Modeling Cardiac Function

Raimond L. Winslow

The Center for Cardiovascular Bioinformatics and Modeling
The Johns Hopkins University School of Medicine and
Whiting School of Engineering

Correspondence:
Raimond L. Winslow, PhD
Rm. 201B Clark Hall
The Johns Hopkins University
3400 N. Charles St. Baltimore MD 21218
410-516-5417 (Office); 410-516-5294 (FAX)
rwinslow@bme.jhu.edu

Chapter to be included in:

1. Introduction

Cardiac electrophysiology is a field with an extensive history of integrative modeling that is closely coupled with both the design and interpretation of experiments. The first models of the cardiac action potential (AP) were developed shortly after the Hodgkin-Huxley model of the squid AP\textsuperscript{1,2}, and were formulated in order to explain the experimental observation that unlike the neuronal AP, cardiac APs exhibit a long duration plateau phase. It was not long after the formulation of these early myocyte models that initial models of electrical conduction in cardiac tissue were formulated and applied to yield clinically useful insights into mechanisms of arrhythmia\textsuperscript{3}. This close interplay between experiment and integrative modeling continues today, with new model components and applications being developed in close coordination with the emergence of new sub-cellular, cellular and whole-heart data describing cardiac function in health and disease.

This chapter will review the current state of integrative modeling of the heart, focusing on three topics. First, we will review the integration of experimental data into the most commonly used class of ventricular myocyte models –common pool models. These models take the form of coupled systems of ordinary differential-algebraic equations. We will exam both the successes and failures of these common pool models. Second, we will review the formulation of a new class of myocyte models known as local-control models. These models take the form of coupled systems of stochastic differential equations, whose properties are evolved in time using a combination of Monte Carlo simulation and numerical integration. While these models are more computationally intensive than are common pool models, they are able to capture critically important aspects of single channel behaviors that have a profound impact on myocyte function, and which cannot be described using common pool models. Finally, we will review how cellular
models may be integrated with imaging data on heart geometry and micro-anatomic structure to formulate computational models of cardiac ventricular electrical conduction.

2. Cellular Models

2.1 The Cardiac Action Potential

In order to understand properties of modern computational models of the cardiac myocyte, it is necessary to review the ionic mechanisms giving rise to the cardiac AP. In this and all other sections of this chapter, we will focus exclusively on description and models of the properties of cardiac ventricular myocytes, as the properties of these cells figure so importantly in the genesis of heart disease.

Figure 1A shows a schematic illustration of the large mammalian cardiac AP. The currents mediating the AP upstroke (Phase 0) are the fast inward sodium (Na$^+$) current ($I_{Na}$, for review see 4), and to a lesser extent the L-Type Ca$^{2+}$ current ($I_{Ca,L}$, for review see 5). The Phase 1 notch which is apparent in ventricular myocytes isolated from epi- and mid-myocardial regions, but which is largely absent in those isolated from the endo-cardium, is produced by activation of the voltage-dependent transient outward potassium (K$^+$) current ($I_{to,1}$). In the canine, a transient voltage-independent Ca$^{2+}$-modulated Cl$^-$ current contributes to the Phase 1 notch ($I_{to,2}$), however, this current is not known to be expressed in human. The Phase 2 plateau is a time during which membrane conductance is very low, with potential being determined by a delicate balance between small inward and outward currents. The major inward plateau current is $I_{Ca,L}$, and major outward plateau currents are generated by the rapid and slow-activating delayed outward rectifier K$^+$ currents $I_{Kr}$ and $I_{Ks}$, respectively, and the plateau K$^+$ current $I_{Kp}$. Finally, repolarization Phase 3 is produced by the hyperpolarizing activated inward rectifier K$^+$ current $I_{K1}$. 
Three major ion transporters and exchangers play a critically important role in shaping properties of the cardiac AP, Ca\(^{2+}\) transient, and in long term regulation of intracellular ion concentrations. These are the sarcolemmal Na\(^+\)-K\(^+\) pump, the sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchanger and the SR Ca\(^{2+}\)-ATPase. The sarcolemmal Na\(^+\)-K\(^+\) pump, present in virtually all mammalian cell membranes, extrudes 3 Na\(^+\) ions while importing 2 K\(^+\) ions on each cycle. This pump functions to keep intracellular Na\(^+\) low, thereby maintaining the external versus internal gradient of Na\(^+\), by extruding Na\(^+\) that enters during each AP. Cycling of this pump requires hydrolysis of 1 ATP molecule, and generates a net outward movement of 1 positive charge, thus contributing to outward membrane current and influencing resting membrane potential.

The sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchanger imports three Na\(^+\) ions for every Ca\(^{2+}\) ion extruded, yielding a net charge movement. It is driven by both transmembrane voltage and intra- and extracellular Na\(^+\) and Ca\(^{2+}\) ion concentrations. It functions in forward mode during diastole, in which case it extrudes Ca\(^{2+}\) and imports Na\(^+\) thus generating a net inward current. It is the principal means by which Ca\(^{2+}\) is extruded from the myocyte following each AP, particularly during the diastolic interval. Due to the voltage- and Ca\(^{2+}\)-sensitivity of the exchanger, experimental evidence indicates that it can function in reverse mode during the plateau phase of the AP, in which case it extrudes Na\(^+\) and imports Ca\(^{2+}\) thus generating a net outward current.

A second major cytoplasmic Ca\(^{2+}\) extrusion mechanism is the SR Ca\(^{2+}\)-ATPase. This ATPase pumps Ca\(^{2+}\) from the cytosol into the NSR. The SR Ca\(^{2+}\)-ATPase has both forward and reverse components, with the reverse component serving to prevent overloading of the SR with Ca\(^{2+}\) at rest. An additional Ca\(^{2+}\) extrusion mechanism is the sarcolemmal Ca\(^{2+}\)-ATPase. This pump hydrolyzes ATP to transport Ca\(^{2+}\) out of the cell. However, it contributes a sarcolemmal
current which is small relative to that of the Na\(^+\)-Ca\(^{2+}\) exchanger, with estimates indicating perhaps that as little as 3% of Ca\(^{2+}\) extrusion from the myocyte being mediated by this pump.

2.2 The Structure of Myocyte Models

Development of myocyte models began in the early 1960’s with publication of Purkinje fiber AP models. Subsequent elaboration of these models led to development of the first biophysically-based cell model describing interactions between voltage-gated membrane currents, membrane pumps and exchangers that regulate Ca\(^{2+}\), Na\(^+\) and K\(^+\) levels, and additional intracellular Ca\(^{2+}\) cycling processes in the cardiac myocyte - the DiFrancesco-Noble model of the Purkinje fiber\(^8\). This important model established the conceptual framework from which all subsequent models of the myocyte have been derived (ventricular myocytes\(^9\)-\(^12\); SA node cells\(^13\)-\(^16\) and atrial myocytes\(^17\),\(^18\)). These models have proven reproductive and predictive properties and have been applied to advance our understanding of myocyte function in both health and disease.

Each of the integrative models of the myocyte cited above are of a type known as “common pool” models\(^19\), the structure of which is shown in Fig. 1B. In such models, Ca\(^{2+}\) flux through both L-type Ca\(^{2+}\) channels (LCCs) and ryanodine sensitive Ca\(^{2+}\) release channels (RyRs) in the JSR membrane is directed into a single common Ca\(^{2+}\) compartment referred to as the subspace. The subspace represents the total volume of the ~ 5,000 diadic spaces present in the ventricular myocyte. Stern demonstrated that common pool models are structurally unstable, exhibiting all-or-none Ca\(^{2+}\) release except (possibly) over some narrow range of model parameters\(^20\). This instability occurs because Ca\(^{2+}\) release from JSR produces a large, rapid increase of Ca\(^{2+}\) concentration in the subspace. This in turn results in a very strong positive feedback effect in which increased binding of Ca\(^{2+}\) to RyR induces further RyR channel opening.
and release of Ca\(^{2+}\). Despite this inability to reproduce experimentally measured properties of graded JSR Ca\(^{2+}\) release, common pool models have been very successful in reproducing and predicting a range of myocyte behaviors. This includes properties of interval-force relationships that depend heavily on proper dynamic modeling of intracellular Ca\(^{2+}\) uptake and release mechanisms\(^{21}\). In the following sections we describe the components from which common pool models are formed.

2.3 Model Components - Ion Channels & Currents

For many years Hodgkin-Huxley models have been the standard for describing membrane current kinetics\(^{22}\). However, data obtained using new experimental approaches, in particular those for producing recombinant channels by co-expression of genes encoding pore-forming and accessory channel subunits in host cells, have shown these models have significant limitations. First, while these models can be expanded to an equivalent Markov chain representation having multiple closed and inactivated states\(^{23}\), many single channel behaviors such as mean open time, first latency and a broad range of other kinetics behaviors cannot be described using these equivalent Markov models\(^{6,24}\). Second, where it has been studied in detail, as is the case for cardiac Na channels, Hodgkin-Huxley models are insufficient for reproducing behaviors that may be critically state-dependent, such as how ionic channels interact with drugs and toxins\(^{25,26}\). Accordingly much recent effort in modeling of cardiac ionic currents has focused on development of biophysically detailed Markov chain models of channel gating. We will therefore illustrate the generic concepts involved in modeling of ion channel function and membrane currents by reviewing our recent efforts to model the cardiac sodium (Na\(^{+}\)) channel\(^{27}\). This model is able to reproduce and predict a wide range of single channel and whole-cell
current properties\textsuperscript{27}, and the ways in which this model is formulated and constrained is illustrative of modern approaches to ion channel and current modeling.

Structure of the model is shown in Fig. 2A. The channel can occupy any of 13 states. The top row of states corresponds to zero to four voltage sensors being activated (C\textsubscript{0} through C\textsubscript{4}) plus an additional conformational change required for opening (C\textsubscript{4}→O\textsubscript{1} and C\textsubscript{4}→O\textsubscript{2}). The bottom row of states corresponds to channel inactivation. Affinity of the inactivation particle binding site is hypothesized to increase by a scaling factor (a) as the channel activates and to decrease by the same factor as the channel deactivates. Closed-closed and closed-open transitions (horizontal transitions) are voltage dependent and closed-inactivated transitions (vertical transitions) are voltage independent. Transition rates are of a form given by Eyring rate theory\textsuperscript{23}, and include explicit temperature dependence:

\[
\lambda = \frac{kT}{h} \exp\left(\frac{-\Delta H_\lambda}{RT} + \frac{\Delta S_\lambda}{R} + \frac{z_\lambda FV}{RT}\right)
\]

where \(k\) is the Boltzmann constant, \(T\) is the absolute temperature, \(h\) is the Planck constant, \(R\) is the gas constant, \(F\) is Faraday’s constant, \(\Delta H_\lambda\) is the change in enthalpy, \(\Delta S_\lambda\) is the change in entropy, \(z_\lambda\) is the effective valence (i.e., the charge moved times the fractional distance the charge is moved through the membrane), and \(V\) is the membrane potential in volts.

The probability of occupying any particular channel state is described by a set of ordinary differential equations, written in matrix notation as

\[
\frac{\partial \mathbf{P}(t)}{\partial t} = \mathbf{WP}(t),
\]

where \(\mathbf{P}(t)\) is a vector state occupancy probabilities and \(\mathbf{W}\) is the state transition matrix. \(\mathbf{W}\) is in general a function of voltage and time. For the voltage-clamp conditions generally used to constrain ion current models, \(\mathbf{W}\) is piece-wise time-independent, thus Eq. 2 has the analytic
solution

\[ P(t) = \exp(Wt)P(0). \] (3)

Current through an ensemble of Na channels is calculated as

\[ I_{Na}(t) = NG_{Na}P_{open}(t)(V(t) - E_{Na}(t)) \] (4)

where \( I_{Na}(t) \) is Na current, \( N \) is the number of Na channels, \( G_{Na} \) is single channel conductance, \( P_{open}(t) \) is the probability of occupying the open states \((O_1 + O_2)\), \( V(t) \) is membrane potential, and \( E_{Na}(t) \) is the reversal potential for Na given by the Nernst equation.

The number of coupled differential equations, and hence the number of parameters that need to be constrained for the model may be reduced through application of two fundamental principles. First, the state occupancy probabilities for a Markov chain model must sum to one. Second, there are several loops in the model that must satisfy the principle of microscopic reversibility. Microscopic reversibility is derived from the law of conservation of energy and states that the product of rate constants when traversing a loop clockwise must be equal to the product when traversing the same loop counterclockwise\(^{23}\). For the closed-closed-inactivated loops, satisfying microscopic reversibility requires that the transitions among the closed-inactivated states be scaled by \( a \), the same factor used to scale the transitions between rows. Microscopic reversibility is preserved around the closed-open-inactivated loop by isolating the \( \Delta H, \Delta S, \) and \( z \) terms in the product and satisfying each term separately using the following equations:

\[ \Delta H_{\gamma \gamma} = \Delta H_{\gamma} + \Delta H_{on} + \Delta H_{\delta \delta} + \Delta H_{of} + 8RT \ln a - \Delta H_{\delta} - \Delta H_{cn} - \Delta H_{of} \] (5)

\[ \Delta S_{\gamma \gamma} = \Delta S_{\gamma} + \Delta S_{on} + \Delta S_{\delta \delta} + \Delta S_{of} - \Delta S_{\delta} - \Delta S_{cn} - \Delta S_{of} \] (6)

\[ z_{\gamma \gamma} = z_{\gamma} + z_{on} + z_{\delta} + z_{of} - z_{\delta \delta} \] (7)

Similarly, microscopic reversibility is preserved around the closed-open-open loop using the
following equations for $\Delta H_\eta$, $\Delta S_\eta$, and $z_\eta$:

$$\Delta H_\eta = \Delta H_\gamma + \Delta H_\zeta + \Delta H_\nu - \Delta H_\delta - \Delta H_\omega$$  \hspace{1cm} (8)

$$\Delta S_\eta = \Delta S_\gamma + \Delta S_\zeta + \Delta S_\nu - \Delta S_\delta - \Delta S_\omega$$  \hspace{1cm} (9)

$$z_\eta = z_\gamma + z_\zeta - z_\nu.$$ \hspace{1cm} (10)

These microscopic reversibility constraints thus reduce the dimension of the parameter estimation problem, as transition rates $\gamma$, $\zeta$, and $\eta$ are fully constrained.

The model of Fig. 2A may also be viewed as a Markov chain description of single channel behavior. Single channel gating may be simulated using the method of Clay and DeFelice. In this method, the length of time a channel stays in its current state (i.e., its dwell time denoted as $T_j$) is calculated according to the formula

$$T_j = -(\ln r)\sqrt{\sum_{k=1}^{x} \lambda_{jk}}$$ \hspace{1cm} (11)

where $r$ is a random variable drawn from a uniform distribution on the interval [0,1] and $\lambda_{jk}$ is the transition rate from state $j$ to state $k$. The sum is over the $x$ pathways out of state $j$. The resulting dwell time $T_j$ is an exponentially distributed random variable with parameter $\lambda = \sum_{k=1}^{x} \lambda_{jk}$. At the end of the dwell time, the new state of the channel is determined by assigning random numbers to a portion of the interval [0,1] based on the probabilities of changing to neighboring states. These probabilities are equal to the rate constant for a particular transition divided by the sum of the rate constants for all possible transitions. Once the new state is determined, another random number is used to calculate the dwell time in the new state. At an instantaneous voltage step, channels remain in their current state, but the dwell times are recalculated.

Extensive experimental data were required to fully determine the model parameters. The
majority of data were taken from human SCN5A-encoded Na channels. Experimental data obtained at temperatures of 13° and 21°C were used to constrain the model, and the ability of the model to predict data collected at 17°C was tested. Constraining data included: a) ionic currents in response to voltage-clamp; b) gating charge accumulation; c) steady-state inactivation curve; d) rate of tail current relaxation; e) time course of recovery from inactivation; and f) single channel open times. A cost function defined as the squared error between simulated and experimental data (including both whole-cell current and single-channel data) was minimized to determine an optimal model parameter set. A simulated annealing algorithm was needed to perform this minimization, as the cost function exhibited many local minima. The resulting model was able to reproduce a broad range of membrane current data, and Figs. 2B-E demonstrates the ability of the model to predict channel/current properties at 17°C as well as single channel data not included in the fitting process. A similar methodology has been used to develop quantitative models of other myocyte membrane currents, most notably I_{Kr}, I_{Ks}, I_{CaL}, and I_{to}.\(^{12,30-32}\)

2.4 Model Components – Intracellular Ion Concentration Changes

We illustrate the process of modeling time-varying changes of intracellular ion concentration with reference to the common pool model architecture shown in Fig. 1B. In the common pool model formulation, there are four distinct Ca\(^{2+}\) compartments (the cytosol, subspace, NSR and JSR) and one Na\(^+\) and potassium (K\(^+\)) compartment (the cytosol). Note that in present common pool myocyte models, the cytosolic concentrations of both Na\(^+\), K\(^+\) and Ca\(^{2+}\) are assumed to be uniform. The time rate of change of concentration \(C_i\) of the \(i\)th ionic species in a given compartment is given by
where \( C_i(t) \) is concentration (typically mM) of species \( i \), \( t \) is time (typically mSec), \( I_i(t) \) is net current into the compartment carried by species \( i \) (typically in pA), \( z_i \) is valence of the \( i^{th} \) species, \( F \) is Faraday's constant and \( V \) is compartment volume (typically in units of pL). One such equation may be defined for the concentration of each ionic species in each model compartment.

Ion flux between compartments, related to the term \( I_i(t) \) in Eq. (12), is produced either by: a) diffusion due to differences in ion species concentration between adjacent compartments (as is the case for the flux term \( J_{xfer} \) in Fig. 1B representing \( Ca^{2+} \) diffusion from the subspace to the cytosol); b) gating of ion channels in the sarcolemmal or JSR membrane (as is the case for \( Ca^{2+} \) flux \( J_{rel} \) in Fig. 1B from the JSR into the subspace through RyR channels); or c) the action of membrane transporters and exchangers (for example, \( Ca^{2+} \) flux through the SR \( Ca^{2+}\)-ATPase, labeled \( J_{up} \) in Fig. 1B). The form of the algebraic equations describing function of membrane transporters and exchangers, including their concentration-, voltage- and in some instances ATP-dependence, may be found in the published equations for a number of myocyte models. In addition, buffering of \( Ca^{2+} \) by negatively charged phospholipids head groups in the sarcolemmal and JSR subspace membrane, by cytosolic myofilaments (troponin) and calsequestrin in the JSR is modeled. Buffering due to mechanisms other than myofilaments is described using the rapid buffer approximation of Wagner and Keizer\(^{33}\).

### 2.5 Composite Equations for Common Pool Models

Common pool models of the cardiac myocyte consist of systems of nonlinear ordinary differential-algebraic equations describing the time evolution of model state variables. These state variables are: a) probability of occupancy of ion channel states (Eq. 2) and current flux
through open channels (Eq. 4); b) concentrations of ion species in model compartments (Eq. 12); and c) time-evolution of membrane potential. Currently, all biophysically detailed model of the myocyte assume that since these cells are spatially compact, they are isopotential, with time-rate-of-change of membrane potential given by:

$$\frac{dv(t)}{dt} = -\left\{ \sum_i I_{i^{\text{ion}}}[v(t)] + \sum_i I_{i^{\text{pump}}}[v(t), c(t)] \right\}$$  \hspace{1cm} (13)

where $v(t)$ is membrane potential, $I_{i^{\text{ion}}}[v(t)]$ is current carried by the $i^{\text{th}}$ membrane current, and $I_{i^{\text{pump}}}[v(t), c(t)]$ is current through the $i^{\text{th}}$ membrane pump/exchanger, which can depend on both membrane potential $v(t)$ and the relevant ion concentration $c(t)$. Figure 3 shows examples of simulated normal APs (solid line, Fig. 3C) and Ca\textsuperscript{2+} transients (solid line, Fig. 3D) compared with those measured from isolated canine ventricular myocytes (AP – solid line, Fig. 3A; Ca\textsuperscript{2+} transient – solid line, Fig. 3B). These data demonstrate that common pool models have been quite successful in reconstruction of the AP and in reconstructing some aspects (the time-varying waveform) of the Ca\textsuperscript{2+} transient. In the following section, we illustrate how such models may be used to gain insights into cardiovascular disease mechanisms.

2.6 Application – Modeling the Molecular Basis of Heart Failure

Heart failure (HF), the most common cardiovascular disorder, is characterized by ventricular dilatation, decreased myocardial contractility and cardiac output. Prevalence in the general population is over 4.5 million, and increases with age to levels as high as 10%. New cases number approximately 400,000 per year. Patient prognosis is poor, with mortality roughly 15% at one year, increasing to 80% at six years subsequent to diagnosis. It is now the leading cause of Sudden Cardiac Death (SCD) in the U.S. An increased understanding of the molecular
basis of this disease therefore offers the possibility of improved treatments that can reduce the risk of SCD.

Experimental studies have now identified two major features of the cellular phenotype of heart failure. First, ventricular myocytes isolated from failing human\textsuperscript{34} and canine\textsuperscript{35,36} hearts exhibit significant AP prolongation. An example of this AP prolongation recorded from normal versus failing canine ventricular myocytes is shown in Fig. 3A (normal and failing APs shown in solid and dashed lines, respectively). Duration of the failing AP (~ 660 mSec) is roughly twice that of the normal (~ 330 mSec). AP duration is controlled by the balance between inward and outward membrane currents during the plateau phase of the AP. Possible explanations for this prolongation are therefore HF-induced up-regulation of inward currents, and/or down-regulation of outward currents. Second, failing ventricular myocytes exhibit altered Ca\textsuperscript{2+} transients. An example of normal and failing Ca\textsuperscript{2+} transients obtained simultaneously with the AP recordings of Fig. 3A is shown in Fig. 3B. Differences between normal (solid line) and failing (dotted line) Ca\textsuperscript{2+} transients include: a) reduced amplitude; and b) reduced rate of decline of the Ca\textsuperscript{2+} transient subsequent to repolarization of the AP.

There is little evidence to support the idea that an up-regulation of inward currents is responsible for prolongation of AP duration in HF, as the majority of measurements of whole-cell Na\textsuperscript{+} and Ca\textsuperscript{2+} current density show no change in the density of these currents\textsuperscript{36}. However, down-regulation of voltage-gated K currents is known to occur in HF. Measurements of whole-cell inward rectifier current $I_{K1}$ show that current density at hyperpolarized membrane potentials is reduced in HF by ~ 50\% in human\textsuperscript{37}, and by ~ 40\% in dog\textsuperscript{35}. Measurements of $I_{ol1}$ show that in end-stage HF human and canine tachycardia pacing-induced HF indicate current density is reduced by up to 70\% in HF\textsuperscript{38}. Human and canine Ca\textsuperscript{2+}-independent transient outward current
$I_{\text{to1}}$ is a combination of currents encoded by the KCND3 and KCNA4 genes$^{38,39}$, and KCND3 expression has been shown to be reduced in HF$^{40}$. There appears to be no change in expression of the HERG or KCNQ1 gene encoding $\alpha$-subunits of the $I_{Kr}$ and $I_{Ks}$ channels, respectively, in HF.

Expression of diverse proteins involved in the processes of EC coupling have also been measured in normal and failing myocytes. These proteins include: a) the SR Ca$^{2+}$-ATPase encoded by the SERCA2 gene; b) the phospholamban protein encoded by the PLN gene; and c) the sodium-calcium (Na$^+$-Ca$^{2+}$) exchanger protein encoded by the NCX1 gene. Measurements indicate there is an approximate 50% reduction of SERCA2 mRNA$^{36,41,42}$, expressed SR Ca$^{2+}$-ATPase protein level and direct SR Ca$^{2+}$-ATPase uptake rate$^{36}$ during HF. There is a 55% increase in NCX1 mRNA levels, and an approximate factor of two increase in Na$^+$-Ca$^{2+}$ exchange activity in human$^{42,43}$ and canine HF$^{36}$. There is uncertainty as to whether mRNA and expressed protein level of phospholamban is decreased$^{44}$ or unchanged$^{45}$ in human HF, and evidence that expressed protein level is decreased by a percentage amount equal to that of the SR Ca$^{2+}$-ATPase in failing canine heart$^{36}$.

It is therefore critically important to understand how these changes in gene expression, protein levels and current densities measured experimentally impact on the morphology of the AP and Ca$^{2+}$ transient. In particular, it is key to know which of these changes have the greatest functional effect. To answer this question, we developed a computational model of the failing ventricular myocyte$^{27}$. The above data suggest the following minimal model of altered repolarization and Ca$^{2+}$ handling in ventricular cells from the failing canine heart: a) reduced expression of $I_{Ks}$ and $I_{\text{to1}}$; b) down-regulation of the SR Ca$^{2+}$-ATPase; and c) up-regulation of the electrogenic Na$^+$-Ca$^{2+}$ exchanger. Since the density, but not the kinetic behavior, of each of the...
four transporters and ion currents comprising the minimal model appears altered in HF, we incorporated information on this altered gene and protein expression in the canine cell model by varying the density of these four membrane transporters ($I_{\text{to1}}$, $I_{\text{K1}}$, SR Ca$^{2+}$-ATPase, and Na$^+$-Ca$^{2+}$ exchanger) within experimentally derived limits$^{36}$.

The model has been used to test the hypothesis that this minimal set of heart failure induced changes can account for prolongation of AP duration, as well as decreased peak amplitude and decay rate of the Ca$^{2+}$ transient observed in failing myocytes. Figures 3C and 3D demonstrate the ability of the model to reconstruct APs and Ca$^{2+}$ transients measured in both normal and failing canine midmyocardial ventricular myocytes. Figure 3C shows a normal model AP (solid line), and model APs corresponding to the additive effects of sequential down-regulation of $I_{\text{to1}}$ (by 62%; dot-dashed line), $I_{\text{K1}}$ (by 32%; long-dashed line), and SR Ca$^{2+}$-ATPase (by 62%; rightmost short-dashed bold line), followed by up-regulation of Na$^+$-Ca$^{2+}$ exchanger (by 75%; dotted line). Changes of transporter amplitude were based on average values derived from experiments using mid-myocardial failing canine ventricular myocytes. Model simulations indicate that down-regulation of $I_{\text{to1}}$ produces a modest shortening, not lengthening, of AP duration. On first consideration, this seems an anomalous effect since $I_{\text{to1}}$ is an outward K current, but is one which agrees with the experimental results of Zygmunt et al$^{46}$ in canine myocytes (see their Fig. 2). The mechanism of this AP duration shortening has been investigated in detail using computational models$^{32}$, and results show that reduction of the Phase 1 notch depth through down-regulation of $I_{\text{to1}}$ reduces the electrical driving force on inward Ca$^{2+}$ current and hence shortens AP duration. The additional down-regulation of $I_{\text{K1}}$ (long-dashed line) produces modest AP prolongation, consistent with the fact that outward current through $I_{\text{K1}}$ is activated primarily at potentials which are hyperpolarized relative to the plateau potential. The
most striking result is shown by the short-dashed line in Fig. 3C - significant AP prolongation occurs following down-regulation of SR Ca\textsuperscript{2+}-ATPase. This down-regulation results in a near doubling of AP duration that is similar to that observed experimentally (Fig. 3A). Finally, the model predicts that up-regulation of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger, when superimposed on these other changes, contributes to modest APD shortening due to reverse mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange and generation of a net outward current during the plateau phase of the AP.

This modeling has provided important insights into the mechanism of AP prolongation and altered Ca\textsuperscript{2+} transients in heart failure. Prior to this work, the consensus was that down-regulation of the genes encoding the I\textsubscript{to1} and I\textsubscript{K1} outward K currents was responsible for AP prolongation – a very intuitive and reasonable hypothesis. The model indicates that this is not likely to be the case. Rather, the main contributor to AP prolongation involves down-regulation of the gene encoding the SR Ca\textsuperscript{2+}-ATPase. Subsequent model simulations have shown that down-regulation of this transport process alone has a severe effect on prolongation of the AP – a prediction confirmed by experiments in which cyclopiazonic acid is used to block SR Ca\textsuperscript{2+}-ATPase transport\textsuperscript{30}. This modeling illustrates the value of using quantitative models to interpret the consequences of changes in gene and protein expression on cell function. It also points out how prediction of a cellular phenotype using knowledge of underlying molecular changes must be based on interpretations derived from quantitative experimentally-based models.

2.7 A New Class of Myocyte Models

While common pool models are able to reconstruct APs with high fidelity, they are unable to reproduce a very fundamental behavior of cardiac myocytes – SR Ca\textsuperscript{2+} release that is smoothly and continuously graded with influx of trigger Ca\textsuperscript{2+} through sarcolemmal LCCs. This
failure is demonstrated in Fig. 3E. This figure shows normalized peak Ca\(^{2+}\) flux through RyR channels (ordinate) as a function of membrane potential (mV; abscissa). Filled circles are experimental measurements from the work of Weir et al\(^{47}\), showing that release flux increases smoothly to a maximum flux at about 0 mV, and then decreases to near zero at more depolarized potentials. Release flux increases from -40 to 0 mV since over this potential range, open probability of LCCs increases very steeply reaching a maximum value. Release flux decreases over the potential range greater than 0 mV because electrical driving force on Ca\(^{2+}\) decreases monotonically. The solid line shows release flux for the Jafri-Rice-Winslow guinea pig ventricular myocyte model. Release is all-or-none, with regenerative release initiated at a membrane potential causing opening of a sufficient number of LCCs (~-15 mV), and release terminating at potential for which electrical driving force is reduced to a critical level (~ +40 mV).

This all-or-none behavior of Ca\(^{2+}\) release in common pool models has very important implications for common pool model dynamics. LCCs not only undergo voltage- but also Ca\(^{2+}\)-dependent inactivation\(^{48,49}\). Inactivation depends on local subspace Ca\(^{2+}\) concentration, and occurs as Ca\(^{2+}\) binding to calmodulin\(^{49}\), which is tethered to the LCC, induces the channel to switch from a normal mode of gating to a mode in which transitions to open states are extremely rare. Recent experimental data have demonstrated that voltage-dependent inactivation of LCCs is a slow and weak process, whereas Ca\(^{2+}\)-dependent inactivation is relatively fast and strong\(^{49,50}\) (see Fig. 4C). This implies in turn that there is a very strong coupling between Ca\(^{2+}\) release from JSR into the local subspace, and regulation of inactivation of the LCC. When this newly revealed balance between voltage- and Ca\(^{2+}\)-dependent inactivation is incorporated into common pool models, the models become unstable, exhibiting alternating short and long duration APs\(^{30,51}\) (see
Fig. 4D). The reason for this is intuitively clear – since JSR Ca$^{2+}$ release is all or none in these models, Ca$^{2+}$-dependent inactivation of LCCs is all-or-none, depending on whether release has or has not occurred. Since L-type Ca$^{2+}$ current is a major contributor to inward current during the plateau phase of the AP, its biphasic inactivation leads to instability of AP duration. This, unfortunately, constitutes a fatal weakness of common pool models.

The fundamental failure of common pool models described above suggests that more biophysically-based models of excitation-contraction coupling must be developed and investigated. Understanding of the mechanisms by which Ca$^{2+}$ influx via LCCs triggers Ca$^{2+}$ release from the JSR has advanced tremendously with the development of experimental techniques for simultaneous measurement of LCC currents and Ca$^{2+}$ transients and detection of local Ca$^{2+}$ transients, and this has given rise to the local control hypothesis of EC coupling$^{19,47,52,53}$. As illustrated schematically in Fig. 4A, this hypothesis asserts that opening of an individual LCC in the T-tubular membrane triggers Ca$^{2+}$-release from the small cluster of RyRs located in the closely apposed (~ 12 nm) JSR membrane. Thus, the local control hypothesis asserts that release is all-or-none at the level of these individual groupings of LCCs and RyRs (referred to as the functional unit). However, LCC:RyR clusters are physically separated at the ends of the sarcomeres$^{54}$. These clusters therefore function in an approximately independent fashion. The local control hypothesis asserts that graded control of SR Ca$^{2+}$ release, in which Ca$^{2+}$-release from JSR is a smooth, continuous function of Ca$^{2+}$ influx, is achieved by the statistical recruitment of elementary Ca$^{2+}$ release events in these independent diadic spaces. Thus, central to the local-control hypothesis is the assertion that the co-localization of LCCs and RyRs is a structural component that is fundamental to the property of graded Ca$^{2+}$ release and force generation at the level of the cell. This concept of channel co-localization contributing in
fundamental ways to cell behavior is a general theme of biophysical signal processing in excitable cells.

We have recently implemented a local-control model of myocyte function\textsuperscript{51}. As a compromise between structural and biophysical detail versus tractability, a “minimal model” of local control of Ca\textsuperscript{2+} release, referred to as the Ca\textsuperscript{2+} release unit (CaRU) model, was developed. Fig. 4\textsuperscript{B} shows a schematic of the CaRU model. This model is intended to mimic the properties of Ca\textsuperscript{2+} sparks in the T-tubule/SR (T-SR) junction (Ca\textsuperscript{2+} sparks are elementary SR Ca\textsuperscript{2+} release events arising from opening of a cluster of RyRs\textsuperscript{55}). Fig. 4\textsuperscript{B} shows a cross-section of the model T-SR cleft, which is divided into four individual diadic subspace compartments arranged on a 2 × 2 grid. Each subspace (SS) compartment contains a single LCC and 5 RyRs in its JSR and sarcolemmal membranes, respectively. All 20 RyRs in the CaRU communicate with a single local JSR volume. The 5:1 RyR to LCC stoichiometry is chosen to be consistent with recent estimates indicating that a single LCC typically triggers the opening of 4-6 RyRs\textsuperscript{56}. Each subspace is treated as a single compartment in which Ca\textsuperscript{2+} concentration is uniform, however Ca\textsuperscript{2+} may diffuse passively to neighboring subspaces within the same CaRU. The division of the CaRU into four subunits allows for the possibility that an LCC may trigger Ca\textsuperscript{2+} release in adjacent subspaces (i.e., RyR recruitment) under conditions where unitary LCC currents are large. Since LCC:RyR clusters are physically separated\textsuperscript{54}, each model CaRU is assumed to function independently of other CaRUs. Upon activation of RyRs, subspace Ca\textsuperscript{2+} concentration will become elevated. This Ca\textsuperscript{2+} will freely diffuse to either adjacent subspace compartments ($J_{\text{iss}}$), or into the cytosol ($J_{\text{fer}}$) along its concentration gradient. The local JSR compartment is refilled via passive diffusion of Ca\textsuperscript{2+} from the network SR (NSR) compartment ($J_{\text{tr}}$).
The local control simulation algorithm is described in detail in the Appendix of Greenstein and Winslow\textsuperscript{51}. Simulation of the dynamics of each CaRU requires both numerical integration of the ordinary differential equations describing local subspace and JSR Ca\textsuperscript{2+} balance, as well as Monte Carlo simulation of LCC and RyR channel gating in the approximately \(\sim 12,500\) CaRUs in the cell (there are \(\sim 50,000\) LCCs per ventricular myocyte). The state of each channel is described by a set of discrete valued random variables that evolve in time as described by Markov processes. Time steps for CaRU simulations are adaptive and are chosen to be sufficiently small based on channel transition rates. The CaRU simulations occur within the (larger) time step used for the numerical integration of the system of ordinary differential equations describing the time-evolution of global state variables. As a result of the embedded Monte Carlo simulation, all model state variables and ionic currents/fluxes will contain a component of stochastic noise. These fluctuations introduce a degree of variability to simulation output.

Figures 4C-F show macroscopic properties of APs and SR Ca\textsuperscript{2+} release in this hybrid stochastic/ODE model. Figure 4C shows the relative balance between the fraction of LCCs not voltage-inactivated (dotted line) and not Ca\textsuperscript{2+}-inactivated (dashed line) during the AP. These fractions were designed to fit the experimental data of Linz and Meyer\textsuperscript{50}. The solid line shows a local-control model AP. This AP should be contrasted with those produced by the common pool model when the same relationship between LCC voltage- and Ca\textsuperscript{2+}-dependent inactivation as shown in Fig. 4C is used. Clearly, the local-control model exhibits stable APs whereas the common pool model does not. Fig. 4E shows the voltage dependence of peak LCC Ca\textsuperscript{2+} influx \((F_{LCC(max)}\) - filled circles, ordinate) and peak RyR Ca\textsuperscript{2+} release flux \((F_{RyR(max)}\) - open circles, ordinate) in response to voltage-clamp steps to the indicated potentials (mV, abscissa). Ca\textsuperscript{2+}
release flux is a smooth and continuous function of membrane potential, and hence trigger \( \text{Ca}^{2+} \), as shown by the experimental data in Fig. 4D. EC coupling gain may be defined as by Wier et al\(^4\), as the ratio \( F_{\text{RyR}(\text{max})} / F_{\text{LCC}(\text{max})} \), and is plotted as a function of voltage in Fig. 4F (triangles). EC coupling gain is monotonically decreasing with increasing membrane potential, and agrees with corresponding experimental measurements made by Wier\(^4\). The role of inter-subspace coupling on gain is demonstrated in Fig. 4F, by comparison of control simulations (triangles) to those in the absence of inter-subspace coupling (squares). With inter-subspace coupling intact, EC coupling gain is greater at all potentials, but the increase in gain is most dramatic at more negative potentials. In this negative voltage range, LCC open probability is sub-maximal, leading to sparse LCC openings. However, unitary current magnitude is relatively high, so that in the presence of \( \text{Ca}^{2+} \) diffusion within the CaRU, the rise in local \( \text{Ca}^{2+} \) due to the triggering action of a single LCC can recruit and activate RyRs in adjacent subspace compartments within the same T-SR junction. The net effect of inter-subspace coupling is therefore to increase the magnitude and slope of the gain function preferentially in the negative voltage range. These simulations therefore offer an intriguing glimpse of how the co-localization and stochastic gating of individual channel complexes can have a profound effect on the overall, integrative behavior of the cell.

### 3. Models of the Cardiac Ventricles

Computational models of the cardiac myocyte have contributed greatly to our understanding of myocyte function. This is in large part due to a rich interplay between experiment and modeling - an interplay in which experiments inform modeling, and modeling suggests new experiments. However, modeling of cardiac ventricular conduction has to a large
Modeling Cardiac Function

extent lacked this interplay. While it is now possible to measure electrical activation of the epicardium at relatively high spatial resolution, the difficulty of measuring the geometry and fiber structure of hearts which have been electrically mapped has limited our ability to relate ventricular structure to conduction via quantitative models. As described in the following sections, we are approaching this problem by: a) mapping ventricular activation using high-density epicardial electrode arrays; b) measuring and modeling ventricular geometry and fiber orientation at high spatial resolution using diffusion tensor magnetic resonance imaging (DTMRI); c) constructing computational models of the imaged hearts; and d) comparing simulated conduction properties with those measured experimentally in the same heart. This is one approach to “closing the loop” between experiment and modeling at the whole-heart level.

3.2 Mapping of Epicardial Conduction in Canine Hearts

We have recently performed electrical mapping studies in which epicardial conduction in response to various current stimuli have been measured using electrode arrays consisting of a nylon mesh with 256 electrodes and electrode spacing of ~5 mm sewn around its surface. Bipolar epicardial twisted-pair pacing electrodes were sewn onto the right atrium (RA) and the right ventricular (RV) free-wall. Four to ten glass beads filled with gadolinium-DTPA (~5 mM) were attached to the sock as localization markers, and responses to different pacing protocols we recorded. Figure 5A shows an example of measurement of activation time (color bar – in mSec) measured in response to an RV stimulus pulse applied at the epicardial locations marked in red. After all electrical recordings are obtained, the animal is euthanized with a bolus of potassium chloride, and the heart is then scanned with high-resolution T1-weighted imaging in order to locate the gadolinium-DTPA filled beads in scanner coordinates. The heart is then excised, sock
electrode locations are determined using a 3D digitizer (MicroScribe 3DLX), and the heart is formalin-fixed in preparation for DTMRI.

### 3.3 Measuring the Fiber Structure of the Cardiac Ventricles using DTMRI

DTMRI is based on the principle that proton diffusion in the presence of a magnetic field gradient causes signal attenuation, and that measurement of this attenuation in several different directions can be used to estimate a diffusion tensor at each image voxel\(^57,58\). Several studies have now confirmed that the principle eigenvector of the diffusion tensor is locally aligned with the long-axis of cardiac fibers\(^59-61\).

Use of DTMRI for reconstruction of cardiac fiber orientation provides several advantages over traditional histological methods. First, DTMRI yields estimates of the absolute orientation of cardiac fibers, whereas histological methods yield estimates of only fiber inclination angle. Second, DTMRI performed using formalin-fixed tissue: a) yields high resolution images of the cardiac boundaries, thus enabling precise reconstruction of ventricular geometry using image segmentation software; and b) eliminates flow artifacts present in perfused heart, enabling longer imaging times, increased signal-to-noise (SNR) ratio and improved spatial resolution. Third, DTMRI provides estimates of fiber orientation at more than one order of magnitude more points than possible with histological methods. Fourth, reconstruction time is greatly reduced (~60 hours versus weeks to months) relative to that for histological methods.

DTMRI data acquisition and analysis for ventricular reconstruction has been semi-automated. Once image data is acquired, software written in the MatLab programming language is used to estimate epi-cardial and endo-cardial boundaries in each short-axis section of the image volume using either the method of region growing or the method of parametric active contours\(^62\). Diffusion tensor eigenvalues and eigenvectors are computed from the DTMRI data.
sets at those image voxels corresponding to myocardial points, and fiber orientation at each image voxel is computed as the primary eigenvector of the diffusion tensor.

Representative results from imaging of one normal and one failing canine heart are shown in Fig. 6. Figures 6A & C are short-axis basal sections taken at approximately the same level in normal (6A) and failing (6C) canine hearts. These two plots show regional anisotropy according to the indicated color code. Figures 6B & D show the angle of the primary eigenvector relative to the plane of section (inclination angle), according to the indicated color code, for the same sections as in Figs. 6A & C. Inspection of these data show: a) the failing heart (HF: panels C & D) is dilated relative to the normal heart (N: panels A & B); b) left ventricular (LV) wall thinning (average LV wall thickness over 3 hearts is 17.5 ± 2.9 mm in N, and 12.9 ± 2.8 mm in HF); c) no change in RV wall thickness (average RV wall thickness is 6.1 ± 1.6 mm in N, and 6.3 ± 2.1 mm in HF); d) increased septal wall thickness HF versus N (average septal wall thickness is 14.7 ± 1.2 mm N, and 19.7 ± 2.1 mm HF); e) increased septal anisotropy in HF versus N (average septal thickness is .71 ± .15 N, and .82 ± .15 HF); and f) changes in the transmural distribution of septal fiber orientation in HF versus N (contrast panels B & D, particularly near the junction of the septum and RV).

3.4 Finite-Element Modeling of Cardiac Ventricular Anatomy

Structure of the cardiac ventricles can modeled using finite-element modeling (FEM) methods developed by Nielson et al. The geometry of the heart to be modeled is described initially using a pre-defined mesh with 6 circumferential elements and 4 axial elements. Elements use a cubic Hermite interpolation in the transmural coordinate (λ), and bilinear interpolation in the longitudinal (µ) and circumferential (θ) coordinates. Voxels in the 3D DTMR
images identified as being on the epi-cardial and endo-cardial surfaces by the semi-automated contouring algorithms described above are used to deform this initial FEM template. Deformation of the initial mesh is performed to minimize an objective function $F(n)$.

$$F(n) = \sum_{d=1}^{D} \gamma_d \|v(e_d) - v_d\|^2 + \int_{\Omega} \left\{ \alpha \nabla^2 n + \beta (\nabla^2 n)^2 \right\} \partial E,$$  \hspace{1cm} (14)

where $n$ is a vector of mesh nodal values, $v_d$ are the surface voxel data, $v(e_d)$ are the projections of the surface voxel data on the mesh, and $\alpha$ and $\beta$ are user defined constants. This objective function consists of two terms. The first describes distance between each surface image voxel ($v_d$) and its projection onto the mesh $v(e_d)$. The second, known as the weighed Sobelov norm, limits stretching (first derivative terms) and the bending (second derivative terms) of the surface. The parameters $\alpha$ and $\beta$ control the degree of deformation of each element. The weighted Sobelov norm is particularly useful in cases where there is an un-even distribution of surface voxels across the elements. A linear least squares algorithm is used to minimize this objective function.

After the geometric mesh is fit to DTMRI data, the fiber field is defined for the model. Principle eigenvectors lying within the boundaries of the mesh computed above are transformed into the local geometric coordinates of the model using the following transformation.

$$V_G = [F \quad G \quad H]^T [R] V_S \hspace{1cm} (15)$$

where $R$ is a rotation matrix that transforms a vector from scanner coordinates ($V_S$) into the FEM model coordinates $V_G$ and F, G, H are orthogonal geometric unit vectors computed from the ventricular geometry as described by LeGrice et al$^{64}$. Once the fiber vectors are represented in geometric coordinates, DTMRI inclination and imbrication angles ($\alpha$ and $\phi$) are fit using a bilinear interpolation in the local $\varepsilon_1$ and $\varepsilon_2$ coordinates, and a cubic Hermite interpolation in the
ε3 coordinate. A graphical user interface for fitting FEMs to both the ventricular surfaces and fiber field data has been implemented using the MatLab programming language. Figure 7 shows FEM fits to the epi- and endo-cardial surfaces of a reconstructed normal canine heart obtained using this software tool. FEM fits to the fiber orientation data are shown on these surfaces as short line segments. We have also developed relational database and data analysis software named HeartScan to facilitate analysis of cardiac structural and electrical data sets obtained from populations of hearts. HeartScan enables users to pose queries (in standard query language, or SQL) on a wide range of cardiac data sets by means of a graphical user interface (Fig. 8). These data sets include: a) DTMRI imaging data; b) FEMs derived from DTMRI data; c) electrical mapping data obtained using epicardial electrode arrays; d) model simulation data. Query results are either: a) displayed on a 3D graphical representation of the heart being analyzed; or b) piped to data processing scripts, the results of which are then displayed visually. Queries may be posed by direct entry of an SQL command into the Query Window (Fig. 8B). This query is executed, and the set of points satisfying this condition are displayed on a wire frame model of the heart being studied (shown in green in Fig. 8C). Queries operating on a particular region of the heart may also be entered by graphically selecting that region (Fig. 8D). SQL commands specifying the coordinates of the selected voxels are then automatically entered into the Query Window. One example of such a pre-defined operation is shown in Fig. 8E, which shows computation of transmural inclination angle for the region enclosed by the box in Fig. 8D.

3.5 Generation of Computational Models from DTMRI Data

The bidomain equations describe the flow of electrical current within the myocardium, between the intracellular and extracellular domains. This approach treats each domain of the
myocardial tissue as a continuum, rather than as being composed of discrete cells connected by
gap junctions and surrounded by the extracellular milieu. Thus, quantities such as conductivity
and transmembrane voltage represent spatial averages. Several excellent reviews have been
published detailing the assumptions in, structure of, and solution methods for the bidomain
equations\textsuperscript{65-67}. The following is a brief review of the origins of these equations.

The bidomain equations are derived by applying conservation of current between the
intra- and extracellular domains. The equations consist of parabolic (Eq. 16) and elliptic (Eq. 17)
equations that must be satisfied within the myocardium, (a region designated as $H$)

\[
\frac{\partial \nu}{\partial t}(\mathbf{x},t) = \frac{1}{C_m} \left[ -I_{ion}(\mathbf{x},t) - I_{app}(\mathbf{x},t) - \frac{1}{\beta} \left( \nabla \cdot M_e(\mathbf{x}) \nabla \phi_i(\mathbf{x},t) \right) \right], \quad \forall \mathbf{x} \text{ in } H, \tag{16}
\]

\[
\nabla \cdot M_i(\mathbf{x}) \nabla \nu(\mathbf{x},t) = -\nabla \cdot M(\mathbf{x}) \nabla \phi_i(\mathbf{x},t), \quad \forall \mathbf{x} \text{ in } H, \tag{17}
\]

and an additional elliptic equation (Eq. 18) that must be satisfied in the bath or tissue
surrounding the heart (a region designated as $B$)

\[
\nabla \cdot M_b(\mathbf{x}) \nabla \phi_b(\mathbf{x},t) = 0, \quad \forall \mathbf{x} \text{ in } B, \tag{18}
\]

where $\mathbf{x}$ is spatial position, $\phi_i(\mathbf{x},t)$ and $\phi_e(\mathbf{x},t)$ are the transmembrane intra- and extracellular
potentials, respectively; $\nu(\mathbf{x},t) = \phi_i(\mathbf{x},t) - \phi_e(\mathbf{x},t)$ is the transmembrane voltage; $C_m$ is the
membrane capacitance per unit area; $I_{ion}(\mathbf{x},t)$ is the sum of the ionic currents per unit area through
the membrane (positive outward); $I_{app}(\mathbf{x},t)$ is an applied cathodal extracellular current per unit
area; $\beta$ is the ratio of membrane area to tissue volume; $M_e(\mathbf{x})$ and $M_i(\mathbf{x})$ are the extracellular and
intracellular conductivity tensors, with $M(\mathbf{x}) = M_e(\mathbf{x}) + M_i(\mathbf{x})$; $\phi_b(\mathbf{x},t)$ is the bath potential; $M_b(\mathbf{x})$
is the bath conductivity tensor. These parameters may be set, in models of the normal heart,
using values described by Pollard et al\textsuperscript{65} and Henriquez et al\textsuperscript{67}. Additionally, boundary
Modeling Cardiac Function

conditions on the interface between the heart and the surrounding tissue, \( \delta H \), and the body surface, \( \delta B \), must be specified. The first boundary condition specifies continuity of potential:

\[
\phi_e = \phi_b \quad \text{on} \quad \delta H
\]

The second specifies continuity of current at the interface:

\[
\sigma_i \frac{\partial \phi_i}{\partial n} + \sigma_e \frac{\partial \phi_e}{\partial n} = \sigma_b \frac{\partial \phi_b}{\partial n} \quad \text{on} \quad \delta H,
\]

where the \( \sigma \)'s are the conductivities normal to the interface, and the \( \partial / \partial n \) is the normal derivative operator. In order for the problem to be well posed, a third boundary condition on \( \delta H \) is required. While the first two follow necessarily for all electrical phenomena, there are a number of ways to formulate the third boundary condition. Typically, we specify:

\[
\sigma_i \frac{\partial \phi_i}{\partial n} = 0 \quad \text{on} \quad \delta H,
\]

which has the physical interpretation that at the heart/body interface, all intracellular current must flow first through the extracellular space before it flows into the surrounding tissue. A boundary condition at \( \delta B \) for the Laplace equation in \( \phi_b \) is also required. Given that air is a poor conductor, this is simply,

\[
\sigma_b \frac{\partial \phi_b}{\partial n} = 0 \quad \text{on} \quad \delta B.
\]

Finally, an initial condition on the transmembrane voltage must be specified, \( v(\bar{x}, t=0) = V(\bar{x}) \). Then from this, initial conditions on \( \phi_e(\bar{x}, t=0) \) and \( \phi_b(\bar{x}, t=0) \) can be found by solving the appropriate elliptic equation. Equations 16 - 22 specify the bidomain problem.

Under some restrictive assumptions, the bidomain equations can be simplified dramatically. If the surrounding tissue is taken to be a good insulator, then \( \sigma_b = 0 \) in \( B \). Then we have
Modeling Cardiac Function

\[ \phi_e = 0 \quad \text{on } \partial H, \]  \hspace{1cm} (23)

\[ \frac{\partial \phi_e}{\partial \eta} = 0 \quad \text{on } \partial H, \]  \hspace{1cm} (24)

\[ \frac{\partial \phi_i}{\partial n} = 0 \quad \text{on } \partial H, \]  \hspace{1cm} (25)

and the Laplace equation for \( \phi_0 \) need not be considered. Additionally, under the assumption of equal anisotropy, namely that

\[ M_i(x) = \frac{1}{\kappa} M_e(x), \]  \hspace{1cm} (26)

where \( k \) is called the anisotropy ratio, Eqs. 16 and 17 uncouple, requiring then only solution of the parabolic equation,

\[ \frac{\partial \nu}{\partial t}(x, t) = \frac{1}{C_m} \left[ -I_{\text{ion}}(x, t) - I_{\text{app}}(x, t) + \frac{1}{\beta} \left( \frac{\kappa}{\kappa + 1} \right) \nabla \cdot (M_i(x) \nabla v(x, t)) \right] \quad \text{on } H. \]  \hspace{1cm} (27)

Equation 27 is referred to as the monodomain equation.

The conductivity tensors at each point within the heart are specified by fiber orientation and by specific conductivities in each of the local coordinate directions. The conductivity tensor in the local coordinate system, \( G_i(x) \), is defined as

\[ G_i(x) = \begin{bmatrix} \sigma_{1,i} \\ \sigma_{2,i} \\ \sigma_{3,i} \end{bmatrix}, \]  \hspace{1cm} (28)

where \( \sigma_{1,i} \) is the longitudinal and \( \sigma_{2,i} \) and \( \sigma_{3,i} \) are the transverse intracellular conductivities, respectively. This local tensor may be expressed in global coordinates to give the conductivity tensor of Eqn. (27) using the transformation

\[ M_i(x) = P(x) G(x) P^T(x), \]  \hspace{1cm} (29)
where $P(x)$ is the coordinate transformation matrix from local to global coordinates. $P(x)$ is in turn determined by the underlying fiber organization of the heart, and is obtained using DTMRI as described in Sect. 3.3. If only fiber direction information is available, then it is appropriate that conductivities transverse to the fiber long-axis be assumed equal ($\sigma_{2,i} = \sigma_{3,i}$).

Figure 5B shows the results of applying these methods to the analysis of conduction in a normal canine heart. As described previously, Fig. 5A shows activation time (color bar – in mSec) measured experimentally in response to an RV stimulus pulse applied at the epi-cardial locations marked in red. Following electrical mapping, this heart was excised, imaged using DTMRI, and an FEM was then fit to the resulting geometry and fiber orientation data sets. Figure 5A shows activation time displayed on this FEM. The stimulus wave front can be seen to follow the orientation of the epicardial fibers, which is indicated by the dark line segments in Fig. 5A. Figure 5B shows results of simulating conduction using a computational model of the very same heart that was mapped electrically in Fig. 5A. Results can be seen to agree qualitatively, however model conduction is more rapid in the region where the RV and LV join. Nonetheless, these results demonstrate the feasibility of combined experimentation and modeling of electrical conduction in specific imaged and reconstructed hearts.

4. Summary – Challenges for the Future

This paper has reviewed modeling research in three broad areas: (1) models of single ventricular myocytes; (2) methods for the reconstruction and modeling of ventricular geometry and microanatomy; and (3) integrative modeling of the cardiac ventricles. We have seen that the level of biophysical detail, and hence the accuracy and predictability of current ventricular myocyte models, is considerable. Nonetheless, much remains to be done.

One emerging area of research is modeling of mitochondrial energy production. Approximately 2% of cellular ATP is consumed on each heartbeat. The major processes consuming ATP in the myocyte
are muscle contraction, activity of the SR Ca\textsuperscript{2+}-ATPase and Na-K pumping. Cellular ATP levels also influence ion channel function including the sarcolemmal ATP-modulated K channel\textsuperscript{68}. Recently, we have formulated an integrated thermokinet model of cardiac mitochondrial energetics comprising the tricarboxylic acid (TCA) cycle, oxidative phosphorylation and mitochondrial Ca\textsuperscript{2+} handling\textsuperscript{69}. This model describes dynamics of key regulatory effectors of TCA cycle enzymes and the production of NADH and FADH\textsubscript{2}. These molecules are used by the electron transport chain to establish a proton motive force (Δμ\textsubscript{H}) which then drives the F\textsubscript{1}F\textsubscript{0}-ATPase. Mitochondrial matrix Ca\textsuperscript{2+} is also a model state variable. Mitochondrial Ca\textsuperscript{2+} concentration is determined by the Ca\textsuperscript{2+} uniporter and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activities, and regulates activity of the TCA cycle enzymes isocitrate dehydrogenase (IDH) and α-ketoglutarate dehydrogenase (KGDH). The model is described by twelve ordinary differential equations that represent ΔΨ\textsubscript{m} (mitochondrial membrane potential) and matrix concentrations of Ca\textsuperscript{2+}, NADH, ADP, and TCA cycle intermediates. The model is able to reproduce experimental data concerning mitochondrial bioenergetics, Ca\textsuperscript{2+} dynamics and respiratory control, relying only on the fundamental properties of the system. The time-dependent behavior of the model, under conditions simulating an increase in workload, closely reproduce the experimentally observed mitochondrial NADH dynamics in heart trabeculae subjected to changes in pacing frequency. The steady-state and time-dependent behavior of the model support the role of mitochondrial matrix Ca\textsuperscript{2+} in matching energy supply with demand in cardiac cells. Further development and testing of this model, its integration into models of the myocyte, and the use of these models to investigate myocyte responses to ischemia, are required.

In real cardiac myocytes, there exist a diversity of mechanisms that act to modulate cellular excitability. This includes α- and β-adrenergic signaling pathways acting through G protein-coupled membrane receptors to modulate properties of LCCs, various K\textsuperscript{+} channels, and Ca\textsuperscript{2+} transporters such as the SR Ca\textsuperscript{2+}-ATPase. The addition of these modulatory mechanisms to the cell models remains an important goal for the future.
As we have shown, magnetic resonance imaging now offers a relatively rapid way to measure ventricular fiber structure at high spatial resolution. The ability to rapidly acquire fiber orientation data throughout the ventricles in large populations of normal and diseased hearts will enable quantitative statistical comparison of normal and abnormal cardiac structure, and will provide insights into the possible structural basis of arrhythmia in heart disease. Unfortunately, a detailed understanding of the spatial heterogeneities within the heart, such as variation of intercellular coupling, regional expression of ionic currents and Ca\(^{2+}\) handling proteins is still unavailable, although significant progress has certainly been made. Understanding and modeling of these spatial heterogeneities remains a challenge for the future.

Finally, the complexity of biological models, including those of the cardiac myocyte, is increasing rapidly. This complexity makes the reliable publication and exchange of models difficult. XML-based markup languages such as CellML\(^{70}\) and the Systems Biology Markup Language (SBML)\(^{71}\) are being developed to support the error-free exchange of models independently of the hardware and software architectures on which these models will run. An application programming interface for CellML is being developed, and several groups are developing software for automated source code generation from CellML files.

These are indeed exciting times for cardiovascular biology. A national infrastructure supporting the acquisition, distribution and analysis of cardiovascular genomic and proteomic data is now in the formative stage (in particular, the Programs for Genomic Applications and Innovative Proteomics Centers supported by the National Heart, Lung and Blood Institute of the National Institutes of Health). The data and models produced from these efforts will without question enhance our understanding of myocyte and whole-heart function in both health and disease. Major challenges in data collection, representation, storage, dissemination and
modeling remain. If these challenges are met, we will have the opportunity to create a truly integrated cardiovascular *research community*, the whole of which is far greater than the sum of its parts.

5. Acknowledgements

This work was supported by grants from the NIH (RO1 HL-61711, RO1 HL-60133, RO1 HL-72488, P50 HL-52307, N01 HV-28180), The Falk Medical Trust, The Whitaker Foundation and IBM Corporation.

6. Bibliography


<table>
<thead>
<tr>
<th></th>
<th>Author(s)</th>
</tr>
</thead>
</table>


Figure Legends

Figure 1: **A**) Schematic illustration of the large mammalian cardiac ventricular myocyte action potential (membrane potential in mV as a function of time) illustrating depolarizing and repolarizing current (left) and alias gene names (right) encoding each of these currents. Reprinted from Tomaselli and Marban (1999). Electrophysiological remodeling in hypertrophy and heart failure. *Circ. Res.* 42: 270-283 by permission of the American Heart Association. **B**) Schematic illustration of the structure of common pool ventricular myocyte models.

Figure 2: **A**) Markov chain model of the human cardiac Na channel. States C0-4 are closed states, states O1,2 are open, conducting states, and states C1-4t and I are inactivated states. **B**) Normalized peak Na current (ordinate) as a function of membrane potential (mV; abscissa). Open and filled symbols are experimental and model data at 17°C, respectively. **C**) Time to peak Na current (msec; ordinate) as a function of membrane potential (mV; abscissa). Experimental and model data are compared at 13, 17 and 21°C. Data at 13, and 21°C are model fits, and data at 17°C is a model prediction. **D**) Model predictions (solid, dashed and dotted lines) at 13, 17 and 21°C, respectively, of single channel open time (msec; ordinate) as a function of membrane potential (mV; abscissa). Filled symbols are experimental data.

Figure 3: Model versus experimental action potentials and Ca^{2+} transients. Each action potential and Ca^{2+} transient is in response to a 1 Hz pulse train, with responses measured in the steady-state. **A**) Experimentally measured membrane potential (mV – ordinate) as a function of time (mSec – abscissa) in normal (solid) and failing (dotted) canine myocytes. **B**) Experimentally measured cytosolic Ca^{2+} concentration (nmol/L – ordinate) as a function of time (mSec –
absissa) for normal (solid) and failing (dotted) canine ventricular myocytes. C. Membrane potential (mV – ordinate) as a function of time (mSec – abscissa) simulated using the normal canine myocyte model (solid), and with the successive down-regulation of $I_{\text{to}}$ (dot-dashed, 66% down-regulation), $I_{K1}$ (long-dashed – down-regulation by 32%), SERCA2 (rightmost short-dashed - down-regulation by 62%) and NCX1 (dotted - up-regulation by 75%). D. Cytosolic $\text{Ca}^{2+}$ concentration (nmol/L – ordinate) as a function of time (mSec – abscissa) simulated using the normal (solid) and heart failure (dotted) model. Reprinted from Winslow et al (2001)(Winslow and others 2001). Computational models of the failing myocyte: Relating altered gene expression to cellular function. 359: 1187-1200 by permission of the Royal Society of London.

**Figure 4:** A) Structure of the LCC-RyR complex, denoted as the functional unit (FU). A single LCC in the sarcolemmal membrane is associated with 5 RyR in the closely apposed JSR membrane. ClCh denotes a single $\text{Ca}^{2+}$-modulated Cl⁻ channel which is thought to be co-located in the dyadic space. B) Structure of the $\text{Ca}^{2+}$ Release Unit (CaRU). Each CaRU consists of 4 FUs, with $\text{Ca}^{2+}$ diffusion between adjacent FUs and into the surrounding cytosolic space. C) Solid line is an action potential (membrane potential in mV, left ordinate; time in msec, abscissa) predicted by the local-control myocyte model. Dotted line is the fraction of channels (right ordinate) not voltage inactivated, and dashed line is the fraction not $\text{Ca}^{2+}$-inactivated during the action potential shown by the solid line. D) Behavior of the common pool myocyte model when the balance between voltage- and $\text{Ca}^{2+}$-inactivation is as shown in panel C). Note instability of action potentials. E) Peak $\text{Ca}^{2+}$ flux (ordinate) through RyRs (open symbols) and LCCs (filled symbols) as a function of membrane potential (mV; abscissa). F) EC coupling gain (ordinate – ratio of peak RyR to LCC flux) as a function of membrane potential (mV; abscissa).
Figure 5: A) Electrical activation times (indicated by color bar) in response to right RV pacing as recorded using electrode arrays. Data was obtained from a normal canine heart that was subsequently reconstructed using DTMRI. Activation times are displayed on the epi-cardial surface of a finite-element model fit to the DTMRI reconstruction data. Fiber orientation on the epi-cardial surface, as fit to the DTMRI data by the FEM model, is shown by the short line segments. B) Activation times predicted using a computational model of the heart mapped in A).

Figure 6: Fiber anisotropy $A(x)$ estimated from DTMRI data, and calculated as

$$A(x) = \sqrt{\frac{[λ_1(x) - λ_2(x)]^2 + [λ_1(x) - λ_3(x)]^2 + [λ_2(x) - λ_3(x)]^2}{λ_1(x)^2 + λ_2(x)^2 + λ_3(x)^2}}$$

where $λ_{1,2,3}(x)$ are diffusion tensor eigenvectors at voxel $x$, in normal (A) and failing (C) canine heart. Fiber inclination angle computed using DTMRI in normal (B) and failing (D) heart. Panels A and B are the same normal, and panels C and D the same failing heart.

Figure 7: Finite-element model of canine ventricular anatomy showing the epi-cardial (red), LV endo-cardial (green) and RV endo-cardial surfaces. Fiber orientation on each surface is shown by short line segments.

Figure 8: “Screenshot” of the windows by which the user interacts with HeartScan. A) window for viewing data tables; B) SQL query window; C) window for interactive 3-D display of heart data; D) pull-down window for user selection of heart regions to query. E) statistics display window.
Figure 1

A

<table>
<thead>
<tr>
<th>Current</th>
<th>Probability clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$</td>
<td>SCN5A</td>
</tr>
<tr>
<td>$I_{Ca_L}$</td>
<td>DHP receptor</td>
</tr>
<tr>
<td>$I_{Na/Ca}$</td>
<td>NCX</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>Kir2.x</td>
</tr>
<tr>
<td>$I_{to,1}$</td>
<td>Kv4.x</td>
</tr>
<tr>
<td>$I_{to,2}$</td>
<td>HERG</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>KvLQT1/minK</td>
</tr>
<tr>
<td>$I_{Kp}$</td>
<td></td>
</tr>
</tbody>
</table>

depolarizing

B

L-type channel $I_{Ca}$ Sarcolemma $I_{NaCa}$ $I_{p(Ca)}$ $I_{Membrane}$

Subspace Calmodulin Calseq JSR $J_{rel}$ $J_{xfer}$ $J_{tr}$ NSR $J_{up}$ $J_{leak}$

Sarcoplasmic Reticulum Myofilaments
Figure 3
Figure 4

A. Calcium flux from NSR ($J_{nf}$) and to cytosol ($J_{sf}$). JSR, RyRs, T-SR cleft, LCC, CICCh, and T-tubule lumen.

B. Diagram showing the exchange terms ($J_{xfer,i,j}$).

C. Membrane potential and probability of not being inactivated as a function of time.

D. Membrane potential over time with superimposed traces.

E. Peak Ca$^{2+}$ flux as a function of membrane potential.

F. EC coupling gain as a function of membrane potential.
Figure 7
Figure 8

A, B, C, D, E represent different sections of the cardiac function modeling.