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

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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 the 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.

1. Introduction

The cardiac action potential (AP) and Ca^{2+} 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 Ca^{2+} current, and several K^+ currents [1–3]. Each of these currents has a complex dependence on time, voltage, and ion concentrations, each of which in turn depends on a large number of other players. The CaT is perhaps even more complex, as it depends greatly on the Ca^{2+} influx during the AP as well as a complex intracellular system of Ca^{2+} handling. Ca^{2+} influx through L-type channels locally triggers Ca^{2+} release through ryanodine receptors, and the dynamics of Ca^{2+} release depend on both Ca^{2+} fluxes across the cell membrane and the amount of Ca^{2+} 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

Abbreviations: AP, action potential; APD_{50}, 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/F_0, relative fluorescence; CV, coefficient of variation; CM, cardiomyocyte; PSA, parameter sensitivity analysis.

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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 distil 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 multivariable 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 such unexpected results 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.

2. 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 (Fig. 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 (Fig. S9). Each parameter was independently varied with a log-normal distribution (CV = 0.2). Simulations were run to a steady state at rest (1000s), followed by pacing at 1 Hz until a new steady state was reached (1000s).

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 (Fig. S1).

For comparison with experiments, dynamic binding of Ca^{2+} to fluo-3 was included assuming [Fluo-3] = 5 μM, $K_{off} = 90\ s^{-1}$ and $K_{on} = 80\ \mu M^{-1}\ s^{-1}$ [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 [Fluo-3] in our experiments was not measured, but the model effects observed were largely independent of [Fluo-3] (Fig. S8).

Over the course of this study, discrepancies were noted between the Pandit model and experimental results. We subsequently made adjustments to Ca^{2+} fluxes 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. A bundle containing Matlab code to reproduce each result in the paper is included as part of the Supplemental Data.

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 the 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 min.

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 min. 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.