td>Ideal gas constant</td>
<td>8.314 μJ·mmol⁻¹·K⁻¹</td>
</tr>
<tr>
<td>T</td>
<td>Absolute temperature</td>
<td>310 K</td>
</tr>
<tr>
<td>[Ca²⁺]o</td>
<td>Extracellular Ca²⁺ concn</td>
<td>1.8 mM</td>
</tr>
<tr>
<td>[K⁺]o</td>
<td>Extracellular K⁺ concn</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>[Na⁺]o</td>
<td>Extracellular Na⁺ concn</td>
<td>132.0 mM</td>
</tr>
<tr>
<td>[Mg²⁺]o</td>
<td>Free intracellular Mg²⁺ concn</td>
<td>1.0 mM</td>
</tr>
</tbody>
</table>

Values represent default values. SR, sarcomplasmatic reticulum. *Value that is modified at least once.

The rat cardiac myocyte, Ca²⁺ removal is accomplished mostly (97%) by SERCA2a; in the canine cardiac myocyte; however, Ca²⁺ removal is mostly divided between the Na⁺/Ca²⁺ exchanger (NCX) (27%) and SERCA2a (73%) (4). To reflect the rat cardiac myocyte, we modified the canine model of Winslow et al. (37) by reducing NCX density and SERCA2a (73%) (4). To reflect the rat cardiac myocyte, we modified the canine model of Winslow et al. (37) by reducing NCX density and increasing the number of SERCA2a pumps. We elected to exclude the mitochondrial contribution to Ca²⁺ removal, as such it is believed to have little effect on a beat-to-beat basis under normal conditions (4). Changes relative to NCX and other Ca²⁺ sarcoplasmic currents are discussed in Tables 1–6, whereas changes in SR Ca²⁺ handling, Ca²⁺ buffering, and genetic manipulations are described below.

**SR Ca²⁺ Handling**

The SERCA2a model proposed by Shannon has been extensively used in the last few years (27, 32–34, 37). However, the values of the SERCA2a parameters vary among these studies. We set the Hill coefficient to 2 and the affinity of the forward mode of the pump to 230 nM to reflect measurements obtained in rodents (5, 32). The other parameters were set to maintain ~0.2 mM and 100 nM free Ca²⁺ (4) in the SR and cytosol, respectively, and to match relaxation rates as obtained in our experimental study (10).

The equations for the ryanodine receptor (RyR) remain as reported by Winslow et al. (37), except for the maximal release rate (increased to match higher buffering, see Buffering and Ca²⁺ Indicator) and the off-rate (koff) from the closed state (Pc2) to the open state (Pon; increased to reduce the refractory period). We also included a small leak between the junctional SR and the subspace compartments to reflect data obtained previously (34). This leak remained small compared with the other fluxes (~15% of the SERCA2a reverse mode at rest). Finally, we decreased the transfer time constant between the subspace and the cytosol to achieve the fast rising phase of the fluorescence signal obtained in our experimental study.

**Buffering and Ca²⁺ Indicator**

Cytosolic buffering was set to combine two terms: troponin and other generic buffers. The sum of the two buffers was adjusted such that the buffering affinity and capacity were in the range of data available in the literature (4). Finally, the effect of the Ca²⁺ indicator fura 2-AM was added to the model (binding properties slightly modified from Ref. 3) to compare simulation results with data published in our experimental study (10).

**Genetic and Chemical Manipulations**

To model the addition of Parv, we used the law of mass action (9). The Parv on- and off-rates were slightly modified from a previous study (9) to obtain a better data match with our experimental study (10). To model the SERCA2a overexpression, we set a multiplying factor (KSR) for forward and reverse modes. The forward-mode dissociation constant (K0a) of the pumps under overexpression conditions was also decreased to reflect an increase in the SERCA2a-to-PLB ratio (5, 32). Application of TG was modeled as an allosteric inhibitor of SERCA2a (36). Affinity for TG is very high (K0a < 1 nM) (17); therefore, most of the TG in a solution will diffuse and bind to available SERCA2a sites. Under our experimental conditions (10) (~1.0 ml of solution and 20,000 myocytes), 150 nM TG gave a maximal effect on Ca²⁺ transient dose-response measurements (unpublished data). Finally, for caffeine tests, the open probability for RyR was set to 1.0 during the drug application.

**Force Production Formulation**

Negroni and Lascano (24) presented a relatively concise model that includes force development and sarcomere shortening. However, the cooperative nature of the force-pCa relation and the sarcomere length dependence are largely unaccounted for in that model. To incorporate these components, we kept the shortening strategy of that model and combined it with a more complete (still relatively simple) cooperative cross-bridge model of force production (29). The cross-bridge model (model 3 in Ref. 29) is a four-state model in which the transition rate parameters are adjusted to match the steady-state conditions (10). To model the SERCA2a overexpression, we set a multiplying factor (KSR) for forward and reverse modes. The forward-mode dissociation constant (K0a) of the pumps under overexpression conditions was also decreased to reflect an increase in the SERCA2a-to-PLB ratio (5, 32). Application of TG was modeled as an allosteric inhibitor of SERCA2a (36). Affinity for TG is very high (K0a < 1 nM) (17); therefore, most of the TG in a solution will diffuse and bind to available SERCA2a sites. Under our experimental conditions (10) (~1.0 ml of solution and 20,000 myocytes), 150 nM TG gave a maximal effect on Ca²⁺ transient dose-response measurements (unpublished data). Finally, for caffeine tests, the open probability for RyR was set to 1.0 during the drug application.

**Table 2. Membrane current parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNa</td>
<td>Peak INa conductance</td>
<td>12.8 mS/μF</td>
</tr>
<tr>
<td>GK</td>
<td>Peak IK conductance</td>
<td>0.5 mS/μF</td>
</tr>
<tr>
<td>Gs</td>
<td>Peak IC conductance</td>
<td>0.170 mS/μF</td>
</tr>
<tr>
<td>GK</td>
<td>Peak IK conductance</td>
<td>0.087 mS/μF</td>
</tr>
<tr>
<td>GKS</td>
<td>Peak IKs conductance</td>
<td>0.024 mS/μF</td>
</tr>
<tr>
<td>GAT</td>
<td>Peak ICAT conductance</td>
<td>0.066 mS/μF</td>
</tr>
<tr>
<td>GAT</td>
<td>Half-saturation constant for effect of 4-AP on INa</td>
<td>0.20 mV</td>
</tr>
<tr>
<td>GAT</td>
<td>Half-saturation constant for effect of 4-AP on IKs</td>
<td>0.10 mM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[4-AP]</th>
<th>4-AP concn</th>
<th>0 mM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_Na,Na</td>
<td>Maximum Na⁺-K⁺ pump current</td>
<td>1.505 μA/μF</td>
</tr>
<tr>
<td>I_Na,K</td>
<td>Half-saturation constant for INa,K</td>
<td>10.0 mM</td>
</tr>
<tr>
<td>I_K</td>
<td>Half-saturation constant for IK</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>I_Ka,Na</td>
<td>Half-saturation constant for IKa,Na</td>
<td>2.0 mV/μF</td>
</tr>
<tr>
<td>I_Ca</td>
<td>Half-saturation constant for ICa</td>
<td>87.5 mM</td>
</tr>
<tr>
<td>I_Ca</td>
<td>Ca²⁺-half saturation constant for ICa</td>
<td>13.8 mM</td>
</tr>
<tr>
<td>I_Ks</td>
<td>Na⁺/Ca²⁺ exchange saturation factor</td>
<td>0.2</td>
</tr>
<tr>
<td>η</td>
<td>Voltage dependence control parameter for ICa</td>
<td>0.35</td>
</tr>
<tr>
<td>I_Ca</td>
<td>Maximum sarcolemmal Ca²⁺ pump current</td>
<td>0.10 μA/μF</td>
</tr>
<tr>
<td>I_Ca</td>
<td>Half-saturation constant for ICa</td>
<td>2.5 × 10⁻⁴ mM</td>
</tr>
<tr>
<td>I_Ca</td>
<td>Peak ICa conductance</td>
<td>0.00032 mS/μF</td>
</tr>
<tr>
<td>I_K</td>
<td>Peak IK conductance</td>
<td>0.0070 mS/μF</td>
</tr>
<tr>
<td>f</td>
<td>ICa, transition rate into open state</td>
<td>0.3 ms⁻¹</td>
</tr>
<tr>
<td>g</td>
<td>ICa, transition rate out of open state</td>
<td>2.0 ms⁻¹</td>
</tr>
<tr>
<td>f'</td>
<td>ICa, transition rate into open state for Ca²⁺</td>
<td>0.005 ms⁻¹</td>
</tr>
<tr>
<td>g'</td>
<td>ICa, transition rate out of open state for Ca²⁺</td>
<td>7.0 ms⁻¹</td>
</tr>
</tbody>
</table>

*Values that is modified at least once.*
The amount of force. After a certain delay, the rest of the filament
In the model, the active element compresses to a length
Table 3. SR parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{SR}$</td>
<td>SERCA2a overexpression factor</td>
<td>1.0*</td>
</tr>
<tr>
<td>$K_{fb}$</td>
<td>Forward-mode half-saturation constant for SERCA2a</td>
<td>0.00023 mM*</td>
</tr>
<tr>
<td>$K_{rb}$</td>
<td>Reverse-mode half-saturation constant for SERCA2a</td>
<td>0.57 mM</td>
</tr>
<tr>
<td>$N_{fb}$</td>
<td>Forward-mode cooperativity for SERCA2a</td>
<td>2.0</td>
</tr>
<tr>
<td>$N_{rb}$</td>
<td>Reverse-mode cooperativity for SERCA2a</td>
<td>2.0</td>
</tr>
<tr>
<td>$v_{maxf}$</td>
<td>Forward-mode maximal rate for SERCA2a</td>
<td>0.00036 mM/ms</td>
</tr>
<tr>
<td>$v_{maxr}$</td>
<td>Reverse-mode maximal rate for SERCA2a</td>
<td>0.00036 mM/ms</td>
</tr>
<tr>
<td>$t_{er}$</td>
<td>Time constant for Ca$^{2+}$ transfer from SERCA2a to JSR</td>
<td>0.5747 ms</td>
</tr>
<tr>
<td>$t_{sarc}$</td>
<td>Time constant for Ca$^{2+}$ transfer from subspace to myoplasm</td>
<td>9.0 ms</td>
</tr>
<tr>
<td>$K_{m,TG}$</td>
<td>TG, half-saturation constant on SERCA2a</td>
<td>0.00015 mM</td>
</tr>
<tr>
<td>$K_{leak}$</td>
<td>Leak from RyR</td>
<td>0 mM*</td>
</tr>
<tr>
<td>$k^+$</td>
<td>Maximum RyR channel Ca$^{2+}$ flux</td>
<td>3.87 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_-$</td>
<td>RyR $P_{O1} - P_{O2}$ rate constant</td>
<td>$1.215 \times 10^{10}$ m$^{-4}$ms$^{-3}$</td>
</tr>
<tr>
<td>$k_+$</td>
<td>RyR $P_{O1} - P_{C1}$ rate constant</td>
<td>0.567 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_0$</td>
<td>RyR $P_{O1} - P_{O2}$ rate constant</td>
<td>$4.05 \times 10^{9}$ m$^{-4}$ms$^{-3}$</td>
</tr>
<tr>
<td>$k_0$</td>
<td>RyR $P_{O1} - P_{O2}$ rate constant</td>
<td>1.93 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_0$</td>
<td>RyR $P_{O1} - P_{O1}$ rate constant</td>
<td>0.1 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_0$</td>
<td>RyR $P_{O2} - P_{O1}$ rate constant</td>
<td>0.0016 ms$^{-1}$</td>
</tr>
<tr>
<td>$n$</td>
<td>RyR Ca$^{2+}$ cooperativity parameter for $P_{C1}$</td>
<td>4</td>
</tr>
<tr>
<td>$m$</td>
<td>RyR Ca$^{2+}$ cooperativity parameter for $P_{O1}$</td>
<td>3</td>
</tr>
</tbody>
</table>

TG, thapsigargin; RyR, ryanodine receptor; $P_{O1}$, open probability; $P_{C1}$, closed probability. *Values that are modified at least once.

between nonpermissive and permissive cross-bridge states is con-
trolled by the portion of tropomyosin C occupied by Ca$^{2+}$ (Fig. 1, bottom left). Cross-bridge transition rates are also a function of sarcomere length. The original model (29) reflects the strong cooperativity
typical of the force-pCa relations observed experimentally (4). The values were adjusted to match the shortening data in our experimental study (10) and other parameters found in the literature, such as the rate of isometric force redevelopment in cardiac muscle (2, 26), twitch force in intact myocytes (38), and the force-pCa curve in intact cardiac muscle (1) (see Fig. B2, E and F, in supplemental data for this article, which may be found at http://ajpheart.physiology.org/cgi/content/full/ 00425.2004.DC1).

Sarcomere-Shortening Formulation

The Negroni-Lascano model (24) is a simplified version of the Huxley model (18). In the Negroni-Lascano model, the basic mechanical subunit is represented by an active elastic element (the myosin heads) in parallel with a passive elastic element (cytoskeletal proteins plus, in our case, myocyte-laminin attachment resistance). In the Huxley model, a probabilistic distribution of cross-bridge attachment is integrated along the longitudinal axis to evaluate the total force production at a given time. In the Negroni-Lascano model, the population of cross bridges responding to a probabilistic distribution is replaced by a single equivalent cross bridge.

The basic functionality of this model is depicted in Fig. 1 (bottom right). In isometric conditions, the active elastic element is at a stable position of length $h_L$ ($Eqs. A125$ and A126; with $h_L = L - x_L$), producing a force proportional to the number of cross bridges attached and to the distance $h_L$. When the sarcomere shortens, some cross bridges detach and reattach at a different position with a given delay. In the model, the active element compresses to a length $h$, reducing the amount of force. After a certain delay, the rest of the filament slides back to its resting position, restoring the active element length
to $h_L$. In addition, to match experimental observations, shortening (or lengthening) velocity reduces the number of attached cross bridges and releases Ca$^{2+}$ from tropomyosin C (Fig. 1, bottom left). The passive element force equation, originally represented by a fifth-order polynomial equation, was replaced by three second-order polynomial equations (see Appendix A) to simplify the computation of the inverse problem (see Algorithm and Numerical Methods).

Algorithm and Numerical Methods

The program was written in C++ and implemented on a personal computer. The differential equations were solved using a fourth-order Runge-Kutta-Merson algorithm with adaptive time steps (14). The

Table 4. Buffering parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
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<tr>
<td>$[Parv]_{tot}$</td>
<td>Parv concn</td>
<td>0 mM*</td>
</tr>
<tr>
<td>$N_{Parv}$</td>
<td>No. of binding sites per Parv molecule</td>
<td>2</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Ca$^{2+}$-on rate for Parv sites</td>
<td>366 mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Ca$^{2+}$-off rate for Parv sites</td>
<td>0.002879 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Mg$^{2+}$-on rate for Parv sites</td>
<td>0.2571 mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Mg$^{2+}$-off rate for Parv sites</td>
<td>0.007557 ms$^{-1}$</td>
</tr>
<tr>
<td>$[LTrpn]_{tot}$</td>
<td>Total tropomin low-affinity site concn</td>
<td>0.070 mM</td>
</tr>
<tr>
<td>$[HITrpn]_{tot}$</td>
<td>Total tropomin high-affinity site concn</td>
<td>0.140 mM</td>
</tr>
<tr>
<td>$K_{Trpn}$</td>
<td>Ca$^{2+}$-on rate for high-affinity tropomin sites</td>
<td>20.0 mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{Trpn}$</td>
<td>Ca$^{2+}$-off rate for high-affinity tropomin sites</td>
<td>0.000066 ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{Trpn}$</td>
<td>Ca$^{2+}$-on rate for low-affinity tropomin sites</td>
<td>100.0 mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{Trpn}$</td>
<td>Ca$^{2+}$-off rate for low-affinity tropomin sites</td>
<td>0.05 ms$^{-1}$</td>
</tr>
<tr>
<td>$[C_{BufMyo}]_{tot}$</td>
<td>Total equivalent buffering capacity for other myoplasm buffers</td>
<td>0.050 mM</td>
</tr>
<tr>
<td>$[C_{BufMyo}]_{tot}$</td>
<td>Ca$^{2+}$-half saturation constant for equivalent myoplasm buffering</td>
<td>0.001 mM</td>
</tr>
<tr>
<td>$[C_{Sqp}]_{tot}$</td>
<td>Total NSR calsequestrin concn sites</td>
<td>15.0 mM</td>
</tr>
<tr>
<td>$K_{Sm,Csp}$</td>
<td>Ca$^{2+}$- half saturation constant for calsequestrin</td>
<td>0.7 mM</td>
</tr>
<tr>
<td>$[Fura 2]_{tot}$</td>
<td>Total fura 2-AM concn sites</td>
<td>0.0001 mM</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Ca$^{2+}$-on rate for fura 2-AM sites</td>
<td>180.0 mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{Ca}$</td>
<td>Ca$^{2+}$-off rate for fura 2-AM sites</td>
<td>0.04333 ms$^{-1}$</td>
</tr>
</tbody>
</table>

Parv, parvalbumin. *Values that are modified at least once.

to $h_L$. In addition, to match experimental observations, shortening (or lengthening) velocity reduces the number of attached cross bridges and releases Ca$^{2+}$ from tropomyosin C (Fig. 1, bottom left). The passive element force equation, originally represented by a fifth-order polynomial equation, was replaced by three second-order polynomial equations (see Appendix A) to simplify the computation of the inverse problem (see Algorithm and Numerical Methods).

Algorithm and Numerical Methods

The program was written in C++ and implemented on a personal computer. The differential equations were solved using a fourth-order Runge-Kutta-Merson algorithm with adaptive time steps (14). The

Table 5. Shortening and force production parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_L$</td>
<td>Sliding cross-bridges velocity</td>
<td>1.2 ms$^{-1}$</td>
</tr>
<tr>
<td>$h_L$</td>
<td>Resting cross-bridges length</td>
<td>0.010 μm</td>
</tr>
<tr>
<td>$g_{XB}$</td>
<td>Force transition off-rate</td>
<td>0.033 ms$^{-1}$</td>
</tr>
<tr>
<td>$f_{XB}$</td>
<td>Force transition on-rate</td>
<td>0.018 ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{Im}$</td>
<td>Transition rate between nonpermissive and permissive states</td>
<td>0.20 ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{X, Ca}$</td>
<td>Force-Ca$^{2+}$ sensitivity parameter</td>
<td>0.0005 mM</td>
</tr>
<tr>
<td>$A_F$</td>
<td>Force generation scaling factor</td>
<td>6.000 mN•mm$^{-2}$•μm$^{-1}$</td>
</tr>
<tr>
<td>$L_{p0}$</td>
<td>Passive force length boundary 0</td>
<td>1.8 μm</td>
</tr>
<tr>
<td>$L_{p1}$</td>
<td>Passive force length boundary 1</td>
<td>2.2 μm</td>
</tr>
<tr>
<td>$L_{p2}$</td>
<td>Passive force scaling factor for length &lt; $L_{p0}$</td>
<td>16.6 mN•mm$^{-2}$</td>
</tr>
<tr>
<td>$L_{p3}$</td>
<td>Passive force scaling factor for length &gt; $L_{p1}$</td>
<td>10.0 mN•mm$^{-2}$</td>
</tr>
<tr>
<td>$L_{p4}$</td>
<td>Passive force scaling factor for length &gt; $L_{p2}$</td>
<td>100 mN•mm$^{-2}$</td>
</tr>
<tr>
<td>$Y_{velX}$</td>
<td>Cross-bridge detachment rate due to movement</td>
<td>1.200.0 ms/μm$^2$</td>
</tr>
<tr>
<td>$Y_{velCAs}$</td>
<td>Ca$^{2+}$ detachment rate from tropomin due to movement</td>
<td>700.0 ms/μm$^2$</td>
</tr>
</tbody>
</table>
only unusual difficulty arises from solving the force-movement relation. The algorithm is summarized in Fig. 2. During isometric simulations, the length is fixed, and the total force is the sum of the forces produced by the active and passive elements. During isotonic simulations, an external load (Fmax) is applied to the myocyte. If the “test” force (passive + active) is smaller than or equal to Fmax, the length remains fixed at its original value, and the test force becomes the total force. If the test force (passive + active) is larger than Fmax, the sarcomere length is adjusted such that the sum of passive and active forces equals Fmax. The inverse quadratic passive force-length relation is solved to find the resulting length. In unloaded shortening conditions, no external load is applied to the myocytes, and Fmax = 0.

**RESULTS AND DISCUSSION**

**Validation**

To test our intact isolated rat cardiac myocyte mathematical model, we compared and contrasted simulation results obtained under different experimental conditions with previously published data. In particular, we simulated adult rat cardiac myocytes 1) action potential, membrane currents, and Ca2+ fluxes during electrical pacing, 2) Ca2+ fluxes during caffeine-induced contractions, 3) the force-Ca2+ relation in intact myocytes with use of an artificial Ca2+ clump or TG to slow the kinetics in a quasi-steady-state mode, and 4) isotonic force contractions. The results are presented in supplemental data for this article (see above). In general, there was good agreement between the simulation results and literature data.

The model reproduced the Ca2+ fluorescence and sarcomere-shortening data from our experimental study (10) for control, Parv-transduced, and SERCA2a-transduced myocytes (Fig. 3). The representative experimental traces were obtained from our experimental study. They were selected (among a set of ~30 traces in each group) as the most representative of the average of the different kinetic parameters reported in our experimental study. For the sarcomere length data, the representative traces were rescaled to have a baseline of 1.8 μm and an amplitude that matched the average measurements performed in that study. The parameters of the model were adjusted, each within the limits of related published data, to match the control myocyte data. These parameters remained fixed and were then used for the rest of the simulations presented in this mathematical study (unless specified otherwise). For Parv myocytes, when the on- and off-rates of Ca2+ and Mg2+ binding to Parv were fixed, only the Parv concentration was allowed to change; 0.062 mM Parv gave the best data match. For SERCA2a overexpression, only the overex-

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**Table 6. Initial conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Membrane voltage</td>
<td>−84.29 mV</td>
</tr>
<tr>
<td>m</td>
<td>I&lt;sub&gt;Na&lt;/sub&gt; activation parameter</td>
<td>0.001741</td>
</tr>
<tr>
<td>h</td>
<td>I&lt;sub&gt;Na&lt;/sub&gt; inactivation parameter</td>
<td>0.9823</td>
</tr>
<tr>
<td>j</td>
<td>I&lt;sub&gt;Ca&lt;/sub&gt; activation parameter</td>
<td>0.9889</td>
</tr>
<tr>
<td>x&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>I&lt;sub&gt;Ca&lt;/sub&gt; activation parameter</td>
<td>0.001275</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>I&lt;sub&gt;Ca&lt;/sub&gt; inactivation parameter</td>
<td>0.3017</td>
</tr>
<tr>
<td>x&lt;sub&gt;K&lt;/sub&gt;</td>
<td>I&lt;sub&gt;K&lt;/sub&gt; activation parameter</td>
<td>0.00004569</td>
</tr>
<tr>
<td>y&lt;sub&gt;K&lt;/sub&gt;</td>
<td>I&lt;sub&gt;K&lt;/sub&gt; inactivation parameter</td>
<td>1.000</td>
</tr>
<tr>
<td>x&lt;sub&gt;passive&lt;/sub&gt;</td>
<td>Passive activation parameter</td>
<td>0.001413</td>
</tr>
<tr>
<td>y&lt;sub&gt;passive&lt;/sub&gt;</td>
<td>Passive inactivation parameter</td>
<td>0.9999</td>
</tr>
<tr>
<td>y&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>Na&lt;sup&gt;+&lt;/sup&gt; activation parameter</td>
<td>0.0001894</td>
</tr>
<tr>
<td>y&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>Na&lt;sup&gt;+&lt;/sup&gt; inactivation parameter</td>
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<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; activation parameter</td>
<td>0.00009081 mM</td>
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<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; inactivation parameter</td>
<td>0.1976 mM</td>
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<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C1</td>
<td>0.9955</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C2</td>
<td>0.004494</td>
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<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C3</td>
<td>0.9982</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C4</td>
<td>0.0001519</td>
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<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C5</td>
<td>0.0</td>
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<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C6</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C7</td>
<td>0.0</td>
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<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C8</td>
<td>0.0</td>
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<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C9</td>
<td>0.0</td>
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<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C10</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode C11</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca1</td>
<td>0.01779</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca2</td>
<td>0.000001083</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca3</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca4</td>
<td>0.0</td>
</tr>
<tr>
<td>y&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel closed: mode Ca5</td>
<td>0.0999</td>
</tr>
<tr>
<td>[LTrpnCa]</td>
<td>Conc of Ca2+ bound to low-affinity troponin site</td>
<td>0.00176 mM</td>
</tr>
<tr>
<td>[HTrpnCa]</td>
<td>Conc of Ca2+ bound to high-affinity troponin site</td>
<td>0.1351 mM</td>
</tr>
<tr>
<td>PVCa</td>
<td>Fraction of Parv sites occupied by Ca2+ in myoplasm</td>
<td>0.2502</td>
</tr>
<tr>
<td>PV Mg</td>
<td>Fraction of Parv sites occupied by Mg2+ in myoplasm</td>
<td>0.7201</td>
</tr>
<tr>
<td>PV Mg</td>
<td>Fraction of Parv sites occupied by Ca2+ in subspace</td>
<td>0.3873</td>
</tr>
<tr>
<td>PV Mg</td>
<td>Fraction of Parv sites occupied by Mg2+ in subspace</td>
<td>0.5884</td>
</tr>
<tr>
<td>fura2Ca</td>
<td>Fraction of fura 2-AM sites occupied by Ca2+ in myoplasm</td>
<td>0.2739</td>
</tr>
<tr>
<td>fura2Ca</td>
<td>Fraction of fura 2-AM sites occupied by Ca2+ in subspace</td>
<td>0.4164</td>
</tr>
<tr>
<td>fura2Ca</td>
<td>Fraction of sarcomere length and cross-bridge length</td>
<td>1.790</td>
</tr>
<tr>
<td>n0XB</td>
<td>Fraction of cross bridges in nonforce/force-producing state</td>
<td>0.99999</td>
</tr>
<tr>
<td>p0XB</td>
<td>Fraction of cross bridges in nonforce/force-producing state</td>
<td>8.034 × 10&lt;sup&gt;−6&lt;/sup&gt;</td>
</tr>
<tr>
<td>p1XB</td>
<td>Fraction of cross bridges in nonforce/force-producing state</td>
<td>6.206 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
</tr>
<tr>
<td>L</td>
<td>Sarcomere length</td>
<td>1.8 μm</td>
</tr>
<tr>
<td>F&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Total force</td>
<td>0.0 mN/mm²</td>
</tr>
</tbody>
</table>

**Isometric Force (L=L<sub>1</sub>):**
- L is fixed at value L<sub>1</sub>
- Ftotal=Fact+Fpass

**Isotonic Force (Ftotal ≤ Fmax):**
- Ftest=Fact+Fpass
- If Ftest ≤ Fmax
  - Ftotal=Fact+Fpass
- If Ftest > Fmax
  - Ftotal=Fmax
  - Find L such that:
  - Fact+Fpass=Fmax

**Note:** Fmax=0 Unloaded Shortening
pression factor ($K_{SR}$) and the forward-mode affinity constant ($K_{fb}$) were allowed to vary (for the rationale, see Modeling SERCA2a Overexpression): the best data match was obtained with $K_{SR}/H11005/2.0$ and $K_{fb}/H11005/0.00022$ mM.

The Ca$^{2+}$/H11001 fluorescence signals were calculated as the fraction of Ca$^{2+}$/H11001 bound to fura 2-AM and normalized with respect to their amplitude. The simulation fit the experimental data closely (Fig. 3, A and C). Control myocytes exhibited a slow monophasic decay. Parv myocytes had a very rapid early decay phase followed by a slower late phase. This slower phase has been attributed to the slow release from Parv of the Ca$^{2+}$/H11001 absorbed earlier during the decay (9). Eventually, the slow phase became even slower than in control myocytes in the very late part of the decay phase, as seen experimentally. SERCA2a-transduced myocytes had an initial decay phase only slightly slower than that of Parv myocytes, while maintaining the fastest kinetics among all groups in the late phase of the decay.

The sarcomere-shortening simulations match the experimental data in the control, Parv, and SERCA2a myocyte groups (Fig. 3, B and D). The sarcomere-shortening amplitude was matched in all groups within 0.005 μm. However, the modeling results revealed some limitations. In control myocytes, there were three main differences. The rising phase of sarcomere shortening was delayed by ~8 ms in simulations compared with experimental data. Moreover, the experimental data clearly exhibited a biphasic relengthening, whereas in simulation the relengthening phase was clearly monophasic. The largest difference was in Parv myocyte simulations, where the simulation relengthening kinetic properties were slower than the experimental properties.

Aside from the differences in sarcomere shortening, we also sought to understand the differences between expression of Parv and SERCA2a in our experimental study (10) and that in the model. In our experimental study, an experimentally estimated value of $0.138 \pm 0.046$ mM was obtained for Parv concentration 3 days after gene transfer. This estimated value is somewhat higher than the value used in the model. Several factors could account for these differences, including experimental variations, use of an indirect method for experimentally estimating expression of these proteins, and uncertainties in the model parameters that might influence the value used for Parv concentration.

Similarly, SERCA2a overexpression was estimated to be 46 ± 7% (corresponding to $K_{SR} = 1.46 \pm 0.07$), which is, in this case, lower than the value used in the model. Again, the model assumptions, such as equivalent forward and reverse rates and a cooperativity factor of 2 for the SERCA2a pumps, might be partly responsible for this discrepancy. Perhaps more importantly, the spatial distribution of the SERCA2a pumps on the junctional SR relative to the RyR Ca$^{2+}$/H11001 release sites and whether this distribution is modified during overexpression might not be fully accounted for in the simple common pool model used here.

**Mechanistic Insights**

**Modeling SERCA2a overexpression.** When SERCA2a is overexpressed, at least two factors influence the model. First, the pump density is increased. In the model, this is achieved by multiplying the SR uptake flux equation by an overexpression factor, $K_{SR}$. Second, in most of the studies where SERCA2a has been overexpressed, no change in PLB has been reported.
Therefore, the SERCA2a-to-PLB ratio is expected to increase, and with that change, the SERCA2a forward-mode affinity for Ca\(^{2+}\) is expected to increase (resulting in a decreased dissociation constant \(K_{\text{fb}}\)) (5). As mentioned previously, we included both of these changes to model SERCA2a-overexpressing myocytes (Fig. 3).

In Fig. 4, we examined the individual contribution of these two factors to myocyte performance. In particular, we examined the effect of varying \(K_{\text{SR}}\) and \(K_{\text{fb}}\) on the Ca\(^{2+}\) transient and on the SR free Ca\(^{2+}\) content. Decreasing \(K_{\text{fb}}\) (increasing affinity) from 230 to 180 nM increased the baseline SR Ca\(^{2+}\) content from 0.20 to 0.25 mM (Fig. 4B). The net result was a large increase in the Ca\(^{2+}\) transient amplitude (Fig. 4A). When normalized (not shown), the increase in decay rate was small.

In contrast, the main effect of increasing \(K_{\text{SR}}\) was acceleration of the Ca\(^{2+}\) transient decay (Fig. 4C). The Ca\(^{2+}\) transient amplitude initially increased \((K_{\text{SR}} = 1.5, 2.0)\) but was reduced in the case of the larger overexpressing factor \((K_{\text{SR}} = 3.0)\). Two factors contributed to these small changes in Ca\(^{2+}\) transient amplitude. First, there was only a moderate increase in SR Ca\(^{2+}\) content from 0.20 mM at \(K_{\text{SR}} = 1.0\) to 0.23 mM at \(K_{\text{SR}} = 3.0\) (Fig. 4D). More importantly, the rate of uptake was so high at \(K_{\text{SR}} = 3.0\) that some of the released Ca\(^{2+}\) was reabsorbed by the SR without having time to accumulate in the myoplasm. This could be seen in the smaller peak decrease in the SR content (Fig. 4D). As shown in Fig. 3, when these two effects were combined \((K_{\text{SR}} = 2.0\) and \(K_{\text{fb}} = 220\) nM), we were able to reproduce the effect of SERCA2a overexpression in rat cardiac myocytes, as demonstrated in our experimental study (10).

Two questions arise from these simulations. First, is this uncoupling effect of adding new pumps (accelerating kinetics) vs. increasing the SERCA2a-to-PLB ratio (increasing amplitude) truly reflective of intact cardiac myocytes, or is it just an artifact of the model? Second, is this behavior dependent on experimental conditions such as temperature and animal species? Several experiments affecting the number of SERCA2a pumps and/or the SERCA2a-to-PLB ratio in different species have been published. In rabbit myocytes at 37°C, Chaudhri et al. (6) directly compared SERCA2a overexpression (increased number of pumps and SERCA2a-to-PLB ratio) with results using a PLB antisense (increase in SERCA2a-to-PLB ratio only). Under baseline conditions, they found a very similar increase in sarcomere-shortening amplitude in both groups, especially with stimulation at higher frequency. The effect on sarcomere relaxation was more pronounced in the SERCA2a-overexpressing myocytes than with the PLB antisense, although PLB antisense myocytes still relaxed more rapidly than control myocytes. One of the primary effects of β-adrenergic
agonists, such as Iso, is initiation of PLB phosphorylation and, therefore, an increase in the functional ratio of SERCA2a to PLB. When 10 nM Iso was applied at 37°C to control rat cardiac myocytes in our experimental study (10), the sarcomere-shortening amplitude was greatly increased (by 107.9%). However, when normalized with respect to their amplitude, the myocytes treated with Iso were only slightly faster than the controls (13.2% difference) (10). Similar results were obtained in another study (11). However, much larger increases in relaxation rates are seen with Iso at lower temperatures (10) or in larger mammals (6). It seems that the results predicted by the model, although more pronounced, qualitatively reflect those documented experimentally in rodent myocytes at physiological temperatures.

Postrest potentiation. Postrest potentiation (PRP) is postulated to reflect SR reuptake of Ca$^{2+}$ after a rest period and the refractory period of the Ca$^{2+}$ release channels (4). To analyze the mechanisms behind PRP, we simulated the same protocol used in our experimental study (10), namely, 10 conditioning pulses (1 s apart), a 120-s rest period, 10 prepulses (1 s apart), a variable rest period, and 10 postpulses (1 s apart). The PRP simulation results are presented in Fig. 5 (control myocytes) and Fig. 6 (Parv and SERCA2a myocytes).

The PRP simulations and experimental data in control myocytes were in good agreement (Fig. 5A). To analyze the behavior of the PRP, as well as the behavior of the prepulses, we traced the SR free Ca$^{2+}$ content for two different rest periods (5 and 10 s; Fig. 5B). There is progressive exponential decay of the baseline SR content during the prepulses (from 0.22 to 0.18 mM), which is likely responsible for some of the decay in sarcomere-shortening amplitude observed during the prepulses. The second point is the recovery of Ca$^{2+}$ reuptake by the SR after the prepulse sequence. When stimulated after a 5-s rest period, the SR reloading was incomplete, which, in turn, led to a reduced sarcomere length amplitude. For longer periods, the recovery became more complete and the sarcomere amplitude potentiation became larger. Although the SR Ca$^{2+}$ load could explain most sarcomere-shortening data, it still does not explain why there is a significant jump between prepulse 1 and 2. To investigate this issue, we plotted the open probability of RyR (Ca$^{2+}$ release channel; Fig. 5C). The results show that the open probability falls from a maximal value of 1.0 for prepulse 1 to ~0.8 for prepulse 2. This difference is in agreement with the 20% loss of amplitude observed experimentally and in the model for sarcomere

![Fig. 5. Postrest potentiation (PRP) in control myocytes. Traces, time-dependent signals; symbols, peak values. A: comparison between simulations (traces) and experimental normalized sarcomere-shortening data (□ and ■) from our experimental study (10). Gray traces and □, prepulses 1–10; black traces and □, postpulse 1, obtained after rest periods. B: effects of SR Ca$^{2+}$ reloading with 5-s (solid trace) and 10-s (dotted trace) rest intervals. C and D: effects of modifying ryanodine receptor (RyR) refractory period on ryanodine open probability (C) and normalized sarcomere length (SL, D). Solid traces, effect of prolonged RyR refractory period; v and ■, peak values obtained from original model (same as A); gray traces, prepulses 1–10; black traces, postpulse 1, obtained after rest periods; dotted traces, control myocytes.](http://ajpheart.physiology.org/)}
shortening. To confirm this finding, the RyR refractory period was increased by reducing $k_c$ by one-half (Eqs. A78–A81). This parameter controls the rate of recovery from the nonexcitable closed state ($P_{c2}$) to the excitable closed state ($P_{c1}$) through the open state ($P_{o1}$) (19). The results are presented in Fig. 5C. As seen experimentally, the attenuation between prepulses 1 and 2 was twice as large as for the control case. Interestingly, even with a longer refractory period, the open probability of the RyR had almost fully recovered at 5 s. The expected effects on sarcomere length should be a stronger attenuation for prepulses 2–10, with almost no change in PRP, at least for a >5-s rest period. As expected (Fig. 5D), the simulations with the extended refractory period showed a stronger attenuation in prepulses 2–10 than with the normal refractory period. Surprisingly, PRP was increased with the longer refractory period (gray traces and filled triangles), even for >5-s rest periods. On further investigation, this increase in PRP was attributed to an elevated SR Ca$^{2+}$ during the rest period (not shown). In turn, this increase in SR Ca$^{2+}$ during the rest period appeared to be caused by the reduced SR Ca$^{2+}$ depletion during...
the prepulses (due to lower RyR open probability). This shows the complexity of the interactions involved in the PRP response.

Next, we studied the effect of Parv on PRP (Fig. 6, A–C). As in Fig. 3, we used 0.062 mM Parv. Figure 6A shows the comparison between data collected in our experimental study and the simulation results. For additional comparisons, we also included the simulation results of control myocytes. The main difference between Parv myocyte simulation and experimental data was in the amplitude of prepulse 1 before normalization. Experimentally, the amplitude of prepulse 1 in Parv myocytes was almost identical to that in control myocytes. In contrast, in the simulations, prepulse 1 in Parv myocytes was only 58% of that in control myocytes. However, once normalized, Parv myocytes exhibited some of the same qualitative behavior that was observed experimentally. Indeed, Parv myocytes showed a greater amplitude deficit between prepulses 1 and 2 than control myocytes, followed by a flatter response. However, most interestingly, Parv myocytes, as seen experimentally, had the strongest early PRP (rest periods of 2–10 s) of all groups. The effect is attributable to a much faster SR Ca2+ buffering capacity of Parv. Indeed, during every cycle, Parv binds and releases a large amount of Ca2+. At a stimulation rate of 1 Hz, release of Ca2+ from Parv is incomplete between each cycle (Fig. 6C). However, after the final prepulse, all this extra Ca2+ is released back into the myoplasm to be rapidly sequestered by the SR, explaining the rapid reloading mentioned above.

We also examined the effect of PRP in SERCA2a-overexpressing myocytes (Fig. 6, D–F). Here, the simulations (Fig. 6D) are in good agreement with the experimental data, showing that SERCA2a-overexpressing myocytes were the least sensitive, among all groups, to change in stimulation rate. To explain this behavior, we plotted the SR Ca2+ content (Fig. 6E). The results show that the SERCA2a-overexpressing myocytes exhibited less baseline Ca2+ depletion than the control myocytes during the prepulses. This can be explained by the higher rate of sequestration in the SERCA2a myocytes, which allows almost all of the Ca2+ to be reloaded between each pulse at a stimulation frequency of 1.0 Hz.

Finally, in our experimental study (10), we showed that some of the SERCA2a-transduced myocytes exhibited a response with increasing sarcomere-shortening amplitude from prepulse 1 to prepulse 10 and were labeled “atypical.” These myocytes showed an early strong PRP between rest periods of 2 and 10 s and exhibited negative PRP at longer rest periods. One possible explanation is that, in some myocytes, SERCA2a overexpression favors a leak from the SR. In the main model (Eqs. A89, A110, and A111), a small leak already exists between the junctional SR and the subspace, to reflect a leak flux measured experimentally from the RyR (34). Increasing this leak flux fourfold was not sufficient to reproduce the experimental results; however, increasing it fivefold augmented the Ca2+ in the subspace domain sufficiently to create spontaneous contractions (results not shown). As an alternative mechanism, we added another leak between the network SR and the myoplasm in these SERCA2a-transduced myocytes ($K_{SR} = 2.0$ and $K_{mb} = 220$ nM). The resulting PRP simulation results are presented in Fig. 6F. The simulation results followed the same trend observed experimentally in these atypical SERCA2a myocytes. Interestingly, the same SR-to-myoplasm leak in control myocytes ($K_{SR} = 1.0$ and $K_{mb} = 230$ nM) also followed the same trend, although with a maximal shortening amplitude of 7.2 nm in control myocytes vs. 37.1 nm in SERCA2a myocytes, which would be difficult to detect experimentally. Whether such a leak would be caused by SERCA2a overexpression or whether SERCA2a overexpression would “rescue” a portion of the population naturally bearing that kind of leak remains to be investigated experimentally.

**TG as a chemical tool to mimic cellular diastolic dysfunction.** In our experimental study (10), we used TG to mimic impairment in Ca2+ removal as seen in heart failure (23). Indeed, TG is a specific high-affinity blocker of SERCA2a (17). We showed that Parv-transduced myocytes, in contrast to control myocytes, were able to maintain their diastolic function in the presence of TG. We also mentioned that use of TG alone to model pure diastolic dysfunction was not ideal, because TG also impaired contractile function. However, use of elevated amounts of external Ca2+ helped correct this situation. Here, we will investigate some of these aspects, first in control myocytes (Fig. 7) and then in Parv-transduced myocytes (Fig. 8).

The normalized Ca2+ fluorescence and normalized sarcomere shortening for control myocytes at 0, 50, 150, and 350 nM TG are presented in Fig. 7. The model simulation results at 150 nM TG gave a good match to the data, and increasing TG levels also resulted in greater diastolic dysfunction. As seen experimentally, along with the cellular diastolic dysfunction came a reduction in shortening amplitude. Figure 7C shows the summary of the simulation in control myocytes for sarcomere-shortening amplitude and index of relaxation rate ($1/T_{1/2}$). This response shows that the amplitude is more sensitive to TG than are the relaxation kinetics (11). This decrease in amplitude is associated with a progressive emptying of the SR Ca2+ content. The baseline free Ca2+ level in the network SR was 0.20 mM in the absence of TG and was reduced to 0.17 mM at 150 nM TG and to 0.085 mM at 1.000 nM TG. To create a pure diastolic dysfunction model (without systolic impairment), it was necessary to compensate for the extra Ca2+ leaving the myocyte, by reducing sarcolemmal Ca2+ removal or by allowing more Ca2+ to enter the myocyte. In Fig. 7D, we used three different strategies to return the sarcomere-shortening amplitude to the level of the control myocytes in the absence of TG while preserving diastolic dysfunction. First, external Ca2+ was elevated to allow more Ca2+ entry, mostly through the background Ca2+ current ($I_{Ca,b}$). Second, Ca2+ removal capacity of NCX was reduced to 72% of its original value. Third, 4-Aminopyridine (4-AP, 0.66 mM) was used to partially block transient outward and voltage-dependent K+ currents ($I_a$ and $I_{Ks}$) to elongate the action potential, allowing more Ca2+ entry through the L-type Ca2+ channel. In this case, the action potential duration at 90% repolarization increased from 53.6 ms in the absence of 4-AP to 97.1 ms in the presence of 4-AP. All three strategies gave nearly identical results.

Next, we studied the effect of TG on Parv-transduced myocytes. The model was not able to match the fast kinetics observed experimentally in Parv-transduced sarcomere lengthening in the absence of TG (Fig. 3). However, as seen experimentally, Parv-transduced myocytes showed no diastolic dysfunction in sarcomere-shortening data (Fig. 8B) when up to 350 nM TG was applied. Again, as observed experimentally, Ca2+ fluorescence in Parv myocytes exhibited diastolic dys-
function in the presence of TG only in the late part of the cycle (Fig. 8A).

From these results, two questions arise: 1) Where does Ca\textsuperscript{2+} go when TG is present? 2) How is Parv able to maintain myocyte relaxation function in the presence of TG? To answer these questions, we tracked the Ca\textsuperscript{2+} sequestration fluxes into the SR (SERCA2a pumps; Fig. 8C) and through the sarcolemma (NCX\textsuperscript{2+}-ATPase; Fig. 8D). TG at 150 nM reduced the peak SR uptake flux to about one-half of its value in the absence of TG in control myocytes (Fig. 8C).

The total Ca\textsuperscript{2+} uptake was also reduced. The differential Ca\textsuperscript{2+} fluxes went through the sarcolemma, not by way of an increase in Ca\textsuperscript{2+} flux peak value but, rather, an increase in the duration of the transient removal (Fig. 8D). When the different Ca\textsuperscript{2+} fluxes were integrated over a 1-s period after stimulation, distribution in the absence of TG was 94.1% for SERCA2a, 4.4% for NCX, and 1.5% for sarcolemmal Ca\textsuperscript{2+}-ATPase (see Fig. B2 in supplemental data). In the presence of 150 nM TG, the distribution was 90.6% for SERCA2a, 7.3% for NCX, and 2.1% for sarcolemmal Ca\textsuperscript{2+}-ATPase. This change in the relative contribution of each removal mechanism is often seen in heart failure (4). Interestingly, in transduced myocytes, some of the sequestration demand on SERCA2a was eased by Parv during most of the Ca\textsuperscript{2+} transient decay and redistributed later when the demand was less. The total distribution in Parv myocytes in the presence of TG was 91.6% for SERCA2a, 6.2% for NCX, and 2.3% for sarcolemmal Ca\textsuperscript{2+}-ATPase.

Model Predictions

Optimizing Parv-binding characteristics. The law of mass action was used to model Parv interaction with Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. This results in two first-order differential equations (Eqs. A95 and A96) that are controlled by the four binding rate constants to Ca\textsuperscript{2+} and Mg\textsuperscript{2+}: \( k_{PvCa} \), \( k_{PvCa}^{-1} \), \( k_{PvMg} \), and \( k_{PvMg}^{-1} \). In turn, the concentration of Parv and the fraction of its sites occupied by Ca\textsuperscript{2+} determine its effect on the Ca\textsuperscript{2+} decay rate (Eqs. A93 and A109). In an experimental context, where a given Parv molecule has a predetermined set of binding rate constants, the only controllable variable is the concentration. As predicted with the use of a much simpler model and as observed experimentally in a previous study (9), increasing Parv concentration resulted in an acceleration of the Ca\textsuperscript{2+} transient decay and a decrease in its amplitude (Fig. 9A).
However, with the set of parameters used in the present study, the resulting Parv concentration range where attenuation is negligible compared with the increased relaxing effects on the kinetics (Fig. 9D) is rather small compared with that observed experimentally (8). Nonetheless, one can ask the following question: Is it possible, at least theoretically, to modify the binding characteristics of Parv to broaden the concentration range where relaxation rates are increased with little or no effect on sarcomere-shortening and Ca\(^{2+}\) transient amplitude?

We proposed that a “gain in relaxation,” without a compromise in sarcomere-shortening or Ca\(^{2+}\) transient amplitude, could be achieved if the amount of Ca\(^{2+}\) buffered during the rising phase of the Ca\(^{2+}\) transient is maintained while the amount of Ca\(^{2+}\) buffered during the decay phase of the Ca\(^{2+}\) transient is increased. One way to achieve this goal is to decrease the off-rate of Mg\(^{2+}\) (\(k_{PvMg}\)), such that more time is required for the Ca\(^{2+}\) to dislodge Mg\(^{2+}\) early in the Ca\(^{2+}\) transient. To test this hypothesis, we divided the value of the off-rate for Mg\(^{2+}\) by 2.5 \([k_{PvMg(modified)} = k_{PvMg}/2.5]\), and to maintain the balance between the two metal ions binding, we also divided the off-rate for Ca\(^{2+}\) by the same amount \([k_{PvCa(modified)} = k_{PvCa}/2.5]\). Figure 9B shows the change in the amount of Ca\(^{2+}\) buffered during one cycle by wild-type Parv and by modified Parv with new off-rate characteristics. Application of these changes to the off-rates impacted two parameters at 0.062 mM Parv: 1) the initial Ca\(^{2+}\) binding slope in modified Parv is smaller than that of original wild-type Parv, resulting in a smaller amount of Ca\(^{2+}\) buffered during the rising phase of the Ca\(^{2+}\) transient (therefore, less attenuation), and 2) the maximal amount of Ca\(^{2+}\) buffered during the Ca\(^{2+}\) transient decay was also reduced, diminishing acceleration of the Ca\(^{2+}\) transient. To optimize this outcome, we next increased modified Parv concentration until the initial Ca\(^{2+}\) binding slope was similar to the wild-type model. We found that a concentration of 0.200 mM with modified Parv gave the desired results. In this case, the maximal amount of Ca\(^{2+}\) buffered during the Ca\(^{2+}\) transient decay was greater than for wild-type Parv; therefore, it was expected to further accelerate the decay. This expectation was confirmed (Fig. 9C) when the Ca\(^{2+}\) transient produced under this condition (modified, 0.200 mM Parv) was compared with wild-type (0.062 mM Parv). Indeed, the amplitude was maintained while the transient was accelerated. As illustrated in Fig. 9D, Parv with modified binding characteristics had a broader optimal Parv concentration range than wild-type Parv. Whether such a modification would be demonstrated experimentally is outside the scope of this study. We also tested other conditions and found that 1) increasing the binding affinity for only one ion (Ca\(^{2+}\) or Mg\(^{2+}\)) would saturate Parv with that ion and reduce its buffering efficiency; 2) lowering the affinity for both ions would increase the amount of free Parv and is, therefore, expected to increase...
instant Ca\textsuperscript{2+} buffering during the initial transient rise in Ca\textsuperscript{2+}, thereby reducing the amplitude of the Ca\textsuperscript{2+} transient; and f) increasing the on-rates would not significantly affect Parv performance. The characteristics of the Ca\textsuperscript{2+} transient and Parv closely interact with each other, and, consequently, the optimal Parv kinetic and concentration properties may vary with experimental conditions (e.g., stimulus frequency and animal species).

Energetics and diastolic Ca\textsuperscript{2+} levels. Two important issues that have not been addressed experimentally are investigated using the mathematical model. First, in heart failure, energy production is often impaired (13, 39), raising the following important question: What happens to energy utilization when Parv is expressed de novo or when SERCA2a is overexpressed? Second, the diastolic levels of free Ca\textsuperscript{2+} in the myoplasm influence the level of Ca\textsuperscript{2+} bound to calmodulin. In turn, Ca\textsuperscript{2+}-calmodulin can initiate a cascade of signaling events that can lead to maladaptive hypertrophy (7, 28). The question is: Does Parv or SERCA2a affect the diastolic Ca\textsuperscript{2+} levels?

The main ATP-dependent proteins involved in the cardiac contraction cycle are the myosin heavy chain, the SERCA2a, the Na\textsuperscript{+}-K\textsuperscript{+} pump, and, to a much lesser extent, the sarcoplasmic Ca\textsuperscript{2+}-ATPase. Unfortunately, because of the formulation of the model, it is not possible to assess ATP consumption by the myosin heavy chain, but it is possible to do so for the three other processes. Indeed, the SERCA2a transports two Ca\textsuperscript{2+} per ATP; the sarcoplasmal Ca\textsuperscript{2+}-ATPase requires one ATP per Ca\textsuperscript{2+} carried across the membrane, and the Na\textsuperscript{+}-K\textsuperscript{+} pump allows three Na\textsuperscript{+} out and two K\textsuperscript{+} in, for each molecule of ATP used (4). Using this information, we traced the combined instantaneous ATP consumption of these three processes, as a function of time during a 0.2-Hz contraction (Fig. 10A). The peak level of ATP utilization was greatest in SERCA2a myocytes, whereas levels for Parv and control myocytes were similar. As explained previously (Fig. 8C), when expressed, Parv dominates Ca\textsuperscript{2+} sequestration during the early part of the Ca\textsuperscript{2+} transient decay, reducing the energy demand during that period and redistributing it later in the cycle, where the demand is less. Baseline ATP use was mostly controlled by the Na\textsuperscript{+}-K\textsuperscript{+} pump and was similar in all groups. For that reason, we focused on the transient consumption of ATP by first removing baseline ATP use and then integrating over time to obtain the total amount of ATP used by the three processes (Fig. 10B). Most of the transient ATP use occurs during the first 500 ms (Fig. 10A). When the integration was performed.
during that period (0–500 ms), SERCA2a myocytes showed the greatest total consumption among the three groups, whereas Parv myocytes used slightly less total ATP than control myocytes. When the integration was performed over the entire cycle (0–5,000 ms), the results were the same, except the Parv level of ATP use reached that of control myocytes. This confirms that Parv myocytes reduce ATP utilization during the transient and redistribute this utilization in the late part of the cycle at a much lower rate.

To address the second question regarding the effect of Parv and SERCA2a on the diastolic levels of Ca\(^{2+}\) in the myoplasm, we ran simulations at six different stimulation frequencies (Fig. 10C). For the 0-Hz case (quiescent myocyte), we let the simulation run for 120 s (of myocyte model time); for the other frequencies, we stimulated 24 beats and examined Ca\(^{2+}\) level just before the onset of the last stimulation. In the quiescent state (0 Hz), the level of free Ca\(^{2+}\) was the same in all groups. When the frequency increased, the level of free Ca\(^{2+}\) in control myocytes initially decreased, mostly because of a decrease in baseline SR Ca\(^{2+}\) content. The increase in diastolic Ca\(^{2+}\) at the highest frequencies was due to the inability of the myocyte to rapidly sequester Ca\(^{2+}\) during these short beats. The effect of SERCA2a was to flatten this response because of its smaller SR content depletion at low frequencies and its better ability to sequester Ca\(^{2+}\) at a high rate at high frequencies. Parv myocytes initially followed the SERCA2a traces for the same reason: less SR Ca\(^{2+}\) depletion. However, at intermediate frequencies (0.5–2.0 Hz), Parv myocytes showed an elevated diastolic Ca\(^{2+}\) level compared with the other groups. This is most likely a residual effect of the slow Ca\(^{2+}\) release by Parv toward the end of the cycle. Although variations are present in these Ca\(^{2+}\) diastolic levels, they are rather small (∼10% for ≤2 Hz), and it is not certain whether this is sufficient to influence the level of Ca\(^{2+}\)-calmodulin complex formation.

Model Limitations

One approach to study the performance of the myocardium is to first isolate individual myocytes and then perform electrophysiological, Ca\(^{2+}\) transient fluorescence, and/or unloaded sarcomere-shortening measurements. Isolated myocytes can be used to investigate pathological conditions, pharmacological treatments, biological diversity (myocyte lineage, species, gender, and age), environmental conditions, and genetic modifications. In this study, we developed, for the first time to our knowledge, a mathematical model that simultaneously addresses these three modalities (electrophysiology, Ca\(^{2+}\)-handling, and unloaded sarcomere shortening) in genetically engineered rat ventricular myocytes at physiological temperature.

Although the model was able to replicate several experimental results, such as PRP, inhibition of SERCA2a by TG, and Ca\(^{2+}\)-handling protein genetic manipulations, it has limitations. In particular, the force-generation/sarcomere-shortening component does not always reveal all the details seen experimentally. For example, the experimental sarcomere-relengthening traces clearly exhibit a first rapid relaxation followed by a somewhat slower return to baseline. Alternatively, the simulation results simply follow a monoexponential time course of relaxation (Fig. 3). Moreover, for equivalent increases in decay rate of Ca\(^{2+}\) fluorescence data, the model was not able to reproduce the sarcomere-shortening fast relaxation rates observed in Parv myocytes experimentally. These two points are most likely related and could potentially be attributed to several factors. First, the myofilament compartment of the model is an oversimplification of a more complex system, where millions of independent cross bridges attach and detach, producing force and sarcomere length movement. Moreover, this system is influenced by sarcomere length, availability of ATP, pH, and inorganic phosphate. Second, the lack of force-
related data in intact isolated myocytes made the development of that section of the model difficult. Indeed, several parameters had to be based on data from permeabilized myocytes at low temperature or from entire cardiac muscle bundles. Recently, a new technique to measure force in intact cardiac myocytes has been developed using special carbon-fiber force transducers (38). With this technical advancement, it is hoped that tests such as force-pCa measurements [using TG (1); see Fig. B2 in supplemental data], slack tests, isometric and isotonic twitches, and force-frequency relation could provide more insights specific to intact single cardiac myocytes and help enhance the model.

Implications

Along with revealing underlying mechanisms behind the experimental results presented in our experimental study (10), the model simulations led to new insights related to the use of Ca²⁺-handling proteins to correct diastolic dysfunction. From the experimental and mathematical modeling studies presented here, it is apparent that there are different secondary consequences of using Parv or modifying SERCA2a function to increase relaxation rates. Interestingly, the way in which SERCA2a function is modified might also influence the functional outcome of myocyte contractile performance (see Modeling SERCA2a overexpression and Fig. 4). Indeed, Fig. 4 shows that modifying Ca²⁺ sensitivity of SERCA2a (as seen when SERCA2a-to-PLB ratio changes) mostly influences Ca²⁺ transient amplitude in rat myocytes, whereas increasing the number of pumps (without changing the SERCA2a-to-PLB ratio) mostly influences the Ca²⁺ sequestration rate. This result implies that using SERCA2a overexpression, which affects the SERCA2a-to-PLB ratio and the maximal number of pumps, might not be equivalent to only modifying the SERCA2a-to-PLB ratio, as with PLB-knockout and antisense strategies. This may have implications for potential SERCA2a-to-PLB strategies in human heart failure.

β-Adrenergic stimulation has three main effects on the heart in vivo: 1) increase in contractility (positive inotropy), 2) increase in relaxation (positive lusitropy), and 3) increase in heart rate (positive chronotropy) (4). In our experimental study (10), we showed that, in SERCA2a-overexpressing myocytes, the β-adrenergic response was blunted at stimulation at 0.2 Hz. However, the PRP simulation and experimental results obtained in this study, as well as frequency response obtained in other studies (6, 31), show that SERCA2a overexpression has a positive inotropic effect at increased stimulation pacing compared with control myocytes (see prepulses in Fig. 6D at 1.0 Hz). It is therefore conceivable that SERCA2a overexpression in vivo could appear to have normal behavior in the presence of a β-adrenergic agonist, because the positive inotropic response caused by an elevation in heart rate in vivo could possibly compensate for the blunted β-adrenergic response.

Results from the PRP simulations also revealed an interesting interplay between Ca²⁺ unloading from Parv and Ca²⁺ reloading in the SR. Indeed, during the first 1 or 2 s after the prepulses, SR Ca²⁺ reloading was much more rapid in Parv-transduced than in control myocytes. Is it possible that, in Parv myocytes, early Ca²⁺ sequestration renders the competition for final removal between NCX and SERCA2a biased toward the latter, allowing less Ca²⁺ to leave the myocyte and more to be taken up by the SR? It seems that this could be the case, because the basal SR Ca²⁺ load is slightly greater in Parv than in control myocytes at the end of prepulse 10 of PRP (Fig. 6B) and during steady-state pacing at 0.2 Hz (basal SR Ca²⁺ = 207 mM for Parv and 200 mM for control). Additionally, TG simulations (Fig. 8, C and D) show that Parv myocytes have an amplitude of SR Ca²⁺ flux similar to that in control myocytes, whereas sarcolemmal Ca²⁺ uptake was lower in myocytes expressing Parv. This may be important, because in heart failure the opposite situation is favored; i.e., the tendency is for Ca²⁺ to leave the myocyte because of a higher NCX-to-SERCA2a sequestration ratio.

In addition, we found through the model that energy consumption was more elevated in SERCA2a-transduced than in control or Parv-transduced myocytes (Fig. 10, A and B). It is estimated that, under normal conditions, the rate of ATP synthesis in cardiac myocytes is ~1–2 mM/s (22, 30). If it is assumed that pacing frequency is 1 Hz, the difference observed in our model represents ~1% of the total ATP synthesis in normal conditions. However, this difference might become more important in human failure, where ATP synthesis and transfer efficiency are greatly impaired (35).

In summary, this modeling study and our experimental study (10) provide a direct comparison between two genetic strategies that have been proposed to improve relaxation rate in myocardial tissue: Parv expression and SERCA2a overexpression. Moreover, the model allowed accessibility to variables such as distribution of Ca²⁺ fluxes (SR vs. sarcolemma), instantaneous SR Ca²⁺ load under all conditions, and assessment of energy utilization. Overall, the results showed that these two methods (Parv vs. SERCA2a), or even modification of the SERCA2a-to-PLB ratio using PLB antisense, are not fully equivalent. The best method may be dependent on the cause, the molecular symptoms, and the state of the cardiac disease. Importantly, in vivo experimentation in larger mammalian systems, under normal and diverse pathological conditions, would be required before these results could be extrapolated to possible human therapies.

APPENDIX A

Equations. Na⁺ current (I_{Na})

\[ I_{Na} = G_{Na} m^{hj} (V - E_{Na}) \]  (A1)

\[ E_{Na} = \frac{RT}{F} \ln \left( \frac{[Na^{+}]}{[Na^{-}]}, \right) \]  (A2)

\[ \frac{dm}{dr} = \alpha_{m}(1 - m) - \beta_{m}m \]  (A3)

\[ \frac{dh}{dr} = \alpha_{h}(1 - h) - \beta_{h}h \]  (A4)

\[ \frac{dj}{dr} = \alpha_{j}(1 - j) - \beta_{j}j \]  (A5)

\[ \alpha_{m} = 0.32 \frac{V + 47.13}{1 - e^{-0.11(V+47.13)}} \]  (A6)

\[ \beta_{m} = 0.08 e^{-\frac{V}{111}} \]  (A7)

For \( V \approx -40 \) mV

\[ \alpha_{s} = 0.0 \]  (A8)
\[
\beta_h = \frac{1}{0.13[1 + e^{(V+10.66)/-11.7}]} \quad (A9)
\]
\[
\alpha_j = 0.0 \quad \beta_j = 0.3 \quad e^{-5.35 \times 10^{-7}V} \quad (A11)
\]
For \( V < -40 \text{ mV} \)
\[
\alpha_s = 0.135 e^{0.80 + V/-6.8} \quad (A12)
\]
\[
\beta_s = 3.56 e^{0.707V} + 3.1 \times 10^7 e^{0.35V} \quad (A13)
\]
\[
\alpha_j = (-127.140 e^{0.2444V} - 3.474 \times 10^{-5} e^{-0.04391V}) \quad (A14)
\]
\[
\beta_j = 0.1212 e^{-0.1052V} \quad (A15)
\]
Inward rectifier K⁺ current (\(I_{K1}\))
\[
E_K = \frac{RT}{F} \ln \left( \frac{[K^+]_o}{[K^+]_i} \right) \quad (A16)
\]
\[
I_{K1} = \tilde{G}_{K1} \frac{[K^+]_o}{5.4} k_{1o} (V - E_K) \quad (A17)
\]
\[
k_{1o} = \frac{\alpha_{K1}}{\alpha_{K1} + \beta_{K1}} \quad (A18)
\]
\[
\alpha_{K1} = \frac{1.02}{1.0 + e^{(0.2393x - 69.2155)}} \quad (A19)
\]
\[
\beta_{K1} = 0.49124 e^{0.00832(V - E_K + 5.476)} + 0.006735 e^{-0.464(V - E_K - 0.31)} \quad (A20)
\]
Transient outward current (\(I_{to}\))
\[
I_{to} = \frac{1.0}{1.0 + ([4-AP]/K_{m4-AP})} \tilde{G}_{to} x_{to} y_{to} (V - E_K) \quad (A21)
\]
\[
\frac{dx_{to}}{dt} = \frac{x_{to} - x_{to}}{\tau_{sto}} \quad (A22)
\]
\[
\frac{dy_{to}}{dt} = \frac{y_{to} - y_{to}}{\tau_{sto}} \quad (A23)
\]
\[
x_{to} = \frac{1}{1 + e^{-V/1.13}} \quad (A24)
\]
\[
y_{to} = \frac{1}{1 + e^{-(V+38.89)/38.89}} \quad (A25)
\]
\[
\tau_{sto} = 0.33 + 3.67 e^{-[V+303/60]} \quad (A26)
\]
\[
\tau_{sto} = 11.67 + 15.00 e^{-[V+209/900]} \quad (A27)
\]
Delayed rectifier current (\(I_K\))
\[
I_K = \tilde{G}_K x_K y_K (V - E_K) \quad (A28)
\]
\[
\frac{dx_K}{dt} = \frac{x_K - x_K}{\tau_K} \quad (A29)
\]
\[
\frac{dy_K}{dt} = \frac{y_K - y_K}{\tau_K} \quad (A30)
\]
\[
x_{K} = \frac{1}{1 + e^{-V/9.914}} \quad (A31)
\]
\[
\sigma = \frac{1}{7} (e^{(N\text{a}^+/19.37) - 1}) \quad (A48)
\]
\[
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\]
\[
\tau_{sto} = 0.33 + 3.67 e^{-[V+303/60]} \quad (A26)
\]
\[
\tau_{sto} = 11.67 + 15.00 e^{-[V+209/900]} \quad (A27)
\]
Delayed rectifier current (\(I_K\))
\[
I_K = \tilde{G}_K x_K y_K (V - E_K) \quad (A28)
\]
\[
\frac{dx_K}{dt} = \frac{x_K - x_K}{\tau_K} \quad (A29)
\]
\[
\frac{dy_K}{dt} = \frac{y_K - y_K}{\tau_K} \quad (A30)
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x_{K} = \frac{1}{1 + e^{-V/9.914}} \quad (A31)
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\]
\[
\tau_{sto} = 11.67 + 15.00 e^{-[V+209/900]} \quad (A27)
\]
\[ I_{Na,b} = \bar{G}_{Na,b}(V - E_{Na}) \]  \hspace{1cm} (A53)

L-type Ca\(^{2+}\) current \((I_{Ca})\)
\[ \alpha = 0.4 e^{(V - 2)/10} \]  \hspace{1cm} (A54)
\[ \beta = 0.05 e^{(V - 2)/13} \]  \hspace{1cm} (A55)
\[ \alpha' = \alpha a \]  \hspace{1cm} (A56)
\[ \beta' = \beta b \]  \hspace{1cm} (A57)
\[ \gamma = 0.10375 (\text{Ca}^{2+})_s \]  \hspace{1cm} (A58)
\[ \frac{dC_0}{dt} = \beta C_1 + \omega C_{Cal} - (4\alpha + \gamma) C_0 \]  \hspace{1cm} (A59)
\[ \frac{dC_1}{dt} = 4\alpha C_0 + 2\beta C_2 + \omega \frac{b}{b'} C_{Cal} - (\beta + 3\alpha + \gamma) C_1 \]  \hspace{1cm} (A60)
\[ \frac{dC_2}{dt} = 3\alpha C_1 + 3\beta C_3 + \omega \frac{b}{b'} C_{Cal} - (2\beta + 2\alpha + \gamma) C_2 \]  \hspace{1cm} (A61)
\[ \frac{dC_3}{dt} = 2\alpha C_2 + 4\beta C_4 + \omega \frac{b}{b'} C_{Cal} - (3\beta + \alpha + \gamma) C_3 \]  \hspace{1cm} (A62)
\[ \frac{dC_4}{dt} = \alpha C_3 + gO + \omega \frac{b}{b'} C_{Cal} - (4\beta + f + \gamma) C_4 \]  \hspace{1cm} (A63)
\[ \frac{dO}{dt} = fC_4 - gO \]  \hspace{1cm} (A64)
\[ \frac{dC_{Cal}}{dt} = \beta' C_{Cal} + \gamma C_0 - (4\alpha' + \omega) C_{Cal} \]  \hspace{1cm} (A65)
\[ \frac{dC_{Cal}}{dt} = 4\alpha' C_0 + 2\beta' C_{Cal} + \gamma' C_1 - (\beta' + 3\alpha' + \omega') C_{Cal} \]  \hspace{1cm} (A66)
\[ \frac{dC_{Cal}}{dt} = 3\alpha' C_0 + 3\beta' C_{Cal} + \gamma' C_2 - (2\beta' + 2\alpha' + \omega') C_{Cal} \]  \hspace{1cm} (A67)
\[ \frac{dC_{Cal}}{dt} = 2\alpha' C_2 + 4\beta' C_{Cal} + \gamma' C_3 - (3\beta' + \alpha' + \omega') C_{Cal} \]  \hspace{1cm} (A68)
\[ \frac{dC_{Cal}}{dt} = \alpha' C_{Cal} + g'O + \omega' \frac{b}{b'} C_{Cal} - (4\beta' + f' + \gamma) C_{Cal} \]  \hspace{1cm} (A69)
\[ \frac{dO}{dt} = f'C_{Cal} - g'O \]  \hspace{1cm} (A70)
\[ I_{Ca} = \bar{I}_{Ca} \left( \frac{4VF^2}{C_{Sc}} \right) \frac{0.001 e^{2V_{PT}} - 0.341 [\text{Ca}^{2+}]_L}{e^{2V_{PT}} - 1} \]  \hspace{1cm} (A71)
\[ I_{Ca} = \bar{I}_{Ca} y (O + O_{ca}) \]  \hspace{1cm} (A72)
\[ I_{Ca,k} = \frac{P_k}{C_{Sc}} \left( \frac{y (O + O_{ca})}{RT} \right)^2 \left( [\text{K}^+]_e - [\text{K}]_o \right) \]  \hspace{1cm} (A73)
\[ P_k = \frac{\bar{I}_{Ca}}{I_{Ca,off}} \]  \hspace{1cm} (A74)
\[ \frac{dy}{dt} = \frac{y - y}{\tau_y} \]  \hspace{1cm} (A75)
\[ y = \frac{0.8}{1 + e^{(V + 2.29)/35}} + 0.2 \]  \hspace{1cm} (A76)

\[ \tau_y = 20 + \frac{600}{1 + e^{(V + 20)/35}} \]  \hspace{1cm} (A77)

RyR channel
\[ \frac{dP_{Cl}}{dt} = -k_u [C_{Cl}]_{SS} P_{Cl} + k_a P_{Cl} \]  \hspace{1cm} (A78)
\[ \frac{dP_{Cl}}{dt} = k_u [C_{Cl}]_{SS} P_{Cl} - k_a P_{Cl} - k_b [C_{Cl}]_{SS} P_{Cl} + k_P \]  \hspace{1cm} (A79)
\[ \frac{dP_{Cl}}{dt} = k_u [C_{Cl}]_{SS} P_{Cl} - k_a P_{Cl} \]  \hspace{1cm} (A80)
\[ \frac{dP_{Cl}}{dt} = k_u [C_{Cl}]_{SS} P_{Cl} - k_a P_{Cl} \]  \hspace{1cm} (A81)

RyR open probability:
\[ J_{rel} = \nu_1 (\text{RyR probability}) ([\text{Ca}^{2+}]_{SS} - [\text{Ca}^{2+}]_{LS}) \]  \hspace{1cm} (A82)

SERCA2a pump
\[ f_b = ([\text{Ca}^{2+}]_{LS}[\text{K}]_{vis}) \]  \hspace{1cm} (A83)
\[ f_b = ([\text{Ca}^{2+}]_{LS}[\text{K}]_{vis}) \]  \hspace{1cm} (A84)
\[ f_b = ([\text{Ca}^{2+}]_{LS}[\text{K}]_{vis}) \]  \hspace{1cm} (A85)
\[ J_{up} = K_{SR} \frac{1.0}{1 + ([\text{Ca}^{2+}]_{LS} - [\text{Ca}^{2+}]_{SS})} \]  \hspace{1cm} (A86)

Intracellular Ca\(^{2+}\) fluxes
\[ J_u = [\text{Ca}^{2+}]_{LS} - [\text{Ca}^{2+}]_{SS} \]  \hspace{1cm} (A87)
\[ J_{sler} = [\text{Ca}^{2+}]_{LS} - [\text{Ca}^{2+}]_{SS} \]  \hspace{1cm} (A88)
\[ J_{leak} = K_{Ca} ([\text{Ca}^{2+}]_{LS} - [\text{Ca}^{2+}]_{SS}) \]  \hspace{1cm} (A89)
\[ J_{Trpn} = \left[ \frac{d[\text{HTrpnCa}]}{dt} + \frac{d[\text{LTrpnCa}]}{dt} \right] \]  \hspace{1cm} (A90)
\[ \frac{d[\text{HTrpnCa}]}{dt} = k_{Trpn}[\text{Ca}^{2+}]_{LS}([\text{HTrpnCa}]_{SS} - [\text{HTrpnCa}]_{LS}) \]  \hspace{1cm} (A91)
\[ \frac{d[\text{LTrpnCa}]}{dt} = k_{Trpn}[\text{Ca}^{2+}]_{LS}([\text{LTrpnCa}]_{SS} - [\text{LTrpnCa}]_{LS}) \]  \hspace{1cm} (A92)
\[ \frac{d[\text{LTrpnCa}]}{dt} = k_{Trpn}[\text{Ca}^{2+}]_{LS}([\text{LTrpnCa}]_{SS} - [\text{LTrpnCa}]_{LS}) \]  \hspace{1cm} (A93)
\[ J_{parv} = N_{parv} \text{sin}(\text{Parv}_{SS} \frac{dy}{dt}) \]  \hspace{1cm} (A94)
\[ J_{parv,SS} = N_{parv} \text{sin}(\text{Parv}_{SS} \frac{dy}{dt}) \]  \hspace{1cm} (A95)

[HANDLING IN MYOCYTES. II. MODEL]

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\[
\frac{dPvM_{SS}}{dt} = k_{PvM}[^{2+}\text{Ca}]_{JSS}(1.0 - PvM_{SS}) - k_{PvM}PvM_{SS}
\]  
(A98)

\[
J_{Fura2} = [\text{Fura2}]_{JSS} \frac{d\text{Fura2Ca}}{dt} \quad (A99)
\]

\[
J_{Fura2, SS} = [\text{Fura2}]_{JSS} \frac{d\text{Fura2Ca}_{SS}}{dt} \quad (A100)
\]

\[
\frac{d\text{Fura2Ca}}{dt} = k_{\text{Fura2}}[^{2+}\text{Ca}](1.0 - \text{Fura2Ca}) - k_{\text{Fura2,Fura2Ca}} \quad (A101)
\]

\[
\frac{d\text{Fura2Ca}_{SS}}{dt} = k_{\text{Fura2}}[^{2+}\text{Ca}]_{SS}(1.0 - \text{Fura2Ca}_{SS}) - k_{\text{Fura2,Fura2Ca}_{SS}} \quad (A102)
\]

Intracellular ion concentrations and membrane voltage:

\[
\frac{dV}{dt} = -[I_{Na} + I_{Na\theta} + I_{NaCa} + I_{K} + I_{K\theta} + I_{SS} + I_{Kl} + I_{Ca\theta} + I_{Ca} + I_{Ca\theta} + I_{pCa}]  
\]

\[
+ \frac{d[Na^+]}{dt} = -(I_{Na} + I_{Na\theta} + 3I_{NaCa} + 3I_{NaK}) \frac{A_{cap} C_{m}}{V_{myoF}} \quad (A103)
\]

\[
\frac{d[K^+]}{dt} = -(I_{K} + I_{K\theta} + I_{SS} + I_{Kl} + I_{Ca\theta} - 2I_{NaK}) \frac{A_{cap} C_{m}}{V_{myoF}} \quad (A104)
\]

\[
\beta_i = \left[1 + \frac{C_{CaMyo} K_{CaMyo}}{(K_{CaMyo} + [^{2+}\text{Ca}])^{-1}} \right]^{-1} \quad (A105)
\]

\[
\beta_{SS} = \left[1 + \frac{C_{CaMyo} K_{CaMyo}}{(K_{CaMyo} + [^{2+}\text{Ca}]_{SS})^{-1}} \right]^{-1} \quad (A106)
\]

\[
\beta_{SR} = \left[1 + \frac{C_{SR} K_{SR}}{(K_{SR} + [^{2+}\text{Ca}]_{SR})^{-1}} \right]^{-1} \quad (A107)
\]

\[
\frac{d[^{2+}\text{Ca}]}{dt} = \beta_i \left[ (J_{act} - J_{aq} - J_{trp} - J_{pass} - J_{pass,2})  
\right.
\]

\[
- (I_{Ca\theta} - 2I_{NaCa} + I_{pCa}) \frac{A_{cap} C_{m}}{2V_{myoF}} \quad (A108)
\]

\[
\frac{d[^{2+}\text{Ca}]_{SS}}{dt} = \beta_{SS} \left[ (J_{act} + J_{Ca\theta}) \frac{V_{SR}}{V_{SS}} - J_{trp} \frac{V_{myo}}{V_{SS}} - J_{pass,SS} - J_{pass,SS,2} \right.  
\]

\[
- (I_{Ca\theta} - 2I_{NaCa} + I_{pCa}) \frac{A_{cap} C_{m}}{2V_{SSF}} \quad (A109)
\]

Cross-bridge movement model (X_c):

\[
\frac{dx_c}{dt} = B_l(L - x_c - h_c) \quad (A110)
\]

Cross-bridge states:

\[
T_{Ca} = \frac{[LTrpCa][LTrp]_{tot}}{[LTrpCa][LTrp]_{tot}} \quad (A111)
\]

REFERENCES

