Published in final edited form as:

J Mol Cell Cardiol. 2016 July ; 96: 38–48. doi:10.1016/j.yjmcc.2015.07.016.

There and back again: iterating between population-based modeling and experiments reveals surprising regulation of calcium transients in rat cardiac myocytes

Ryan A. Devenyi^a and Eric A. Sobie^a

^aDepartment of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, 1 Gustave L Levy Place, New York, NY, USA

Abstract

While many ion channels and transporters involved in cardiac cellular physiology have been identified and described, the relative importance of each in determining emergent cellular behaviors remains unclear. Here we address this issue with a novel approach that combines population-based mathematical modeling with experimental tests to systematically quantify the relative contributions of different ion channels and transporters to the amplitude of the cellular Ca^{2+} transient. Sensitivity analysis of a mathematical model of the rat ventricular cardiomyocyte quantified the response of cell behaviors to changes in the level of each ion channel and transporter, and experimental tests of these predictions were performed to validate or invalidate the predictions. The model analysis found that partial inhibition of transient outward current in rat ventricular epicardial myocytes was predicted to have a greater impact on Ca^{2+} transient amplitude than either: (1) inhibition of the same current in endocardial myocytes, or (2) comparable inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA). Experimental tests confirmed the model predictions qualitatively but showed some quantitative disagreement. This guided us to recalibrate the model by adjusting the relative importance of several Ca^{2+} fluxes, thereby improving the consistency with experimental data and producing a more predictive model. Analysis of human cardiomyocyte models suggests that the relative importance of outward currents to Ca^{2+} transporters is generalizable to human atrial cardiomyocytes, but not ventricular cardiomyocytes. Overall, our novel approach of combining population-based mathematical modeling with experimental tests has yielded new insight into the relative importance of different determinants of cell behavior.

Graphical Abstract

Corresponding Author: Eric A Sobie. ; Email: eric.sobie@mssm.edu

Conflicts of Interest: None.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

cardiac myocytes; parameter sensitivity analysis; cardiac Ca²⁺ transients; sarcoplasmic/endoplasmic-reticulum Ca²⁺ ATPase; cardiac potassium channels

Introduction

The cardiac action potential (AP) and Ca²⁺ transient (CaT) are key aspects of the electrical and mechanical functions of the heart. The cardiac AP is orchestrated by a number of different currents, most notably the fast Na⁺ current, the L-type Ca²⁺ current, and several K⁺ currents [1–3]. Each of these currents has complex dependence on time, voltage, and ion concentrations, each of which in turn depend on a large number of other players. The CaT is perhaps even more complex, as it depends greatly on the Ca²⁺ influx during the AP as well as a complex intracellular system of Ca²⁺ handling. Ca²⁺ influx through L-type channels locally triggers Ca²⁺ release through ryanodine receptors, and the dynamics of Ca²⁺ release depend on both Ca²⁺ fluxes across the cell membrane and the amount of Ca²⁺ stored in the sarcoplasmic reticulum (SR) [4–7]. Overall, emergent behaviors of the cardiomyocyte result from the dynamic integration of many ion channels, pumps and transporters, creating a need for quantitative mathematical models to allow for rigorous understanding of the system.

Mathematical models have provided a key tool to gain quantitative insight into a wide variety of complex systems in biology, such as cell signaling cascades [8], drug response in cancer [9], and behavior of neural networks [10, 11]. The long history of mathematical modeling of cardiomyocytes began with studies of the action potential [12], and has been extended to a huge range of physiological behaviors [13–15], such as excitation-contraction coupling [16], beta-adrenergic signaling [17], and arrhythmogenesis at both cellular [18] and tissue [19] levels. The ability to manipulate model components at will allows for inferences about causation and mechanism even in highly convoluted systems. This makes mathematical models an excellent playground for both developing and testing hypotheses. Nonetheless, experimental validation of key findings has always been a crucial complement to model analysis and can help drive further model development.

As mathematical models increase in complexity they increasingly resemble a “black box” from which it is difficult to intuit how high-level model behaviors (such as the cellular CaT) arise from model inputs (such as the levels of ion channels and transporters). “Population-based modeling” methods globally probe complex biological systems by studying a large number of model variants with broadly varied parameters to understand how the component parts work together to generate higher-level behaviors. One common application of this

strategy, parameter sensitivity analysis [20–28], can be used to distill the complex underlying model into readily interpretable sensitivity coefficients that quantify how changes in model parameters affect key behaviors.

Here we utilize a method previously developed by our group [20] (see Fig S1) to perform a sensitivity analysis of the model of the rat LV cardiomyocyte developed by Pandit and colleagues [29], a landmark model in rat cardiac electrophysiology and one that, along with several closely related models [30, 31], remains the primary model of rat action potentials and calcium transients in use today. We generated a population of model variants by randomly scaling the levels of 14 ion channels, pumps, and transporters, and used regression to quantitatively relate these model parameters to measurable model outputs such as Ca^{2+} transient amplitude (CaTA). This analysis gave rise to the novel prediction that membrane currents rather than Ca^{2+} pumps and transporters can primarily determine CaTA, especially in epicardial cells with shorter APs.

Experimental validation of unexpected novel results such as this can be very useful in determining whether they reflect true physiology or merely a quirk of the model. As each parameter sensitivity coefficient represents a quantitative prediction of how changes to an ion channel, pump or transporter will affect a measurement (e.g. CaTA), these coefficients can be experimentally tested by measuring the effect of partial pharmacological inhibition of a corresponding target. We proceeded to experimentally test our model-driven hypothesis in this manner. Our results qualitatively supported our hypothesis that membrane currents can have greater impact on CaTA than Ca^{2+} transporters, although there were significant quantitative differences between experimental results and model predictions. We used these differences to drive a targeted adjustment of the model to better match experimental findings. The work therefore demonstrates how experimental tests inspired by sensitivity analysis can both guide model improvement and yield new physiological insight.

Materials and Methods

2.1. Mathematical modeling

We performed parameter sensitivity analysis of the 2001 Pandit model of the rat LV cardiomyocyte [29] and the Grandi models of the human atrial [32] and ventricular [33] cardiomyocytes (Figure S1). A population of 300 model variants was generated for each model by randomly varying each of 14 parameters representing the activity levels of ion channels, pumps and transporters (Table 1), a population size more than sufficiently large to allow for convergence of sensitivity coefficients (Figure S9). Each parameter was independently varied with a log-normal distribution ($\text{CV}=0.2$). Simulations were run to a steady state at rest (1000 s), followed by pacing at 1 Hz until a new steady state (1000 s).

CaTA and action potential duration at 50% repolarization (APD_{50}) (collectively termed model “outputs”) were measured for each model variant. Multivariable regression (non-linear iterative partial least squares method) on log-transformed values was used to generate a matrix of sensitivity coefficients indicating the importance of each model parameter in determining that output. The accuracy of the regression was validated by strong agreement

between measured outputs with those predicted by multiplying the sensitivity coefficients by model parameters (Figure S1).

For comparison with experiments, dynamic binding of Ca^{2+} to fluo-3 was included assuming $[\text{Fluo-3}] = 5 \mu\text{M}$, $K_{\text{off}} = 90 \text{ s}^{-1}$ and $K_{\text{on}} = 80 \mu\text{M}^{-1}\text{s}^{-1}$ [34], which was compared to experiments assuming fluorescence only in the Ca^{2+} -bound state. Experimental conditions and stimulation protocols were mirrored in simulations for direct comparison. The true intracellular $[\text{Fluo-3}]$ in our experiments was not measured, but the model effects observed were largely independent of $[\text{Fluo-3}]$ (Figure S8).

Over the course of this study, discrepancies were noted between the Pandit model and experimental results. We subsequently made adjustments to the Ca^{2+} balance in the Pandit model to make it more consistent with experimental results. In this process we implemented an alternate formulation for SERCA developed by Tran and Crampin [35] as used in Williams *et al.* [36]. We also decreased the levels of NCX and PMCA and made additional modifications to preserve reasonable Ca^{2+} balance, as detailed in the Supplement.

Simulations were performed in Matlab (MathWorks, Natick MA) using the ode15s solver, with absolute tolerance 10^{-3} and relative tolerance 10^{-6} for each state variable. Code for running these simulations is included as a Supplemental file.

2.2. Solutions and Chemicals

Cell storage and experiments were carried out in modified Tyrode solution containing (in mM) NaCl 140, KCl 5.4, CaCl_2 1, MgCl_2 1, NaH_2PO_4 1, HEPES 10, and glucose 5 (pH 7.4, NaOH). Cell isolation was carried out in otherwise identical solutions with different concentrations of CaCl_2 , as noted. The Ca^{2+} -free digestion solution contained 0.35–0.5 mg/mL type II collagenase from *Clostridium histolyticum* (Worthington CLS-2, concentration adjusted for each lot) and 0.03 mg/mL type XIV protease from *Streptomyces griseus* (Sigma P5147). Cells were loaded with fluo-3 in a solution of 5 μM Fluo-3, acetoxymethyl (AM) ester (Biotium 50013) and 0.2% pluronic F-127 (Sigma P2443) in modified Tyrode solution with 0.8% DMSO. Cells were stained with di-8-ANEPPS in a solution of 10 μM di-8-ANEPPS (Biotium 61012) in 0.2% DMSO. AP measurements were made in presence of 10 μM blebbistatin (Sigma B0560) with 0.1% DMSO. Drugs applied during experiments were 4-aminopyridine (4-AP, Sigma-Aldrich A78403), cyclopiazonic acid (CPA) from *Penicillium cyclopium* (Sigma-Aldrich C1530), and caffeine (Sigma-Aldrich C7731). Solutions for CPA experiments contained 0.06% DMSO.

2.3. Cardiomyocyte isolation

Rat LV cardiomyocytes were isolated from 6–8 week old male Sprague-Dawley rats (Charles River) as previously described [37]. Briefly, rats were injected intraperitoneally with 20,000 U/kg heparin and 120 mg/kg sodium pentobarbital, and once the animal was unresponsive cervical dislocation was performed. The heart was excised and placed in cold modified Tyrode solution with 2 mM Ca^{2+} . After aortic cannulation, hearts were perfused in retrograde with, in order, Tyrode with 2 mM Ca^{2+} , nominally Ca^{2+} -free Tyrode, Ca^{2+} -free Tyrode containing collagenase and protease, and Tyrode with 0.1 mM Ca^{2+} . Digestion proceeded until the heart noticeably softened, about 6–10 minutes.

The LV wall was minced to release cells in 0.1 mM Ca^{2+} Tyrode. For epicardial and endocardial isolations, thin tissue slices were removed from the inner and outer surfaces of the LV wall. Ca^{2+} was slowly adjusted to 1 mM over 30 minutes. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai.

2.4. Experimental studies

Cells were loaded with Fluo-3-AM for 30 minutes. For experiments with CPA only, cells were washed for 15 minutes to allow for de-esterification of the dye. Recordings were made using a Zeiss confocal microscope with excitation by a 488 nm argon laser, and emission recorded through a 505 nm longpass filter. For AP measurements in epicardial and endocardial cells, cells were stained with di-8-ANEPPS for 17 minutes. Recordings were made with excitation by 543 nm HeNe laser, and emission recorded through a 560 nm longpass filter. Exponential decay was subtracted from raw traces to allow for AP analysis. All experiments were carried out at room temperature.

The effect of 4-AP on the CaT of an individual cell was measured by pacing that cell for 80 s at 1 Hz in the absence of drug, and then applying 66.7 μM 4-AP for 60s while continuing to pace. The CaTA for each treatment was taken to be the average of the final 20 CaTs recorded for each condition. Cells with unstable amplitudes at baseline or in 4-AP were excluded. To measure the effect of CPA on CaTs, CaTs were measured in groups of cells each treated with 0, 2, 3.5, or 6 μM CPA. Measurements were made from 10 cells in each group on each of 4 experimental days, and the order of treatments varied across experimental days to account for any possible effects of cell age on measurements. Each cell was paced for 35 s, and CaTA was taken as the average from the final 5 CaTs. The decay rate of Ca^{2+} transients was fit to an exponential in order to estimate K_{decay} for both electrically-evoked CaTs treated with different doses of CPA, as well as in the presence of 10–20 mM caffeine to functionally disable Ca^{2+} removal via SERCA. Data were processed in Matlab using custom scripts. Further details of the experimental protocol and analysis are provided in the Supplement. Statistical analysis was performed in SPSS. Error bars throughout represent 95% confidence interval (CI) of the mean.

Results

3.1. Parameter sensitivity analysis reveals contributions of ion channels, pumps and transporters to cell behaviors

We performed parameter sensitivity analysis to determine how the levels of 14 different currents and transporters (Table 1) influence the amplitude and shape of the CaT and AP. Two populations (based on the epicardial and endocardial versions of the Pandit model [29]) of 300 model variants were created by simultaneously randomizing these 14 parameters independently with a log-normal distribution. Separate epicardial and endocardial models were studied as epicardial cells display much shorter APs than endocardial cells due to greater density of the transient outward current (I_{to}) [3, 38]. The models were run to a steady-state after pacing at 1 Hz (1000 stimulations), and key outputs were measured, such as CaTA and APD_{50} , with each showing significant variability (Fig 1B,D). Multivariable

regression was used to generate sensitivity coefficients that represent quantitative, directional metrics of how each model parameter influences the output, such as CaTA (Fig S1).

The results of this analysis are shown in Figure 1. Unsurprisingly, the L-type Ca^{2+} current (I_{CaL} , the primary source of Ca^{2+} influx) and sodium-potassium ATPase (NKA, the target of inotropic cardiac glycosides) are important determinants of CaTA (Fig 1A). Also as expected, the main determinants of APD_{50} were I_{CaL} and I_{to} , the primary depolarizing and repolarizing currents for most of the AP, respectively (Fig 1B). However, the analysis also makes the surprising prediction that the conductance of the transient outward current (G_{to}) is also a predominant determinant of CaTA in epicardial cells while having little to no impact on CaTA in endocardial cells (Fig 1A). The effect in epicardial cells is also surprisingly much larger than the effects of the Ca^{2+} handling transporters, SERCA and NCX. SERCA, for instance, is predicted to have surprisingly little effect on CaTA. The effects of separately changing each parameter agree with the sensitivity coefficients (Fig 1E). We therefore sought to enable direct comparisons by measuring the effects of a relatively small degree of inhibition of SERCA and I_{to} under similar experimental conditions.

3.2. I_{to} inhibition increases CaTA in rat LV cardiomyocytes

We first set out to test the surprising prediction that I_{to} is a primary determinant of CaTA in epicardial cells only. Experiments were performed on cells separately isolated from LV epicardium and endocardium. Endocardial cells displayed longer APs (Fig S2) and delayed CaT time-to-peak (Fig 2F), consistent with the model and previous published work [3, 38, 39]. 4-aminopyridine (4-AP) is a selective blocker of I_{to} with $\text{IC}_{50} = 200 \mu\text{M}$ in rat ventricular cardiomyocytes [40]. We partially inhibited I_{to} using $66.7 \mu\text{M}$ 4-AP, which will inhibit the current by 25% assuming a linear relationship between drug binding and channel block. We compared this to a 25% reduction in the maximal conductance of I_{to} in the model (G_{to}).

As shown in Figure 2, 25% reduction in G_{to} leads to a 40.2% increase in CaTA (as measured by fluo-3 F/F₀) in the epicardial model (Fig 2A) but only a 0.9% increase in the endocardial model (Fig 2B). Experiments revealed that $66.7 \mu\text{M}$ 4-AP lead to a highly significant increase in CaTA (Fig 2E) not only in epicardial cells (Fig 2C,E, mean increase 36.2%, 95% CI $\pm 11.6\%$, $p < 0.001$ by paired t-test), but also in endocardial cells (Fig 2D,E, 15.1% $\pm 5.6\%$, $p < 0.001$), with a significantly larger effect in epicardial cells (Fig 2G, $p = 0.003$, independent t-test). Experimental tests therefore supported the idea that I_{to} can have a major impact on CaTA in epicardial cells, more so than in endocardial cells. However, while the model prediction for epicardial cells was well within the experimentally observed range, experiments found a much larger effect in endocardial cells than the model predicts, with the model result well outside the 95% confidence interval of experimental results. This indicates that I_{to} does have a major impact on CaTs, even in endocardial cells, though the effect is much smaller than in epicardial cells.

3.3. Differential effects of I_{to} inhibition on CaTA depend on dynamics of I_{CaL} gating

After experimentally confirming that I_{to} block has a greater effect on CaTA in epicardial cells than endocardial cells, we returned to the model to understand the underlying mechanisms. In Figure 3 we demonstrate that the dynamics of I_{CaL} gating account for this difference. Reduction in G_{to} prolongs the AP in both models, but only substantially increases the duration of I_{CaL} in the epicardial model, where the baseline AP is much shorter (Fig 3A,D). Therefore reduction in G_{to} greatly increases the total Ca^{2+} influx integrated over the course of an AP (Q_{CaL}) in the epicardial model, but not in the endocardial model (Fig 3B,E).

This is due to differences in the relative importance of activation and inactivation gating in driving I_{CaL} closure in the two models. In the epicardial model, as a result of the shorter AP I_{CaL} is terminated largely due to closure of the voltage-dependent activation gate as the cell repolarizes, which is of course dependent on I_{to} . Prolonging the AP by reducing G_{to} delays the closure of this activation gate, prolonging I_{CaL} (Fig 3C). In contrast, in the endocardial model the AP is long enough that I_{CaL} largely self-terminates by inactivation, which is independent of APD. Therefore prolonging the AP by reducing G_{to} has little effect on I_{CaL} (Fig 3F). This suggests a hypothesis for why there is in fact an effect in endocardial cells that is not demonstrated in the model. If I_{CaL} is in reality partially constrained by I_{to} -mediated repolarization in endocardial cells, this could explain why experiments demonstrated a small increase in CaTA after I_{to} block.

3.4. SERCA inhibition decreases CaTA more than the model predicts

We next set our sights on the model prediction that moderate changes in SERCA activity have only a minor impact on CaTA. This is a surprising prediction given the established importance of SERCA under both physiological and pathophysiological conditions [7]. This experimental test not only assesses the specific prediction related to SERCA, but also the more general hypothesis that the major membrane currents play a larger role in determining CaTA in rat than the Ca^{2+} handling apparatus.

The results of this analysis are shown in Figure 4. The model shows only extremely minor decreases in CaTA with significant reductions in SERCA (Figs 4A, S4). Note that conversion to fluo-3 F/F₀ for comparison to experiments reduces the apparent effect size of SERCA inhibition, but the effects on Ca^{2+} remain quite small. Since there are no reports quantifying dose-dependent inhibition of SERCA by CPA in rat cardiomyocytes, we performed a dose-response curve and compared the dose-response relationship to the model by the effect of CPA on $K_{decay,SERCA}$, defined as the difference in the rate of decay of CaT in the presence and absence of caffeine (Fig S3). SERCA levels in Figure 4A were chosen to match the experimentally-observed decrease in $K_{decay,SERCA}$ seen with each concentration of CPA. CPA significantly decreased CaTA and K_{decay} and prolonged CaT duration in a dose-dependent manner (Fig 4C–E).

The effect on CaTA is much larger than the model predicts (Fig 4F), so the model greatly under-estimates the importance of SERCA in determining CaTA. Nonetheless, although the effect of SERCA inhibition on CaTA is greater than the model predicted, the effect is still

smaller than the experimentally measured effect of I_{to} inhibition. Therefore, these experiments support the qualitative hypothesis that I_{to} actually has a greater impact on CaTA in epicardial cells

3.5. Ca^{2+} fluxes in the Pandit model are improperly balanced

Figure 5 demonstrates how closer examination of the model reveals that the balance of Ca^{2+} fluxes in this model is strikingly inconsistent with experimental results. In this model SERCA accounts for only a relatively small proportion of the removal of Ca^{2+} from the cytosol (44% in the endocardial model (Fig 5A), 30% in the epicardial (Fig S6) model), whereas it has been estimated from experimental data to account for 92% of Ca^{2+} removal in rats [5]. In addition, the concentration of Ca^{2+} in the SR during pacing is much lower in the model (18 or 11 $\mu\text{mol/L}$ cytosol, endocardial and epicardial, respectively, see Fig 5B) than has been measured experimentally (73–210 $\mu\text{mol/L}$ cytosol) [2, 41–43]. Therefore, the Pandit model greatly underestimates the contribution of SR Ca^{2+} release to the CaT, which results from an under-representation of the contribution of SERCA to Ca^{2+} removal.

3.6. Adjusting Ca^{2+} fluxes in the model produces more physiological behaviors

Given these discrepancies with experimental results, we chose to improve the model by altering the balance of various Ca^{2+} fluxes (Fig 5, S6). We began by increasing the contribution of SERCA to Ca^{2+} efflux by increasing the levels of SERCA and decreasing the levels of NCX and PMCA (Fig 5A). After these adjustments, the original SERCA formulation in the Pandit model was unable to maintain diastolic $[Ca^{2+}]$ lower than 300 nM. We therefore replaced this with the formulation developed by Tran and Crampin [35], which demonstrates more physiological dependence of SERCA pumping on SR load (Fig S5). After additional modifications to prevent Ca^{2+} overload and maintain reasonable CaT and AP characteristics (see Supplement), Ca^{2+} fluxes and SR Ca^{2+} levels are much more in line with experimental measurements (Fig 5).

In this adjusted model, the contribution of SERCA to Ca^{2+} removal is increased to values more consistent with experimental estimates (90.2% and 87.2% in endocardial and epicardial models, respectively), and SR load is increased into a more physiological range (136 and 102 $\mu\text{mol/L}$ cytosol). As a result, the effect of SERCA inhibition on CaTA more closely matches what is experimentally observed, with the model predictions falling well within the experimental observed range (Figure 6A,C).

We repeated our parameter sensitivity analysis on this adjusted model (Fig 6D). In this model CaTA is more sensitive to changes in SERCA compared to the original model, but nonetheless this contribution remains significantly smaller than that of I_{to} in the epicardial model. This analysis therefore represents an improved quantitative assessment of the contribution of each parameter to cell behavior that is more consistent with experimental results and therefore has improved predictive power.

3.7. Outward membrane currents are similarly important in a model of human atrial cells

As the shape and underlying currents of the human ventricular AP are very different in rat, there is no reason to expect that this mechanism will generalize to human ventricular cells.

However, the human atrial AP is shorter and has a shape more similar to rat ventricular cardiomyocytes, and we sought to determine if outward currents could similarly influence CaTs in these cells.

In Figure 7 we show the results of sensitivity analysis of the Grandi models of human atrial [32] and ventricular [33] cardiomyocytes. In the ventricular model, the primary repolarizing current is the rapid delayed rectifier current (I_{Kr} , Fig 7A) [44, 45], which has distinctly slower kinetics than I_{to} . As a consequence, I_{to} has no significant impact on CaTA, although I_{Kr} does have a relatively minor effect (Fig 7B). However, in the atrial model, the primary repolarizing currents are I_{to} and the ultrarapid outward current (I_{Kur}), another current that activates early following AP upstroke (Fig 7C) [46]. These two currents both have significant impact on CaTA in the atrial model (Fig 7D), greater than the impact of SERCA and comparable to the impact of NKA and NCX. Therefore, the major role played by K^+ currents in determining CaTA that we demonstrated in rat also has potential relevance for human atrial cells.

Discussion

4.1. Quantitative importance of I_{to} and SERCA in determining CaTA

Our approach here has involved an innovative iteration between population-based model analysis and experimental tests in order to gain insight into mechanisms controlling CaTs in cardiac myocytes. This has led us to the conclusion that in rat epicardial cells, the repolarizing current I_{to} is actually more important in determining CaTA than SERCA, a key Ca^{2+} handling protein. I_{to} exerts its influence on the CaT by constraining Ca^{2+} influx, an effect that is more prominent when APs are short and I_{to} -mediated repolarization shuts off I_{CaL} via deactivation rather than inactivation of the L-type Ca^{2+} channel. The magnitude of the effect is therefore greater in epicardial compared with endocardial cells. Although the roles that I_{to} and SERCA play in excitation-contraction coupling have been previously studied, the fact that our experimental tests were made under common conditions and linked by our quantitative model predictions allows us to draw novel conclusions about the relative importance of the two.

Many published studies have shown that strong inhibition of I_{to} can increase CaTA [3, 39, 47–49] and inhibition of SERCA can decrease it [50, 51]. Some studies have also further shown that I_{to} inhibition can have greater effects in epicardial cells [3, 39]. However, due to the fact that in general these studies made these assessments using relatively drastic perturbations (such as near-complete block by very high concentrations of drug) under varying experimental conditions makes it impossible to draw conclusions about the relative importance of the two. Here we quantified how relatively small partial block of I_{to} and SERCA affects CaTA under similar experimental conditions, which in coordination with model analysis allowed us to draw the general conclusion that in epicardial cells CaTA is more sensitive to changes in the level of I_{to} than SERCA.

One recent study has performed a careful quantification of how varying degrees of SERCA block affects CaTA. Bode *et al.* [51] demonstrated that when voltage-clamped rat CMs were excited with 100 ms square depolarizing pulses, partial SERCA inhibition has a near-linear

relationship with CaTA, a much stronger relationship than predicted by the Pandit model. However, in the absence of the endogenous AP waveform, it is impossible to draw conclusions about the relative importance of membrane currents and Ca^{2+} handling proteins. Despite drastic differences in the experimental protocol, these findings are roughly in agreement with our experimental results, further supporting our findings and reinforcing the physiological relevance of the work of Bode *et al.*

4.2. Implications for other species

Given that AP shape and the role played by I_{to} in rat ventricle differs drastically from humans and other large mammals, these findings cannot be directly related to human ventricular physiology. Indeed, analysis of a ventricular model [33] shows that I_{to} has essentially no impact on CaTA (Figure 7A,B). However, human atrial CMs have much shorter APs in which I_{to} and a second early K^+ current, the ultrarapid outward current I_{Kur} , play an important role in repolarization [46, 52, 53]. Analysis of a human atrial model [32] suggests that the influence of these early outward currents in human atria may be analogous to the important role we have demonstrated in rat ventricular myocytes. This result highlights that results obtained in rodent ventricular myocytes may yield important lessons for understanding human atrial physiology, even if a direct comparison with human ventricular physiology is frequently not valid.

4.3. Benefits of PSA methods

Although many modeling studies focus on testing specific hypotheses with targeted model changes, they can also be used for more global study of the behavior of complex systems. “Population-based” modeling approaches have broad applicability in generating new physiological insight in a systematic, undirected manner, which can then be followed up by more detailed study. As these populations of models exhibit highly variable behavior, these approaches provide an ideal tool for studying how behavioral variability can originate from differences between individual samples in the levels of ion channels and transporters [20–27]. Here we have exploited a second benefit of this modeling strategy, which is that these approaches produce systematic, quantitative predictions regarding the relative importance of different ion channels and transporters in determining physiological behaviors. These strategies are highly applicable to other aims as well, such as identifying novel drug targets [26] and understanding the integration of complex changes in diseases such as heart failure [54].

4.4. Importance of experimental tests of model predictions

Continuous interplay between simulations and experiments is essential. Here we accentuate the importance of experimentally testing predictions made by model analysis. Parameter sensitivity analysis, as we have performed here (Fig 1), can lead to exciting new predictions but can also potentially expose model flaws that had previously been overlooked. Experimental tests are necessary to differentiate between these two possibilities for any novel prediction. Here we undertook one of the first attempts to test the predictions of a sensitivity analysis, and found a middle ground between these two extremes: the qualitative prediction that I_{to} plays a greater role than SERCA in determining CaTA held true, but

quantitative disagreements between simulations and data identified model weaknesses and suggested corresponding improvements.

Many experimental approaches involve drastic changes to protein activity levels, such as knockout or overexpression of proteins, or near-complete block with high doses of drug. However, in order to test predictions about more moderate changes in activity levels, a different approach with only partial inhibition was needed. Additionally, as a membrane K^+ channel and an intracellular Ca^{2+} pump are functionally very different, to quantitatively assess the relative importance of the two in determining CaTA it was essential to compare their effects with the same assay under the same conditions, as we have done here. Together with the model analysis, this approach allows for quantitative assessment of the role played by different ion channels and transporters under physiological conditions, and can be extrapolated to applications such as natural variability between individuals and cells.

4.5. Model adjustments in response to experimental results

George Box famously stated that “All models are wrong, but some are useful” [55]. The results presented here effectively demonstrate both sides of this aphorism. For instance, despite the fact that sarcolemmal and SR Ca^{2+} fluxes in the original Pandit model were inconsistent with experimental data, the model nonetheless generated the accurate prediction that K^+ currents can have a greater impact on CaTA than SERCA in rat epicardial cardiomyocytes. The inaccurate predictions from this model were also useful, since these results guided us to adjust Ca^{2+} fluxes, producing better agreement with both our experimental results and several previous studies [5, 56]. After replacing the SERCA formulation with one that would function properly at physiological SR loads [35], we adjusted the balance of Ca^{2+} fluxes to match previously reported experimental data [5], thereby producing a model that predicted effects of SERCA inhibition more accurately (Fig 6). This approach is similar in spirit to prior studies in mouse myocyte models that have re-parameterized Ca^{2+} fluxes to better reflect cellular physiology [57, 58].

Our findings therefore demonstrate how globally probing a model with population-based model analysis can be used as a driver for model improvement, especially when combined with experimental tests of predictions. Even the most sophisticated models are generally validated through a limited number of model behaviors, and population-based modeling provides a further lens to study the model for weaknesses. This iterative process allows for the development of a model that is useful under more circumstances, even if some aspects of the model remain incorrect.

4.6. Limitations and Future Work

Our adjustments do not represent a systematic approach to model improvement but instead are somewhat limited in scope and based on hand-tuning the model to match new experimental data. Although this targeted strategy has proven successful, the procedure also leaves features of the model that remain to be updated. For example, Ca^{2+} -induced Ca^{2+} release assumes release from a homogeneous population of ryanodine receptors, rather than more realistic stochastic triggering of local release events (Ca^{2+} sparks) [59], a property that is essential for proper modeling of graded CICR [4]. Additionally, the phenomenological

calcium release mechanism does not simulate realistic local depletion of SR $[Ca^{2+}]$, an effect that is known to be important for proper termination of Ca^{2+} release [60, 61]. However, as the experimental results demonstrate, phenomenological models that lack mechanistic details of Ca^{2+} release can still be useful for addressing questions that depend primarily on the balance of fluxes across the cellular and SR membranes [21].

The model could also be further improved by the addition of a local subsarcolemmal space where membrane proteins such as NCX are exposed to higher Ca^{2+} concentrations [62, 63]. Additionally, the fact that in contrast to experimental results in the endocardial model I_{to} inhibition has essentially no effect on CaTA suggests that the formulation for I_{CaL} is imperfect. Finally, although we tested several key predictions of our sensitivity analysis, additional predictions remain untested, which could guide further model improvement. However, despite these limitations our adjustments greatly improved agreement with experimental results.

Our parameter-sensitivity analysis methodology quantifies the effect of variability over a fairly wide range of parameter values, but three potential limitations of this approach should be noted. One is that the importance of a parameter may change when expression levels change drastically, as may occur during knockout or overexpression experiments, or in some genetic diseases. Second, parameter sensitivities could also interact with each other. For instance, a given parameter may become more important when another parameter that performs a partially redundant function is reduced. Third, in performing our analysis we assume that all parameters vary independently and to the same relative degree. These assumptions may not hold, as for example in neuronal systems it has been demonstrated that ion channel expression levels are often correlated with each other [64–66]. Nonetheless, while inaccuracies in these assumptions would change the distribution of behaviors in the population, the consequences for a given degree of variability in a parameter would remain unchanged.

Conclusion

Our population-based parameter sensitivity analysis systematically probed the quantitative contributions of 14 ion channels, pumps and transporters in determining the AP and CaT. We focused on the model prediction that, in determining CaTA, I_{to} is of greater importance than SERCA in epicardial cells, but is of little importance in endocardial cells. This hypothesis was qualitatively supported by experimental tests that measured the effect of partial inhibition of I_{to} and SERCA on Ca^{2+} transients, but there were significant quantitative differences between simulations and experiments. These quantitative differences guided model improvement, namely a more realistic representation of the balance of Ca^{2+} fluxes and the contribution of SR Ca^{2+} release. This represents a novel manner of exploring the emergent properties of the model under a range of parameters, and has allowed us to both generate and test novel physiological hypotheses and find and address model weaknesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health grants R01 HL076230 and R21 HL122564 to EAS. Mr. Devenyi was previously supported by National Institutes of Health training grant T32 GM007280. The authors would like to thank Christine Falce for assistance with experiments.

Abbreviations

AP	action potential
APD₅₀	action potential duration at 50% repolarization
CaT	Ca ²⁺ transient
CaTA	Ca ²⁺ transient amplitude
LV	left ventricle
I_{to}	transient outward current
I_{Kur}	ultra-rapidly rectifying current
I_{CaL}	L-type Ca ²⁺ current
SERCA	sarco/endoplasmic reticulum Ca ²⁺ ATPase
NCX	Na ⁺ /Ca ²⁺ exchanger
PMCA	plasma membrane Ca ²⁺ ATPase
NKA	Na ⁺ /K ⁺ ATPase
RyR	ryanodine receptor
K_{decay}	decay rate of CaT
K_{decay,caff}	CaT decay rate in presence of caffeine
K_{decay,SERCA}	SERCA-dependent CaT decay rate
SR	sarcoplasmic reticulum
4-AP	4-aminopyridine
CPA	cyclopiazonic acid
F/F₀	relative fluorescence
CV	coefficient of variation

Works Cited

1. Apkon M, Nerbonne JM. Characterization of two distinct depolarization-activated K⁺ currents in isolated adult rat ventricular myocytes. *J Gen Physiol.* 1991; 97:973–1011. [PubMed: 1865177]
2. Varro A, Lathrop DA, Hester SB, Nanasi PP, Papp JG. Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes. *Basic Res Cardiol.* 1993; 88:93–102. [PubMed: 8389123]

3. Clark RB, Bouchard RA, Salinasstefanon E, Sanchezchapula J, Giles WR. Heterogeneity of action-potential wave-forms and potassium currents in rat ventricle. *Cardiovasc Res.* 1993; 27:1795–9. [PubMed: 8275526]
4. Cannell MB, Kong CH. Local control in cardiac E-C coupling. *J Mol Cell Cardiol.* 2012; 52:298–303. [PubMed: 21586292]
5. Bassani JW, Bassani RA, Bers DM. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J Physiol.* 1994; 476:279–93. [PubMed: 8046643]
6. Wier WG, Egan TM, Lopez-Lopez JR, Balke CW. Local control of excitation-contraction coupling in rat heart cells. *J Physiol.* 1994; 474:463–71. [PubMed: 8014907]
7. Eisner D, Bode E, Venetucci L, Trafford A. Calcium flux balance in the heart. *J Mol Cell Cardiol.* 2013; 58:110–7. [PubMed: 23220128]
8. Orton RJ, Sturm OE, Vyshemirsky V, Calder M, Gilbert DR, Kolch W. Computational modelling of the receptor-tyrosine-kinase-activated MAPK pathway. *Biochem J.* 2005; 392:249–61. [PubMed: 16293107]
9. Tyson JJ, Baumann WT, Chen C, Verdugo A, Tavassoly I, Wang Y, et al. Dynamic modelling of oestrogen signalling and cell fate in breast cancer cells. *Nat Rev Cancer.* 2011; 11:523–32. [PubMed: 21677677]
10. Marder E, Prinz AA. Modeling stability in neuron and network function: the role of activity in homeostasis. *Bioessays.* 2002; 24:1145–54. [PubMed: 12447979]
11. Williams AH, Kwiatkowski MA, Mortimer AL, Marder E, Zeeman ML, Dickinson PS. Animal-to-animal variability in the phasing of the crustacean cardiac motor pattern: an experimental and computational analysis. *J Neurophysiol.* 2013; 109:2451–65. [PubMed: 23446690]
12. Noble D. A modification of the Hodgkin–Huxley equations applicable to Purkinje fibre action and pace-maker potentials. *J Physiol.* 1962; 160:317–52. [PubMed: 14480151]
13. Niederer SA, Smith NP. At the heart of computational modelling. *J Physiol.* 2012; 590:1331–8. [PubMed: 22271869]
14. Roberts BN, Yang PC, Behrens SB, Moreno JD, Clancy CE. Computational approaches to understand cardiac electrophysiology and arrhythmias. *Am J Physiol Heart Circ Physiol.* 2012; 303:H766–83. [PubMed: 22886409]
15. Carusi A, Burrage K, Rodriguez B. Bridging experiments, models and simulations: an integrative approach to validation in computational cardiac electrophysiology. *Am J Physiol Heart Circ Physiol.* 2012; 303:H144–55. [PubMed: 22582088]
16. Shannon TR, Wang F, Puglisi J, Weber C, Bers DM. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. *Biophys J.* 2004; 87:3351–71. [PubMed: 15347581]
17. Saucerman JJ, Brunton LL, Michailova AP, McCulloch AD. Modeling beta-adrenergic control of cardiac myocyte contractility in silico. *J Biol Chem.* 2003; 278:47997–8003. [PubMed: 12972422]
18. Chudin E, Goldhaber J, Garfinkel A, Weiss J, Kogan B. Intracellular Ca(2+) dynamics and the stability of ventricular tachycardia. *Biophys J.* 1999; 77:2930–41. [PubMed: 10585917]
19. Sato D, Shiferaw Y, Garfinkel A, Weiss JN, Qu Z, Karma A. Spatially discordant alternans in cardiac tissue: role of calcium cycling. *Circ Res.* 2006; 99:520–7. [PubMed: 16902177]
20. Sobie EA. Parameter sensitivity analysis in electrophysiological models using multivariable regression. *Biophys J.* 2009; 96:1264–74. [PubMed: 19217846]
21. Lee YS, Liu OZ, Sobie EA. Decoding myocardial Ca(2+)(+) signals across multiple spatial scales: a role for sensitivity analysis. *J Mol Cell Cardiol.* 2013; 58:92–9. [PubMed: 23026728]
22. Maltsev VA, Lakatta EG. Numerical models based on a minimal set of sarcolemmal electrogenic proteins and an intracellular Ca2+ clock generate robust, flexible, and energy-efficient cardiac pacemaking. *J Mol Cell Cardiol.* 2013; 59:181–95. [PubMed: 23507256]
23. Cummins MA, Devenyi RA, Sobie EA. Yoga for the sinoatrial node: Sarcoplasmic reticulum calcium release confers flexibility. *J Mol Cell Cardiol.* 2013; 60
24. Britton OJ, Bueno-Orovio A, Van Ammel K, Lu HR, Towart R, Gallacher DJ, et al. Experimentally calibrated population of models predicts and explains intersubject variability in cardiac cellular electrophysiology. *PNAS.* 2013; 110

25. Sarkar AX, Sobie EA. Quantification of repolarization reserve to understand interpatient variability in the response to proarrhythmic drugs: A computational analysis. *Heart Rhythm*. 2011; 8:1749–55. [PubMed: 21699863]
26. Cummins MA, Dalal PJ, Bugana M, Severi S, Sobie EA. Comprehensive analyses of ventricular myocyte models identify targets exhibiting favorable rate dependence. *PLoS Comput Biol*. 2014; 10:e1003543. [PubMed: 24675446]
27. Gemmell P, Burrage K, Rodriguez B, Quinn TA. Population of computational rabbit-specific ventricular action potential models for investigating sources of variability in cellular repolarisation. *PLoS ONE*. 2014; 9:e90112. [PubMed: 24587229]
28. Lee YS, Liu OZ, Hwang HS, Knollmann BC, Sobie EA. Parameter sensitivity analysis of stochastic models provides insights into cardiac calcium sparks. *Biophys J*. 2013; 104:1142–50. [PubMed: 23473497]
29. Pandit SV, Clark RB, Giles WR, Demir SS. A mathematical model of action potential heterogeneity in adult rat left ventricular myocytes. *Biophys J*. 2001; 81:3029–51. [PubMed: 11720973]
30. Padmala S, Demir SS. Computational model of the ventricular action potential in adult spontaneously hypertensive rats. *J Cardiovasc Electrophysiol*. 2003; 14:990–5. [PubMed: 12950545]
31. Niederer SA, Smith NP. A mathematical model of the slow force response to stretch in rat ventricular myocytes. *Biophys J*. 2007; 92:4030–44. [PubMed: 17369410]
32. Grandi E, Pandit SV, Voigt N, Workman AJ, Dobrev D, Jalife J, et al. Human atrial action potential and Ca²⁺ model: sinus rhythm and chronic atrial fibrillation. *Circ Res*. 2011; 109:1055–66. [PubMed: 21921263]
33. Grandi E, Pasqualini FS, Bers DM. A novel computational model of the human ventricular action potential and Ca transient. *J Mol Cell Cardiol*. 2010; 48:112–21. [PubMed: 19835882]
34. Smith GD, Keizer JE, Stern MD, Lederer WJ, Cheng H. A simple numerical model of calcium spark formation and detection in cardiac myocytes. *Biophys J*. 1998; 75:15–32. [PubMed: 9649364]
35. Tran K, Smith NP, Loiselle DS, Crampin EJ. A thermodynamic model of the cardiac sarcoplasmic/endoplasmic Ca(2+) (SERCA) pump. *Biophys J*. 2009; 96:2029–42. [PubMed: 19254563]
36. Williams GS, Chikando AC, Tuan HT, Sobie EA, Lederer WJ, Jafri MS. Dynamics of calcium sparks and calcium leak in the heart. *Biophys J*. 2011; 101:1287–96. [PubMed: 21943409]
37. Ramay HR, Liu OZ, Sobie EA. Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity. *Cardiovasc Res*. 2011; 91:598–605. [PubMed: 21613275]
38. Shimoni Y, Severson D, Giles W. Thyroid status and diabetes modulate regional differences in potassium currents in rat ventricle. *J Physiol*. 1995; 488(Pt 3):673–88. [PubMed: 8576857]
39. Volk T, Nguyen TH, Schultz JH, Ehmke H. Relationship between transient outward K⁺ current and Ca²⁺ influx in rat cardiac myocytes of endo- and epicardial origin. *J Physiol*. 1999; 519(Pt 3):841–50. [PubMed: 10457095]
40. Castle NA, Slawsky MT. Characterization of 4-aminopyridine block of the transient outward K⁺ current in adult rat ventricular myocytes. *J Pharmacol Exp Ther*. 1993; 265
41. Callewaert G, Cleemann L, Morad M. Caffeine-induced Ca²⁺ release activates Ca²⁺ extrusion via Na⁺-Ca²⁺ exchanger in cardiac myocytes. *Am J Physiol*. 1989; 257:C147–52. [PubMed: 2750887]
42. Bassani RA, Bers DM. Rate of diastolic Ca release from the sarcoplasmic reticulum of intact rabbit and rat ventricular myocytes. *Biophys J*. 1995; 68:2015–22. [PubMed: 7612843]
43. Trafford AW, Diaz ME, Eisner DA. A novel, rapid and reversible method to measure Ca buffering and time-course of total sarcoplasmic reticulum Ca content in cardiac ventricular myocytes. *Pflugers Arch*. 1999; 437:501–3. [PubMed: 9914410]
44. Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier K⁺ current. Differential sensitivity to block by class III antiarrhythmic agents. *J Gen Physiol*. 1990; 96:195–215. [PubMed: 2170562]

45. Konarzewska H, Peeters GA, Sanguinetti MC. Repolarizing K⁺ currents in nonfailing human hearts. Similarities between right septal subendocardial and left subepicardial ventricular myocytes. *Circulation*. 1995; 92:1179–87. [PubMed: 7648663]
46. Wang Z, Fermini B, Nattel S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K⁺ current similar to Kv1.5 cloned channel currents. *Circ Res*. 1993; 73:1061–76. [PubMed: 8222078]
47. Sah R, Ramirez RJ, Kaprielian R, Backx PH. Alterations in action potential profile enhance excitation-contraction coupling in rat cardiac myocytes. *J Physiol (Lond)*. 2001; 533:201–14. [PubMed: 11351028]
48. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, Zobel C, et al. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I_{to}). *J Physiol*. 2003; 546:5–18. [PubMed: 12509475]
49. Bouchard RA, Clark RB, Giles WR. Effects of action-potential duration on excitation-contraction coupling in rat ventricular myocytes. Action-potential voltage-clamp measurements. *Circ Res*. 1995; 76:790–801. [PubMed: 7728996]
50. Li SY, Golden KL, Jiang Y, Wang GJ, Privratsky JR, Zhang X, et al. Inhibition of sarco(endo)plasmic reticulum Ca²⁺-ATPase differentially regulates contractile function in cardiac myocytes from normotensive and spontaneously hypertensive rats: role of Ca²⁺ regulatory proteins. *Cell Biochem Biophys*. 2005; 42:1–12. [PubMed: 15673924]
51. Bode EF, Briston SJ, Overend CL, O'Neill SC, Trafford AW, Eisner DA. Changes of SERCA activity have only modest effects on sarcoplasmic reticulum Ca²⁺ content in rat ventricular myocytes. *J Physiol (Lond)*. 2011; 589:4723–9. [PubMed: 21825024]
52. Escande D, Coulombe A, Faivre JF, Deroubaix E, Coraboeuf E. Two types of transient outward currents in adult human atrial cells. *Am J Physiol*. 1987; 252:H142–8. [PubMed: 3028160]
53. Shibata EF, Drury T, Refsum H, Aldrete V, Giles W. Contributions of a transient outward current to repolarization in human atrium. *Am J Physiol*. 1989; 257:H1773–81. [PubMed: 2557769]
54. Walmsley J, Rodriguez JF, Mirams GR, Burrage K, Efimov IR, Rodriguez B. mRNA expression levels in failing human hearts predict cellular electrophysiological remodeling: a population-based simulation study. *PLoS ONE*. 2013; 8:e56359. [PubMed: 23437117]
55. Box, GEP.; Draper, NR. Empirical model-building and response surfaces. New York: Wiley; 1987.
56. Bers, DM. Excitation-contraction coupling and cardiac contractile force. 2. Dordrecht; Boston: Kluwer Academic Publishers; 2001.
57. Li L, Niederer SA, Idigo W, Zhang YH, Swietach P, Casadei B, et al. A mathematical model of the murine ventricular myocyte: a data-driven biophysically based approach applied to mice overexpressing the canine NCX isoform. *Am J Physiol Heart Circ Physiol*. 2010; 299:H1045–63. [PubMed: 20656884]
58. Li L, Louch WE, Niederer SA, Andersson KB, Christensen G, Sejersted OM, et al. Calcium dynamics in the ventricular myocytes of SERCA2 knockout mice: A modeling study. *Biophys J*. 2011; 100:322–31. [PubMed: 21244828]
59. Cheng H, Lederer WJ. Calcium sparks. *Physiol Rev*. 2008; 88:1491–545. [PubMed: 18923188]
60. Radwanski PB, Belevych AE, Brunello L, Carnes CA, Gyorke S. Store-dependent deactivation: cooling the chain-reaction of myocardial calcium signaling. *J Mol Cell Cardiol*. 2013; 58:77–83. [PubMed: 23108187]
61. Sobie EA, Lederer WJ. Dynamic local changes in sarcoplasmic reticulum calcium: physiological and pathophysiological roles. *J Mol Cell Cardiol*. 2012; 52:304–11. [PubMed: 21767546]
62. Trafford AW, Diaz ME, O'Neill SC, Eisner DA. Comparison of subsarcolemmal and bulk calcium concentration during spontaneous calcium release in rat ventricular myocytes. *J Physiol*. 1995; 488(Pt 3):577–86. [PubMed: 8576849]
63. Weber CR, Ginsburg KS, Philipson KD, Shannon TR, Bers DM. Allosteric regulation of Na/Ca exchange current by cytosolic Ca in intact cardiac myocytes. *J Gen Physiol*. 2001; 117:119–31. [PubMed: 11158165]
64. Schulz DJ, Goillard J-M, Marder E. Quantitative expression profiling of identified neurons reveals cell-specific constraints on highly variable levels of gene expression. *Proceedings of the National Academy of Sciences*. 2007; 104:13187–91.

65. Tobin A-E, Cruz-Bermudez ND, Marder E, Schulz DJ. Correlations in ion channel mRNA in rhythmically active neurons. PLoS ONE. 2009; 4:e6742. [PubMed: 19707591]
66. Temporal S, Lett KM, Schulz DJ. Activity-dependent feedback regulates correlated ion channel mRNA levels in single identified motor neurons. Curr Biol. 2014; 24:1899–904. [PubMed: 25088555]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

1. Sensitivity analysis was performed on a model of the rat left ventricular myocyte.
2. I_{to} has greater impact on Ca^{2+} transients than SERCA in the epicardial model.
3. This model prediction was qualitatively supported by experimental tests.
4. The model was adjusted to better quantitatively match our experimental results.
5. K^+ currents also have major impact on Ca^{2+} transients in a human atrial model.

Figure 1.

Parameter sensitivity analysis on the Pandit model. 14 parameters were independently varied to create a population of 300 variants based on each of the epicardial and endocardial formulations, and steady-state CaTA and APD₅₀ were measured. **a,c.** Sensitivity coefficients demonstrating the quantitative importance of each parameter in determining CaTA (**a**) and APD₅₀ (**c**) in the epicardial (green) and endocardial (blue) models. **b,d.** Distributions of CaTA (**b**) and APD₅₀ (**d**) in population of model variants (epicardial in green, endocardial in blue) plotted on a log scale. **e.** Predictions of the effect of scaling parameters using sensitivity coefficients (dashed lines) match the effect of scaling each parameter individually (shapes). Missing values in endocardial model are due to the failure of the model to produce an AP that repolarizes appropriately.

Figure 2.

Model predictions and experimental tests of importance of I_{to} in determining CaTA. Reducing G_{to} by 25% leads to a 40% increase in CaTA in the Pandit epicardial model (a) but very little change in the endocardial model (b). Experimental inhibition of I_{to} by $\approx 25\%$ with $66.7 \mu\text{M}$ 4-AP leads to a 36.2% (95% CI $\pm 11.6\%$) increase in CaTA in epicardial cells (c) and a 15.1% ($\pm 5.6\%$) increase in endocardial cells (d). e. 4-AP leads to significant increases in CaTA in both epicardial cells ($p < 0.001$, paired t-test) and endocardial cells ($p < 0.001$). The difference in baseline CaTA between epicardial and endocardial cells is not significant (independent two-way t-test). f. 4-AP leads to significant prolongation of CaT time to peak in both epicardial ($p < 0.001$) and endocardial ($p < 0.001$) cells. Endocardial cells show a significantly later baseline time to peak than epicardial cells ($p = 0.005$), reflecting longer Ca^{2+} influx. g. The 4-AP dependent increase in CaTA in epicardial cells is significantly larger than in endocardial cells (two-way independent samples t-test, $p = 0.003$). Asterisks represent significance levels, with * for $p < 0.05$, ** for $p < 0.005$, *** for $p < 0.001$). Error bars: 95% CI.

Figure 3.

Mechanism of differential effects of I_{to} block in the model. **a,d.** In the epicardial model (**a**), AP prolongation in response to 25% reduction in G_{to} also leads to greatly prolonged activation of I_{CaL} , whereas in the endocardial model (**d**) I_{CaL} activation is only slightly altered. **b,e.** Total I_{CaL} flux over the course of an AP is greatly increased by AP prolongation that results from G_{to} reduction in the epicardial model (**b**), but is essentially unchanged by the same perturbation in the endocardial model (**e**). **c.** In the epicardial model, I_{CaL} terminates largely due to closure of the activation gate during AP repolarization, and therefore I_{CaL} remains open longer when the AP is prolonged by 25% reduction in G_{to} . **f.** In the endocardial model, I_{CaL} terminates primarily due to time-dependent inactivation, and therefore its open probability is essentially unchanged by AP prolongation.

Figure 4.

Effects of partial SERCA inhibition by CPA dose-response. When the effect on $K_{\text{decay, SERCA}}$ is matched to experimental results in the CPA dose-response, the endocardial version of the model (a) shows little effect of SERCA reduction on CaTA, in contrast to a significant effect seen in experiments (b). CPA titration leads to significant decreases in CaTA (c) and rate of CaT decay (d) and significantly prolongs CaTD (e) (one-way-ANOVA, $p < 0.001$ for each). f. Dose-response relationship of SERCA inhibition (as estimated by $K_{\text{decay, SERCA}}$) shows a much larger effect with experimental inhibition by CPA than in simulations with reductions in K_{SERCA} . $n = 40$ for each group. Asterisks represent

significance with Tukey post-hoc test, with * for $p<0.05$, ** for $p<0.005$, *** for $p<0.001$).
Error bars: 95% CI.

Figure 5.

Adjustments to improve balance of Ca^{2+} flux in the endocardial model **a**. Model adjustments to improve the balance of Ca^{2+} fluxes via different transport mechanisms over the course of one Ca^{2+} transient at the steady state. **b**. Ca^{2+} flux adjustments produce a model with greater SR load and slightly larger Ca^{2+} transient with faster decay of the CaT, but similar AP.

Figure 6.

Effect of SERCA inhibition and parameter sensitivities in model with adjusted Ca^{2+} balance. When the effect on $K_{\text{decay, SERCA}}$ is matched to experimental results in the CPA dose-response, the endocardial version of the model with adjusted Ca^{2+} fluxes (a) shows significant effects of SERCA reduction on CaTA, similarly to what is seen in experiments (b). c. Dose-response of the effect of SERCA inhibition (as measured by $K_{\text{decay, SERCA}}$) on CaTA in experiments as well as simulated in the adjusted model, in both epicardial (green) and endocardial (blue) formulations. d. Parameter sensitivities of the adjusted model. Error bars: $\pm$ 95% CI

Figure 7.

Parameter sensitivities in models of human cardiomyocytes. **a.** In a human ventricular model, I_{K_r} is the primary repolarizing current, while the transient outward currents have only a small effect. **b.** In ventricular cells, CaTA is primarily determined by I_{CaL} in conjunction with NKA and NCX, with I_{K_r} having a small role. **c.** In a human atrial model, the primary repolarizing currents are I_{to} and I_{Kur} , with I_{K_r} and I_{Ks} playing smaller roles. **d.** In human atrial cells, the primary determinant of CaTA is I_{CaL} , but I_{to} and I_{Kur} make significant contributions, as do NKA and NCX.

Table 1

Parameters varied in sensitivity analysis

Pandit model parameters:	
Parameter	Meaning
G_{Na}	Fast Na^+ current, max. conductance
G_{CaL}	L-type Ca^{2+} current, max. conductance
G_{to}	Transient outward K^+ current, max. conductance
G_{ss}	Steady-state outward K^+ current, max. conductance
G_{K1}	Inwardly rectifying K^+ current, max. conductance
G_f	Hyperpolarization-activated current, max. conductance
G_{NaB}	Background Na^+ current, conductance
G_{KB}	Background K^+ current, conductance
G_{CaB}	Background Ca^{2+} current, conductance
v_{RyR}	Ryanodine receptor flux, max. velocity
v_{NKA}	Na^+/K^+ ATPase current, max. velocity
v_{PMCA}	Plasma membrane Ca^{2+} ATPase current, max. velocity
v_{NCX}	Na^+/Ca^{2+} exchanger current, max. velocity
v_{SERCA}	Sarco/endoplasmic reticulum Ca^{2+} ATPase flux, max. velocity
Additional parameters for Grandi models:	
Parameter	Meaning
G_{toS}	Transient outward K^+ current, slow component, max. conductance
G_{toF}	Transient outward K^+ current, fast component, max. conductance
G_{Kur}	Ultra-rapid rectifying K^+ current, max. conductance
G_{Kr}	Rapid delayed rectifying K^+ current, max. conductance
G_{Ks}	Slow delayed rectifying K^+ current, max. conductance
G_{Kp}	Plateau K^+ current, max. conductance
G_{ClCa}	Ca^{2+} -activated Cl^- current, max. conductance