LOCAL CONTROL OF CALCIUM RELEASE AND ITS IMPLICATIONS FOR CARDIAC MYOCYTE PROPERTIES

by

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ABSTRACT

The studies presented in this dissertation develop experimentally-based models of the canine ventricular myocyte and apply these models to better understand the role of the interaction between intracellular Ca$^{2+}$ dynamics and membrane currents in determining AP configuration in both healthy and diseased states.

To help resolve the role of the transient outward current (I_{to1}) in modulating AP duration (APD), Markov state models of the human/canine Kv4.3- and Kv1.4-encoded currents are developed based on experimental measurements. A model of canine I_{to1} is formulated as the combination of the Kv4.3 and Kv1.4 currents, and is incorporated into a canine ventricular myocyte model. Simulations demonstrate strong coupling between L-type Ca$^{2+}$ current and $I_{Kv4.3}$, and predict a bimodal relationship between $I_{Kv4.3}$ density and APD, whereby perturbations in $I_{Kv4.3}$ density may produce either prolongation or shortening of APD depending on baseline $I_{to1}$ current level.

The canine ventricular myocyte model is reformulated to conform to local control theory, which asserts that L-type Ca$^{2+}$ current tightly controls Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) via local interaction of closely apposed L-type Ca$^{2+}$ channels (LCCs) and ryanodine receptors (RyRs). The model formulation incorporates details of microscopic excitation-contraction (EC) coupling properties in the form of Ca$^{2+}$ release units (CaRUs) in which individual sarcolemmal LCCs interact in a stochastic manner with nearby RyRs. The CaRUs are embedded within and interact with the global systems of the myocyte. The model can reproduce both the detailed properties of EC
coupling, such as variable gain and graded SR Ca\(^{2+}\) release, and whole-cell phenomena, such as modulation of AP duration by SR Ca\(^{2+}\) release.

The local control myocyte model is applied to the scenarios of β-adrenergic stimulation and heart failure. Incorporation of phosphorylation dependent effects on model membrane currents and Ca\(^{2+}\)-cycling proteins yields altered AP configuration consistent with APs measured experimentally in the presence of β-adrenergic agonists. Moreover, discrete effects of β-adrenergic stimulation on LCCs and RyRs are correlated with specific changes in the voltage dependence of EC coupling gain which have been observed in experiments. Heart failure associated alterations in expression level of K\(^+\) membrane currents and Ca\(^{2+}\)-handling proteins yield defective EC coupling and profound prolongation of model APD. Additional analyses suggest that SR Ca\(^{2+}\) load plays a more significant role in heart failure related AP prolongation than altered LCC availability and kinetics.

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## Table of Contents

Chapter 1: Introduction .................................................................................................. 1

- Integrative Modeling of the Myocyte ................................................................. 6
- Heart Failure ........................................................................................................ 12
- Genetically Based Structural Channel Models .................................................. 16
- Local Control of Excitation Contraction Coupling............................................ 19
- Regulatory Role of Sympathetic Inputs............................................................... 22

Chapter 2: Role of the Ca\(^{2+}\) Independent Transient Outward Current \(I_{to1}\) in Shaping Action Potential Morphology and Duration ................................................. 28

- Introduction ........................................................................................................ 29
- Methods ............................................................................................................... 31
  - Composition of \(I_{to1}\) ................................................................................ 31
  - Characterization of \(I_{Kv4.3}\) ...................................................................... 32
  - Characterization of \(I_{Kv1.4}\) ...................................................................... 35
  - Modeling \(I_{to1}\) Effects on the Action Potential ........................................ 35
- Results ................................................................................................................ 35
  - Functional Expression of \(hKv4.3\) and \(I_{Kv4.3}\) Model Validation .......... 35
  - Model Validation of \(I_{Kv1.4}\) and Canine \(I_{to1}\) ....................................... 39
  - Effect of \(I_{to1}\) Density on Canine AP Shape and Duration ...................... 45
  - Mechanism of \(I_{Kv4.3}\) influence on AP Shape........................................ 47
- Discussion ......................................................................................................... 51
- Appendix: Model Development ........................................................................ 60
Chapter 3: An Integrative Model of the Cardiac Ventricular Myocyte Incorporating Local Control of Ca²⁺ Release ................................................................. 67

Introduction........................................................................................................ 68

Methods.............................................................................................................. 71

The Ca²⁺ Release Unit Model........................................................................... 71

Model State Variables.................................................................................... 79

Local Control Model Simulation Algorithm.................................................. 80

Results................................................................................................................ 81

L-type Ca²⁺ Current ....................................................................................... 81

Excitation Contraction Coupling .................................................................. 89

Action Potentials............................................................................................. 103

Discussion........................................................................................................... 116

Conclusions...................................................................................................... 128

Appendix I ....................................................................................................... 131

Local Ionic Fluxes ....................................................................................... 131

Local Ca²⁺ Balance ....................................................................................... 140

Global Ionic Fluxes ...................................................................................... 141

Global Ionic Flux Balance and Membrane Potential ..................................... 141
List of Tables

Table 2.1: Optimized Markov Model parameter Values at 35°C................................. 66

Table 3.1: Physical Constants and Cell Geometry Parameters................................. 143
Table 3.2: Standard Ionic Concentrations................................................................. 144
Table 3.3: L-type Ca^{2+} Channel and Ca^{2+}-dependent Cl^- Channel Parameters...... 145
Table 3.4: Ryanodine Receptor Parameters............................................................... 146
Table 3.5: Ca^{2+} Flux and Buffering Parameters..................................................... 147
Table 3.6: Ionic Current and Flux Formulations from previous Studies..................... 148
Table 3.7: State Variable Initial Conditions.............................................................. 149

Table 4.1: Adjusted Control Model Parameters......................................................... 176
Table 4.2: β-AR Stimulation Parameters................................................................. 177
Table 4.3: Heart Failure Model Parameters............................................................. 178
## List of Figures

| Figure 1.1: | Ionic Currents Underlying the Cardiac Action Potential | 5 |
| Figure 1.2: | Schematic of Jafri et al. (1998) Cardiac Myocyte Model | 11 |
| Figure 1.3: | β-Adrenergic Signaling in the Cardiac Myocyte | 23 |
| Figure 2.1: | State Diagram of the Kv4.3 Channel Model | 34 |
| Figure 2.2: | Expressed and Model-simulated Kv4.3 Currents | 37 |
| Figure 2.3: | Peak Current and Steady-state Availability for Model I_{Kv4.3} | 40 |
| Figure 2.4: | Canine I_{to1} Model Electrophysiological Characteristics | 43 |
| Figure 2.5: | Effect of I_{Kv4.3} on AP Shape and Duration | 46 |
| Figure 2.6: | Role of IKv4.3 on AP, L-type Ca^{2+} Channel Open Probability, and I_{CaL} | 48 |
| Figure 2.7: | APs and Ca^{2+} Transients Following a Brief Pause in Pacing | 54 |
| Figure 2.8: | Effect of I_{Kv4.3} on Guinea Pig Model APs | 56 |
| Figure 3.1: | Schematic of the Ca^{2+} Release Unit | 73 |
| Figure 3.2: | Single L-type Channel Model Properties | 83 |
| Figure 3.3: | Properties of Macroscopic I_{CaL} | 87 |
| Figure 3.4: | Response of a Single CaRU | 90 |
| Figure 3.5: | Whole Cell Ca^{2+} Dynamics Under Voltage Clamp | 94 |
| Figure 3.6: | Microscopic Properties of Ca^{2+} Spikes | 97 |
| Figure 3.7: | Voltage Dependent Excitation Contraction Coupling Properties | 101 |
| Figure 3.8: | Variable EC Coupling Gain | 104 |
CHAPTER 1:

Introduction
Introduction

The proper and efficient function of all organ systems relies on a healthily functioning heart. The human heart works continuously and tirelessly throughout the lifetime of an individual. It never pauses to rest from the moment it starts beating until the moment it stops. In the average human lifetime, the heart beats more than two and a half billion times. On each and every beat, the heart must relax to allow its chambers to fill with blood, and then contract to eject the blood, and send it to the rest of the body.

The pumping action of the heart moves blood through the vessels of the circulatory system, delivering essential elements and removing harmful wastes throughout the body. As blood courses through the vessels, it sustains life by transporting oxygen from the lungs to the body tissues and carbon dioxide from the body tissues to the lungs. It sustains growth by transporting nourishment from digestion and hormones from glands throughout the body, and it sustains health by transporting disease fighting substances to the tissue and wastes to the kidneys. The continuous movement of blood through the circulation is made possible by the fact that cardiac muscle contraction is automatic. That is, heart tissue will spontaneously contract at a periodic rate without any neural or hormonal inputs. This arises from the fact that individual cardiac myocytes spontaneously generate action potentials at a periodic rate. The specialized pacemaker cells of the sinoatrial node normally determine the heart rate. The electrical activity of these pacemaker cells is transmitted in an orderly fashion throughout the rest of the heart via the syncitial properties of heart muscle (whereby electrical excitation spreads from one cell to its immediate neighbors by current flowing through gap junctions) and via
specialized conduction systems. Heart rate must be regulated so that in times of metabolic need the tissues can be supplied with a greater flow of blood. Both the sympathetic and parasympathetic nervous systems alter the activity of the pacemakers to regulate the heart rate. Sympathetic stimulation increases the rate at which pacemaker cells generate action potentials and also speeds conduction from the atria to the ventricles, while parasympathetic stimulation has the opposite effect. The positive and negative influences of these inputs are balanced in order to regulate cardiac behavior.

The absolute necessity of the heart for life, as well as its complex and dynamic behavior has made it the focus of intense investigation. The heart is a complex electrical, chemical, and mechanical system which is designed to pump blood efficiently based on the metabolic needs of the body. Many of the properties of the whole heart arise from the properties of individual myocytes, each of which may be considered a miniature, but still rather complex, electrical, chemical, and mechanical system. The contraction of heart tissue is a direct consequence of a process known as excitation-contraction coupling. The electrical excitation of a single myocyte produces an action potential, the characteristic depolarization of the cell membrane that occurs as a result of the passage of electrical currents across the cell membrane. In response to excitation, intracellular Ca\(^{2+}\) concentration rises, which in turn leads to activation of the myofilaments, and ultimately results in cell shortening. The coordinated activation and shortening of myocytes throughout the heart produce contraction of the whole organ. Defects in excitation-contraction coupling are thought to play an important role in reducing the ability of the heart to pump effectively in diseased states. Since whole heart function, both in normal
and diseased states, often reflects the processes that occur in individual myocytes, it is critical to understand the physiological properties of single cardiac myocytes.

A characteristic feature of the cardiac myocyte is its action potential (AP). The AP is a depolarization of the cell membrane which arises as a result of the dynamic behavior of a diverse population of membrane ion channels. A prototypical ventricular myocyte AP is shown in Fig. 1.1 (Marban, 2002). The AP exhibits a steep upstroke, followed by a sustained slowly decaying plateau phase, which eventually gives way to repolarization. Above the AP are shown the associated depolarizing currents, which are carried by Na\(^+\) and Ca\(^{2+}\) ions. Under normal conditions, the Na\(^+\) current (I\(_{Na}\)) activates rapidly, producing the AP upstroke, and then inactivates completely (Marban et al., 1998). L-type Ca\(^{2+}\) current (I\(_{CaL}\)) inactivates a bit more slowly, and incompletely, allowing for the inward Ca\(^{2+}\) current to maintain the plateau phase of the AP (Zeng and Rudy, 1995). The influx of Ca\(^{2+}\) via the L-type Ca\(^{2+}\) channels (LCCs) triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR), an internal Ca\(^{2+}\) storage compartment. This excitation-contraction (EC) coupling event is known as Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). The SR is a subcellular organelle that releases the majority of activator Ca\(^{2+}\) during each heartbeat (Bers, 2001). The rise in cytosolic Ca\(^{2+}\) ultimately leads to cell contraction. The SR also actively sequesters Ca\(^{2+}\), which is the primary mechanism by which Ca\(^{2+}\) is removed from the cytosol in order to allow relaxation in between heartbeats (Bassani et al., 1994). The intracellular Ca\(^{2+}\) signal is not only triggered by membrane depolarization, but also feeds back on the L-type Ca\(^{2+}\) channel, mediating inactivation of the current, and therefore plays a role in influencing AP shape. The lower part of Fig 1.1 shows the various types of K\(^+\) channels (and a Cl\(^-\) channel) that are
Figure 1.1: Ion currents that underlie the cardiac action potential. Top, depolarizing currents as a function of time; Middle, a prototypical ventricular action potential; Bottom, repolarizing currents as a function of time. Figure reprinted from Marban (2002).
involved in membrane repolarization. The inward rectifier current ($I_{K1}$) maintains and stabilizes the resting potential, the transient outward currents ($I_{to1}$ and $I_{to2}$), thought to be carried by $K^+$ and $Cl^-$ respectively (Näbauer et al., 1993; Collier et al., 1996), contribute to the notch that follows the upstroke, and the components of the delayed rectifier current ($I_{Kr}$ and $I_{Ks}$) as well as the plateau $K^+$ current ($I_{Kp}$) display voltage- and time-dependent properties that contribute to the repolarization of the AP (Yue and Marban, 1988). In general, membrane potential, extracellular and intracellular ion concentrations, as well as regulatory proteins that modulate the activity of specific channel types influence ionic currents. The shape and duration of the AP is therefore an integrative cellular feature that is influenced by the interaction of many underlying cellular processes. For example, AP configurations display characteristic features in different regions of the mammalian heart as a result of the regional variation in underlying currents (reviewed in Katz, 1992).

**Integrative Modeling of the Myocyte**

Given the highly integrative nature of the dynamic processes which occur during excitation and contraction of a cardiac myocyte, the approach of integrative modeling is used in order to address fundamental questions about the function of the heart. This is accomplished by developing experimentally based, biophysically detailed, mathematical descriptions of individual cellular components, such as ion channels, pumps, exchangers, and subcellular compartments. Mathematical descriptions and models of each cellular component are developed based on separate sets of experiments obtained under conditions designed to isolate and characterize a particular current or other subcellular...
component. Models of each individual system component are then incorporated into a single integrative myocyte model in which these subcellular systems can interact. The power and utility of an integrative model stems from the fact that it is “transparent” under all conditions. Transparency refers to the fact that all variables of interest (e.g. membrane potential, ionic concentrations, ionic currents, channel open probability) can be monitored simultaneously at all times during any simulation, i.e. one can “see” into the cell and observe every aspect of the subcellular processes. This is obviously not possible in experimental approaches. Experimental techniques generally isolate and characterize the properties of a single subcellular component. The manipulations that are necessary to perform such experiments often require the presence of pharmacological agents, exogenous buffers, and/or non-physiological solutions. Integrative modeling can therefore be an important tool in interpreting experimental data by helping to elucidate mechanisms underlying phenomena which may otherwise be difficult to understand based on experiments alone. Moreover, models can be used as an exploratory tool to make quantitative predictions, and to guide the design of future experiments.

While the transparency of integrative models is their key empowering feature, it is important to recognize that the predictive ability of any model is limited by the fact that there are likely to be mechanisms and/or components that are missing or incompletely characterized. Taken together, the components of a fully comprehensive integrative model represent the collection of the current body of knowledge obtained from experiments (and possibly previous models). The inability of a model to reproduce phenomena observed in experiments indicates that there are gaps in our knowledge of the system. However, the model may yield clues as to where these gaps are, and
consequently would help guide the necessary experiments designed to obtain new data and fill such gaps. The model can then be updated based on newly obtained data, and may then be used to make further predictions. This iterative interaction between experiment and simulation has been key to the broadening of our knowledge of the underlying mechanisms of the cardiac ventricular action potential (reviewed in Noble, 2001).

Much of the current knowledge regarding ion channel kinetics and functional current density in excitable tissues has been obtained using the technique of voltage clamp. This approach was pioneered by Hodgkin, Huxley, and Katz (1949) now more than five decades ago, and continues to be the best biophysical technique for the study of ion channels (Hille, 1992). “Clamping” of the membrane potential to a constant value using a feedback amplifier with current passing electrodes allows for the quantitative characterization of voltage dependent ion channel gating kinetics. The development of this technique paved the way for the use of integrative modeling as an approach to understanding excitable cells.

In 1952, Hodgkin and Huxley published a series of groundbreaking papers (Hodgkin and Huxley, 1952d; Hodgkin and Huxley, 1952b; Hodgkin and Huxley, 1952a; Hodgkin and Huxley, 1952c) describing both voltage clamp measurements of membrane currents and an integrative model of the action potential for the squid giant axon. The researchers successfully formulated simple quantitative descriptions of Na⁺ and K⁺ channels. Their modeling approach postulated that ion channel permeability was regulated by gating mechanisms, whereby distinct entities (i.e., gates) controlled the opening and closing of both Na⁺ and K⁺ channels. The position of these gates defined
three functional states of the channel: a closed resting state, an open state, and a closed refractory state. By combining their descriptions of the Na\(^+\) and K\(^+\) currents with a leak conductance into an integrative model, Hodgkin and Huxley were able to describe in detail how the interaction of these elements combine to generate an AP. The spectacular success of the Hodgkin-Huxley (HH) model is evident in the fact that, to this day, it serves as a paradigm for describing ion channel behavior.

Soon after Hodgkin and Huxley laid the foundation for the use of integrative models in biology, the first cardiac cell models were developed using a similar approach. The models of Fitzhugh (1960) and Noble (1962) addressed the issue of whether Na\(^+\) and K\(^+\) current descriptions similar to those of Hodgkin and Huxley could be employed to account for the long plateau of the cardiac AP in Purkinje fibers (reviewed in Noble, 2001). Modifications to Na\(^+\) and K\(^+\) channel kinetics were able to generate a plateau, which was supported by the inward Na\(^+\) current. These early models could therefore reproduce APs, but not other relevant features of cardiac cells, in part, due to the fact that Ca\(^{2+}\) currents had not yet been discovered.

The continued interactive iteration between experiments and simulations has led to improved mechanistic insights into cardiac myocyte electrophysiology. Aspects of intracellular Ca\(^{2+}\) handling were introduced in the DiFrancesco-Noble Purkinje cell (DiFrancesco and Noble, 1985) and the Luo-Rudy ventricular cell (Luo and Rudy, 1994) models. New features of these models were the inclusion of the intracellular SR compartment, time varying intracellular and extracellular ion concentrations, and ion pumps and exchangers. Whereas each of these models generate APs using detailed kinetic descriptions of membrane currents, the Ca\(^{2+}\) subsystem in each is represented by a
phenomenological model that mimics the process of \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release, but fails to capture the biophysical details involved. A recent model of the guinea pig cardiac ventricular myocyte (Jafri et al., 1998) attempted to improve upon it predecessors by incorporating detailed descriptions of \( \text{Ca}^{2+} \) handling processes. A schematic diagram of this model, developed by Jafri et al. (1998), is shown in Fig. 1.2. In addition to the membrane currents described in the Luo-Rudy model, important features of this model include: 1) a formulation of the L-type \( \text{Ca}^{2+} \) channel (LCC) based on mode-switching behavior observed in experiments by Imredy and Yue (1994); 2) a biophysically detailed model of the SR \( \text{Ca}^{2+} \) release channel (ryanodine receptor, RyR) developed by Keizer and Levine (1996); 3) \( \text{Ca}^{2+} \) efflux through the RyRs and \( \text{Ca}^{2+} \) influx through the LCCs are assumed to empty into a common restricted subspace located between the junctional SR (JSR) and the cell membranes; and 4) detailed kinetic descriptions of \( \text{Ca}^{2+} \) buffering by the myofilament protein troponin. The arrows in Fig. 1.2 indicate the various ion fluxes produced by membrane currents, pumps, and exchangers that are incorporated into the model.

The integrative features of the Jafri et al. (1998) model capture many of the details of \( \text{Ca}^{2+} \) cycling in the sequence of events that occur during an excitation-contraction-relaxation cycle. Inward currents originating from a neighboring cell via gap junctions (in vivo) or from a current passing electrode (in vitro) depolarize the membrane to threshold, whereby the fast sodium current (\( I_{\text{Na}} \), included with \( I_{\text{Membrane}} \) in Fig 1.2) is activated and produces a rapid, regenerative depolarization, generating the fast upstroke of the AP. In response to depolarization, the L-type \( \text{Ca}^{2+} \) current is activated leading to an influx of \( \text{Ca}^{2+} \) ions into the restricted subspace (shown as \( I_{\text{Ca}} \), locally raising the \( \text{Ca}^{2+} \)
Figure 1.2: Schematic diagram of Ca$^{2+}$ cycling in the ventricular myocyte model of Jafri et al. (1998). In response to depolarization, the L-type Ca$^{2+}$ current (shown as $I_{Ca}$) is activated leading to an influx of Ca$^{2+}$ ions into the restricted subspace. The rise in subspace Ca$^{2+}$ concentration triggers RyR opening leading to Ca$^{2+}$ release from the SR ($J_{rel}$). The influx Ca$^{2+}$ as well as the released Ca$^{2+}$ diffuse from the restricted subspace into the myoplasm ($J_{xfer}$). The termination of the AP occurs as the cell membrane repolarizes, primarily by the action of outward K$^+$ currents (included in $I_{Membrane}$). Ca$^{2+}$ removal occurs by Ca$^{2+}$ reuptake into the network SR (NSR) by the SR Ca$^{2+}$ pump ($J_{up}$) and by Ca$^{2+}$ extrusion via the Na$^+$-Ca$^{2+}$ exchanger ($I_{NaCa}$) and the sarcolemmal Ca$^{2+}$ pump ($I_{p(Ca)}$). The JSR is refilled by Ca$^{2+}$ flux from the NSR ($J_{trp}$). Ca$^{2+}$ is buffered in the SR by calsequestrin, in the subspace by calmodulin, and in the myoplasm by both calmodulin and troponin (myofilaments). Figure reprinted from Jafri et al. (1998).
concentration. The rise in subspace \( \text{Ca}^{2+} \) concentration is sensed by the RyRs, triggering a substantial amount of \( \text{Ca}^{2+} \) release from the SR \( (J_{\text{rel}}) \) in the process known as \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release. The influx \( \text{Ca}^{2+} \) as well as the released \( \text{Ca}^{2+} \) diffuse from the restricted subspace into the myoplasm raising the cytosolic \( \text{Ca}^{2+} \) concentration by approximately an order of magnitude from \( \sim 0.1 \) \( \mu \text{M} \) to \( \sim 1 \) \( \mu \text{M} \), leading to \( \text{Ca}^{2+} \) binding to multiple cytosolic \( \text{Ca}^{2+} \) buffers, including calmodulin and troponin. When \( \text{Ca}^{2+} \) binds to troponin, it activates the myofilaments in a cooperative manner producing contraction.

The termination of the AP occurs as the cell membrane repolarizes, primarily by the action of outward \( K^+ \) currents. For relaxation to occur, cytosolic \( \text{Ca}^{2+} \) must decline in order to allow for \( \text{Ca}^{2+} \) to dissociate from troponin, thereby turning off the contractile machinery. This is accomplished mainly by \( \text{Ca}^{2+} \) reuptake into the network SR (NSR) by the SR \( \text{Ca}^{2+} \) pump \( (J_{\text{up}}) \) and by \( \text{Ca}^{2+} \) extrusion across the sarcolemma via the \( \text{Na}^+\text{-Ca}^{2+} \) exchanger \( (I_{\text{NaCa}}) \).

**Heart Failure**

Congestive heart failure (CHF) affects over 2 million Americans and is a major cause of death around the world (Ghali et al., 1990). CHF often results from dilated cardiomyopathy (DCM), a primary cardiac disease characterized by ventricular dilatation, decreased myocardial contractility and cardiac output. In the U.S., new cases number about 400,000 per year and prevalence is over 4.5 million (Parmley et al., 1991). CHF is now the leading cause of Sudden Cardiac Death (SCD) in the U.S. Nearly 50% of CHF patient deaths are sudden and unexpected (Packer, 1987; Investigators, 1991;
Group, 1987). In heart failure patients, SCD is commonly attributed to ventricular tachycardia or fibrillation, however the underlying mechanisms of these fatal arrhythmic events are poorly understood. Alterations in myocyte Ca\(^{2+}\) regulation may be a critical factor in the arrhythmogenesis associated with CHF (O'Rourke et al., 1999b; Pogwizd et al., 1999).

Individual cardiac cells extracted from failing hearts reveal changes in the level of expression of genes and proteins involved in electrical membrane repolarization and intracellular calcium (Ca\(^{2+}\)) handling. There is substantial experimental evidence indicating altered expression levels of outward K\(^+\) currents I\(_{K1}\) and I\(_{to1}\), the SR Ca\(^{2+}\)-ATPase (SERCA2a), and the Na\(^+\)-Ca\(^{2+}\) exchanger (NCX) in failing myocytes (Beuckelmann et al., 1993; Koumi et al., 1995; Kääb et al., 1996; Näbauer et al., 1996; Studer et al., 1994; Hasenfuss et al., 1994; Limas et al., 1987; Flesch et al., 1996; Reinecke et al., 1996). These changes correlate with alterations in cellular phenotype including AP prolongation, reduced intracellular Ca\(^{2+}\) transient amplitude, and slowed removal of Ca\(^{2+}\) from the cell. The resulting destabilization of the AP repolarization process increases the likelihood of secondary, potentially arrhythmogenic, depolarizations (Tomaselli et al., 1994).

In order to study the quantitative role of heart failure related alteration in functional expression levels of K\(^+\) currents and Ca\(^{2+}\) cycling proteins, Winslow et al. (1999) developed an integrative model of the canine AP. Both the membrane currents and the Ca\(^{2+}\) handling subsystems of the canine AP model are derived from the Jafri et al. (1998) model of the guinea pig ventricular myocyte described above. The primary modifications necessary to represent properties of canine midmyocardial ventricular cells
include: 1) the addition of the Ca\(^{2+}\)-independent transient outward K\(^+\) current (I\(_{to1}\)) which is absent in guinea pig myocytes (Campbell et al., 1993); 2) the separation of the delayed rectifier current into its rapid- and slowly-activating components, I\(_{Kr}\) and I\(_{KS}\) respectively (Zeng et al., 1995); and 3) a new formulation of SR Ca\(^{2+}\) ATPase which incorporates both a forward and a reverse Ca\(^{2+}\) flux (Shannon et al., 1997) (see also Shannon, 2000).

The development of the canine AP model (Winslow et al., 1999) was motivated by the observation that studies using the canine tachycardia pacing-induced model of heart failure (e.g., Spinale et al., 1992; Kääb et al., 1996) demonstrated that changes in cellular electrophysiological and EC coupling processes are qualitatively similar to those observed in cells isolated from failing human heart (e.g., Beuckelmann et al., 1993; Studer et al., 1994; Mercadier et al., 1990). In this experimental model, the heart is paced rapidly (~250 min\(^{-1}\), 2x the normal resting heart rate) for 3-5 weeks. Dogs subjected to this protocol exhibit features of heart failure similar to those seen in humans such as altered end-systolic pressure-volume relations and reduced fractional shortening (Wolff et al., 1992; Williams et al., 1994), chamber dilatation and wall thinning (Komamura et al., 1992; Armstrong et al., 1986; Spinale et al., 1995; Wolff et al., 1992), chronic neuro-humoral activation and decreased β-adrenergic receptor density and adenylate cyclase activity (Calderone et al., 1991; Moe et al., 1989). Approximately 25% of dogs subjected to the tachycardia pacing protocol die suddenly, and Holter recordings in these animals indicate episodes of polymorphic ventricular tachycardia prior to death (Pak et al., 1994).

Use of the canine tachycardia pacing-induced model also eliminates complications such as diversity of disease origin and drug treatment present in human material.
Recent experimental studies have demonstrated that SR Ca\(^{2+}\) uptake is functionally impaired while Na\(^{+}\)-Ca\(^{2+}\) exchange current is enhanced in pacing induced heart failure of the dog (O'Rourke et al., 1999b). Therefore, in failing myocytes, there is a greater dependence on the function of NCX1 than on SERCA2a for cytosolic Ca\(^{2+}\) removal compared to control myocytes. Model-based analyses of canine myocytes were performed to estimate functional down- and up-regulation of SERCA2a and NCX1 respectively (Winslow et al., 1999). These analyses indicated that reduced expression of SERCA2a and simultaneous up-regulation of NCX1, to the degree that they have been measured experimentally, are sufficient to account for the reduced amplitude, altered shape, and slowed relaxation of Ca\(^{2+}\) transients in the failing canine heart. Moreover, model predictions suggest that the altered functional expression of these Ca\(^{2+}\) cycling proteins may be an important factor in prolongation of AP duration in heart failure. According to this hypothesis, AP prolongation results from the dynamic behavior of a complex negative feedback loop regulating L-type Ca\(^{2+}\) current. The properties of this feedback loop are determined by the amount of JSR Ca\(^{2+}\) available for release during an AP, and the extent to which Ca\(^{2+}\) in the local environment of the LCC induces inactivation. When JSR Ca\(^{2+}\) levels are reduced, then the local Ca\(^{2+}\) in the environment of the LCC and hence LCC inactivation during the AP are also reduced, leading to an enhanced inward LCC current and AP prolongation. Results of this study further revealed that reduction of both I_{NaL} and I_{K1} has only modest effects on AP duration. While the down-regulation of K\(^{+}\) currents associated with heart failure has long been suspected as playing an important role in AP prolongation (reviewed in Tomaselli and
Marban, 1999), these recent studies make a strong argument indicating that alterations in Ca\(^{2+}\) homeostasis may in fact be a key mechanism.

**Genetically Based Structural Channel Models**

The experimental findings of the last five years has brought to light a large quantity of new data which has revealed novel perspectives about integrative cardiac myocyte electrophysiology and Ca\(^{2+}\) handling (reviewed in Bers, 2002). In keeping with the theme of interactive iteration between experiments and simulations, and in order to help interpret these recent findings, even the most recent models require improved formulations of subcellular processes and the incorporation of additional mechanisms. The studies described in this dissertation stem from the recent modeling work of Winslow et al. (1999), and focus on improving our understanding of the detailed interactions between sarcolemmal membrane channels, Ca\(^{2+}\) cycling proteins, and regulatory cell signaling systems in both normal and failing myocytes.

While the traditional Hodgkin-Huxley approach to modeling channels is still considered a powerful tool, it is generally limited to describing macroscopic current behavior. The HH formalism can accurately capture the ensemble behavior of a population of ion channels, however it is limited in its ability to describe the underlying channel kinetics (Aldrich et al., 1983). With the development of experimental techniques to obtain single ion channel recordings, and the accumulation of knowledge regarding the relationship between ion channel structure and function, the need for biophysically detailed channel models has become evident in order to capture behaviors that are state-
specific. The necessary detailed kinetic models have been developed by using Markov process based descriptions of channel gating to replace traditional HH formulations. The necessity for this approach has been demonstrated when considering mechanisms of drug action. In one study (Liu and Rasmusson, 1997), an HH model and a Markov model of the cardiac transient outward current, \( I_{\text{to1}} \), were compared. While the two models behaved similarly under control conditions, simulation of the presence of an open state specific channel blocker led to substantially different degrees of channel block in each model. Another motivation for this approach is the challenge to integrate molecular level data on structure/function and the role of genetic defects into cardiac myocyte models. Recent efforts to link genetic defects in the cardiac \( \text{Na}^+ \) channel (Clancy and Rudy, 1999) and \( \text{K}^+ \) channels (Mazhari et al., 2001) by developing Markov state models of these genetically-encoded currents and incorporating them into integrative myocyte models has demonstrated how mutant ion channels in myocytes of patients with long QT syndrome modulate the AP and lead to cellular arrhythmias in the form of AP prolongation and early after depolarizations (EADs).

The myocyte models of Winslow et al. (1999) and Jafri et al. (1998) were among the first to incorporate detailed Markov process based descriptions of ionic currents. As described above, both of these cell models have incorporated a formulation of the L-type \( \text{Ca}^{2+} \) current based on mode-switching (Imredy and Yue, 1994) and a biophysically detailed model of the RyR (Keizer and Levine, 1996). However, the remaining membrane currents are described by traditional HH formalisms. Of particular interest is the voltage-dependent \( \text{Ca}^{2+} \)-independent transient outward current, \( I_{\text{to1}} \). Although the evidence linking \( I_{\text{to1}} \) magnitude to characteristics of the early repolarization phase (phase
1) of the cardiac AP is strong (Liu et al., 1993; Yu et al., 2000; Beuckelmann et al., 1993; Kääb et al., 1996), the role of \( I_{to1} \) on AP duration (APD) remains unclear because experiments designed to reveal its role on APD have yielded conflicting results (Beuckelmann et al., 1993; Kääb et al., 1996; Litovsky and Antzelevitch, 1989; Zygmunt et al., 1997; Hoppe et al., 1999). Moreover, a reduction in the current density of \( I_{to1} \) is the most consistent change in ionic current density observed in hypertrophy and heart failure (reviewed in Tomaselli and Marban, 1999). Recent experiments have demonstrated that two distinct gene products underlie \( I_{to1} \) in human, and that these currents exhibit distinct kinetic behavior (Kääb et al., 1998; Po et al., 1992). The uncertainty of the role of \( I_{to1} \) on APD in both normal and failing hearts, coupled with the availability of these data signaled that these questions may be addressed by updating the description of \( I_{to1} \) in the model of Winslow et al. (1999).

Chapter 2 describes a genetically based biophysically detailed model of canine \( I_{to1} \). The mathematical description of \( I_{to1} \) is developed by formulating two separate Markov state models of the \( Kv4.3 \)- and the \( Kv1.4 \)-encoded currents (\( I_{Kv4.3} \) and \( I_{Kv1.4} \), respectively), and then combining these current components based on the ratio measured in canine myocytes (Kääb et al., 1996). The \( I_{to1} \) model is then incorporated into the model of Winslow et al. (1999) in order to determine the mechanisms by which \( I_{to1} \) influences AP shape and duration. Simulations demonstrate strong coupling between L-type \( Ca^{2+} \) current and \( I_{Kv4.3} \) and predict a bimodal relationship between \( I_{Kv4.3} \) density and APD whereby perturbations in \( I_{Kv4.3} \) density may produce either prolongation or shortening of APD, depending on baseline \( I_{to1} \) current level. The modeling results suggest that the main impact of \( I_{to1} \) on APD is secondary to its effects on \( I_{CaL} \). A
reduction of Ito1 density from normal levels tends to produce modest shortening of APD, contrary to the belief that loss of Ito1 may be responsible for the extreme AP prolongation observed in heart failure. Implicit in this finding is that Ito1 may not play a critical role in APD prolongation-induced arrhythmias such as EADs.

Local Control of Excitation Contraction Coupling

While the myocyte models of Winslow et al. (1999) and Jafri et al. (1998) include biophysical details of the Ca2+ handling subsystems that were absent from prior efforts, these models do not exhibit the property of graded SR Ca2+ release. Graded Ca2+ release refers to the fact that Ca2+ release from JSR has been observed to be a smooth increasing function of LCC Ca2+ influx (Beuckelamnn and Wier, 1988; Stern, 1992). In these models, all Ca2+ influx through sarcolemmal LCCs and Ca2+ release flux through RyRs is directed into a common Ca2+ compartment (the restricted subspace, see Fig 1.2). This type of model has been referred to as a “common pool” model. It has been theoretically demonstrated that the result of this physical arrangement is that once RyR Ca2+ release is initiated, the resulting increase of Ca2+ concentration in the common pool stimulates regenerative, all-or-none rather than graded Ca2+ release in a positive feedback manner (Stern, 1992). In light of this scenario, it remains unclear how CICR exhibits the properties of high gain (i.e., a small amount of LCC influx Ca2+ is amplified by triggering SR Ca2+ release that is an order of magnitude greater, Isenberg, 1995; Fabiato, 1985a), gradedness, and stability.
The lack of understanding regarding the mechanism of stability of CICR has led some modelers to resort to unphysiological, phenomenological formulations for EC coupling which exhibit graded release “by design” (Luo and Rudy, 1994; Faber and Rudy, 2000). $Ca^{2+}$ release in these models is an explicit function of L-type current magnitude or total integrated $Ca^{2+}$ entry. Such models lack mechanistic descriptions of the process that are the underlying basis for CICR, and therefore lack predictive ability.

Understanding of the mechanisms by which $Ca^{2+}$ influx via voltage-gated 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 (Wier et al., 1994; Cannell et al., 1987; Nabauer et al., 1989), and detection of local $Ca^{2+}$ transients (Cannell et al., 1984; Lopez-Lopez et al., 1994; Lopez-Lopez et al., 1995; Cheng et al., 1995). This has given rise to the local control theory of EC coupling (Sham, 1997; Stern, 1992; Bers, 2001; Wier et al., 1994), which asserts that opening of an individual LCC located in the transverse tubular membrane triggers $Ca^{2+}$-release from a small cluster of RyRs located in the closely apposed JSR membrane (Fabiato, 1985b; Cheng et al., 1993; Cannell et al., 1995; Santana et al., 1996; Sham et al., 1995; Collier et al., 1999; Wang et al., 2001). Tight regulation of this CICR is made possible by the fact that LCCs and RyRs are sensitive to $Ca^{2+}$ levels in the local microdomain (the dyad) rather than global $Ca^{2+}$ levels. The local control theory also asserts that graded control of SR $Ca^{2+}$ release is achieved by statistical recruitment of elementary SR $Ca^{2+}$ release events by trigger $Ca^{2+}$ entering via single LCCs (Stern, 1992; Beuckelamnn and Wier, 1988; Wier and Balke, 1999). As discussed above, increases of local $Ca^{2+}$ also promote $Ca^{2+}$-mediated inactivation of LCCs (Peterson et al., 1999; Bers and Perez-Reyes, 1999).
Since $I_{CaL}$ plays a primary role in determining AP shape and duration, local control theory therefore implies that the microscopic properties of Ca$^{2+}$ release are likely to contribute to macroscopic electrophysiological responses of the cardiac myocyte.  

The improved understanding of the microscopic aspects of EC coupling has made it clear that it is important to think about CICR in terms of microdomains and local control, suggesting that common pool models may be inadequate for detailed study of Ca$^{2+}$ handling and its interaction with other cellular systems. Several computational models have been developed to investigate properties of local Ca$^{2+}$ release at the level of the cardiac dyad (Rice et al., 1999; Stern et al., 1999; Langer and Peskoff, 1996; Cannell and Soeller, 1997; Soeller and Cannell, 1997). Isolated EC coupling models such as these, however, cannot elucidate the nature of the interaction between local Ca$^{2+}$ dynamics and integrative cellular behavior. It is therefore hypothesized that the development of a new comprehensive model of the cardiac ventricular myocyte, based closely on the theory of local control, will yield new insights into the mechanisms that underlie the experimentally observed properties of CICR. In addition, the hypothesis that bi-directional interactions between local Ca$^{2+}$ dynamics and membrane potential play a central role in establishing the integrative electrophysiological properties of the normal cardiac myocyte can be tested in this type of model.  

Chapter 3 describes a novel integrative model of the normal canine ventricular myocyte that incorporates detailed mechanisms accounting for local control of EC coupling. This is accomplished by updating and extending the model of Winslow et al. (1999) to include a population of dyadic Ca$^{2+}$ release units. Local interactions of individual sarcolemmal LCCs with nearby RyRs in the JSR membrane are simulated
stochastically, with these local simulations embedded within the numerical integration of the differential equations describing ionic and membrane pump/exchanger currents, SR Ca\(^{2+}\) cycling, and time-varying cytosolic ion concentrations. This model faithfully reproduces experimentally observed features of LCC voltage- and Ca\(^{2+}\)-dependent gating (Linz and Meyer, 1998; Sipido et al., 1995; Hobai and O'Rourke, 2001; Rose et al., 1992; Herzig et al., 1993), microscopic EC coupling (Wier et al., 1994; Sham et al., 1998; Song et al., 2001), and macroscopic whole cell AP and Ca\(^{2+}\) cycling properties (O'Rourke et al., 1999b). Simulations elucidate the nature of the cross-talk that occurs between Ca\(^{2+}\) cycling and membrane potential, in that SR Ca\(^{2+}\) release plays an important role in determining AP properties, and conversely, that AP profile influences SR Ca\(^{2+}\) release.

**Regulatory Role of Sympathetic Inputs**

The sympathetic neurotransmitter norepinephrine and the adrenal hormone epinephrine stimulate the heart via cell surface β-adrenergic receptors (β-ARs). The β-AR is a member of the GTP-binding protein (G-protein)-coupled receptor family of membrane proteins. A schematic of the events that occur as a result of β-AR stimulation is shown in Fig. 1.3 (Bers, 2002). β-adrenergic signal transduction begins upon binding of the β-AR to agonist. The interaction of the β-AR with the stimulatory G-protein (G\(_s\)) activates adenylyl cyclase, leading to the production of the signaling molecule cyclic AMP (cAMP). The cAMP subsequently activates cAMP-dependent protein kinase A (PKA), which leads to the phosphorylation of a number of proteins that are directly involved in EC coupling.
Figure 1.3: β-adrenergic signaling cascade in the cardiac myocyte. AC, adenylyl cyclase; ACh, acetylcholine; AKAP, A kinase (PKA) anchoring protein; β-AR, β-adrenergic receptor; M2-Rec, M2-muscarinic receptor; PLB, phospholamban; Reg, PKA regulatory subunit; SR, sarcoplasmic reticulum. Figure reprinted from Bers (2002).
L-type Ca$^{2+}$ channels, the SR membrane protein phospholamban (PLB), RyRs, and myofilament proteins are all targets of PKA-mediated phosphorylation. Ca$^{2+}$ current is increased by PKA, which therefore increases the trigger signal for CICR, and the overall cellular Ca$^{2+}$ content (Hussain and Orchard, 1997). Under normal conditions, PLB acts as an inhibitory regulator of the function of SERCA2a. The phosphorylation of PLB relieves SERCA2a inhibition, thereby enhancing the SR Ca$^{2+}$ uptake rate which increases SR Ca$^{2+}$ content and accelerates the rate of decline of the cytosolic Ca$^{2+}$ transient (Simmerman and Jones, 1998). The role PKA-mediated phosphorylation in modulation of RyR function is less clear. In single channel recording made from heavy SR-enriched microsomes, steady-state RyR open probability has been shown to be decreased in the presence of PKA while the transient open probability in response to an abrupt increase in Ca$^{2+}$ concentration has been shown to be increased with PKA (Valdivia et al., 1995). In bilayer measurements, however, the steady-state RyR open probability was enhanced by PKA (Marx et al., 2000). In contrast, measurements obtained from intact cellular systems revealed no significant effect of PKA-dependent RyR phosphorylation on resting SR Ca$^{2+}$ leak under conditions where SR load was unchanged by knockout of PLB (Li et al., 2002). PKA-dependent modulation of LCC and/or RyR function would be expected to alter properties of EC coupling gain. Recent measurements of altered gain in response to β-AR stimulation have demonstrated both an increase (Viatchenko-Karpinski and Gyorke, 2001) and a decrease (Song et al., 2001) in gain.

While detailed studies of LCC availability and gating kinetics have demonstrated an increase in L-type Ca$^{2+}$ current in response to β-adrenergic stimulation (Yue et al., 1995).
the ventricular AP becomes shorter under these conditions (Tomaselli and Marban, 1999). This likely occurs because an increase in β-adrenergic signaling results in a functional increase in repolarizing K⁺ currents as well. It has recently been demonstrated that IKr is enhanced by β-adrenoreceptor stimulation via a reduction in channel inactivation, and hence a reduction in rectification of the current (Heath and Terrar, 2000). In addition, isoproterenol (a β-AR agonist) has been shown to increase the current magnitude of Iks 2- to 3-fold (Kathofer et al., 2000).

Understanding the role of β-AR signaling in the heart is further complicated by the fact that there are genetically and pharmacologically distinct receptor subtypes, which display physiologically differing effects (reviewed in Xiao et al., 1999). The activation of β1-ARs enhance ICaL, SERCA2a function, and speed the dissociation of Ca²⁺ from myofilaments, while the effect of β2-AR activation seems to be targeted to regulation of events that occur in the dyadic space, affecting mainly LCCs and RyRs. The co-assembly of the β2-AR signaling cascade components, including PKA, with the LCC (Davare et al., 2001) and the RyR (Marx et al., 2000) by way of regulatory subunits and anchoring proteins (see Fig 1.3) form a structural basis for the local signaling associated exclusively with β2-ARs. In failing hearts, the expression of β1-ARs is decreased while there is only little or no loss of β2-ARs in both human and animal models (reviewed in Xiao et al., 1999). In addition, constitutive activation of PKA has been shown to predispose mice to arrhythmias and SCD (Antos et al., 2001), RyRs from failing human myocytes have been found to be hyperphosphorylated (Marx et al., 2000), and a defect in
dephosphorylation of LCCs possibly due to decreased phosphatase activity has been identified in human heart failure (Schroder et al., 1998).

The complex nature and broad cellular influence of the β-AR signaling cascade, along with the altered expression of signaling proteins associated with heart failure, suggests that an integrative modeling approach is of necessity for the quantification of the mechanisms by which β-adrenergic inputs modulate whole-cell AP and Ca\(^{2+}\) cycling in normal and failing myocytes. As described above, recent data suggest that localized signaling within subcellular microdomains is a key feature in the β-AR-mediated regulation of EC coupling. A myocyte model, based on the theory of local control, can therefore be used to test the hypothesis that the discrete effects of β-adrenergic inputs on each of the mechanisms that are subject to regulation (e.g., LCC, RyR, SERCA2a, I\(_{Kr}\)) can explain myocyte responses at the cellular level.

In chapter 4, the local control myocyte model described in chapter 3 is expanded to form cellular models of β-adrenergic stimulation and heart failure. The β-AR-stimulated model is formulated by incorporating PKA-mediated effects on LCCs, PLB, RyRs, I\(_{Kr}\), and I\(_{Ks}\) as described above. This requires the inclusion of both active and inactive populations of LCCs as well as defining high activity modes of LCC gating that are thought to be enhanced by PKA-mediated channel phosphorylation (Yue et al., 1990). The model of the failing myocyte is developed by adjusting K\(^{+}\) channel and Ca\(^{2+}\) cycling protein levels based on the functional up or down regulation measured experimentally (Kääb et al., 1996; O'Rourke et al., 1999b). In addition, the impact of heart failure related alterations of β-adrenergic activity on myocyte properties, such as increased RyR
Ca$^{2+}$-sensitivity due to hyperphosphorylation (Marx et al., 2000) and altered LCC properties arising from defective dephosphorylation of the channels (Schroder and Herzig, 1999), is investigated. The model results indicate that heart failure associated impairment of the ability to load the SR with Ca$^{2+}$ leads to dramatic prolongation of AP and severely blunted cytosolic Ca$^{2+}$ transients, whereas altered LCC availability and open probability (Schroder et al., 1998), and a decreased number of LCCs (He et al., 2001) similar to that measured in failing myocytes have relatively modest effects on AP configuration and cytosolic Ca$^{2+}$ transients.
CHAPTER 2:

Role of the Ca\(^{2+}\) Independent Transient Outward Current I\(_{\text{to1}}\) in

Shaping Action Potential Morphology and Duration
Introduction

The voltage-dependent calcium (Ca$^{2+}$)-independent transient outward current $I_{to1}$ is a key contributor in shaping the early phase of the cardiac ventricular action potential (AP). Recordings obtained from single ventricular myocytes isolated from different transmural depths have shown a correlation between $I_{to1}$ density and prominence of the phase 1 notch (Liu et al., 1993; Näbauer et al., 1996; Li et al., 1998; Lukas and Antzelevitch, 1993; Yu et al., 2000). $I_{to1}$ magnitude is also reduced substantially in ventricular myocytes isolated from failing human and canine hearts (Beuckelmann et al., 1993; Kääb et al., 1996), and APs recorded from these cells exhibit a decreased phase 1 notch depth. In addition, blockers of $I_{to1}$ such as 4-aminopyridine (4-AP) reduce or eliminate the phase 1 notch (Litovsky and Antzelevitch, 1989; Zygmunt et al., 1997; Beuckelmann et al., 1993; Kääb et al., 1996).

While the evidence linking $I_{to1}$ magnitude to phase 1 notch depth is strong, the role of $I_{to1}$ on AP duration (APD) remains unclear. Heart failure-induced reduction of $I_{to1}$ density in canine and human myocytes is accompanied by significant prolongation of APD (Kääb et al., 1996; Beuckelmann et al., 1993). However, heart failure is also accompanied by altered expression of genes encoding the inward rectifier potassium (K$^+$) current $I_{K1}$ (Beuckelmann et al., 1993), the sarcoplasmic reticulum (SR)-Ca$^{2+}$ ATPase (Studer et al., 1994; Meyer et al., 1995), and the sodium-calcium exchanger (Studer et al., 1994; Flesch et al., 1996). Recently, a model of the failing canine ventricular myocyte was developed and used to investigate mechanisms influencing APD in heart failure (Winslow et al., 1999). Model predictions are that reduction of both $I_{to1}$ and $I_{K1}$
magnitude, based on the average decrease in current densities measured in terminal heart failure (Kääb et al., 1996), have only modest effects on APD, and that AP prolongation occurs mainly due to altered expression of intracellular Ca$^{2+}$-handling proteins and the accompanying reduction of both SR Ca$^{2+}$ concentration and Ca$^{2+}$-mediated inactivation of the L-type Ca$^{2+}$ current ($I_{\text{Cal}}$) (Winslow et al., 1999).

Experiments designed to reveal the role of $I_{\text{to1}}$ on APD have yielded conflicting results. In the absence of Ca$^{2+}$ buffers, low concentrations of 4-AP (1 mmol/L) shorten APD in isolated canine midmyocardial (Zygmunt et al., 1997) and epicardial (Litovsky and Antzelevitch, 1989) ventricular cells. Higher doses of 4-AP (3-5 mmol/L) prolong the AP in Ca$^{2+}$ buffered canine (Kääb et al., 1996) and human (Beuckelmann et al., 1993) ventricular midmyocardial cells. Interpretation of these findings is complicated by the lack of specificity of 4-AP for $I_{\text{to1}}$ and the use of Ca$^{2+}$ buffers. AP prolongation may result from modest block of delayed rectifier K$^+$ currents in response to higher concentrations of 4-AP. In guinea pig myocytes, the introduction of $I_{\text{to1}}$ by cell fusion techniques produces a reduction of APD which is correlated with increasing $I_{\text{to1}}$ density (Hoppe et al., 1999). The presence of sustained inward currents, however, may have influenced APD in these studies. Functional knockout of a major component of $I_{\text{to1}}$ ($I_{\text{to,f}}$) in mouse also prolongs APD (Barry et al., 1998; Guo et al., 2000). However, $I_{\text{to1}}$ density is much larger in mouse than in canine or human myocytes, and APD is significantly shorter (Barry et al., 1998; Kääb et al., 1996; Näbauer et al., 1996).

These data highlight the uncertainty of the role of $I_{\text{to1}}$ in controlling APD. To help clarify this role, we have: a) functionally expressed and characterized the human
Kv4.3-encoded current (long splice variant, denoted as hKv4.3-2) at 35°C; b) developed a Markov state model of the hKv4.3-2 encoded current based on these data; c) developed a Markov state model of the human Kv1.4 encoded current based on the data of Po et al. (1992, 1993) and combined the hKv1.4 and hKv4.3-2 models to form a model of canine Ito1; d) incorporated the Ito1 model into a computational model of a canine midmyocardial ventricular cell (Winslow et al., 1999); and e) determined the mechanisms by which Ito1 influences AP shape and duration.

Methods

Composition of Ito1

Canine and human Ito1 is likely a combination of Kv4.3- and Kv1.4-encoded currents (I_{Kv4.3} and I_{Kv1.4} respectively). Each component has different kinetics of recovery from inactivation (Brahmajothi et al., 1999; Näbauer et al., 1996; Yu et al., 2000; Wickenden et al., 1999). The Kv4.3 encoded current (Kong et al., 1998; Dilks et al., 1999; Dixon et al., 1996) has kinetics and pharmacological sensitivity similar to the Ito1 component with fast recovery (Näbauer et al., 1996). Reduction in Kv4.3 mRNA transcript level is also correlated with reduction in Ito1 density in human and canine heart failure (Kääb et al., 1998; Kääb et al., 1996). The Kv1.4 current has kinetics similar to the slowly recovering component of Ito1 (Po et al., 1992; Näbauer et al., 1996). In addition, Kv1.4 mRNA transcripts have been detected in canine (Dixon et al., 1996) and human (Kääb et al., 1998) myocytes at levels 16% and 72% as abundant as those of Kv4.3 respectively.
On the basis of these data, the model of \( I_{\text{to1}} \) is constructed as a combination of \( I_{\text{Kv4.3}} \) and \( I_{\text{Kv1.4}} \). The balance of \( I_{\text{Kv4.3}} \) and \( I_{\text{Kv1.4}} \) (77% and 23% respectively) is based on the relative magnitudes of fast vs. slow recovery time constants measured by Kääb et al. (1996, estimated from their Fig. 7D) in canine midmyocardial cells. The strategy for modeling \( I_{\text{Kv4.3}} \) and \( I_{\text{Kv1.4}} \) is to: a) express hKv4.3 in cell culture and characterize and model properties of human \( I_{\text{Kv4.3}} \); b) adjust parameters of this human \( I_{\text{Kv4.3}} \) model to better correspond to properties of the rapidly recovering component of \( I_{\text{to1}} \) measured in isolated canine myocytes; c) formulate a human \( I_{\text{Kv1.4}} \) model using published data on expressed hKv1.4 current; and d) adjust parameters of this human \( I_{\text{Kv1.4}} \) model to better correspond to properties of the slowly recovering component of \( I_{\text{to1}} \) measured in isolated canine myocytes.

**Characterization of \( I_{\text{Kv4.3}} \)**

The hKv4.3 gene has two splice variants with quantitatively similar biophysical properties in the basal state. In this study, only the long splice variant, denoted hKv4.3-2, is expressed. Full-length cDNA encoding hKv4.3-2 (Kong et al., 1998) were subcloned into the pIRES-GFP vector for bicistronic expression of the hKv4.3-2 channel and green fluorescence protein in mouse Ltk⁻ fibroblasts (ATCC). Transient transfection was performed using the lipofectamine method (GIBCO-BRL) as previously described (Kong et al., 1998). Cells were transferred to the stage of an inverted microscope (Nikon Diaphot) and selected by epifluorescence for patch-clamp experiments using the whole-cell configuration (Hamill et al., 1981).
All currents were recorded at 35°C using an Axopatch 200A amplifier (Axon Instruments). Glass pipettes had 1-2MΩ tip resistance when filled with an internal solution containing (in mmol/L) 115 KCl, 10 HEPES, 1 EGTA, 5 MgATP adjusted to pH 7.2 with KOH to yield a final K⁺ concentration of 130 mmol/L. Cells were perfused with Tyrode’s solution containing: 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose adjusted to pH 7.4. Cell capacitance was estimated by integrating the area under an uncompensated 10 mV depolarizing voltage step from -80 mV. The cell capacitance was 15.9 ± 0.9 pF (n = 11). Currents were low-pass filtered at 2 kHz and digitized at 10 kHz through a Digidata 1200 analog-to-digital interface (Axon Instruments) for off-line analysis. The measured hKv4.3-2 current was defined as the difference between the peak transient current and steady state current at the end of a 500 ms clamp. Pooled data are presented as mean ± SE.

Fig. 2.1 shows the Markov state model structure for the hKv4.3-2 channel, which is assumed to be homotetrameric. Rightward transitions represent activation, while transitions into the lower row represent inactivation. Kv4.3 model parameters are optimized to accurately reproduce experimentally measured peak current, time-to-peak, time constant of inactivation, steady-state availability, and recovery from inactivation in response to standard voltage clamp protocols. The $I_{Kv4.3}$ time constant of inactivation was reduced slightly in order to insure behavior consistent with native currents (see Appendix) (Liu et al., 1993; Näbauer et al., 1996; Li et al., 1998).
Figure 2.1: State diagram of the hKv4.3-2 encoded K⁺ channel model. Model structure consists of four closed states (C₀ – C₃), four closed-inactivated states (CI₀ – CI₃), one open state (O), and one open-inactivated state (OI). Transition rates \( \alpha_a, \beta_a, \alpha_i, \) and \( \beta_i \) are voltage dependent, and scaling factors \( f_1 – f_4 \) and \( b_1 – b_4 \) allow for coupling of inactivation to activation.
Characterization of \( I_{\text{Kv1.4}} \)

The \( I_{\text{Kv1.4}} \) model uses the same structure as shown in Fig. 2.1 and is based on the data of Po et al. (1992, 1993). Inactivation is assumed to be voltage independent (Zagotta and Aldrich, 1990). The \( I_{\text{Kv1.4}} \) recovery time constant was reduced in order to insure behavior consistent with native currents (Näbauer et al., 1996; Näbauer et al., 1993; Kääb et al., 1996; Li et al., 1998). Parameters were determined at room temperature and then scaled to 35°C (see Appendix).

Modeling \( I_{\text{to1}} \) effects on the AP

The canine \( I_{\text{to1}} \) is incorporated into the Winslow-Rice-Jafri (WRJ) (Winslow et al., 1999) canine ventricular cell model to investigate its interaction with other membrane currents. Additional myocyte models are implemented to test robustness of simulation results (see Discussion). APs are simulated at 1 Hz and 2 Hz periodic pacing to steady state. For brevity, only those at 1 Hz are shown.

Results

Functional expression of hKv4.3 and \( I_{\text{Kv4.3}} \) model validation

Representative normalized whole cell currents elicited by a family of depolarizing voltage steps from 0 mV to 60 mV in 20 mV increments are shown in Fig. 2.2A (solid lines) with corresponding model simulated currents (dashed lines). Experimental currents are normalized by the peak current measured at 60 mV (2118 pA). Successive current traces are displaced vertically by 0.1 normalized units (212 pA) for clarity. The
current activates and inactivates rapidly, decaying within 100 ms. Time-to-peak decreases monotonically from ≈6.5 ms at –10 mV to ≈2 ms at 60 mV (Fig. 2.2B). The time constant of inactivation becomes nearly voltage independent with a time constant of ≈13.5 ms at potentials greater than 10 mV (Fig 2.2C). The current activates in the range of –40 mV to –30 mV and peak current increases nearly linearly over more positive potentials (Fig. 2.2D). In all cases, experimental data (symbols) are well fit by the model (lines).

The steady-state availability curve is shown in Fig. 2.2E. Boltzmann function fits to experimental data (filled circles, data; dashed line, fit) and to the model (open triangles, data; fit not shown) both yield half-maximal current at (V_{1/2}) –51.1 ± 0.7 mV, with slope factor (k) of 5.6 ± 0.4 mV (±SE values and n = 6 for experiments), in agreement with previous measurements (Hoppe et al., 1999; Dilks et al., 1999; Kong et al., 1998). The current inactivates fully at potentials more positive than –10 mV. Recovery kinetics were determined at –100 mV and –80 mV (Fig. 2.2F). The currents recover mono-exponentially with time constants of 20.23 ± 1.72 ms (filled circles, n = 5) and 37.69 ± 1.76 ms (filled squares, n = 6) for experiments and 20 ms (open triangles, simulated current; dashed line, fit) and 38 ms (open diamonds, simulated current; solid line, fit) for the model at –100 mV and –80 mV respectively. These data demonstrate the ability of the model to reproduce properties measured experimentally.
Figure 2.2: Electrophysiological characteristics of the expressed hKv4.3-2 current and model-simulated currents at 35°C. (A) Representative normalized whole cell currents (solid lines) and model simulated currents (dashed lines) elicited by voltage steps from –80 mV to 0, 20, 40, and 60 mV. Current traces at each test potential are displaced along the ordinate for clarity. (B) Time-to-peak current, (C) time constant of decay, and (D) normalized peak current are shown as functions of membrane potential (●, experiment; △, model). (E) Steady state availability curves are obtained by stepping to a 50 mV test potential from various conditioning prepulse potentials (●, experiment; △, model). A Boltzmann function fit to both data and model (dashed line) yields half-maximal availability voltage ($V_{1/2}$) and slope factor (k) of $-51.1 \pm 0.7$ mV and $5.6 \pm 0.4$ mV respectively (±SE values and n=6 for experiments). (F) For recovery curves obtained at $-100$ mV (●, experiment; △, model) and $-80$ mV (■, experiment; ◇, model) respectively, recovery time constants are $20.23 \pm 1.72$ ms (n = 5) and $37.69 \pm 1.76$ ms (n = 6) for experiments and 20 ms (dashed line) and 38 ms (solid line) for the model.
Figure 2.2
Model validation of $I_{Kv1.4}$ and canine $I_{to1}$

Fig. 2.3 shows features of the $I_{Kv1.4}$ model at 22°C. Fig. 2.3A shows peak $I_{Kv1.4}$ model current (normalized to the +100 mV peak current) with the corresponding model current traces in the inset. The current activates at potentials greater than –50 mV and the current-voltage relation shows slight outward rectification in agreement with experimental data (Po et al., 1992). The time constant of inactivation is nearly voltage independent at potentials greater than –10 mV, ranging from 52.4 ms at –10 mV to 49.1 ms at +100 mV. Time-to-peak is 10-17 ms depending on clamp potential (not shown). Steady-state availability of $I_{Kv1.4}$ (Fig. 2.3B: symbols, model; line, fit) exhibits $V_{1/2}$ of –66.3 mV and k of 4.0 mV, in close agreement with experiments (Po et al., 1993).

Peak $I_{to1}$ current in response to depolarizing voltage steps is shown in Fig. 2.4A, with corresponding current traces shown in the inset. Currents are normalized by peak $I_{to1}$ magnitude at 60 mV. Model $I_{to1}$ activates at ≈–40 mV and the peak current-voltage relation increases monotonically. The steady-state availability curve (Fig. 2.4B: symbols, model; line, fit) exhibits a $V_{1/2}$ of –55.5 mV, with k of 6.8 mV. The features of Figs. 2.4A and 2.4B agree well with native $I_{to1}$ measured in both canine (Kääb et al., 1996; Liu et al., 1993) and human (Näbauer et al., 1993; Li et al., 1998; Wettwer et al., 1994) myocytes. Currents in these experiments activate in the range of –20 mV to –10 mV (Kääb et al., 1996; Liu et al., 1993; Näbauer et al., 1993; Li et al., 1998; Wettwer et al., 1994), and have $V_{1/2}$ in the range of –23 mV to –37 mV (Kääb et al., 1996; Liu et al., 1993; Li et al., 1998; Wettwer et al., 1994). The ≈20 mV difference in both the voltage where $I_{to1}$ first activates and in $V_{1/2}$ is accounted for by the presence of extracellular...
Figure 2.3: Voltage dependence of peak current and steady state availability for the $I_{Kv1.4}$ model at room temperature. (A) Peak current-voltage relationship normalized to the peak current at $+100$ mV with corresponding model current traces in the inset. (B) Steady-state availability curve (○, model currents; line, Boltzmann fit) with $V_{1/2}$ and $k$ of $–66.3$ mV and $4.0$ mV respectively.
Figure 2.3
divalent cations (usually 0.1-0.3 mmol/L Cd^{2+}), which produce a 15-25 mV positive shift in both the peak current-voltage relation and the steady-state inactivation curve of native I_{\text{to1}} (Wettwer et al., 1994; Stengl et al., 1998) and expressed Kv4.3 currents (Faivre et al., 1999).

The time constant of inactivation for model I_{\text{to1}} is nearly voltage independent at potentials greater than –10 mV, ranging from 10.8 ms at –10 mV to 8.4 ms at +60 mV, and the current reaches its peak value in 2-5 ms depending on test potential. The inactivation kinetics agree with values obtained at 35°C for human subendocardial and subepicardial myocytes (7.0 ms and 7.9 ms respectively, Näbauer et al., 1993) and for canine midmyocardial cells (9.4 ms, estimated from Fig. 8 of Liu et al., 1993). Fig. 2.4C shows the time course of recovery from inactivation at –80 mV. Normalized peak currents (open circles) in response to a two-pulse protocol with 200 ms steps to +40 mV are fit to the biexponential recovery function, 1 – a_1 e^{-t/\tau_1} – a_2 e^{-t/\tau_2} (solid line). These data are replotted on a log scale to illustrate the clearly biexponential nature of the model recovery process (Fig. 2.4C, inset). The fit yields values of 37.0 ms and 583.6 ms for \tau_1 and \tau_2 respectively, where the relative amplitude of \tau_1 (i.e. a_1 / (a_1 + a_2)) is 0.779. These time constants and their relative weights have the values expected based on the individual recovery properties and the combination ratio of the component currents, I_{\text{Kv4.3}} and I_{\text{Kv1.4}}, and are in agreement with those measured experimentally at or near 35°C in both canine (Liu et al., 1993; Kääb et al., 1996) and human (Näbauer et al., 1993; Li et al., 1998) ventricular midmyocardial cells.
Figure 2.4: Canine \( I_{\text{ol}} \) model electrophysiological characteristics at 35°C. (A) Peak current-voltage relationship simulated from a holding potential of –80 mV and normalized to the peak current at +60 mV. Inset shows corresponding current traces. (B) Steady-state availability curve (○, model currents; line, Boltzmann fit) with \( V_{1/2} \) and \( k \) of –55.5 mV and 6.8 mV respectively. (C) Recovery curve for \( I_{\text{ol}} \) at –80 mV (○) using a protocol identical to that of Liu et al., 1993 (see text). The best fit to a biexponential recovery function (line) yields time constants of 37.0 ms (77.9%) and 583.6 ms (22.1%). The inset depicts the same data (○) and fit (line), both subtracted from unity, on a logarithmic scale.
Figure 2.4
Effect of $I_{to1}$ density on canine AP shape and duration

Downregulation of Kv4.3, without an associated reduction in Kv1.4 level, is believed to be the basis for the reduction of $I_{to1}$ observed in failing canine myocytes (Kääb et al., 1998). Therefore, the $I_{to1}$ model was incorporated into the WRJ canine ventricular cell model to study the impact of Kv4.3 downregulation on AP properties. The effect of varying the density of the Kv4.3 component of $I_{to1}$ on model AP shape (Fig. 2.5A) and duration (Fig. 2.5B, filled circles) is multifaceted. With complete elimination of $I_{Kv4.3}$, APD at 90% repolarization (APD$_{90}$) is approximately 250 ms. As $I_{Kv4.3}$ current density is increased, phase 1 repolarization becomes more prominent, resulting in an AP with a spike and dome configuration. Hyperpolarization of phase 1 membrane potential due to an early repolarizing current is commonly observed in experiments, however the model reveals an additional effect of $I_{Kv4.3}$ on APD. At relatively low densities of $I_{Kv4.3}$, incremental changes in current density produce progressive prolongation of APD. For example, an $I_{Kv4.3}$ with maximal conductance ($G_{Kv4.3}$) of 0.07 nS/pF (corresponds to 4.6 pA/pF peak current in response to a depolarization to +20 mV from –80 mV) results in an APD of 263 ms. Increasing $G_{Kv4.3}$ to 0.10 and 0.12 nS/pF produces APs with durations of 275 and 300 ms respectively. Further increases in the density of $I_{Kv4.3}$ reveal the presence of a threshold phenomenon, whereby the AP configuration switches from the spike and dome morphology with relatively long duration to a short triangular AP that lacks a plateau phase. The short APs resemble those measured in species normally expressing high levels of $I_{to1}$ such as mouse and rat (Barry et al., 1998). At these relatively high current densities, any further increase in maximal conductance leads to
Figure 2.5: Effect of $I_{Kv4.3}$ current density on canine action potential shape and duration (1 Hz steady-state). (A) APs simulated with the Winslow-Rice-Jafri (Winslow et al., 1999) canine ventricular cell model with increasing values of $G_{Kv4.3}$. (B) $APD_{90}$ as a function of $G_{Kv4.3}$ from simulations described in (A) with normal $I_{Na}$ (●) and $I_{Na}$ reduced by 50% (□).
shortening of APD. The same simulations were repeated with a 50% reduction in density of the fast inward sodium current $I_{Na}$ (Fig. 2.5B, open squares). This decrease in $I_{Na}$ shifts the $G_{Kv4.3}$ vs. APD relationship only slightly toward lower conductance values. The effects of $G_{Kv4.3}$ on APD are seen to depend on the baseline value of $G_{Kv4.3}$ against which perturbations in current density are made. At lower levels of $I_{Kv4.3}$ expression, increasing $G_{Kv4.3}$ prolongs APD, while at higher levels of expression, increasing $G_{Kv4.3}$ shortens APD. Qualitatively similar results were obtained at both 1 Hz (Fig. 2.5) and 2 Hz (not shown) pacing rates.

**Mechanism of $I_{Kv4.3}$ influence on AP shape**

In order to understand mechanisms underlying the influence of $I_{Kv4.3}$ on AP shape, the effect of varying $G_{Kv4.3}$ on individual membrane currents and state variables was examined. L-type Ca$^{2+}$ current ($I_{CaL}$) shape and magnitude is closely coupled to the density of $I_{Kv4.3}$. Fig. 2.6A shows three simulated canine APs. In case 1, $I_{Kv4.3}$ is underexpressed by 70% (dashed line, $G_{Kv4.3} = 0.0358$ nS/pF), in case 2, $I_{Kv4.3}$ is expressed at normal levels (solid line, $G_{Kv4.3} = 0.1194$ nS/pF), and in case 3, $I_{Kv4.3}$ is overexpressed by 20% (dotted line, $G_{Kv4.3} = 0.1432$ nS/pF). The normal current level of case 2 is set such that total $I_{to1}$ density ($\approx 9.5$ pA/pF peak current in response to a step to +20 mV) agrees with that measured in control canine left ventricular midmyocardial cells (5-11 pA/pF, Kääb et al., 1996; Liu et al., 1993) and consists of 77% $I_{Kv4.3}$ (Kääb et al., 1996). Figs. 2.6B, 2.6C, and the inset of Fig. 2.6C show corresponding L-type Ca$^{2+}$ channel open probability ($P_{\text{open}}-I_{CaL}$), $I_{CaL}$, and $I_{to1}$ for each of the three cases. An increase in the
Figure 2.6: Effect of $I_{K_{V4.3}}$ current density on canine action potential, open probability of the L-type Ca$^{2+}$ channel ($P_{\text{open-ICaL}}$), and L-type Ca$^{2+}$ current ($I_{\text{CaL}}$) magnitude. (A) Membrane potential simulated with the WRJ (Winslow et al., 1999) canine ventricular cell model for case 1 (dashed line, underexpression of $I_{K_{V4.3}}$ by 70%), case 2 (solid line, normal expression level of $I_{K_{V4.3}}$), and case 3 (dotted line, overexpression of $I_{K_{V4.3}}$ by 20%). (B) $P_{\text{open-ICaL}}$ for the model parameters described in (A). Inset of (B) depicts $P_{\text{open-ICaL}}$ with case 1 shifted with respect to case 2. (C) $I_{\text{CaL}}$ and $I_{\text{to1}}$ (inset) for the model parameters described in (A).
Figure 2.6
expression level of \( I_{K_{v4.3}} \) leads to an increase in the phase 1 repolarization rate, which results in stronger hyperpolarization of the notch potential (Fig. 2.5A). This decrease in phase 1 notch potential has two effects on \( I_{CaL} \). The rate of decline of \( P_{open-I_{CaL}} \) during phase 1 increases for case 2 vs. case 1 due to a partial deactivation of the L-type channel. There is a concurrent increase in occupation probability of the closed states immediately preceding the open state of the L-type channel (not shown), brought about by activation of \( I_{K_{v4.3}} \). In addition, the driving force for the L-type current is increased for case 2 vs. case 1, increasing peak \( I_{CaL} \) by \( \approx 70\% \) (Fig. 2.6C). Indeed, \( I_{CaL} \) for case 2 remains greater than that for case 1 throughout phase 1 even though \( P_{open-I_{CaL}} \) is decreased (Figs. 2.6B and 2.6C). This increased inward current prevents the relatively large \( I_{K_{v4.3}} \) from truncating the AP, and allows for the subsequent return to activation of the L-type channel following inactivation of \( I_{K_{v4.3}} \), and progression to phase 2 of the AP. The delayed secondary activation of the L-type channel is shown by the increase in \( P_{open-I_{CaL}} \) and \( I_{CaL} \) which occurs between 30 and 50 ms after the upstroke of the AP for case 2 (Fig. 2.6B, arrow). Subsequent to this secondary activation, \( P_{open-I_{CaL}} \) and \( I_{CaL} \) for cases 1 and 2 are time-shifted versions of each other (Fig. 2.6B, inset). Thus, the difference in APD in case 1 vs. case 2 can be attributed to the difference in duration of phase 1. The case 3 AP exhibits an even more rapid early phase 1 rate of hyperpolarization compared to case 2. This results in a more complete deactivation of the L-type channel, thus eliminating the ability of \( I_{CaL} \) to overcome \( I_{K_{v4.3}} \) regardless of the increase in L-type channel driving force. The resulting AP repolarizes rapidly and is therefore lacking a plateau phase.
Discussion

Recent experimental findings suggest that reductions in magnitude of $I_{t01}$, as a consequence of reduced Kv4.3 expression, may be important in modulating APD in normal vs. failing canine and human cardiac myocytes (Kääb et al., 1996; Beuckelmann et al., 1993). In order to investigate the role of $I_{t01}$ in AP profile and duration, a new model of canine $I_{t01}$, built upon descriptions of $I_{Kv4.3}$ and $I_{Kv1.4}$, has been developed in this study. Incorporation of this $I_{t01}$ model into the WRJ canine ventricular cell model (Winslow et al., 1999) reveals a complex interaction between $I_{Kv4.3}$ density and $I_{CaL}$ magnitude which in turn modulates APD. At relatively low levels of $I_{Kv4.3}$, increasing $I_{Kv4.3}$ augments the driving force for $I_{CaL}$ and produces a delay in activation of the late phase of $I_{CaL}$. Both effects contribute to the modest prolongation of APD. Further increasing $I_{Kv4.3}$ density reveals a threshold phenomenon, whereby the early outward current overcomes $I_{CaL}$, thus eliminating phase 2 producing a short AP with triangular shape. Loss of the AP dome resulting from imbalance of membrane currents during phase 1 has been observed previously both experimentally (Lukas and Antzelevitch, 1993) and in simulations (Dumaine et al., 1999). As a consequence of this bimodal phenomenon, increasing $I_{Kv4.3}$ density shortens APD at high baseline densities, while at lower levels, increasing $I_{Kv4.3}$ density produces modest prolongation of APD. Thus, the effect of perturbing $I_{Kv4.3}$ density is dependent on the underlying current level against which the changes are imposed (Fig. 2.5).

This study indicates the relationship between APD and $I_{t01}$ and/or $I_{Kv4.3}$ density is not a simple monotonic correlation. Rather, this relationship exhibits a bifurcation
separating two distinct modes of behavior. In mouse ventricular myocytes, a 57% reduction in mean peak outward current density induced by overexpression of dominant-negative Kv4 α subunits, leads to prolongation of APD (Barry et al., 1998). Since I_{to1} density is significantly greater in mouse than in human or canine cells (Barry et al., 1998; Kääb et al., 1996; Näbauer et al., 1996), model predictions for the APD vs. I_{to1} relationship at high baseline levels of I_{to1} are consistent with these data. However, the complex nature of the relationship between APD and I_{to1} over a wider range of I_{to1} density suggests that extrapolation of the consequences of altering expression levels of I_{to1} in mouse to other species may not be valid. In fact, the WRJ canine model predicts that reduction of I_{to1} from normal levels will lead to modest shortening of APD (Fig. 2.6A). Via this mechanism, a rate dependent decrease in the availability of I_{to1} is expected to contribute to both a shallower phase 1 notch potential and a shorter APD. Such rate dependent changes in AP morphology have been observed in canine epicardial (Liu et al., 1993; Lukas and Antzelevitch, 1993; Litovsky and Antzelevitch, 1989) and midmyocardial (Zygmunt et al., 1997) cells, and human subepicardial cells (Näbauer et al., 1996; Li et al., 1998) and have led to the suggestion that the main impact of I_{to1} on APD is secondary to its effects on I_{CaL} (Litovsky and Antzelevitch, 1989; Zygmunt et al., 1997), consistent with the mechanism described in this study.

Recent experimental evidence for this mechanism has been demonstrated in canine midmyocardial cells (Brian O’Rourke, unpublished data) by presenting a pause in the pacing protocol. In this protocol, steady-state APs are initiated by current injection pulse at 1 Hz. Pacing is then paused for 3 seconds (i.e., 3 stimuli are omitted), and then
resumes. Figs. 2.7A and 2.7B demonstrate canine APs and Ca\(^{2+}\) transients respectively immediately preceding the pause (black) and immediately following the pause (gray). The post-pause AP exhibits both a deeper phase 1 notch and prolongation of APD relative to the pre-pause AP (which is in steady-state). The similarity of the Ca\(^{2+}\) transients corresponding to the pre- and post-pause APs suggests that the 3-second pause does not significantly alter intracellular Ca\(^{2+}\) load, and therefore the modest prolongation in APD is not likely due to altered Ca\(^{2+}\) release and/or Ca\(^{2+}\) dependent currents. Figs. 2.7C and 2.7D show WRJ canine model simulations of APs and Ca\(^{2+}\) transients, respectively, in response to the same pacing protocol. Model simulations agree with experiments, and display both a deeper phase 1 notch and APD prolongation following the pause in pacing, with only minimal differences in properties of the Ca\(^{2+}\) transient. The correlation between phase 1 notch and APD results from the mechanism described in Fig. 2.6. The increased notch depth of the post-pause AP relative to the pre-pause AP occurs due to increased availability of I\(_{\text{to1}}\) as a result of the greater extent of recovery of I\(_{\text{Kv1.4}}\) (data not shown).

To show that the I\(_{\text{Kv4.3}}\) vs. APD behavior is not unique to the WRJ canine cell model, the hKv4.3-2 model was incorporated into the Luo-Rudy Phase II (LRII) (Luo and Rudy, 1994) and the Jafri-Rice-Winslow (JRW) (Jafri et al., 1998) guinea pig ventricular cell models. Descriptions of Ca\(^{2+}\)-handling and I\(_{\text{CaL}}\) differ greatly in the WRJ/JRW vs. the LRII models. Simulations using the guinea pig models (Figs. 2.8A and 2.8B) produced qualitatively similar results to those of the WRJ canine model (Fig. 2.5). The similar effects of I\(_{\text{Kv4.3}}\) density on AP morphology in all three models demonstrate
Figure 2.7: Effect of a brief pause in pacing on experimental and simulated APs and Ca\(^{2+}\) transients. Experimental canine APs (A) and corresponding Ca\(^{2+}\) transients measured using Indo-1 (B) are shown as a function of time. Steady-state (1 Hz) AP and Ca\(^{2+}\) transient are shown in black, and the first AP and Ca\(^{2+}\) transient following a 3 second pause in pacing stimuli are shown in gray. The post-pause AP displays a deeper phase 1 notch and slight AP prolongation whereas pre- and post-pause Ca\(^{2+}\) transients are similar. (Experimental data provided by Brian O’Rourke, unpublished). Simulated APs (C) and corresponding Ca\(^{2+}\) transients (D) using the WRJ model (Winslow et al., 1999) are shown as a function of time for the identical stimulus protocol.
Figure 2.7
Figure 2.8: Effect of $I_{Kv4.3}$ current density on simulated guinea pig APs (1 Hz steady-state). APD$_{90}$ as a function of $G_{Kv4.3}$ for the (A) LRII (Luo and Rudy, 1994) and (B) JRW (Jafri et al., 1998) models. Alternans in APD$_{90}$ were present for the values of $G_{Kv4.3}$ enclosed by the dashed lines in (B). Maximum (▲) and minimum (▼) APDs observed in this region of alternans are shown. (C) APs simulated by introducing $I_{Kv4.3}$ and an instantaneous background inward current ($I_b$, $G_b = 0.1G_{Kv4.3}$ with reversal potential $E_K$) into the JRW (Jafri et al., 1998) model with increasing values of $G_{Kv4.3}$ in 0.2 nS/pF increments.
Figure 2.8
this behavior is not likely to be attributed to any particular mathematical formulation of Ca\textsuperscript{2+}-cycling or detailed representation of ionic currents. Rather, this behavior emerges as a general consequence of interactions between a rapidly activating and inactivating outward current and a rapidly activating and partially inactivating inward current.

The finding that introduction of $I_{Kv4.3}$ to guinea pig ventricular cell models produces APD prolongation (at low $I_{Kv4.3}$ density) contrasts with experimental findings (Hoppe et al., 1999). In these experiments, expressed rat Kv4.3 current was introduced into isolated guinea pig myocytes via cell fusion techniques. Kv4.3 current density had a strong influence on AP plateau potential, and was inversely correlated with APD over the entire range of $I_{Kv4.3}$ densities studied (see Fig. 5 of Hoppe et al., 1999). The presence of a maintained outward current complicates interpretation of these results. The $I_{to1}$-related maintained current magnitude (measured as the difference between the fully activated current and the current following an inactivating prepulse to 0 mV) was not correlated with Kv4.3 current density, consistent with complete inactivation of the Kv4.3 current. However, the possibility remains that a time-independent maintained outward current was correlated with APD. JRW model simulations show that concurrent increases in both $I_{Kv4.3}$ and an instantaneous leak current ($I_b$, with reversal potential $E_K$) produce monotonic decreases in both APD and plateau potential qualitatively similar to that observed experimentally (Fig. 2.8C) (Hoppe et al., 1999). For each AP, conductance of $I_b$ is equal to 10% of $G_{Kv4.3}$, corresponding to a background current that is $\approx$16% of peak $I_{Kv4.3}$, which is in the lower range of those observed experimentally (Hoppe et al., 1999).
Clearly, the $G_{Kv4.3}$ vs. APD relationship described in Figs. 2.8A and 2.8B may be obscured in the presence of background currents.

The properties of $I_{to1}$ and its relationship to AP shape may be modulated by additional factors. Recently, members of the KChIP family of proteins were found to modulate Kv4 currents in oocytes at room temperature (An et al., 2000). Extrapolation of these data to myocytes at 35°C is difficult. At this time, there is insufficient data to explore this issue quantitatively. Moreover, the close similarity in kinetic properties of expressed Kv4.3 and Kv1.4 currents to the two major components of native $I_{to1}$ in human and canine myocytes suggests that the role of accessory proteins may be subtle. Since $I_{CaL}$ (Fig. 2.6) and to a lesser extent $I_{Na}$ (Fig. 2.5B) interact with $I_{to1}$, any factors that modulate these currents, in addition to direct modulators of $I_{to1}$, are likely to influence the impact of $I_{to1}$ on AP shape.

The influence of $I_{to1}$ on the trajectory of $I_{CaL}$ and on the profile and duration of the AP demonstrates the complex interaction of currents that are active during phase 1. A reduction of $I_{to1}$ from normal levels tends to produce modest shortening of APD, contrary to the belief that loss of $I_{to1}$ may be responsible for the extreme APD prolongation observed in heart failure. Implicit in this finding is that $I_{to1}$ may not play a critical role in APD prolongation-induced arrhythmias such as early after depolarizations.
Appendix: Model Development

Markov State Model of the hKv4.3 Channel

The hKv4.3-2 channel Markov model is derived based on the assumption that members of the Kv4 family of K⁺ channels are homotrameric. Each subunit contains a voltage sensor for activation in a similar manner to Kv1 channels (MacKinnon, 1991; Campbell et al., 1993). Therefore, a model consisting of four closed and four closed-inactivated states, one open state, and one open-inactivated state, as shown in Fig. 2.1, is employed. Each transition from left to right within the upper row of states represents progression toward the open state via activation of a single subunit’s voltage sensor. The channel conducts once all four of the subunits have been activated. The scaling of forward and reverse activation rates is based on the assumption that the four subunits activate in a manner that is identical and independent. The forward and reverse rates for the activation process, \( \alpha_a \) and \( \beta_a \) respectively, are exponential functions of voltage in accordance with Eyring rate theory. For each state in the upper row, there is a corresponding inactivated state in the lower row. In a manner similar to activation, transition rates into and out of the inactivated states, \( \beta_i \) and \( \alpha_i \) respectively, are expressed as exponential functions of voltage. The voltage dependent transition rates (in ms⁻¹) are calculated as follows:

\[
\alpha_a(V) = \alpha_{a0}e^{a_1V}
\]

\[
\beta_a(V) = \beta_{a0}e^{-b_1V}
\]

\[
\alpha_i(V) = \alpha_{i0}e^{-a_1V}
\]

\[
\beta_i(V) = \beta_{i0}e^{b_1V}
\]
where \( V \) is membrane potential in mV. The forward and reverse rates between non-inactivated and inactivated states (i.e. transitions between upper and lower rows of Fig. 2.1) are assigned the scaling factors \( f_1 - f_4 \) and \( b_1 - b_4 \) respectively. The scaling factors allow these transition rates to differ as a function of the state of activation of the channel. This assumption of coupling of inactivation to activation provides the necessary additional degrees of freedom to enable the model to accurately fit all aspects of measured macroscopic current behavior. Inactivation is not constrained to be voltage-independent, as it is generally the case for Kv1 channels (Zagotta et al., 1989; Zagotta and Aldrich, 1990). Microscopic reversibility (Hille, 1992) is satisfied by choosing appropriate scaling rates for transitions between inactivated states (in the lower row).

The hKv4.3-2 ionic current is computed as

\[
I_{\text{Kv4.3}}(V,t) = G_{\text{Kv4.3}} \times P_{\text{open}}(V,t) \times \left( V(t) - E_K \right)
\]

(2.5)

where \( I_{\text{Kv4.3}} \) is the hKv4.3-2 current, \( G_{\text{Kv4.3}} \) is the maximal channel conductance, \( P_{\text{open}}(V,t) \) is the probability the channel is in the open state, \( V(t) \) is membrane potential, and \( E_K \) is the potassium reversal potential as determined by the Nernst equation.

**Markov State Model of the hKv1.4 Channel**

The model of \( I_{\text{Kv1.4}} \) is based on the model structure used for \( I_{\text{Kv4.3}} \) (Fig. 2.1) with the additional constraint that inactivation is assumed to be voltage independent (i.e. \( a_i = b_i = 0 \) in Eqs. 2.1 – 2.4) (Zagotta et al., 1989; Zagotta and Aldrich, 1990). Permeation through the Kv1.4 channel exhibits outward rectification, which is described using the
Goldman-Hodgkin-Katz current equation (Po et al., 1992). The Kv1.4 current is therefore computed as

\[
I_{\text{Kv1.4}} = \frac{P_{\text{Kv1.4}}}{C_{sc}} \times \frac{F^2 V}{RT} \times P_{\text{open}}(V,t) \times \frac{[K^+]_i - [K^+]_o e^{-VF/RT}}{1 - e^{-VF/RT}}
\]  

(2.6)

where \(P_{\text{Kv1.4}}\) is the maximum channel permeability, \(C_{sc}\) is the specific membrane capacity \((10^4 \text{ pF/mm}^2)\), \(F\) is the Faraday constant, \(R\) is the gas constant, \(T\) is absolute temperature, \([K^+]_i\) and \([K^+]_o\) are intracellular and extracellular \(K^+\) concentrations respectively, and \(P_{\text{open}}\) is the probability the channel is in the open state.

**Model Fitting Process**

The time evolution of the state occupation probabilities for any finite state Markov process is described by the Kolmogorov forward equations (Ross, 1996). In matrix notation, these equations are

\[
\dot{P}(t) = AP(t)
\]  

(2.7)

where \(P(t)\) is expressed as a column vector of probabilities for occupying each state, and \(A\) is the state transition matrix. Since the rate constants are functions of voltage, \(A\) is generally a voltage-dependent matrix. Under applied voltage clamp protocols, however, this system is a piece-wise linear time invariant system. In this case the form of the analytic solution for an \(n\) state Markov process is

\[
P(t) = \sum_{i=1}^{n} c_i e_i \exp(\lambda_i t)
\]  

(2.8)

where \(\lambda_i\) is the \(i^{th}\) eigenvalue of the matrix \(A\), \(e_i\) is its corresponding eigenvector, and \(c_i\) is a constant of integration which depends on the initial value, \(P(t=0)\).
The free parameters of each model are determined using a Nelder-Mead simplex search algorithm (Nelder and Mead, 1965) implemented by MATLAB (The Mathworks Inc.). In order to compare model behavior to experimental data, Eq. 2.8 is used to calculate model response to the identical voltage clamp protocols used for experimental characterization of the hKv4.3-2 and hKv1.4 expressed currents. For hKv4.3-2, parameter sets were determined at 35°C based on the data obtained in this study. For hKv1.4, parameters are fit at room temperature (21-23°C) based on previous reported data (Po et al., 1992; Po et al., 1993). The quality of fit for any set of parameters is measured by a weighted cost function. The cost function accounts for differences between experiment and simulation by incorporating the mean squared error in time-to-peak (over the range –10 mV to +60 mV for Kv4.3, at +100 mV for Kv1.4), normalized peak current (over the range –40 mV to +60 mV), and time constant of inactivation (over the range –20 mV to +60 mV for Kv4.3, –10 mV to +40 mV for Kv1.4). The cost function accounts for differences in steady state availability by including the squared error of the half maximal availability ($V_{1/2}$) and slope factor ($k$), in Boltzmann function fits (i.e. $[1 + e^{-(V-V_{1/2})/k}]^{-1}$) to model currents vs. experimental currents. An error term (mean steady state availability over the range 0 mV to 55 mV) is included to insure that the current is fully inactivating. The cost function accounts for the time course of recovery from inactivation by including the squared error in time constants from fits to monoeponential recovery curves for model simulations vs. experimental data. Recovery curves are obtained by depolarizing cells to 50 mV for 500 ms, then holding at –100 mV or –80 mV for a variable duration, then depolarizing to 50 mV for 500 ms. The currents
during the second pulse are normalized by the current measured on the first pulse. Table 2.1 gives the optimal set of parameters for the hKv4.3-2 channel expressed in mouse Ltk\(^{-}\) cells at 35°C based on the data obtained in this study.

**Model of Canine I\(_{\text{to1}}\)**

Canine I\(_{\text{to1}}\) is assumed to consist of two distinct currents, I\(_{\text{Kv4.3}}\) and I\(_{\text{Kv1.4}}\). Minor adjustments are made to each of these components to insure agreement of the I\(_{\text{to1}}\) model with native canine I\(_{\text{to1}}\) characteristics. The time constant of inactivation of Kv4.3 is reduced based on fast inactivation time constants measured for native I\(_{\text{to1}}\) in both human and canine ventricular cells (Liu et al., 1993; Näbauer et al., 1996; Li et al., 1998). The inactivation time constant was reduced to \(\approx 9\) ms at depolarized potentials (from \(\approx 13.5\) ms in expressed hKv4.3-2). All other aspects of Kv4.3 behavior were left unchanged.

The Kv1.4 time constant of recovery from inactivation was reduced from that measured by Po et al. (1993) in order to match data from human and canine myocytes. For behavior at 22°C, this time constant was set to 3500 ms and 2000 ms at recovery potentials of –80 mV and –100 mV respectively. These values are in the midrange of time constants for the slow component of recovery from inactivation measured in human left ventricular subendocardial cells (3109±155 ms at –80 mV) (Näbauer et al., 1996), failing human ventricular midmyocardial cells (4425 ms at –80 mV, 2331 ms at –100 mV) (Näbauer et al., 1993), and in canine ventricular midmyocardial cells (1593±323 ms at –100 mV) (Kääb et al., 1996). The parameters of the Kv1.4 model are scaled to 35°C using a Q\(_{10}\) of 3.6 based on measurements of inactivation kinetics made in human native
I_{to1} (Näbauer et al., 1996). In order to maintain steady state behavior, all rates in the model are scaled by the same Q_{10}. This yields a model time constant of recovery from inactivation of 580 ms at 35°C for a recovery potential of −80 mV, a value which is in the midrange of time constants measured by Liu et al. (1993) in canine midmyocardial cells at 37°C (456±212 ms), by Näbauer et al. (1996) in human left ventricular subendocardial cells at 35°C (839±38 ms), and by Li et al. (1998) in human right ventricular endocardial cells at 36°C (490±31 ms).

The canine I_{to1} current is formed by the combination of the canine I_{Kv4.3} and I_{Kv1.4} currents in a 77:23 ratio (based on peak currents elicited by a voltage step from −100 mV to 40 mV). This ratio is based on the relative magnitudes of fast vs. slow recovery time constants measured by Kääb et al. (1996, estimated based on data of their Fig. 7D) in canine midmyocardial cells and corresponds to values of 0.1194 nS/pF and 1.709×10^{-6} mm/s for G_{Kv4.3} and P_{Kv1.4} respectively. Optimized parameter values for the Kv4.3 and Kv1.4 components of the canine I_{to1} model at 35°C are given in Table 2.1.

**Ventricular Myocyte Models**

The canine I_{to1} is incorporated into the Winslow-Rice-Jafri (WRJ) (Winslow et al., 1999) canine ventricular cell model. The dynamical equations are solved using the Merson modified Runge-Kutta fourth-order adaptive step algorithm (Kubicek and Marek, 1983), with a maximum step size of 100 µsec and a maximum normalized truncation error tolerance of 10^{-6}. APs were simulated at 1 Hz and 2 Hz periodic pacing to steady state. APs are initiated using a 100 pA/pF current injection with 500 µs duration.
Table 2.1: Optimized Markov model parameter values at 35°C.

<table>
<thead>
<tr>
<th></th>
<th>$I_{Kv4.3}$</th>
<th>Kv4.3 component of canine $I_{to1}$</th>
<th>Kv1.4 component of canine $I_{to1}$</th>
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<tr>
<td>$\alpha_{a0}$ (ms$^{-1}$)</td>
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<td>0.04984</td>
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CHAPTER 3:

An Integrative Model of the Cardiac Ventricular Myocyte

Incorporating Local Control of Ca$^{2+}$ Release
Introduction

Understanding of the mechanisms by which Ca\(^{2+}\) influx via voltage-gated L-Type Ca\(^{2+}\) channels (LCCs) triggers Ca\(^{2+}\) release from the junctional sarcoplasmic reticulum (SR) has advanced tremendously with the development of experimental techniques for simultaneous measurement of LCC currents and Ca\(^{2+}\) transients (Wier et al., 1994; Cannell et al., 1987; Nabauer et al., 1989), and detection of local Ca\(^{2+}\) transients (Cannell et al., 1984; Lopez-Lopez et al., 1994; Lopez-Lopez et al., 1995; Cheng et al., 1995). This has given rise to the local control theory of excitation contraction (EC) coupling (Sham, 1997; Stern, 1992; Wier et al., 1994; Bers, 2001), which asserts that opening of an individual LCC located in the transverse (T) tubular membrane triggers Ca\(^{2+}\)-release from a small cluster of SR Ca\(^{2+}\) release channels known as ryanodine receptors (RyRs) located in the closely apposed junctional SR membrane (Fabiato, 1985b; Cheng et al., 1993; Cannell et al., 1995; Santana et al., 1996; Sham et al., 1995; Collier et al., 1999; Wang et al., 2001). Tight regulation of this Ca\(^{2+}\)-induced Ca\(^{2+}\)-release (CICR) is made possible by the fact that LCCs and RyRs are sensitive to local rather than global Ca\(^{2+}\) levels. The local control theory also asserts that graded control of SR Ca\(^{2+}\) release, in which Ca\(^{2+}\)-release from junctional SR is a smooth, increasing function of Ca\(^{2+}\) influx, is achieved by statistical recruitment of elementary SR Ca\(^{2+}\) release events by trigger Ca\(^{2+}\) entering via single LCCs (Stern, 1992; Beuckelamnn and Wier, 1988; Wier and Balke, 1999). In addition to triggering SR Ca\(^{2+}\) release, increases of local Ca\(^{2+}\) promote Ca\(^{2+}\)-dependent inactivation of LCCs (Peterson et al., 1999; Bers and Perez-Reyes, 1999). Since L-type Ca\(^{2+}\) current (I\(_{\text{CaL}}\)) plays a primary role in determining action potential (AP)
shape and duration, local control theory therefore implies that the microscopic properties of Ca\textsuperscript{2+} release are likely to contribute to macroscopic electrophysiological responses of the cardiac myocyte.

Several computational models have been developed to investigate properties of local Ca\textsuperscript{2+} release at the level of the cardiac dyad (Rice et al., 1999; Stern et al., 1999; Langer and Peskoff, 1996; Cannell and Soeller, 1997; Soeller and Cannell, 1997). Each of these model formulations incorporates: (1) one or a few LCCs; (2) a cluster of RyRs; (3) the dyadic volume in which the events of CICR occur; and (4) anionic binding sites which buffer Ca\textsuperscript{2+}. In some of these models, detailed descriptions of diffusion and Ca\textsuperscript{2+} binding in the dyadic cleft are employed to demonstrate the effects of geometry, LCC and RyR properties and organization, and surface charge on the spatio-temporal profile of Ca\textsuperscript{2+} within the dyad, and hence on the efficiency of CICR (Langer and Peskoff, 1996; Cannell and Soeller, 1997; Soeller and Cannell, 1997). Stern et al. (1999) have simulated CICR stochastically using numerous RyR schemes to demonstrate conditions necessary for stability of EC coupling, and have suggested a possible role for allosteric interactions between RyRs. The functional release unit model of Rice et al. (1999) has demonstrated that local control of CICR (i.e., graded SR release and high EC coupling gain) can be obtained without including computationally intensive descriptions of Ca\textsuperscript{2+} gradients within the dyadic space. Isolated EC coupling models such as these, however, cannot elucidate the nature of the interaction between dyadic Ca\textsuperscript{2+} release and integrative cellular behavior.
Existing models of the cardiac ventricular myocyte do not incorporate mechanisms of local control of SR Ca\textsuperscript{2+} release (Winslow et al., 1999; Jafri et al., 1998; Luo and Rudy, 1994; Priebe and Beuckelmann, 1998; Pandit et al., 2001; Noble et al., 1998). Rather, in these models all Ca\textsuperscript{2+} influx through sarcolemmal LCCs and Ca\textsuperscript{2+} release flux through RyRs is directed into a common Ca\textsuperscript{2+} compartment. Such models are referred to as “common pool” models (Stern, 1992). If the SR release mechanism is formulated such that RyRs activate in response to a rise in common pool Ca\textsuperscript{2+}, then the result of this physical arrangement is that once RyR Ca\textsuperscript{2+} release is initiated, the resulting increase of Ca\textsuperscript{2+} concentration in the common pool stimulates regenerative, all-or-none rather than graded Ca\textsuperscript{2+} release (Stern, 1992). This “latch up” of Ca\textsuperscript{2+} release can be avoided, and graded SR release can be achieved in a common pool model by formulating Ca\textsuperscript{2+} release flux as an explicit function of sarcolemmal Ca\textsuperscript{2+} influx (Priebe and Beuckelmann, 1998; Luo and Rudy, 1994; Faber and Rudy, 2000; Fox et al., 2002). These phenomenological formulations, however, lack mechanistic descriptions of the processes that are the underlying basis of CICR. Common pool models are therefore inadequate for the study of how detailed microscopic features of EC coupling impact on macroscopic electrophysiological properties of the myocyte such as the whole cell Ca\textsuperscript{2+} transient and AP morphology.

In this study, we develop a comprehensive model of the ventricular myocyte based on the theory of local control of SR Ca\textsuperscript{2+} release. This is accomplished by updating and extending the canine ventricular myocyte model of Winslow et al. (1999) to include a population of dyadic Ca\textsuperscript{2+} release units. Local interactions of individual
sarcolemmal LCCs with nearby RyRs in the JSR membrane are simulated stochastically, with these local simulations embedded within the numerical integration of the differential equations describing ionic and membrane pump/exchanger currents, SR Ca\(^{2+}\) cycling, and time-varying cytosolic ion concentrations. We demonstrate that this model faithfully reproduces experimentally observed features of LCC voltage- and Ca\(^{2+}\)-dependent gating (Linz and Meyer, 1998; Sipido et al., 1995; Sham et al., 1995; Sham, 1997; Hobai and O'Rourke, 2001; Rose et al., 1992; Herzig et al., 1993), microscopic EC coupling (Wier et al., 1994; Sham et al., 1998; Song et al., 2001), and macroscopic whole cell AP and Ca\(^{2+}\) cycling properties (O'Rourke et al., 1999b). Simulations demonstrate that local control is an essential property for stability of APs when the LCC inactivation process depends more strongly on local Ca\(^{2+}\) than on membrane potential, a scenario that is supported by experiments (Linz and Meyer, 1998; Peterson et al., 1999; Peterson et al., 2000) but which cannot be implemented successfully using a common pool model of the type where the inherent positive feedback of rising Ca\(^{2+}\) levels on RyR activation is intact. Modeling support the hypothesis that the robust bi-directional interaction between Ca\(^{2+}\) dynamics and membrane potential in the local control environment plays a central role in establishing the integrative electrophysiological properties of the cardiac myocyte.

**Methods**

The Ca\(^{2+}\) Release Unit Model

We seek to define a model of local control of SR Ca\(^{2+}\) release which captures fundamental properties such as graded release, while at the same time is computationally
tractable so that it may be incorporated within an integrative model of the ventricular myocyte. Models describing diffusion of Ca\(^{2+}\) within the dyadic space, detailed dyad geometry, and surface charge effects (Canell and Soeller, 1997; Soeller and Canell, 1997; Langer and Peskoff, 1996) are too computationally demanding for this application. As a compromise between structural and biophysical detail versus tractability, a “minimal model” of local control of Ca\(^{2+}\) release, referred to as the Ca\(^{2+}\) release unit (CaRU) model, is implemented. A full mathematical description of the stochastic state models, dynamical equations, parameters, and initial conditions defining the myocyte model are given in Appendix I.

Fig. 3.1A shows a schematic of the CaRU model based in part on the previous model of Rice et al. (1999). The CaRU model is intended to mimic the properties of Ca\(^{2+}\) sparks in the T-tubule/SR (T-SR) junction. Ca\(^{2+}\) sparks are elementary SR Ca\(^{2+}\) release events arising from a cluster of RyRs (Cheng et al., 1993). Fig. 3.1B shows a cross-section of the model T-SR cleft, which is divided into four individual dyadic 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 (5 RyRs/SS × 4 SSs/CaRU = 20 RyRs/CaRU) 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 (Wang et al., 2001). Each subspace is treated as a single compartment in which Ca\(^{2+}\) concentration is uniform, however Ca\(^{2+}\) may diffuse passively to neighboring subspaces within the same CaRU. The rate of Ca\(^{2+}\) transfer between two adjacent
Figure 3.1: Schematic representation of the Ca\textsuperscript{2+} release unit model (CaRU). (A) Trigger Ca\textsuperscript{2+} influx through the LCCs enters into the T-SR cleft (dyadic space). The rise in local Ca\textsuperscript{2+} level promotes the opening of RyRs and ClChs. The excess local Ca\textsuperscript{2+} passively diffuses out of the cleft into the cytosol, and JSR Ca\textsuperscript{2+} is refilled via passive diffusion from the NSR. (B) The T-SR cleft (shown in cross-section) is composed of four dyadic subspace volumes, arranged on a 2 × 2 grid, each containing 1 LCC, 1 ClCh, and 5 RyRs. Ca\textsuperscript{2+} in any subspace may diffuse to a neighboring subspace (J\textsubscript{iss}) or to the cytosol (J\textsubscript{xfer}). J\textsubscript{iss,i,j,l} represents Ca\textsuperscript{2+} flux from the j\textsuperscript{th} subspace to the l\textsuperscript{th} subspace within the i\textsuperscript{th} CaRU. Similarly J\textsubscript{xfer,i,j} represents Ca\textsuperscript{2+} flux from the j\textsuperscript{th} subspace to the cytosol from the i\textsuperscript{th} CaRU (see Appendix I).
Figure 3.1
subspace compartments is assumed to be 10-fold slower than that from subspace to cytosol. This yields an intersubspace transfer rate \( (r_{iss}) \) of 20 ms\(^{-1}\) which corresponds to a diffusion coefficient of \( \sim 3.3 \times 10^{-6} \) cm\(^2\) s\(^{-1}\) when the assumed height of the model subspace is 12 nm. This value is similar to estimates for Ca\(^{2+}\) diffusion in the presence of RyR “feet” structures in the restrictive dyadic subspace volume (Soeller and Cannell, 1997). The division of the CaRU into four subunits allows for the possibility of triggering Ca\(^{2+}\) release in adjacent subspaces (i.e., RyR recruitment) under conditions where unitary LCC currents are large. The existence of communication among adjacent subspace volumes is supported by the findings that Ca\(^{2+}\) release sites can be coherent over distances larger than that occupied by a single release site (Parker et al., 1996), and that the mean amplitude of Ca\(^{2+}\) spikes, local SR Ca\(^{2+}\) release events that consist of one or a few Ca\(^{2+}\) sparks (Song et al., 1998), exhibits a bell shaped voltage dependence, indicating synchronization of Ca\(^{2+}\) release events within a T-SR junction (Song et al., 2001). The choice of four subunits allows for semi-quantitative description of dyadic Ca\(^{2+}\) diffusion while retaining minimal model complexity.

One of the bases for local control of SR Ca\(^{2+}\) release is the structural separation of T-SR clefts at the ends of sarcomeres (i.e., RyR clusters are physically separated) (Franzini-Armstrong et al., 1999). Each CaRU is therefore simulated independently in accord with this observation. Upon activation of RyRs, subspace Ca\(^{2+}\) concentration will become elevated. This Ca\(^{2+}\) will freely diffuse to either adjacent subspace compartments \( (J_{iss}) \), or into the cytosol \( (J_{xfer}) \) along its concentration gradient. The local JSR
compartment is refilled via passive diffusion of $\text{Ca}^{2+}$ from the network SR (NSR) compartment ($J_n$).

The model for the L-type $\text{Ca}^{2+}$ channel is identical in structure to the mode-switching model developed previously by Jafri et al. (1998). The following modifications have been made to model parameters: 1) voltage-dependence of forward and reverse activation transition rates ($\alpha$ and $\beta$ respectively) have been adjusted based on recent measurements of $I_{\text{CaL}}$ obtained in canine midmyocardial cells (Hobai and O'Rourke, 2001); 2) voltage-independent transition rates into open states $f$ and $f'$ have been adjusted to yield peak open probability in the range of 5-15% in response to a maximally activating voltage clamp stimulus (Rose et al., 1992; Herzig et al., 1993; Handrock et al., 1998); 3) transition rates between the normal gating mode (Mode Normal) and the $\text{Ca}^{2+}$-inactivation mode (Mode Ca) $\gamma$ and $\omega$ are adjusted to enhance $\text{Ca}^{2+}$ dependent inactivation, while the voltage-dependent steady-state inactivation function ($y_{\infty}$) is modified to have an asymptotic value of 0.6 for large positive membrane potentials. The latter is based on the observation that there is a small sustained component of $\text{Ca}^{2+}$ current in response to voltage clamp stimuli in canine ventricular cells (Kääb et al., 1996; Tseng et al., 1987), and that $\text{Ca}^{2+}$ dependent inactivation dominates the $I_{\text{CaL}}$ inactivation process while voltage-dependent inactivation is relatively weak and incomplete (Linz and Meyer, 1998; Hadley and Hume, 1987; Peterson et al., 1999; Peterson et al., 2000); 4) permeation through the LCC is described by the Goldman-Hodgkin-Katz current equation as originally presented by Luo and Rudy (Luo and Rudy, 1994) where $\text{Ca}^{2+}$ concentration at the inner mouth of the channel is assumed to be
equivalent to Ca\textsuperscript{2+} concentration of the adjacent subspace, rather than assuming it is constant (Jafri et al., 1998; Rice et al., 1999); 5) LCC permeability ($P_{CaL}$) is adjusted to a value of $9.13 \times 10^{-13}$ cm\textsuperscript{3} s\textsuperscript{-1} which yields a single channel slope conductance of 8.2 pS and a unitary current of $\sim -0.12$ pA at 0 mV (see Fig. 3.2G) (Rose et al., 1992; Yue and Marban, 1990).

Whole cell Ca\textsuperscript{2+} current can be described as a function of the total number of channels ($N_{LCC}$), the single channel current magnitude ($i$), the open probability ($p_o$), and the fraction of channels that are available for activation ($f_{active}$, i.e., in a phosphorylated mode), where $I_{CaL} = N_{LCC} \times i \times p_o \times f_{active}$ (Handrock et al., 1998). Under conditions where $f_{active}$ remains constant, $I_{CaL} = N_{active} \times i \times p_o$, where $N_{active} = N_{LCC} \times f_{active}$ is constant. As described above, single channel parameters are based on experimentally obtained constraints on both $i$ and $p_o$. $N_{active}$ is therefore chosen such that the amplitude of the ensemble current summed over all LCCs corresponds to whole cell measurements in canine myocytes (Hobai and O'Rourke, 2001). This approach yields a value of 50,000 for $N_{active}$, which agrees with experimental estimates of LCC density (Rose et al., 1992; McDonald et al., 1986), and which corresponds to 12,500 CaRUs ($N_{CaRU}$). The process of slow cycling between active and inactive states is not included in the model at this time; rather only active LCCs are simulated.

Each RyR channel is represented by the model developed by Keizer and Smith (1998) and later modified by Rice et al. (1999). This model was originally designed to describe the property of RyR adaptation, a slow spontaneous decrease in open probability which has been observed to occur after activation by a step increase in Ca\textsuperscript{2+} when
measured in single channels reconstituted in lipid bilayers (Gyorke and Fill, 1993). A channel in the adapted state can be reactivated by an additional increase in Ca\textsuperscript{2+}. In contrast, the findings of Sham et al. (1998) suggest that RyR inactivation into an absolute refractory state occurs in vivo during EC coupling. It is difficult to incorporate single channel RyR data obtained in vitro into models of EC coupling due to the lack of quantitative information regarding in vivo regulation by accessory proteins and other ligands. Therefore, our approach has been to constrain RyR model parameters based on experimentally obtained properties of EC coupling (Song et al., 2001; Wier et al., 1994), without an explicit attempt to retain the property of adaptation. The Ca\textsuperscript{2+} dependence of the RyR model state transition rates have been adjusted based on the assumption that four Ca\textsuperscript{2+} ions must bind to the channel before it can enter the open state (Zahradnikova et al., 1999). During stochastic simulation of each RyR, two approximations are implemented to reduce simulation time. Descriptions of these approximations are included in Appendix I (see Fig. 3.13). Briefly, the six-state model can be reduced to a five- or four-state model by using the rapid equilibrium assumption under conditions of high subspace Ca\textsuperscript{2+} (Keizer and Smith, 1998). In addition, the Ca\textsuperscript{2+}-dependent transition rates are bounded (in a manner that maintains microscopic reversibility) in order to avoid prohibitively large transition rates during spikes in subspace Ca\textsuperscript{2+} level.

Ca\textsuperscript{2+} buffering in each CaRU is implemented as described previously (Rice et al., 1999) using the rapid buffer approximation (Wagner and Keizer, 1994). It is assumed that Ca\textsuperscript{2+} is buffered in the subspace by the phospholipid anion groups on both the SR and sarcolemmal membranes, and that these buffers are immobile (Smith et al., 1998).
Buffering parameters for JSR Ca\(^{2+}\) are based on measures of Ca\(^{2+}\)-calsequestrin binding (Shannon et al., 2000).

The Ca\(^{2+}\)-dependent transient outward chloride (Cl\(^{-}\)) current (\(I_{\text{to2}}\)) is included as part of the CaRU. Experimental evidence indicates that the Ca\(^{2+}\) binding affinity of this Cl\(^{-}\) channel (ClCh) is low (\(K_{d,\text{ClCh}} \sim 150\) \(\mu\)M) relative to normal cytosolic Ca\(^{2+}\) concentrations (Collier et al., 1996), and that \(I_{\text{to2}}\) is abolished in the presence of caffeine and exhibits rate dependent properties that correlate with those of SR Ca\(^{2+}\) load (Zygmunt, 1994), suggesting that ClChs are activated by subspace Ca\(^{2+}\). Estimates of ClCh density are in the range of 3-4 \(\mu\)m\(^{-2}\) (Collier et al., 1996), a value similar to the density of active LCCs (2-5 \(\mu\)m\(^{-2}\) (Rose et al., 1992; McDonald et al., 1986). Each CaRU therefore includes an equal number of ClChs as LCCs, i.e., one Cl\(^{-}\) channel per subspace (Fig. 3.1B). \(I_{\text{to2}}\) is modeled as a voltage- and time-independent ligand-gated channel, using a simple two-state, closed-open model (see Fig. 3.12C), based on the gating and permeation properties of the unitary current as measured by Collier et al. (1996). For simplicity, intracellular Cl\(^{-}\) concentration is assumed to be constant.

**Model State Variables**

All local control model state variables (SVs) are categorized as members of one of the following three groups: 1) global, 2) CaRU-compartment, and 3) CaRU-random (see Appendix II for details). Global and CaRU-compartment SVs are continuous in value, and are each described by a single ordinary differential equation. Global SVs of the local control myocyte model are based on those of the Winslow et al. (1999) canine
ventricular cell model with the following modifications: 1) the voltage-dependent Ca\(^{2+}\)-independent transient outward potassium (K\(^+\)) current (\(I_{to1}\)) is modeled as described by Greenstein et al. (2000); 2) the rapid delayed rectifier K\(^+\) current (\(I_{Kr}\)) is modeled as described by Mazhari et al. (2001); 3) the SR Ca\(^{2+}\) ATPase (SERCA2a pump) has been updated based on the model and parameter set of Shannon et al. (2000), which accounts for both a forward and a backward Ca\(^{2+}\) pump flux; and 4) some membrane currents and ionic fluxes are scaled to preserve cytosolic ionic concentrations and AP shape (see Appendix I).

There are a small number of channels (1 LCC, 5 RyRs, 1 ClCh) within the local environment of a single CaRU subspace, and channels in physically separate subspaces will be exposed to different local Ca\(^{2+}\) levels. Gating behavior therefore may not be approximated using deterministic descriptions based on continuously varying state occupancy probabilities. Simulation of the local control myocyte model requires that the state solution trajectory for every channel within each CaRU be simulated individually. CaRU-random SVs are therefore discrete valued random variables evolving in time described by Markov processes.

**Local Control Model Simulation Algorithm**

The local control simulation algorithm is comprised of three intercommunicating sub-algorithms, each of which is applied to one of the three groups of SVs. Global SVs are numerically integrated using the Runge-Kutta-Merson 4\(^{th}\)-order adaptive step method (Kubicek and Marek, 1983). CaRU-compartment SVs are integrated using the 2\(^{nd}\)-order
trapezoidal method (at a time-step determined locally within each CaRU). CaRU-random SVs are updated at the end of each local time step by Monte Carlo simulation based on the calculated probability that a transition has occurred into each possible destination state. Local time steps are chosen sufficiently small to insure that there is negligible probability that any channel within a CaRU would undergo multiple transitions within a single time step. The majority of computational time is spent in stochastic simulation of the large number of independent CaRUs. This simulation is therefore highly parallelizable, and is implemented on a SGI Power Challenge symmetric multiprocessing computer configured with 12 R10000 processors and one Gbyte memory. A detailed description of the local control simulation algorithm is given in Appendix II.

As a result of the embedded Monte Carlo simulation, all state variables (e.g., $V_m$) and ionic currents/fluxes (e.g., $I_{\text{CaL}}$) will contain a component of stochastic noise (e.g., Fig. 3.3A). These fluctuations introduce a degree of variability to simulation output. Therefore, where appropriate, simulation data are presented as mean ± SE, where the specified value for n refers to the number of simulation runs.

**Results**

**L-type Ca$^{2+}$ Current**

Since local control of Ca$^{2+}$ release is the central feature of the current myocyte model, it is necessary to verify that the newly parameterized L-type Ca$^{2+}$ channel model behavior is consistent with experimental data for both single LCCs and whole cell currents. Fig. 3.2 demonstrates single LCC properties of the model under normal
physiological conditions (i.e., with EC coupling intact and 2 mM extracellular Ca\(^{2+}\)). Sample LCC unitary currents in response to 200 ms membrane depolarizations to test potentials of –20 mV and 0 mV from a holding potential of –100 mV are shown in Figs. 3.2, A and B respectively. At test pulses ≥ 0 mV, LCC openings seldom occur because: (1) no silent mode behavior (Herzig et al., 1993; Handrock et al., 1998) is implemented in this model; (2) voltage-dependent inactivation is relatively slow and incomplete with respect to activation kinetics; and (3) the likelihood that Ca\(^{2+}\)-mediated inactivation occurs is very low prior to the first opening of an LCC. Multiple openings are common because the steady state probability that an LCC inactivates at depolarized potentials is substantially less than unity in canine myocytes (i.e., inactivation is incomplete) (Kääb et al., 1996; Tseng et al., 1987). Figs. 3.2, C and D show open time histograms and Figs. 3.2, E and F show cumulative first latency distributions, determined at test potentials of –20 mV and 0 mV respectively based on a random sampling of 500 LCCs. Open time histograms are well described by a single exponential (\(\tau_{\text{open}}= 0.481\) ms and 0.492 ms at –20 mV and 0 mV respectively) indicating that mean open time does not vary with test potential. Open durations, as well as first latency distributions are consistent with previous measurements after accounting for differences in experimental conditions (e.g., temperature) (Herzig et al., 1993; Handrock et al., 1998; Schroder et al., 1998; Rose et al., 1992). The fraction of sweeps exhibiting no openings is lower in the model than found in experiments due to the exclusion of LCC silent mode behavior in the model (Handrock et al., 1998). The sharpening of the first latency distribution at depolarized potentials indicates that channel openings become less temporally dispersed with
Figure 3.2: Single L-type Ca\textsuperscript{2+} channel model properties. (A) and (B), Sample unitary Ca\textsuperscript{2+} currents in response to a 200 ms test pulse to –20 mV and 0 mV respectively. The small positive deflection in each current record indicates the time at which voltage is switched to the test potential. (C) and (D), Open duration histograms measured from 500 sweeps at –20 mV and 0 mV respectively. Exponential fits (black line) yield mean open duration of 0.481 ms and 0.492 ms at –20 mV and 0 mV respectively. (E) and (F), First latency distribution measured at –20 mV and 0 mV respectively. (G) Single channel current-voltage relation of model LCCs with 2 mM extracellular Ca\textsuperscript{2+}. Unitary conductance is 8.2 pS when measured in the range of –80 mV to –20 mV.
Figure 3.2
increasing depolarization, in agreement with experimental findings (Rose et al., 1992). Fig. 3.2G shows unitary currents as a function of membrane potential. Single channel slope conductance, as measured in the range between −80 mV and −20 mV, is 8.2 pS and agrees with measurements made in near physiological solutions (Rose et al., 1992; Yue and Marban, 1990).

The summation of all unitary Ca\textsuperscript{2+} currents within the myocyte, such as those shown in Figs. 3.2, A and B, yields macroscopic $I_{CaL}$. Simulated whole cell currents elicited by a family of depolarizing voltage steps from −30 mV to 40 mV in 10 mV increments are shown in Fig. 3.3A. Currents activate rapidly (< 6 ms) and decay over ~100 ms. In Fig. 3.3B, peak $I_{CaL}$ amplitude is plotted as function of test potential (where data points represent the mean of five runs at each potential). Maximum inward Ca\textsuperscript{2+} current is produced at a test potential of 10 mV. ($n = 5$). The bell shaped peak current profile is in agreement with peak currents measured in canine (Hobai and O'Rourke, 2001; Kääb et al., 1996; O'Rourke et al., 1999b), guinea pig (Rose et al., 1992), and human (He et al., 2001) ventricular myocytes. Fig. 3.3C demonstrates the underlying processes that govern the time course of $I_{CaL}$ during the voltage clamp to 0 mV. The quantities shown are LCC open probability ($p_o$, black solid line), the probability of occupancy of Mode Normal ($\text{Prob}\{\text{Norm}\} = 1 – \text{Prob}\{\text{Ca}^{2+}\text{-mediated inactivation has occurred}\}$, gray solid line) and the fraction of channels available for voltage-dependent inactivation ($y = 1 – \text{Prob}\{\text{voltage-dependent inactivation has occurred}\}$, dashed line). LCC $p_o$ reaches a peak value of ~0.1, consistent with studies that indicate that peak $p_o$ with Ca\textsuperscript{2+} as the charge carrier (Rose et al., 1992) and time averaged $p_o$ with Ba\textsuperscript{2+} as the
charge carrier (minimizing $\text{Ca}^{2+}$-mediated inactivation) (Herzig et al., 1993; Handrock et al., 1998) is in the range of 0.05 to 0.15. A comparison of the time progression of \( \text{Prob} \{ \text{Norm} \} \) and \( \gamma \) clearly demonstrates that at 0 mV, $\text{Ca}^{2+}$-mediated inactivation of \( I_{\text{CaL}} \) develops more rapidly and progresses more completely than voltage-dependent inactivation, consistent with recent experiments (Linz and Meyer, 1998; Sipido et al., 1995; Hadley and Hume, 1987; Peterson et al., 1999; Peterson et al., 2000). In addition, $\text{Ca}^{2+}$-mediated inactivation is partially relieved in the latter portion of the pulse (Fig. 3.3C), indicative of decaying local $\text{Ca}^{2+}$ levels.

The feedback of local $\text{Ca}^{2+}$ signals on LCC gating plays an important role in the determination of properties of both CICR and APs, and is a key feature of the local control myocyte model which is explored in more detail below. Steady state inactivation properties of \( I_{\text{CaL}} \) are measured using a double pulse protocol where membrane potential is first stepped from –100 mV to various pre-pulse potentials for 400 ms and then to a 0 mV test potential. Peak currents, normalized by current obtained in the absence of a pre-pulse, are shown as a function of the pre-pulse potential in Fig. 3.3D. Simulations were performed under normal conditions (filled circles) and with subspace $\text{Ca}^{2+}$ clamped to diastolic levels to mimic an alternative charge carrier such as $\text{Ba}^{2+}$ that would not significantly promote $\text{Ca}^{2+}$-mediated inactivation (open circles). Under normal conditions, the \( I_{\text{CaL}} \) steady state inactivation curve is U-shaped. Disabling of $\text{Ca}^{2+}$-mediated inactivation yields an inactivation curve that decreases monotonically with depolarization. Inactivation is incomplete, and asymptotically approaches ~ 50% for highly depolarized pre-pulses. These features agree well with inactivation curves
Figure 3.3: Properties of macroscopic $I_{CaL}$. (A) Simulated whole cell currents as a function of time in response to a family of depolarizing voltage steps from $-30$ mV to $40$ mV in $10$ mV increments. (B) Mean peak current-voltage relation based on five simulations at each potential (i.e., $n = 5$). (C) LCC open probability (black solid line), probability of occupancy of Mode Normal (Prob{Norm}, gray solid line), and probability that voltage-dependent inactivation has not occurred ($y$, dashed line) in response to a voltage clamp to $0$ mV. (D) Steady-state inactivation curve obtained using a double pulse protocol (see text) with (filled circles) and without (open circles) Ca$^{2+}$ as the charge carrier.
Figure 3.3
obtained experimentally in native myocytes (Hadley and Hume, 1987; Linz and Meyer, 1998). Ca\(^{2+}\)-mediated inactivation makes a dominant contribution to the total inactivation of \(I_{\text{CaL}}\) in the range of potentials of –10 mV to +30 mV, consistent with the range of potentials where LCC Ca\(^{2+}\) influx is maximal (Fig. 3.3B). In addition, this is within the range of plateau potentials where inactivation would normally occur during an AP. The U-shape is therefore a consequence of the variation in local Ca\(^{2+}\) transients that arise due to the voltage dependence of LCC Ca\(^{2+}\) influx, and the subsequent graded CICR.

**Excitation Contraction Coupling**

Figs. 3.4, A and B, demonstrate the most elementary model release event, as triggered by a single LCC. A typical response to a 200 ms duration 0 mV voltage clamp pulse is shown. Ca\(^{2+}\) flux through an LCC (gray line) and the net SR Ca\(^{2+}\) release flux through the five adjacent RyRs (black line) are shown in Fig. 3.4A. At onset of the voltage pulse, the LCC first opens after ~ 5 ms, and then exhibits only one additional opening. Local JSR release flux is triggered by the first LCC opening and lasts ~ 20 ms, far longer than the LCC open duration. The amplitude of the release flux varies with the number of open RyRs and the local Ca\(^{2+}\) gradient across the JSR membrane. Individual RyR channel gating events can be discerned as step-like changes in local JSR release flux (Fig. 3.4A, *arrow 1*), while changes due to the time varying local Ca\(^{2+}\) gradient across the RyRs (i.e., effects of JSR depletion) occur more gradually over time (Fig. 3.4A, *arrow 2*). Mean RyR open time varies from ~ 7 ms when subspace Ca\(^{2+}\) is high early in the voltage
Figure 3.4: Sample results for a single CaRU in response to a 200 ms voltage clamp to 0 mV. (A) Ca$^{2+}$ flux through a single LCC (*gray line*) and through the set of five RyRs (*black line*) within a single dyadic subspace compartment. Arrows 1 and 2 highlight changes in SR Ca$^{2+}$ release flux due to RyR gating and JSR depletion respectively. (B) Subspace Ca$^{2+}$ concentration associated with the events of panel A. (C) Ca$^{2+}$ flux through the set of four LCCs (*gray line*) and the set of 20 RyRs (*black line*) within a single CaRU. (D) Mean subspace Ca$^{2+}$ concentration in the four subspace compartments associated with the events in the CaRU described in panel C.
Figure 3.4
clamp pulse to < 1 ms after the subspace Ca\(^{2+}\) has subsided later in the pulse (data not shown), similar to values reported previously (Rice et al., 1999; Lukyanenko et al., 1996). Fig. 3.4B shows the corresponding dyadic subspace Ca\(^{2+}\) concentration, which reaches a peak value of ~ 40 µM. The small amplitude deflections in subspace Ca\(^{2+}\) level that continue to occur following termination of the transient are the result of inter-subspace Ca\(^{2+}\) diffusion (\(J_{iss}\)), and are indicative of trigger events (LCC openings) occurring in neighboring subspace compartments within the CaRU.

A local Ca\(^{2+}\) spike (a localized Ca\(^{2+}\) release event within a single T-SR junction) is modeled by a single CaRU in Figs. 3.4, C and D, for the same voltage clamp stimulus. Total Ca\(^{2+}\) influx through the set of four LCCs (gray line) and the net SR Ca\(^{2+}\) release flux through the set of twenty RyRs (black line) are shown in Fig. 3.4C. LCC Ca\(^{2+}\) influx rises to a level consistent with 2 open channels within a short time following the initiation of the pulse, indicating some degree of temporal synchronization in the onset of trigger Ca\(^{2+}\) influx at 0 mV. Net JSR Ca\(^{2+}\) release flux follows a similar time course to that observed in an individual subspace. The spatial average of Ca\(^{2+}\) concentration in the four subspace compartments of the CaRU is intended to represent a Ca\(^{2+}\) spike (Fig. 3.4D). The peak amplitude of this signal is less than that seen in a single subspace (Fig. 3.4B) due to temporal dispersion of Ca\(^{2+}\) release events. The Ca\(^{2+}\) spike duration is ~ 25 ms (at half-maximal amplitude), similar to that measured in myocytes using confocal imaging techniques (20-50 ms) (Cheng et al., 1993; Song et al., 2001; Sham et al., 1998).

Whole cell Ca\(^{2+}\) signals, which can be explained as the spatial and temporal summation of local elementary Ca\(^{2+}\) release events, are shown in Fig. 3.5. Total LCC
Ca\textsuperscript{2+} influx (*black line*) and RyR Ca\textsuperscript{2+} release flux (*gray line*) in response to a 0 mV voltage clamp are plotted as a function of time in Fig. 3.5A. EC coupling gain, measured as the ratio of peak RyR Ca\textsuperscript{2+} flux to peak LCC Ca\textsuperscript{2+} flux, is \(\sim 12\) at 0 mV. While peak Ca\textsuperscript{2+} flux through RyRs and LCCs occurs within a few milliseconds following the onset of the voltage pulse, there is a relatively small sustained component of flux that lasts throughout the duration of the voltage clamp, indicative of a small number of release events associated with LCC re-openings and/or late openings. Fig. 3.5B demonstrates mean subspace free Ca\textsuperscript{2+} concentration (*solid line*) averaged over all CaRUs, and bulk cytosolic Ca\textsuperscript{2+} concentration (*dashed line*). The peak amplitude of mean subspace Ca\textsuperscript{2+} concentration is \(\sim 18\ \mu\text{M}\), substantially greater than the cytosolic Ca\textsuperscript{2+} level, which peaks at \(< 1\ \mu\text{M}\). The mean subspace Ca\textsuperscript{2+} concentration is however less than that observed for individual simulated Ca\textsuperscript{2+} release events due to temporal dispersion in the occurrence of Ca\textsuperscript{2+} release events, and because Ca\textsuperscript{2+} release fails to occur in some CaRUs. On average, the local Ca\textsuperscript{2+} transient displays fast kinetics. It rises and decays within \(\sim 70\ \text{ms}\) at 0 mV, while the cytosolic Ca\textsuperscript{2+} transient lasts \(\gg 200\ \text{ms}\). The late sustained Ca\textsuperscript{2+} fluxes shown in Fig. 3.5A give rise to a similar sustained component of the subspace Ca\textsuperscript{2+} signal, which lasts for the duration of the voltage clamp pulse. Fig. 3.5C shows corresponding free Ca\textsuperscript{2+} levels in the JSR (*solid black line*, average over all JSR volume compartments) and NSR (*solid gray line*), and total SR Ca\textsuperscript{2+} load (*dashed line*), which includes both free and buffer-bound Ca\textsuperscript{2+} in all SR compartments. JSR and NSR Ca\textsuperscript{2+} pools are at similar levels at all times during the pulse, indicative of the fast Ca\textsuperscript{2+} diffusion rate between these compartments (\(\tau_{tr} = 3\ \text{ms}\)). Preceding the pulse, SR free Ca\textsuperscript{2+} concentration is \(\sim 730\ \mu\text{M}\),
Figure 3.5: Whole-cell Ca\(^{2+}\) fluxes and concentrations in response to a 200 ms voltage clamp to 0 mV. (A) Ca\(^{2+}\) fluxes through the entire population of LCCs (gray line) and the entire population of RyRs (black line) are shown as a function of time. For consistency, fluxes are reported in units of mmol L-cytosol\(^{-1}\) s\(^{-1}\). (B) Average subspace Ca\(^{2+}\) transient (black line, left axis) and bulk cytosolic Ca\(^{2+}\) transient (dashed line, right axis). (C) Free Ca\(^{2+}\) concentration in the NSR (gray solid line, left axis), mean free JSR Ca\(^{2+}\) concentration (averaged over all CaRUs, black solid line, left axis), and total SR Ca\(^{2+}\) concentration (includes both free and bound Ca\(^{2+}\) in the NSR and JSR, dashed line, right axis).
Figure 3.5
which corresponds to total SR Ca$_{2+}$ content of $\sim 118 \mu$mol L-cytosol$^{-1}$, in agreement with measurements of SR load (Hobai and O'Rourke, 2001). Upon CICR, total SR Ca$_{2+}$ is reduced to $\sim 80 \mu$mol L-cytosol$^{-1}$, resulting in $\sim 32\%$ decrease in total SR Ca$_{2+}$ content. Similar values of fractional SR Ca$_{2+}$ release have been obtained in experiments ($\sim 35\%$) (Bassani et al., 1995; DelBridge et al., 1996).

Recently Song et al. (2001) examined voltage dependent recruitment and amplitude of Ca$_{2+}$ spikes. Under control conditions, they found a bell-shaped voltage dependence for the likelihood of Ca$_{2+}$ spike occurrence and a shallower bell-shaped dependence for the amplitude of Ca$_{2+}$ spikes. These data demonstrated that the gradation of SR Ca$_{2+}$ release is predominantly attributable to graded recruitment of T-SR junctions, with a smaller contribution of alterations in the magnitude of local Ca$_{2+}$ release flux. Fig. 3.6 demonstrates the ability of the local control myocyte model to reproduce these findings. Fig. 3.6A shows the fraction of CaRUs that fire at least one Ca$_{2+}$ spike during a 200 ms depolarizing test pulse as a function of test pulse potential. Ca$_{2+}$ spikes are detected by monitoring the mean subspace Ca$_{2+}$ concentration within each CaRU and are considered to have occurred if Ca$_{2+}$ concentration rises above a threshold value of 4 $\mu$M for a time greater than 5 ms. Values reported in Fig. 3.6 were determined by analyzing a minimum of 250 CaRUs at each potential ($n \geq 250$). Under control conditions (filled circles), the model voltage dependence of the probability of firing a Ca$_{2+}$ spike is bell shaped and saturates at $\sim 1.0$ in the range of $-20$ mV to $+20$ mV, indicating that it is extremely rare for SR release not to occur in this voltage range. The same simulations were performed using an altered version of the model in which there is a single dyadic
Figure 3.6: Microscopic properties of model Ca\(^{2+}\) spikes. (A) The fraction of CaRUs that fire a Ca\(^{2+}\) spike as a function of voltage under control conditions is shown (filled circles). For the modified model lacking intersubspace diffusion, the fraction of subspaces that exhibit a Ca\(^{2+}\) release event is shown (open circles). The fraction of CaRUs that fire a Ca\(^{2+}\) spike in the presence of LCC slow cycling between active and silent modes is estimated (see text, triangles), \(n \geq 250\) simulations at each potential. (B) Ca\(^{2+}\) spike amplitude as a function of voltage clamp potential under control conditions (filled circles) and for the modified model lacking intersubspace diffusion (open circles).
subspace per unit (total number of LCCs, RyRs, and subspace compartments per myocyte being conserved) and therefore lacks inter-subspace diffusion (open circles). These results represent the probability of elementary release events within the model, and peak at $\sim 0.9$ in the range of $-10$ mV to $+10$ mV. In either case, the bell shaped voltage dependence indicates that gradation of SR $\text{Ca}^{2+}$ release arises, in part, from voltage-dependent recruitment of T-SR junction (i.e., CaRU) activation. If it is assumed that the results for the modified model are similar to the properties of a single subunit within a control CaRU and that CaRU activation (i.e., a $\text{Ca}^{2+}$ spike) occurs as long as at least one elementary release event occurs within a CaRU, then the probability of CaRU activation in the presence of LCC silent mode behavior can be estimated. The estimate assumes that only 40% of LCCs are active at any given time ($f_{\text{active}} = 0.4$) (Handrock et al., 1998) and produces peak fractional T-SR activation similar to experiments (Song et al., 2001) as shown in Fig. 3.6A (triangles). Fig. 3.6B shows the model voltage dependence of local $\text{Ca}^{2+}$ spike amplitude (as CaRU $\text{Ca}^{2+}$ release flux) averaged over all CaRUs for the same two simulations. For control case (closed circles), $\text{Ca}^{2+}$ spike amplitude has a shallow bell-shaped voltage dependence and peaks at $\sim 170$ nmol L-cytosol$^{-1}$ s$^{-1}$ in the range of $-10$ mV to $+10$ mV. The rise in mean $\text{Ca}^{2+}$ spike amplitude in the central voltage range occurs as a result of enhanced synchronization of RyR release events within CaRUs contributing to gradation of SR $\text{Ca}^{2+}$ release and agrees well with the experiments of Song et al. (2001). In the modified model lacking inter-subspace $\text{Ca}^{2+}$ diffusion (open circles) subspace $\text{Ca}^{2+}$ release flux is smaller at all potentials. This is the case because these signals represent SR $\text{Ca}^{2+}$ release flux from a set of five RyRs, whereas
the control simulations represent flux from all 20 RyRs in the CaRU. More interesting however, is that the shape of the voltage dependence of the Ca$^{2+}$ transient amplitude in the absence of inter-subspace coupling is inverted compared to the control simulations. In the absence of inter-subspace coupling, synchronization within a single unit is not possible since only a single Ca$^{2+}$ release event occurs. There is therefore no enhancement of local Ca$^{2+}$ release signals in the central range of potentials. The depressed release at central voltages in the modified model is due to a reduction in the Ca$^{2+}$ gradient across the SR membrane as a result of SR depletion. In the central voltage range, where SR release is maximal (Fig. 3.6A, open circles), reduction in global SR Ca$^{2+}$ load over the duration of the pulse will lead to a reduction in Ca$^{2+}$ transient amplitude for events that occur late within the pulse (data not shown). In the control case, this effect is masked by the enhancement of Ca$^{2+}$ spike amplitude that occurs within the same range of potentials due to synchronization of release events within CaRUs.

While Fig. 3.6 shows results regarding voltage dependent gradation of SR Ca$^{2+}$ release at the level of the CaRU, Fig. 3.7 demonstrates the macroscopic properties of SR Ca$^{2+}$ release. Previous experimental studies (Wier et al., 1994; Santana et al., 1996) and mathematical models (Stern, 1992; Stern et al., 1999) have shown that there can be significant differences between the voltage dependence of LCC Ca$^{2+}$ influx ($F_{LCC}$) and RyR Ca$^{2+}$ release flux ($F_{RyR}$) even though SR Ca$^{2+}$ release is controlled by Ca$^{2+}$ entry via $I_{CaL}$. These differences are responsible for variable EC coupling gain. We use the definition of gain given by Wier et al. (1994), as the ratio $F_{RyR(max)}/F_{LCC(max)}$. Fig. 3.7A shows the voltage dependence of $F_{LCC(max)}$ (filled circles) and $F_{RyR(max)}$ (open circles)
obtained using the same voltage protocols as in Fig. 3.3B. In Fig. 3.7B, the peak fluxes of Fig. 3.7A have been normalized based on their respective maxima. While both $F_{\text{LCC}(\text{max})}$ and $F_{\text{RyR}(\text{max})}$ are bell shaped functions of membrane potential, they do not share identical voltage dependence. Maximal LCC Ca$^{2+}$ influx occurs at 10 mV whereas maximal RyR Ca$^{2+}$ release flux occurs at 0 mV. EC coupling gain as defined above is plotted as a function of voltage in Fig. 3.7C ($\text{triangles}$), and is monotonically decreasing with increasing membrane potential. The similarity of EC coupling gain to the unitary LCC current ($\text{dashed line}$, scaled for comparison to gain) suggests that these quantities may be related. The simulated data of Fig. 3.7 agrees well with experimentally obtained measurements of whole cell Ca$^{2+}$ fluxes (Wier et al., 1994; Santana et al., 1996; Song et al., 2001). The value of $r_{\text{iss}}$ (inter-subspace Ca$^{2+}$ transfer rate) was chosen to match the model gain function with experiments and to be consistent with estimates of Ca$^{2+}$ diffusion within the dyad (Soeller and Cannell, 1997). The role of inter-subspace coupling on gain can be seen in Fig. 3.7C, by comparison of control simulations ($\text{triangles}$) to those in the absence of inter-subspace coupling where $r_{\text{iss}} = 0$ ($\text{squares}$). In the presence of inter-subspace coupling, EC coupling gain is greater at all potentials, but the increase in gain is most dramatic at more negative potentials. In this voltage range, where LCC $p_o$ is sub-maximal and unitary current magnitude is relatively high, increasing $r_{\text{iss}}$ allows the opening of a single LCC to recruit and activate RyRs in adjacent subspace compartments (within the same T-SR junction), effectively raising the RyR to LCC ratio, and hence increasing the slope of the gain function. The local control myocyte model predicts that Ca$^{2+}$ diffusion among the subspaces is an important
Figure 3.7: Voltage dependence of macroscopic LCC Ca$^{2+}$ influx, SR Ca$^{2+}$ release, and EC coupling gain. (A) Mean peak Ca$^{2+}$ flux amplitudes, $F_{LCC(max)}$ (filled circles) and $F_{RyR(max)}$ (open circles) as a function of membrane voltage, $n = 5$ simulations at each voltage. (B) Peak Ca$^{2+}$ fluxes (data of panel A) normalized by their respective maxima. (C) EC coupling gain as a function of membrane potential defined as $F_{RyR(max)}/F_{LCC(max)}$ under control conditions (triangles) and in the absence of intersubspace coupling within the CaRUs (squares), as well as L-type unitary current (dashed line, scaled to match the gain function at $-40$ mV).
Figure 3.7
mechanism underlying the rate at which gain decreases with increasing voltage. Previous models of EC coupling have similarly achieved high gain at negative potentials by incorporating details of spatial Ca$^{2+}$ gradients in the dyadic space (Stern et al., 1999).

One defining difference between a common pool model and a local control model of EC coupling is that it would be impossible for a common pool model to exhibit different values of gain for macroscopic L-type Ca$^{2+}$ currents of the same amplitude (Stern, 1992). Wier et al. (1994) have explicitly demonstrated that Ca$^{2+}$ currents of similar shape and amplitude can evoke very different responses of SR Ca$^{2+}$ release. The local control myocyte model can reproduce the findings of this experiment as shown in Fig. 3.8. Figs. 3.8, A and B, show $F_{LCC}$, and Figs. 3.8, C and D, show $F_{RyR}$ in response to 200 ms depolarizing pulses to $-20$ mV and $+50$ mV respectively. While the amplitude and time course of macroscopic LCC Ca$^{2+}$ influx is similar for the two test pulses, the SR Ca$^{2+}$ release is triggered effectively in response to the $-20$ mV pulse (where gain is high), but only minimal Ca$^{2+}$ release occurs at $+50$ mV (where gain is low). In addition, upon repolarization to $-100$ mV from $+50$ mV, the brief Ca$^{2+}$ tail current triggers substantial SR Ca$^{2+}$ release (Fig. 3.8D).

**Action Potentials**

Fig. 3.9 demonstrates the ability of the model to reconstruct action potentials and Ca$^{2+}$ transients of normal canine midmyocardial ventricular myocytes. In Fig. 3.9A, a normal 1 Hz steady state AP is shown. AP properties are similar to those measured in experiments (O'Rourke et al., 1999b), with action potential duration (APD) of ~ 300 ms.
Figure 3.8: Variable EC coupling gain. (A) and (B), Whole-cell LCC Ca^{2+} influx ($F_{LCC}$) in response to voltage clamp stimuli of –20 mV and +50 mV respectively. (C) and (D), Whole-cell SR Ca^{2+} release flux ($F_{RyR}$) in response to voltage clamp stimuli of –20 mV and +50 mV respectively. Note that while $F_{LCC}$ is similar in response to each of the two voltage clamp stimuli, the magnitude of $F_{RyR}$ is very different, indicating that EC coupling gain is not determined by properties of macroscopic $I_{CaL}$. In addition, note that the large, but brief, tail of Ca^{2+} influx that occurs following repolarization from +50 mV triggers substantial SR Ca^{2+} release flux.
Figure 3.8
Figure 3.9: The action potential, Ca$^{2+}$ transients, and membrane currents. Signals shown are in response to a 1 Hz pulse train, with responses shown in steady state. (A) Membrane potential as a function of time simulated using the local control myocyte model under normal conditions. (B) Cytosolic (black line, left axis) and mean subspace (gray line, right axis) Ca$^{2+}$ concentrations corresponding to the AP simulated in panel A. (C) L-type Ca$^{2+}$ current ($I_{Ca_L}$, black line) and the Ca$^{2+}$-dependent transient outward Cl$^-$ current ($I_{to2}$, gray line) corresponding to the AP simulated in panel A.
Fig. 3.9B shows cytosolic (*black line*) and mean subspace (*gray line*) Ca$^{2+}$ transients. While the cytosolic Ca$^{2+}$ transient peaks at ~ 0.75 µM, and lasts longer than the duration of the AP, Ca$^{2+}$ in the subspace reaches ~ 11 µM on average, and equilibrates to near cytosolic levels rapidly within ~ 100-150 ms. Fig. 3.9C demonstrates the two model currents which communicate directly with the local subspaces within the CaRUs, $I_{CaL}$ (*black line*) and $I_{to2}$ (*gray line*). $I_{CaL}$ peaks at ~ 4.7 pA pF$^{-1}$ and has a sustained component of ~ 0.7 pA pF$^{-1}$ which lasts for nearly the entire duration of the AP. $I_{to2}$ peaks at ~ 0.6 pA pF$^{-1}$ and also displays a minimal sustained current component. The sustained current appears since subspace Ca$^{2+}$ remains moderately elevated on average throughout the AP due to LCC re-openings and because $I_{to2}$ does not inactivate.

While macroscopic $I_{CaL}$ shown in Fig. 3.9C appears to have a similar shape as that of the common pool Winslow et al. (1999) canine myocyte model, the underlying LCC inactivation process in the local control model has been altered to depend more strongly on local Ca$^{2+}$ than on membrane potential. This adjustment is based on experimental findings obtained from both isolated myocytes (Linz and Meyer, 1998; Sipido et al., 1995) as well as recombinant channels expressed in HEK 293 cells (Peterson et al., 1999; Peterson et al., 2000) which show that LCC voltage-dependent inactivation is slow and incomplete while Ca$^{2+}$-mediated inactivation is strong and dominates the inactivation process (see also Fig. 3.3D). Strong Ca$^{2+}$-dependent inactivation (in the absence of strong voltage-dependent inactivation) is a key mechanism in determining how graded SR Ca$^{2+}$ release influences AP properties and whole cell Ca$^{2+}$ dynamics. Fig. 3.10 demonstrates the differences in $I_{CaL}$ inactivation properties between the Winslow et al.
(1999) common pool model and the local control myocyte model, and their consequences. Fig. 3.10, A and B, shows steady state APs (solid line), \( \text{Prob}\{\text{Norm}\} \) (dashed line), and \( y \) (dotted line), for the common pool and local control models respectively. \( \text{Prob}\{\text{Norm}\} \) and \( y \) are the model quantities indicating the time progression of the \( \text{Ca}^{2+} \)- and voltage-dependent inactivation processes respectively, as previously described in Fig. 3.3C. During the plateau of the AP, \( \sim 70\% \) of LCCs become unavailable due to voltage-dependent inactivation while \( \sim 60\% \) become unavailable due to \( \text{Ca}^{2+} \)-dependent inactivation in the common pool model (Fig. 3.10A). The balance between voltage- and \( \text{Ca}^{2+} \)-dependent inactivation processes in the Winslow et al. (1999) common pool model are therefore in contrast to experimental findings. The roles of these processes are reversed (as they should be) in the local control myocyte model, with only \( \sim 35\% \) of LCCs succumbing to voltage-dependent inactivation while \( \sim 75\% \) are shut down by \( \text{Ca}^{2+} \)-dependent inactivation (Fig. 3.10B, compare to Fig. 11 of Linz and Meyer, 1998). Understanding the fundamental differences in the CICR processes in the common pool vs. the local control models provides the reason why the balance between each of the inactivation processes is incorrectly assigned in the common pool model. In a model where the release of SR \( \text{Ca}^{2+} \) is controlled by sensing \( \text{Ca}^{2+} \) levels in the same pool into which SR \( \text{Ca}^{2+} \) is released, \( \text{Ca}^{2+} \) release will be an all-or-none response (Stern, 1992). If \( \text{Ca}^{2+} \)-dependent inactivation of LCCs were the dominant inactivation process in this type of model, then it follows that \( I_{\text{CaL}} \) inactivation would also exhibit all-or-none behavior, switching on in response to SR \( \text{Ca}^{2+} \) release. The single regenerative SR release event would rapidly and strongly promote \( \text{Ca}^{2+} \)-dependent inactivation of \( I_{\text{CaL}} \), and would
Figure 3.10: Inactivation properties of $I_{\text{CaL}}$ in common pool and local control models of the action potential. (A) and (B), Membrane potential ($\text{solid line}$, left axis), $\text{Prob}\{\text{Norm}\}$ ($\text{dashed line}$, right axis), and $y$ ($\text{dotted line}$, right axis) for the Winslow et al. (1999) common pool myocyte model and the local control myocyte model respectively. $\text{Prob}\{\text{Norm}\}$ and $y$ are the model quantities indicating the time progression of the $\text{Ca}^{2+}$- and voltage-dependent inactivation processes of $I_{\text{CaL}}$ (see text for details). (C) Membrane potential as a function of time for a 10-second simulation of a modified version of the Winslow et al. (1999) model with $I_{\text{CaL}}$ parameterized with strongly $\text{Ca}^{2+}$-dependent and weakly voltage-dependent inactivation (similar to that of the local control model). (D) Membrane potential ($\text{solid line}$, left axis), $\text{Prob}\{\text{Norm}\}$ ($\text{dashed line}$, right axis), and $y$ ($\text{dotted line}$, right axis) for the local control myocyte model where SR $\text{Ca}^{2+}$ load has been reduced to 33% of its normal level.
therefore destabilize the plateau phase of the AP. An attempt at simulating APs using the Winslow et al. (1999) model modified to have strongly Ca\textsuperscript{2+}-dependent and weakly voltage-dependent inactivation of \(I_{\text{CaL}}\) (with equations governing \(y\) identical to that of the local control model) is illustrated in Fig. 3.10C. APs alternate between those with short duration (~150-250 ms) and those with very long duration (> 1000 ms) with unstable oscillatory plateau potentials. The alternans indicate the presence of a bifurcation in AP profile as a function of JSR Ca\textsuperscript{2+} load. Short duration APs occur when the all-or-none SR Ca\textsuperscript{2+} release event strongly inactivates \(I_{\text{CaL}}\), and hence terminates the AP. SR Ca\textsuperscript{2+} load will be gradually diminished following successive short APs due to the imbalance between cellular Ca\textsuperscript{2+} influx (via LCCs) and Ca\textsuperscript{2+} efflux (via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchangers and sarcolemmal Ca\textsuperscript{2+} pumps). When the SR becomes sufficiently depleted, the weak SR Ca\textsuperscript{2+} release flux produces only slight inactivation of \(I_{\text{CaL}}\). In addition, the population of RyRs fails to adequately inactivate/adapt, leading to additional spontaneous release events and a long lasting unstable oscillatory plateau. This unstable behavior occurs over a wide range of LCC inactivation parameters as long as voltage-dependent inactivation of \(I_{\text{CaL}}\) is relatively slow and incomplete (data not shown). Strong voltage-dependent inactivation of \(I_{\text{CaL}}\), although contrary to experimental observations, is therefore necessary to enforce stability of those common pool models that incorporate the regenerative SR release mechanisms of CICR. As seen in Fig. 3.10B, the local control myocyte model does not suffer from the consequences of all-or-none SR Ca\textsuperscript{2+} release (at the whole cell level) and therefore successfully generates stable APs with LCCs whose inactivation process is dominated by local Ca\textsuperscript{2+}-mediated inactivation.
Altered EC coupling in both human (Lindner et al., 1998) and canine (Hobai and O'Rourke, 2001) heart failure is associated with decreased SR Ca\(^{2+}\) content. Both modeling (Winslow et al., 1999) and experimental studies (Ahmmed et al., 2000; O'Rourke et al., 1999b) support the idea that altered Ca\(^{2+}\) handling plays a key role in heart failure associated AP prolongation. Fig. 3.10D shows an AP (solid line), \text{Prob}\{\text{Norm}\} (dashed line), and \text{y} (dotted line) for the local control model under conditions where SR Ca\(^{2+}\) load has been reduced to 33\% of its normal level. The voltage-dependent inactivation mechanism proceeds in a manner similar to that of the control case (dotted line). However, under conditions of reduced SR Ca\(^{2+}\) release, Ca\(^{2+}\)-mediated inactivation of \text{I}_{\text{CaL}} occurs at a far slower rate and to a lesser extent (dashed line), than in the control case (compare to Fig. 3.10B). The resulting increased magnitude of the late sustained component of \text{I}_{\text{CaL}} (not shown) maintains the plateau and dramatically prolongs the AP (solid line). This supports the hypothesis that in heart failure, alterations in Ca\(^{2+}\) handling proteins that decrease SR Ca\(^{2+}\) load and reduce the amplitude of local Ca\(^{2+}\) transients may contribute substantially to prolongation of APD by reducing Ca\(^{2+}\)-mediated inactivation of the L-type current.

In a previous study (Greenstein et al., 2000), we examined the role of \text{I}_{\text{to1}} in shaping AP morphology and duration using the common pool canine myocyte model (Winslow et al., 1999). These simulations predicted that reduction of \text{I}_{\text{to1}} density from normal levels leads to modest shortening of APD. The reduction in \text{I}_{\text{to1}} reduces the depth of the phase 1 AP notch, reducing the driving force for, and hence the peak level of \text{I}_{\text{CaL}}. Because this study was performed in a model displaying all-or-none rather than graded
SR Ca\(^{2+}\) release properties, the altered \(I_{CaL}\) had no ability to modulate \(F_{RyR}\). In Fig. 3.11, the role of \(I_{01}\) is revisited, with particular attention to how an alteration of AP shape influences EC coupling. Under normal 1 Hz steady state conditions (solid black line) the AP has a duration of ~ 315 ms (Fig. 3.11A), peak \(I_{CaL}\) is ~ 4.8 pA pF\(^{-1}\) (Fig. 3.11B), peak \(F_{RyR}\) is ~ 0.8 mmol L-cytosol\(^{-1}\) s\(^{-1}\) (Fig. 3.11C), and peak cytosolic Ca\(^{2+}\) concentration is ~ 0.8 µM (Fig. 3.11D). (Note that slight differences in control simulations in Fig. 3.11 compared to Fig. 3.9 arise due to stochastic noise inherent in the model.) The density of \(I_{01}\) was then reduced by 67%, an amount similar to that observed in failing canine myocytes (Kääb et al., 1996), and simulations were repeated using normal initial conditions in order to demonstrate the role of graded SR Ca\(^{2+}\) release under conditions of identical SR Ca\(^{2+}\) load. The phase 1 notch of the AP becomes less pronounced, and APD is shortened modestly to ~ 255 ms (Fig. 3.11A, gray line). The corresponding peak \(I_{CaL}\) is reduced by 40% to ~ 2.9 pA pF\(^{-1}\) (Fig. 3.11B). The property of graded SR Ca\(^{2+}\) release is evident by observing \(F_{RyR}\) (Fig. 3.11C, gray line). The reduction in trigger Ca\(^{2+}\) reduces peak SR Ca\(^{2+}\) release flux by nearly 50% under conditions where SR load is unchanged. The resulting cytosolic Ca\(^{2+}\) transient is consequently reduced to ~ 0.65 µM (Fig. 3.11D). While this is good demonstration of the effects of graded release during the AP, a sudden decrease in \(I_{01}\) is not a physiologically relevant event. Upon pacing to steady state with a 67% reduction of \(I_{01}\) (dotted line), both graded SR Ca\(^{2+}\) release and the new steady state SR Ca\(^{2+}\) load affect Ca\(^{2+}\) cycling properties. The shortening of the APs results in a decreased steady state SR Ca\(^{2+}\) load compared to control (data not shown) which in turn leads to a further decrease in SR Ca\(^{2+}\) release, reducing the
Figure 3.11: Role of $I_{to1}$ on the events of EC coupling. (A) Membrane potential, (B) L-type Ca$^{2+}$ current ($I_{CaL}$), (C) SR Ca$^{2+}$ release flux ($F_{RyR}$), and (D) Cytosolic Ca$^{2+}$ concentration as a function of time. Each of the signals are shown under normal 1 Hz steady-state conditions (solid black line), with 67% reduction in $I_{to1}$ density using the normal initial conditions (gray line), and with 67% reduction in $I_{to1}$ density under 1 Hz steady state conditions (see text for details). The altered shape of the AP resulting from reduction in $I_{to1}$ reduces trigger influx Ca$^{2+}$ via LCCs leading to decreased SR Ca$^{2+}$ release flux, and therefore a depressed cytosolic Ca$^{2+}$ transient.
Figure 3.11
amplitude of the cytosolic Ca\(^{2+}\) transient to \(\sim 0.6 \, \mu\text{M}, 25\%\) less than control (Fig. 3.11D). The model therefore predicts that reduction in \(I_{\text{to1}}\), similar to that observed in heart failure (Kääb et al., 1996), may contribute to reduced force generation. This model prediction has been verified by recent experiments showing that slowed phase 1 repolarization during the AP reduces temporal synchrony and recruitment of Ca\(^{2+}\) release events, in conjunction with a reduced amplitude of \(I_{\text{CaL}}\) (Sah et al., 2002).

**Discussion**

In this study, we present a biophysically detailed model of the normal canine ventricular myocyte that conforms to the theory of local control of EC coupling in cardiac muscle. Local control theory asserts that L-type Ca\(^{2+}\) current tightly controls SR Ca\(^{2+}\) release because elementary, independent SR Ca\(^{2+}\) release events occur in response to highly localized Ca\(^{2+}\) transients which are produced by the opening of single L-type Ca\(^{2+}\) channels in the vicinity of a small cluster of RyRs (Sham et al., 1995; Stern, 1992; Wier et al., 1994; Bers, 2001). Tight regulation of CICR is made possible by the fact that LCCs and RyRs are sensitive to local submembrane Ca\(^{2+}\), rather than global Ca\(^{2+}\) levels, allowing for graded control of Ca\(^{2+}\) release while maintaining high EC coupling gain (Fabiato, 1985b; Beuckelamnn and Wier, 1988; Sham, 1997). In order to capture these properties of local control, our myocyte model incorporates EC coupling in the form of Ca\(^{2+}\) release units in which individual sarcolemmal L-type Ca\(^{2+}\) channels interact in a stochastic manner with nearby RyRs in localized regions where junctional SR membrane and T-tubular membrane are in close proximity. The CaRUs are embedded within and
interact with the global systems of the myocyte describing ionic and membrane pump/exchanger currents, SR Ca$^{2+}$ uptake, and time-varying cytosolic ion concentrations. The results demonstrate that a single comprehensive model of the cardiac myocyte can reproduce both detailed properties of EC coupling predicted by local control theory, such as variable gain and graded SR Ca$^{2+}$ release, while at the same time reproducing whole-cell phenomena, such as modulation of AP duration by SR Ca$^{2+}$ release. The ability of this model to encompass mechanisms of both microscopic and macroscopic phenomena afford it the unique ability to provide insight into the integrative properties of the myocyte that arise from the interaction among these subcellular processes.

The main goal of this study was to develop a myocyte model incorporating mechanistic descriptions of the processes that underlie local control of SR Ca$^{2+}$ release. This requires the simulation of a large number of individual stochastically gating channels, which poses a substantial computational task. In order to maintain tractability, the CaRU was designed as a “minimal model”, including biophysically detailed descriptions of individual LCCs and RyRs (Jafri et al., 1998; Rice et al., 1999; Keizer and Smith, 1998). Each CaRU contains four subspace compartments in which elementary Ca$^{2+}$ release events may occur, allowing for passive flow of Ca$^{2+}$ to neighboring subspaces providing for the possibility that an initial release event may trigger an additional release event in the neighboring cluster of RyRs (Parker et al., 1996; Song et al., 2001). This geometry allows the summation of release events within a CaRU to serve as a minimal model of a Ca$^{2+}$ spike, while reproducing fundamental properties of EC coupling measured at the whole cell level, such as graded Ca$^{2+}$ release. It is important to
note however, that the individual release events within the CaRU are not intended to reproduce the detailed spatio-temporal properties of experimentally observed Ca\(^{2+}\) sparks (Fig. 3.4A) (Cheng et al., 1993). Such a model would require explicit descriptions of dyadic subspace geometry, Ca\(^{2+}\) diffusion, and surface charge effects. Models accounting for these features have been developed for a single isolated dyad (Cannell and Soeller, 1997; Soeller and Cannell, 1997; Langer and Peskoff, 1996), but would be too computationally demanding for this application.

Stern et al. (1999) have demonstrated that a number of RyR models, with rate constants determined from measurements in lipid bilayers (Zahradnikova and Zahradnik, 1996; Keizer and Levine, 1996; Schiefer et al., 1995), exhibit local instability (failure of SR release termination following activation) or global instability (spontaneous activation by background Ca\(^{2+}\)) when simulated in a local control environment. Further, they have shown that stability of local control requires that RyRs exhibit strong inactivation and cooperative activation by more than one Ca\(^{2+}\) ion, and have suggested that such properties may arise from allosteric interactions between neighboring RyRs. The RyR model formulation presented in this study, which is based on that of Keizer and Smith (1998), exhibits both strong inactivation/adaptation properties consistent with rates observed in the presence of Mg\(^{2+}\) (Valdivia et al., 1995), and requires cooperative binding of four Ca\(^{2+}\) ions in order to open. Although there is mounting evidence that RyR gating properties are influenced by physical coupling of neighboring RyRs (Marx et al., 2001a), the results of this study suggest that the stability of local control of SR Ca\(^{2+}\) release can be achieved without accounting for such interactions explicitly.
The local control myocyte model description requires that \( I_{\text{CaL}} \) be computed based on the ensemble behavior of individually simulated LCCs. This requires that both single channel properties (e.g., unitary conductance, open and closed time distributions) and whole cell current properties (e.g., peak current-voltage relation, steady-state inactivation) are incorporated into the model, unlike typical common pool model formulations which need only describe whole cell properties. Figs. 3.2 and 3.3 demonstrate that the LCC model reproduces both single channel (Herzig et al., 1993; Handrock et al., 1998; Schroder et al., 1998; Rose et al., 1992; Yue and Marban, 1990) and whole cell (Hobai and O'Rourke, 2001; Kääb et al., 1996; O'Rourke et al., 1999b; Rose et al., 1992; He et al., 2001; Linz and Meyer, 1998; Sipido et al., 1995; Sham, 1997; Hadley and Hume, 1987; Peterson et al., 1999) current properties rather well. A model based prediction of \( \sim 50,000 \) was obtained for \( N_{\text{active}} \), the number of active (phosphorylated) LCCs in a cardiac myocyte, which agrees well with experimental estimates (Rose et al., 1992; McDonald et al., 1986). It has been demonstrated that LCCs cycle in a slow multisecound process between an active gating mode, in which channels are phosphorylated and available to open, and a silent gating mode, in which channels are dephosphorylated and remain closed (Herzig et al., 1993). In keeping with the approach of a “minimal model”, only active LCCs were simulated and the process of slow cycling between active and silent modes of behavior was not included in the model. Inclusion of this slow mode switching process would yield CaRUs where only \( \sim 20\% - 40\% \) (Herzig et al., 1993; Handrock et al., 1998) of LCCs are available at rest. In the current CaRU formulation (with an increase in \( N_{\text{CaRU}} \) in order to conserve \( N_{\text{active}} \)), this may reduce EC
coupling gain due to loss of synchronization of release within CaRUs. In addition, the fraction of CaRUs that are active will be reduced by: (1) the occurrence of completely silent CaRUs which contain no active LCCs (estimated in Fig. 3.6A), and (2) the weakening of trigger influx to subthreshold levels at high potentials in CaRUs where only some LCCs are active and unitary LCC current is small. The expected difference between simulations including vs. excluding the slow cycling of LCCs would however be reduced if the dyad of each CaRU were expanded from our minimal representation to include a greater number of LCC-RyR functional groupings (~ 100 RyRs per dyad) as has been observed in structural studies (Franzini-Armstrong et al., 1999).

The $I_{\text{CaL}}$ steady-state inactivation curve (Fig. 3.3D) demonstrates how whole cell properties emerge from mechanistic descriptions of underlying processes. The model steady-state inactivation curve is U-shaped under normal conditions, but monotonically decreases and is incomplete with increasing membrane potential when Ca$^{2+}$ is not the charge carrier, which agrees well with experiments (Hadley and Hume, 1987; Linz and Meyer, 1998). Some previous models of $I_{\text{CaL}}$ have been able to reproduce the phenomena of U-shaped inactivation by incorporating this property directly into the equations describing inactivation (Jafri et al., 1998; Sun et al., 2000). In the local control myocyte model, however, the U-shaped inactivation curve emerges as a consequence of local Ca$^{2+}$ mediated events. The magnitude of the local Ca$^{2+}$ transient is graded as a function of LCC Ca$^{2+}$ influx, which is maximal in the central range of potentials of $-10$ mV to $+30$ mV (Fig. 3.3B). The local rise in Ca$^{2+}$ induces Ca$^{2+}$-mediated inactivation of $I_{\text{CaL}}$, which makes a dominant contribution to the total inactivation of $I_{\text{CaL}}$ when local Ca$^{2+}$ levels are
raised (e.g., Fig. 3.3C). The U-shape therefore emerges as a reflection of the bell-shaped voltage dependence of LCC Ca\(^{2+}\) influx.

By summing over all of the local events that occur in the CaRUs, model simulations are able to faithfully reproduce details regarding the relationship between whole-cell \(I_{\text{Cal}}\) and whole-cell SR Ca\(^{2+}\) release. In response to a depolarizing voltage clamp pulse, the whole-cell SR Ca\(^{2+}\) release flux peaks soon after depolarization and then decays to a small maintained component in a similar manner to signals measured experimentally (Wier et al., 1994). The small amplitude maintained release is supported by the slow/sustained component of \(I_{\text{Cal}}\) and turns off following repolarization to the resting potential (Fig. 3.5A). Control of SR Ca\(^{2+}\) release by \(I_{\text{Cal}}\) has been demonstrated experimentally by showing that SR Ca\(^{2+}\) release can be turned off by termination of Ca\(^{2+}\) current (Wier et al., 1994; Cannell et al., 1987; Cleemann and Morad, 1991). While the SR Ca\(^{2+}\) release flux in response to a short duration pulse has not been shown explicitly in this study, the model does exhibit this behavior. The exemplar Ca\(^{2+}\) spike shown in Fig. 3.4C occurred \(\sim 25\) ms following the onset of the depolarizing voltage clamp pulse, and clearly would not have been triggered if the pulse was truncated to \(< 25\) ms.

Additional evidence for control of SR Ca\(^{2+}\) release by \(I_{\text{Cal}}\) has been demonstrated by the ability of \(I_{\text{Cal}}\) tail currents, occurring upon repolarization to the rest potential following a depolarized voltage clamp, to trigger a release event (Wier et al., 1994; Sham et al., 1998). The local control model readily reproduces this response (Fig. 3.8, B and D). Upon membrane depolarization, the model demonstrates that the mean dyadic subspace Ca\(^{2+}\) concentration reaches levels that are more than 20-fold greater than in the cytosol.
(Fig. 3.5B), consistent with both experimental and theoretical predictions that submembrane \( \text{Ca}^{2+} \) can reach concentrations that are much higher than in the cytosol (Stern, 1992; Sham, 1997; Langer and Peskoff, 1996; Soeller and Cannell, 1997; Jafri et al., 1998; Cheng et al., 1993; Wang et al., 2001; Song et al., 2001). It has been noted recently that the common pool model of Winslow et al. (1999) has a limitation in that the SR is almost \( \frac{2}{3} \) depleted upon a single stimulus (Michailova and McCulloch, 2001). The local control model of this study demonstrates both fractional SR release (\( \sim 32\% \)) and total pre-release SR \( \text{Ca}^{2+} \) content (\( \sim 118 \mu\text{mol L-cytosol}^{-1} \)) consistent with experimental studies (Bassani et al., 1995; DelBridge et al., 1996; Hobai and O'Rourke, 2001).

The key experimental observation that has suggested the process of CICR is locally controlled is that while both \( F_{\text{RyR(max)}} \) and \( F_{\text{LCC(max)}} \) exhibit a bell-shaped dependence on membrane potential, the relationships are not the same (Wier et al., 1994; Santana et al., 1996; Wier and Balke, 1999). At negative potentials, single channel currents are large, but macroscopic current is relatively small due to the fact that LCC openings are infrequent. This implies that LCC openings are sparsely dispersed throughout the membrane. As a result, \( I_{\text{CaL}} \) in this potential range is more effective at triggering SR \( \text{Ca}^{2+} \) release than at positive potentials, where single channel openings are far more frequent but unitary current magnitude is smaller. This behavior reveals itself in the model in the form of a shift in the voltage dependence of \( F_{\text{RyR(max)}} \) vs. \( F_{\text{LCC(max)}} \) (Fig. 3.7B) and in the form of graded SR \( \text{Ca}^{2+} \) release with variable EC coupling gain that decreases with increasing membrane potential (Fig. 3.7C) similar to curves obtained experimentally (Wier et al., 1994; Santana et al., 1996) and theoretically (Stern, 1992;
Stern et al., 1999). Gain decreases with increasing depolarization due to the decrease in local triggering efficiency that occurs as unitary current magnitude decreases. Experimentally measured EC coupling gain functions typically display a steeper slope at negative potentials compared to positive potentials (Wier et al., 1994; Song et al., 2001). Simulations using the local control myocyte model show that coupling among neighboring subspace compartments within the CaRUs is necessary to generate responses with relatively very high gain at the most negative potentials (Fig. 3.7C, triangles). At negative voltages, where the unitary current magnitude is relatively high, the Ca\(^{2+}\) influx from the opening of a single LCC is sufficient to trigger SR release from RyRs that reside in the same dyadic subspace compartment, as well as from RyRs in adjacent subspace compartments, but within the same T-SR junction (i.e., the same CaRU). The number of RyRs that may sense trigger Ca\(^{2+}\) from a particular LCC is effectively increased with increasing unitary LCC current, increasing the slope of the gain function at negative potentials. The fact the SR Ca\(^{2+}\) release is graded is a property that emerges from considering the mechanistic details of local interactions between LCCs and RyRs. Our myocyte model is not the first to exhibit SR Ca\(^{2+}\) release that is graded. Previous myocyte models (Luo and Rudy, 1994; Faber and Rudy, 2000), however, achieved the graded SR release phenomenologically by directly programming a dependence of RyR function on macroscopic current magnitude or total Ca\(^{2+}\) entry during the first few milliseconds of \(I_{\text{CaL}}\) activation. Even though these formulations are capable of generating graded CICR, any SR Ca\(^{2+}\) release formulation which depends upon macroscopic Ca\(^{2+}\) influx cannot exhibit different gain values for Ca\(^{2+}\) currents of the same magnitude (Fig.
3.8), and therefore would be unable to reproduce an EC coupling gain function which decreases monotonically with increasing membrane potential.

Many cardiac Ca\(^{2+}\) cycling proteins that play a role in EC coupling, including LCCs (Herzig et al., 1993; Handrock et al., 1998) and RyRs (Marx et al., 2000), are known target proteins of the β-adrenergic receptor (β-AR)-adenylyl cyclase-cAMP-protein kinase A signaling pathway. An increase in β-adrenergic stimulation, such as that which occurs during heart failure (Handrock et al., 1998; Marx et al., 2000), tends to increase channel availability or promote faster kinetics. The microscopic mechanisms underlying β-AR-mediated modulation of EC coupling are not well understood. Recent experiments suggest that improved synchronization of SR Ca\(^{2+}\) release both within and among T-SR junctions, and hence increased cardiac inotropy, occurs as a result of increased β-adrenergic stimulation (Song et al., 2001). In this study, it has been demonstrated that baseline model Ca\(^{2+}\) spike statistics, both within and among CaRUs, agree well with those measured in experiments (Fig. 3.6) (Song et al., 2001). Additional experiments have demonstrated that heart failure related alterations in LCC properties might not be apparent in whole cell \(I_{CaL}\) measurements. Single LCCs from failing human ventricular myocytes exhibit increased availability and open probability compared to nonfailing myocytes, while whole-cell current measurements do not reveal a change in \(I_{CaL}\) current density, suggesting that the number of functional channels must be reduced in failing myocytes (Schroder et al., 1998). In failing canine myocytes, the number of LCCs has been found to be reduced as well via measurement of intramembrane charge movement, where whole-cell currents were again found to be unchanged (He et al.,
In failing hearts, RyRs have been found to be hyperphosphorylated, leading to dissociation of FKBP12.6 regulatory subunits, which results in both increased Ca\(^{2+}\) sensitivity for activation and elevated channel open probability (Marx et al., 2000). Common pool myocyte models do not account for single channel properties, and are not useful in interpreting these findings. The local control myocyte model, however, provides a means to study detailed mechanisms of \(\beta\)-AR-mediated and/or heart failure related alterations in LCC, RyR, and Ca\(^{2+}\) spike properties, and the role such alterations would have on both microscopic EC coupling as well as whole-cell behavior.

It is widely accepted that \(I_{\text{CaL}}\) inactivates via both voltage-dependent and Ca\(^{2+}\)-mediated processes. Recent experiments have elucidated mechanistic details of the Ca\(^{2+}\)-mediated inactivation process, indicating that calmodulin is tethered to the LCC and acts as a critical Ca\(^{2+}\) sensor, and that targeted mutations which prevent calmodulin binding to the LCC (Zuhlke et al., 1999) or prevent Ca\(^{2+}\) binding to calmodulin (Peterson et al., 1999; Peterson et al., 2000), dramatically reduce the degree of \(I_{\text{CaL}}\) inactivation observed during a depolarizing voltage clamp pulse. It has also been shown that Ca\(^{2+}\)-mediated inactivation plays a dominant role in \(I_{\text{CaL}}\) inactivation under AP clamp protocols (Linz and Meyer, 1998) and under voltage clamp protocols where SR Ca\(^{2+}\) load is varied (Sipido et al., 1995). While experimental evidence indicates that the Ca\(^{2+}\)-mediated inactivation process is dominant and the voltage-dependent process is far slower, mathematical models of \(I_{\text{CaL}}\) have consistently been formulated with strong voltage-dependent inactivation and relatively weaker Ca\(^{2+}\)-mediated inactivation (Winslow et al., 1999; Jafri et al., 1998; Luo and Rudy, 1994; Pandit et al., 2001). This is demonstrated
for the Winslow et al. (1999) canine model (as in Fig. 3.10A) and the guinea pig models of Jafri et al. (1998) and Luo and Rudy (1994) in panels A, B and C of Fig. 3.12, respectively. Steady state APs (solid line), Prob{Norm} (dashed line), and y (dotted line) are shown for each model. In all cases, as the plateau of the AP progresses, a greater fraction of LCCs become unavailable due to voltage-dependent inactivation, than due to Ca\(^{2+}\)-mediated inactivation. The balance between voltage- and Ca\(^{2+}\)-dependent inactivation processes in each of these models is in contrast to experimental findings. The local control myocyte model presented in this study displays strong Ca\(^{2+}\)-mediated LCC inactivation properties consistent with experimental findings (Figs. 3.3D and 3.10B). Moreover, Fig. 3.10 demonstrates that the all-or-none SR Ca\(^{2+}\) release property of common pool models (which include explicit descriptions of CICR mechanisms) precludes stability of simulated APs when the balance between Ca\(^{2+}\)-mediated and voltage-dependent inactivation of \(I_{CaL}\) is adjusted to be consistent with experimental findings. This points to a critical flaw of these common pool model formulations for Ca\(^{2+}\) cycling within the cardiac myocyte. In a number of animal models of heart failure, EC coupling processes have been found to be defective (Lindner et al., 1998; Hobai and O'Rourke, 2001; Ahmmed et al., 2000; Marx et al., 2000; Schroder et al., 1998; He et al., 2001; Gomez et al., 1997; O'Rourke et al., 1999b). A clear and quantitative understanding of Ca\(^{2+}\)-cycling and the microscopic details of CICR are critical to the elucidation of heart failure related alterations in EC coupling. It may be difficult to interpret altered EC coupling process in the context of a common pool model due to the inherent instability of \(I_{CaL}\) in that environment. The local control myocyte model,
Figure 3.12: Imbalance of Ca$^{2+}$-mediated and voltage-dependent inactivation of $I_{\text{CaL}}$ in previous common pool models. Action potentials (solid lines), fraction of LCCs not Ca$^{2+}$-inactivated (dashed lines), and fraction of LCCs not voltage-inactivated (dotted lines) during APs in the ventricular myocyte models of (A) Winslow et al. (1999), (B) Jafri et al. (1998), and (C) Luo and Rudy (1994). In all three examples, voltage-dependent inactivation of $I_{\text{CaL}}$ progresses to a greater degree than Ca$^{2+}$-mediated inactivation, and therefore dominates the overall inactivation of $I_{\text{CaL}}$, particularly in the late plateau phase of each AP.
however, displays robust graded SR Ca\(^{2+}\) release and strong Ca\(^{2+}\)-mediated inactivation of \(I_{\text{CaL}}\) processes that are central to properties of CICR. As an example, the model demonstrates that depressed SR Ca\(^{2+}\) content similar to that measure in heart failure (Lindner et al., 1998; Hobai and O'Rourke, 2001), leads to dramatic AP prolongation by relieving the strong Ca\(^{2+}\)-mediated inactivation of LCCs (Fig. 3.10D). In addition, the coupling of quantitative descriptions of microscopic events of CICR to the macroscopic processes within the myocyte reveals how the integration of these cellular processes gives rise to phenomena that may be difficult to predict or understand without interpretation in the context of a quantitative model (Fig. 3.11).

**Conclusions**

In this study, a biophysically detailed model of the normal canine ventricular myocyte has been presented. The myocyte model is designed to conform to the theory of local control by incorporating details of microscopic EC coupling properties in the form of Ca\(^{2+}\) release units in which individual sarcolemmal L-type Ca\(^{2+}\) channels interact in a stochastic manner with nearby RyRs in localized regions where junctional SR membrane and T-tubular membrane are in close proximity. The CaRUs are embedded within and interact with the global systems of the myocyte describing ionic and membrane pump/exchanger currents, SR Ca\(^{2+}\) uptake, and time-varying cytosolic ion concentrations to form a model of the cardiac AP. The results demonstrate that a single comprehensive model of the cardiac myocyte can reproduce both the detailed properties of EC coupling, such as variable gain and graded SR Ca\(^{2+}\) release, while at the same time reproducing
whole-cell phenomena, such as modulation of AP duration by SR Ca\textsuperscript{2+} release. The local control myocyte model provides a means for studying the interrelationship between microscopic and macroscopic behaviors in a manner that would not be possible in experiments. Moreover, results indicate that proper accounting for EC coupling mechanisms necessitates the incorporation of local control mechanisms in myocyte models because common pool models which incorporate detailed mechanisms of CICR are not stable when Ca\textsuperscript{2+}-mediated inactivation of $I_{\text{CaL}}$ is strong and voltage-dependent inactivation is slow/incomplete as experiments suggest. Furthermore, the need for implementing local molecular interactions poses a unique challenge to the scientific community. More mathematically efficient ways of describing local control of Ca\textsuperscript{2+} release are necessary in order to build models that can be used to rapidly explore hypotheses and/or can be incorporated into larger scale multicellular tissue or whole heart models.

It is likely that the role of local stochastic interactions in determining macroscopic properties applies to physiological systems other than CICR in cardiac myocytes. In a model of phage λ-infection (Arkin et al., 1998), molecular level fluctuations in the rates of gene expression can arise due to stochastic local actions of regulatory proteins. These fluctuations lead to highly erratic time patterns of protein production in individual cells, which determine the phenotypic outcome for the phage λ lysis-lysogeny decision circuit. Recordings from rat hippocampal neurons have demonstrated the existence of a preassembled macromolecular signaling complex, which associates the β-AR with the L-type Ca\textsuperscript{2+} channel (Davare et al., 2001). The complex also contains a G protein, an
adenylyl cyclase, protein kinase A, and a phosphatase. The physical association of the molecular components of these signaling pathway elements suggests that the chain of signaling events from receptor-ligand binding to phosphorylation of the LCC will be determined by interactions between a small number of molecular entities within their local environment. Similarly, it has recently been demonstrated that spatial confinement of specific nitric oxide synthase isoforms in cardiac myocytes plays a critical role in modulating contractility, allowing both inhibition of LCCs and stimulation of RyRs (Barouch et al., 2002). Modeling studies of systems such as these may benefit from an approach, such as the one presented in this study, which combines stochastic and deterministic methods in order to maintain detailed descriptions of local molecular interactions.
Appendix I

Local Ionic Fluxes

Each Ca\(^{2+}\) Release Unit (CaRU) contains a single JSR compartment and four
dyadic subspace compartments, which are arranged on a \(2 \times 2\) grid (Fig. 3.1). Between
each subspace and the JSR compartment there are five RyRs, and between each subspace
and the T-tubule lumen there is one LCC and one ClCh. All model parameter values are
given in Tables 3.1-3.7.

The JSR volume of the \(i^{th}\) CaRU is refilled via passive Ca\(^{2+}\) influx from the NSR:

\[
J_{tr,i} = r_{tr} \left( [Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR,i} \right)
\]

where \(r_{tr}\) is the transfer rate of Ca\(^{2+}\) from the NSR to a JSR compartment; \([Ca^{2+}]_{NSR}\) is
the NSR Ca\(^{2+}\) concentration; and \([Ca^{2+}]_{JSR,i}\) is the JSR Ca\(^{2+}\) concentration in the \(i^{th}\) CaRU.

Total Ca\(^{2+}\) flux from NSR into all CaRU JSR compartments is

\[
J_{tr} = \frac{N_{CaRU}}{N_s} \sum_{i=1}^{N_s} J_{tr,i}
\]

where \(N_{CaRU}\) is the number of CaRUs included in the full model and \(N_s\) is the number of
CaRUs actually simulated (see Appendix II for description of \(N_{CaRU}\) vs. \(N_s\)). Diffusive
Ca\(^{2+}\) flux between the \(j^{th}\) and the \(l^{th}\) contiguous subspaces within the \(i^{th}\) CaRU is defined
by

\[
J_{iss,j,l} = r_{iss} \left( [Ca^{2+}]_{ss,j,l} - [Ca^{2+}]_{ss,l,j} \right)
\]

where \(r_{iss}\) is the inter-subspace Ca\(^{2+}\) transfer rate and \([Ca^{2+}]_{ss,j,l}\) is the subspace Ca\(^{2+}\)
concentration in the \(j^{th}\) subspace of the \(i^{th}\) CaRU. Every subspace compartment has two
neighbors into which or from which Ca$^{2+}$ may flow (Fig. 3.1B). Similarly, Ca$^{2+}$ diffusion out of the $j^{th}$ subspace of the $i^{th}$ CaRU into the cytosol is calculated as

$$J_{\text{xfer},i,j} = r_{\text{xfer}} ([\text{Ca}^{2+}]_{\text{SS},i,j} - [\text{Ca}^{2+}]_{\text{cyto}})$$  (3.4)

where $r_{\text{xfer}}$ is the rate of Ca$^{2+}$ transfer from a single subspace compartment to the cytosol and $[\text{Ca}^{2+}]_{\text{cyto}}$ is the cytosolic Ca$^{2+}$ concentration. The total Ca$^{2+}$ flux entering the cytosol from all CaRUs is

$$J_{\text{xfer}} = \frac{N_{\text{CaRU}}}{N_i} \sum_{i=1}^{N_i} \sum_{j=1}^{4} J_{\text{xfer},i,j}$$  (3.5)

The Markov state model for the L-type Ca$^{2+}$ channel is the mode-switching model developed previously by Jafri et al. (1998) and is shown in Fig. 3.13A. Briefly, the upper row of states comprises Mode Normal, while the lower row of states comprises Mode Ca, the Ca$^{2+}$-mediated inactivation mode. States labeled $O$ and $O_{\text{Ca}}$ (states 6 and 12) are conducting states. Elevation of local Ca$^{2+}$ level promotes transition into Mode Ca, in which transitions to the open state are extremely rare. The rate constants are defined individually for each LCC by the parameter values given in Table 3.3 and

$$\alpha = 2.0 e^{0.012( V_m - 35 )}$$  (3.6)
$$\beta = 0.0882 e^{-0.05( V_m - 35 )}$$  (3.7)
$$\alpha' = \alpha \alpha$$  (3.8)
$$\beta' = \beta / b$$  (3.9)
$$\gamma = \gamma_o [\text{Ca}^{2+}]_{\text{SS}}$$  (3.10)

where $V_m$ is membrane potential in units of mV; $\alpha$, $\beta$, $\alpha'$, $\beta'$, and $\gamma$ are in units of ms$^{-1}$; and $[\text{Ca}^{2+}]_{\text{SS}}$ is the Ca$^{2+}$ concentration in the dyadic subspace adjacent to the inner pore of
Figure 3.13: State diagrams for the LCC and the ClCh channel Markov models. (A) State model of the mode switching and voltage activation processes of the LCC channel model. The states in the upper row encompass Mode Normal and the states in the lower row encompass Mode Ca. Open states are labeled $O$ and $O_{Ca}$ (states 6 and 12). Depolarization promotes transitions from left to right toward the open states. Elevation of $Ca^{2+}$ in the adjacent subspace promotes transitions into Mode Ca, where transitions into $O_{Ca}$ are rare. (For more details see Jafri et al., 1998, and Rice et al., 1999). (B) State model for voltage-dependent inactivation of an LCC. Depolarization promotes transitions from the available state ($y$) to the unavailable state ($1-y$). See Eqs. 3.6-3.16 and Table 3 for equations and parameters describing LCC state transitions. (C) State model for the ClCh ($I_{to2}$). Elevation of $Ca^{2+}$ in the adjacent subspace promotes transitions from the closed state into the open state. See Eqs. 3.17-3.18 and Table 3 for equations and parameters describing ClCh state transitions.
Figure 3.13
the channel. Voltage dependent inactivation of an LCC, indicated by the value of $y_{i,j}$, is modeled by the simple two state gating scheme shown in Fig. 3.13B with rates determined by

$$y_{\infty} = \frac{0.4}{1 + e^{(V_{\text{m}} + 12.5)/5}} + 0.6$$  \hspace{1cm} (3.11)$$

$$\tau_y = 340/\left(1 + e^{(V_{\text{m}} + 30)/12}\right) + 60$$  \hspace{1cm} (3.12)$$

$$k_{b,y} = y_{\infty}/\tau_y$$  \hspace{1cm} (3.13)$$

$$k_{f,y} = (1 - y_{\infty})/\tau_y$$  \hspace{1cm} (3.14)$$

with $\tau_y$ given in ms, and $k_{f,y}$ and $k_{b,y}$ given in ms$^{-1}$. The current passing through the $j^{th}$ LCC of the $i^{th}$ CaRU can then be written as

$$I_{\text{LCC},i,j} = \text{isopen}(\text{LCC}_{i,j}, y_{i,j}) \cdot P_{\text{CaL}} \frac{4F^2 [\text{Ca}^{2+}]_{i,j} e^{2\mu_{\text{f}}/RT - 0.341[\text{Ca}^{2+}]_{o}}}{e^{2\mu_{\text{f}}/RT - 1}}$$  \hspace{1cm} (3.15)$$

where isopen(\text{LCC}_{i,j}, y_{i,j}) indicates when the $j^{th}$ channel of the $i^{th}$ CaRU is open and takes on the value of 1 when it occupies state $O$ or $O_{\text{Ca}}$ and is not voltage-inactivated (i.e., LCC$_{i,j}$ is in state 6 or 12 and $y_{i,j}$ is in state 1), and takes on the value of 0 otherwise; $P_{\text{CaL}}$ is the permeability of a single LCC; $F$ is Faraday’s constant; $R$ is the universal gas constant; $T$ is absolute temperature; $[\text{Ca}^{2+}]_{i,j}$ is the subspace Ca$^{2+}$ concentration adjacent to the $j^{th}$ channel of the $i^{th}$ CaRU; and $[\text{Ca}^{2+}]_{o}$ is extracellular Ca$^{2+}$ concentration. Whole cell $I_{\text{CaL}}$, normalized by whole cell capacitance, is equal to the summation of unitary Ca$^{2+}$ currents over all LCCs

$$I_{\text{CaL}} = \frac{1}{C_{\text{m}}} \sum_{i=1}^{N_{\text{Ca}}} \sum_{j=1}^{N_j} I_{\text{LCC},i,j}$$  \hspace{1cm} (3.16)$$
where \( C_{SA} \) is the capacitance of the cell’s surface area and it has been assumed that specific membrane capacity is 1 \( \mu F \ cm^{-2} \).

The \( Ca^{2+} \)-dependent Cl\(^-\) channel (ClCh) is modeled as a voltage- and time-independent ligand-gated channel, using a simple two-state model as shown in Fig. 3.13C. While detailed \( Ca^{2+} \)-dependent kinetics have not been experimentally measured for this channel, model rates have been chosen to be sufficiently fast (~ 2 ms\(^{-1} \)) to track kinetics of local \( Ca^{2+} \) transients, and to be consistent with the experimentally estimated \( K_{d} \) for \( Ca^{2+} \) binding of ~ 150 \( \mu M \) (Collier et al., 1996). Unitary Cl\(^-\) current through the \( j^{th} \) ClCh of the \( i^{th} \) CaRU is described as

\[
I_{\text{ClCh},i,j} = \text{isopen}(\text{ClCh}_{i,j}) P_{\text{to2}} \frac{v_{e} F^{2} \left[ Cl^{-} \right]_{o} e^{-n_{i,j}/RT}}{e^{v_{e} F/n_{i,j}RT} - 1}
\]

(3.17)

where \( \text{isopen(} \text{ClCh}_{i,j} \)\) indicates when the \( j^{th} \) channel of the \( i^{th} \) CaRU is open and \( P_{\text{to2}} \) is the permeability of a single ClCh. ClCh permeability is adjusted to match unitary Cl\(^-\) currents measured in canine ventricular myocytes (Collier et al., 1996). Since other Cl\(^-\) transport mechanisms are not included in the model, it was assumed that intracellular/subspace Cl\(^-\) concentration remains constant. Whole cell \( I_{\text{to2}} \), normalized by cell surface area, is equal to the summation of unitary Cl\(^-\) currents over all ClChs

\[
I_{\text{to2}} = \frac{1}{C_{SA}} \frac{N_{\text{CaRUs}}}{N_{\text{cells}}} \sum_{i=1}^{N_{\text{CaRUs}}} \sum_{j=1}^{4} I_{\text{ClCh},i,j}
\]

(3.18)

Each RyR channel is represented by the Markov state model developed by Keizer and Smith (1998) and later modified by Rice et al. (1999). Fig. 3.14A shows the baseline RyR model structure. States 3 and 4 correspond to open states in the baseline model. Under conditions of elevated subspace \( Ca^{2+} \), rapid equilibrium approximations are
applied to pairs of states when the transition rates between the pair are ~ 200-fold faster than the rates exiting the pair (Keizer and Smith, 1998). Fig. 3.14, B and C, shows the five- and four-state equivalent models used and the $[\text{Ca}^{2+}]_{\text{SS}}$ ranges in which these reduced order models are considered to be valid. The RyR model representations are dynamically switched among the six, five, and four-state representations during the simulation as local Ca$^{2+}$ levels vary. To further reduce computation time, RyR Ca$^{2+}$-dependent transition rates are bounded below a maximal value corresponding to the rates that occur at a subspace Ca$^{2+}$ level of 50 µM in order to prevent extremely fast and unrealistic transition rates at high local Ca$^{2+}$ levels (Rice et al., 1999). This bound was not applied to rate $k_{12}$ because it is significantly slower than the other Ca$^{2+}$-dependent rates and the constraint of microscopic reversibility does not apply to $k_{12}$ because it is not within a closed loop of states.

The RyR Ca$^{2+}$ release flux in the $j^{th}$ subspace of the $i^{th}$ CaRU is computed as

\[
J_{\text{rel},i,j} = \sum_{k=1}^{5} \text{isopen}(\text{RyR}_{i,j,k})r_{\text{RyR}}(\text{[Ca}^{2+}]_{\text{ISR},i} - \text{[Ca}^{2+}]_{\text{SS},i,j})
\]

(3.19)

where isopen(RyR$_{i,j,k}$) takes on the value of 1 when the $k^{th}$ RyR in the $j^{th}$ subspace of the $i^{th}$ CaRU is open (RyR$_{i,j,k}$ is in state 3, 4, or 3-4) and takes on the value of 0 otherwise; $r_{\text{RyR}}$ is the transfer rate of Ca$^{2+}$ through an open RyR; $[\text{Ca}^{2+}]_{\text{SS},i,j}$ is the subspace Ca$^{2+}$ concentration in the $j^{th}$ subspace of the $i^{th}$ CaRU; and $[\text{Ca}^{2+}]_{\text{ISR},i}$ is the JSR Ca$^{2+}$ concentration in the $i^{th}$ CaRU. $J_{\text{rel}}$, the whole cell SR release flux, can be calculated by applying Eq. 3.16 to $J_{\text{rel},i,j}$. This is necessary only when calculating features such as EC coupling gain.
Figure 3.14: State diagrams for the RyR Markov models. (A) Baseline RyR model where states 1 and 2 are the resting states, states 3 and 4 are the open states, and states 5 and 6 are the refractory states. It is assumed that each Ca$^{2+}$-dependent transition is associated with the binding of two Ca$^{2+}$ ions, therefore requiring that four Ca$^{2+}$ ions bind in order to reach the open state. This model is implemented when subspace Ca$^{2+}$ concentration is less than 0.115 µM. See Table 4 for values of the rate constants. (B) Approximation of the model in panel A where it has been assumed that states 5 and 6 are in rapid equilibrium, and are therefore merged into a single state. This model is implemented when subspace Ca$^{2+}$ concentration is in the range of 0.115 - 36.85 µM. (C) Approximation of the model in panel B where it has been assumed that states 3 and 4 are in rapid equilibrium. This model is implemented when subspace Ca$^{2+}$ concentration is greater than 36.85 µM.
Figure 3.14
Local Ca$^{2+}$ Balance

The Ca$^{2+}$ balance equation for the JSR in the $i$th CaRU is

$$\frac{d[Ca^{2+}_{ls,RJ}]}{dt} = \beta_{JSR,i} \left( J_{tr,i} - \frac{\nu_{rel}}{V_{rel}} \sum_{j=1}^{4} J_{rel,i,j} \right)$$

(3.20)

where

$$\beta_{JSR,i} = \left(1 + \frac{[CSQN]_T}{K_{CSQN}} \right)^{-1}$$

(3.21)

is an implementation of the rapid buffering approximation as previously described by Rice et al. (1999). Calsequestrin concentration and Ca$^{2+}$ binding affinity ([CSQN]$_T$ and $K_{CSQN}$) are based on recent measurements (Shannon et al., 2000). Similarly, Ca$^{2+}$ balance in the 1st, 2nd, 3rd, and 4th subspaces of the $i$th CaRU are respectively described by

$$\frac{d[Ca^{2+}_{ls,1}]}{dt} = \beta_{SS,i,1} \left( J_{LCC,1} + J_{rel,i,1} - J_{xfer,i,1} + J_{iss,i,2,1} + J_{iss,i,4,1} \right)$$

(3.22)

$$\frac{d[Ca^{2+}_{ls,2}]}{dt} = \beta_{SS,i,2} \left( J_{LCC,2} + J_{rel,i,2} - J_{xfer,i,2} + J_{iss,i,3,2} + J_{iss,i,1,2} \right)$$

(3.23)

$$\frac{d[Ca^{2+}_{ls,3}]}{dt} = \beta_{SS,i,3} \left( J_{LCC,3} + J_{rel,i,3} - J_{xfer,i,3} + J_{iss,i,4,3} + J_{iss,i,2,3} \right)$$

(3.24)

$$\frac{d[Ca^{2+}_{ls,4}]}{dt} = \beta_{SS,i,4} \left( J_{LCC,4} + J_{rel,i,4} - J_{xfer,i,4} + J_{iss,i,1,4} + J_{iss,i,3,4} \right)$$

(3.25)

where

$$J_{LCC,i,j} = \frac{-J_{LCC,i,j}}{2F_{SS}}$$

(3.26)

and

$$\beta_{SS,i,j} = \left(1 + \frac{[BSR]_T}{K_{BSR}} + \frac{[BSL]_T}{K_{BSL}} \right)^{-1}$$

(3.27)
describes rapid equilibrium between $\text{Ca}^{2+}$ and membrane bound $\text{Ca}^{2+}$ buffers (Rice et al., 1999).

**Global Ionic Fluxes**

All of the global currents and ionic fluxes that are incorporated into the local control myocyte model have been described fully in previous work. Table 3.6 provides references for previously published mathematical model descriptions and parameters, and any parameters that differ in the present implementation from the referenced version of the model are provided.

**Global Ionic Flux Balance and Membrane Potential**

Intracellular concentrations of $\text{Na}^+$, $\text{K}^+$, NSR $\text{Ca}^{2+}$, and cytosolic $\text{Ca}^{2+}$ are described respectively by

\[
\frac{d[\text{Na}^+]}{dt} = \frac{-C_{\text{Na}}}{F_{\text{cyto}}} (I_{\text{Na}} + I_{\text{Na,b}} + 3I_{\text{NaCa}} + 3I_{\text{NaK}}) \tag{3.28}
\]

\[
\frac{d[\text{K}^+]}{dt} = \frac{-C_{\text{K}}}{F_{\text{cyto}}} (I_{\text{Kr}} + I_{\text{Ks}} + I_{\text{lo1}} + I_{\text{K1}} + I_{\text{Kp}} - 2I_{\text{NaK}}) \tag{3.29}
\]

\[
\frac{d[\text{Ca}^{2+}]_{\text{NSR}}}{dt} = \frac{V_{\text{NSR}}}{F_{\text{NSR}}} J_{\text{up}} - \frac{V_{\text{NSR}}}{F_{\text{NSR}}} J_{\text{tr}} \tag{3.30}
\]

\[
\frac{d[\text{Ca}^{2+}]_{\text{cyto}}}{dt} = \beta_{\text{cyto}} \left\{ \frac{-C_{\text{Ca}}}{F_{\text{cyto}}} \left( I_{\text{Ca,b}} + I_{\text{p(Ca)}} - 2I_{\text{NaCa}} \right) + \frac{V_{\text{Ca}}}{V_{\text{cyto}}} J_{\text{xfer}} - J_{\text{up}} - J_{\text{trp}} \right\} \tag{3.31}
\]

where

\[
\beta_{\text{cyto}} = \left( 1 + \frac{[\text{CMDN}]}{K_{\text{CMDN}} + [\text{Ca}^{2+}]_{\text{cyto}}} \right)^{-1} \tag{3.32}
\]

describes $\text{Ca}^{2+}$ buffering by calmodulin (Winslow et al., 1999).
The membrane potential is calculated as

$$\frac{dV}{dt} = -\left( I_{Na} + I_{CaL} + I_{Kr} + I_{Ks} + I_{tO1} + I_{K1} + I_{Kp} + I_{tO2} + I_{NaK} + I_{NaCa} + I_{p(Ca)} + I_{Ca,b} + I_{Na,b} \right)$$

(3.33)

where all currents are expressed in units of pA pF$^{-1}$. 

Table 3.1: Physical Constants and Cell Geometry Parameters

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<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
<td>96.5 C mmol$^{-1}$</td>
</tr>
<tr>
<td>$T$</td>
<td>Absolute temperature</td>
<td>310 K</td>
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<tr>
<td>$R$</td>
<td>Universal gas constant</td>
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<td>$V_{cyto}$</td>
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<td>$V_{NSR}$</td>
<td>NSR volume</td>
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<td>$22.26 \times 10^{-6}$ pL</td>
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<tr>
<td>$V_{SS}$</td>
<td>SS volume (single compartment)</td>
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<td>$N_{CaRU}$</td>
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<td>Parameter</td>
<td>Definition</td>
<td>Value</td>
</tr>
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<td>-----------</td>
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<td>$[\text{K}^+]_o$</td>
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<td>Extracellular $\text{Na}^+$ concentration</td>
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Table 3.3: L-Type Ca$^{2+}$ Channel and Ca$^{2+}$-dependent Cl$^{-}$ Channel Parameters

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<td>$f$</td>
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<td>0.85 ms$^{-1}$</td>
</tr>
<tr>
<td>$g$</td>
<td>LCC transition rate out of open state</td>
<td>2.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$f'$</td>
<td>LCC transition rate into open state for Mode Ca</td>
<td>0.005 ms$^{-1}$</td>
</tr>
<tr>
<td>$g'$</td>
<td>LCC transition rate out of open state for Mode Ca</td>
<td>7.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$a$</td>
<td>LCC mode transition parameter</td>
<td>2.0</td>
</tr>
<tr>
<td>$b$</td>
<td>LCC mode transition parameter</td>
<td>1.9356</td>
</tr>
<tr>
<td>$\gamma_0$</td>
<td>LCC mode transition parameter</td>
<td>0.44 mM$^{-1}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$\omega$</td>
<td>LCC mode transition parameter</td>
<td>0.02158 ms$^{-1}$</td>
</tr>
<tr>
<td>$P_{CaL}$</td>
<td>LCC permeability to Ca$^{2+}$ (unitary)</td>
<td>$9.13 \times 10^{-13}$ cm$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>$K_{d,ClCh}$</td>
<td>Dissociation constant of ClCh for Ca$^{2+}$</td>
<td>0.1502 mM</td>
</tr>
<tr>
<td>$k_{f,ClCh}$</td>
<td>ClCh transition rate into open state ($k_{b,ClCh}/K_{d,ClCh}$)</td>
<td>13.3156 mM$^{-1}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{b,ClCh}$</td>
<td>ClCh transition rate out of open state</td>
<td>2.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$P_{to2}$</td>
<td>ClCh permeability to Cl$^{-}$ (unitary)</td>
<td>$2.65 \times 10^{-15}$ cm$^3$ s$^{-1}$</td>
</tr>
</tbody>
</table>
### Table 3.4: Ryanodine Receptor Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$</td>
<td>Rate constant: state 1 to state 2</td>
<td>877.5 mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>Rate constant: state 2 to state 1</td>
<td>250.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{23}$</td>
<td>Rate constant: state 2 to state 3</td>
<td>$2.358 \times 10^8$ mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{32}$</td>
<td>Rate constant: state 3 to state 2</td>
<td>9.6 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{34}$</td>
<td>Rate constant: state 3 to state 4</td>
<td>$1.415 \times 10^6$ mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{43}$</td>
<td>Rate constant: state 4 to state 3</td>
<td>13.65 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{45}$</td>
<td>Rate constant: state 4 to state 5</td>
<td>0.07 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{54}$</td>
<td>Rate constant: state 5 to state 4</td>
<td>93.385 mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{56}$</td>
<td>Rate constant: state 5 to state 6</td>
<td>$1.887 \times 10^7$ mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{65}$</td>
<td>Rate constant: state 6 to state 5</td>
<td>30.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{25}$</td>
<td>Rate constant: state 2 to state 5</td>
<td>$2.358 \times 10^6$ mM$^{-2}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$k_{52}$</td>
<td>Rate constant: state 5 to state 2</td>
<td>0.001235 ms$^{-1}$</td>
</tr>
<tr>
<td>$r_{RyR}$</td>
<td>Rate of Ca$^{2+}$ flux through an open RyR</td>
<td>3.92 ms$^{-1}$</td>
</tr>
</tbody>
</table>
Table 3.5: $\text{Ca}^{2+}$ Flux and Buffering Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{\text{xfer}}$</td>
<td>Rate of $\text{Ca}^{2+}$ flux between SS and cytosol</td>
<td>200.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$r_{\text{tr}}$</td>
<td>Rate of $\text{Ca}^{2+}$ flux between NSR and JSR</td>
<td>0.333 ms$^{-1}$</td>
</tr>
<tr>
<td>$r_{\text{iss}}$</td>
<td>Intersubspace $\text{Ca}^{2+}$ flux rate</td>
<td>20.0 ms$^{-1}$</td>
</tr>
<tr>
<td>$[\text{BSR}]_T$</td>
<td>Total subspace SR membrane site concentration</td>
<td>47.0 µM</td>
</tr>
<tr>
<td>$K_{\text{BSR}}$</td>
<td>$\text{Ca}^{2+}$-half saturation constant for BSR</td>
<td>0.87 µM</td>
</tr>
<tr>
<td>$[\text{BSL}]_T$</td>
<td>Total subspace sarcolemma site concentration</td>
<td>1124.0 µM</td>
</tr>
<tr>
<td>$K_{\text{BSL}}$</td>
<td>$\text{Ca}^{2+}$-half saturation constant for BSL</td>
<td>8.7 µM</td>
</tr>
<tr>
<td>$[\text{CSQN}]_T$</td>
<td>Total JSR calsequestrin concentration</td>
<td>13.5 mM</td>
</tr>
<tr>
<td>$K_{\text{CSQN}}$</td>
<td>$\text{Ca}^{2+}$-half saturation constant for calsequestrin</td>
<td>0.63 mM</td>
</tr>
<tr>
<td>$[\text{CMDN}]_T$</td>
<td>Total cytosolic calmodulin concentration</td>
<td>50.0 µM</td>
</tr>
<tr>
<td>$K_{\text{CMDN}}$</td>
<td>$\text{Ca}^{2+}$-half saturation constant for calmodulin</td>
<td>2.38 µM</td>
</tr>
</tbody>
</table>
Table 3.6: Ionic Current and Flux Formulations From Previous Studies

<table>
<thead>
<tr>
<th>Current/Flux</th>
<th>Reference</th>
<th>New Parameter Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast Na(^+) Current ((I_{Na}))</td>
<td>Winslow et al., 1999</td>
<td>No Change</td>
</tr>
<tr>
<td>Rapid Delayed Rectifier K(^+) Current ((I_{Kr}))</td>
<td>Mazhari et al., 2001</td>
<td>(G_{Kr} = 0.024) mS µF(^{-1})</td>
</tr>
<tr>
<td>Slow Delayed Rectifier K(^+) Current ((I_{Ks}))</td>
<td>Winslow et al., 1999</td>
<td>No Change</td>
</tr>
<tr>
<td>Transient Outward K(^+) Current ((I_{to1}))</td>
<td>Greenstein et al., 2000</td>
<td>(G_{Kv4.3} = 0.1389) mS µF(^{-1})</td>
</tr>
<tr>
<td>Time-Independent K(^+) Current ((I_{K1}))</td>
<td>Winslow et al., 1999</td>
<td>(G_{K1} = 3.0) mS µF(^{-1})</td>
</tr>
<tr>
<td>Plateau K(^+) Current ((I_{Kp}))</td>
<td>Winslow et al., 1999</td>
<td>(G_{Kp} = 0.002659) mS µF(^{-1})</td>
</tr>
<tr>
<td>Na(^+)/Ca(^2+) Exchanger Current ((I_{NaCa}))</td>
<td>Winslow et al., 1999</td>
<td>(k_{NaCa} = 0.27) pA pF(^{-1})</td>
</tr>
<tr>
<td>Na(^+)/K(^+) Pump Current ((I_{NaK}))</td>
<td>Winslow et al., 1999</td>
<td>(I_{NaK,max} = 0.901) pA pF(^{-1})</td>
</tr>
<tr>
<td>Sarcolemmal Ca(^2+) Pump Current ((I_{p(Ca)}))</td>
<td>Winslow et al., 1999</td>
<td>(I_{p(Ca),max} = 0.03) pA pF(^{-1})</td>
</tr>
<tr>
<td>Ca(^2+) Background Current ((I_{Ca,b}))</td>
<td>Winslow et al., 1999</td>
<td>(K_{m,p(Ca)} = 0.0005) mM</td>
</tr>
<tr>
<td>Na(^+) Background Current ((I_{Na,b}))</td>
<td>Winslow et al., 1999</td>
<td>(G_{Na,b} = 0.00264) mS µF(^{-1})</td>
</tr>
<tr>
<td>Ca(^2+) Binding to Troponin ((J_{upn}))</td>
<td>Winslow et al., 1999</td>
<td>No Change</td>
</tr>
<tr>
<td>SR Ca(^2+) Pump (SERCA2a Pump, (J_{up}))</td>
<td>Shannon et al., 2000</td>
<td>(V_{max} = V_{maxr} = 209.6) μM s(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(k = 0) (leak term omitted)</td>
</tr>
</tbody>
</table>
Table 3.7: State Variable Initial Conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial Value (Voltage Clamp)*</th>
<th>Initial Value (AP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_m$</td>
<td>-100.0 mV</td>
<td>-91.3258 mV</td>
</tr>
<tr>
<td>$n$ ($I_{Na}$)</td>
<td>$1.21087 \times 10^{-4}$</td>
<td>$5.33837 \times 10^{-4}$</td>
</tr>
<tr>
<td>$h$ ($I_{Na}$)</td>
<td>0.999484</td>
<td>0.996345</td>
</tr>
<tr>
<td>$j$ ($I_{Na}$)</td>
<td>0.999480</td>
<td>0.997315</td>
</tr>
<tr>
<td>$[\text{Na}^+]_{\text{cyto}}$</td>
<td>10.0 mM</td>
<td>10.0 mM</td>
</tr>
<tr>
<td>$[\text{K}^+]_{\text{cyto}}$</td>
<td>133.24 mM</td>
<td>131.84 mM</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{cyto}}$</td>
<td>$1.11074 \times 10^{-4}$ mM</td>
<td>$1.45273 \times 10^{-4}$ mM</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{NSR}}$</td>
<td>0.728737 mM</td>
<td>0.908882 mM</td>
</tr>
<tr>
<td>$[\text{LTRPNCa}]$</td>
<td>7.02394 $\times 10^{-3}$ mM</td>
<td>8.98282 $\times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$[\text{HTRPNCa}]$</td>
<td>0.136915 mM</td>
<td>0.137617 mM</td>
</tr>
<tr>
<td>$x_{K_s}$ ($I_{K_s}$)</td>
<td>$0.104829 \times 10^{-3}$</td>
<td>$0.204717 \times 10^{-3}$</td>
</tr>
<tr>
<td>$C_1$ ($I_{K_r}$)</td>
<td>0.990</td>
<td>0.999503</td>
</tr>
<tr>
<td>$C_2$ ($I_{K_r}$)</td>
<td>0.008</td>
<td>0.413720 $\times 10^{-3}$</td>
</tr>
<tr>
<td>$C_3$ ($I_{K_r}$)</td>
<td>0.002</td>
<td>0.727568 $\times 10^{-4}$</td>
</tr>
<tr>
<td>$O$ ($I_{K_r}$)</td>
<td>0.0</td>
<td>0.873984 $\times 10^{-5}$</td>
</tr>
<tr>
<td>$I$ ($I_{K_r}$)</td>
<td>0.0</td>
<td>0.136159 $\times 10^{-5}$</td>
</tr>
<tr>
<td>$C_0$ ($I_{K_{v4.3}}$)</td>
<td>0.968277</td>
<td>0.953060</td>
</tr>
<tr>
<td>$C_1$ ($I_{K_{v4.3}}$)</td>
<td>0.0133601</td>
<td>0.0253906</td>
</tr>
<tr>
<td>$C_2$ ($I_{K_{v4.3}}$)</td>
<td>$0.691875 \times 10^{-4}$</td>
<td>$0.253848 \times 10^{-3}$</td>
</tr>
<tr>
<td>$C_3$ ($I_{K_{v4.3}}$)</td>
<td>$0.159092 \times 10^{-6}$</td>
<td>$0.112796 \times 10^{-5}$</td>
</tr>
<tr>
<td>$O$ ($I_{K_{v4.3}}$)</td>
<td>0.0</td>
<td>0.187950 $\times 10^{-8}$</td>
</tr>
<tr>
<td>$C_{10}$ ($I_{K_{v4.3}}$)</td>
<td>0.0153235</td>
<td>0.0151370</td>
</tr>
<tr>
<td>$C_{11}$ ($I_{K_{v4.3}}$)</td>
<td>0.00271424</td>
<td>0.00517622</td>
</tr>
<tr>
<td>$C_{12}$ ($I_{K_{v4.3}}$)</td>
<td>$0.243515 \times 10^{-3}$</td>
<td>$0.896600 \times 10^{-3}$</td>
</tr>
<tr>
<td>$C_{13}$ ($I_{K_{v4.3}}$)</td>
<td>$0.115007 \times 10^{-4}$</td>
<td>$0.817569 \times 10^{-4}$</td>
</tr>
<tr>
<td>$O_{1}$ ($I_{K_{v4.3}}$)</td>
<td>$0.163239 \times 10^{-6}$</td>
<td>$0.224032 \times 10^{-5}$</td>
</tr>
<tr>
<td>$C_0$ ($I_{K_{v1.4}}$)</td>
<td>0.824239</td>
<td>0.722328</td>
</tr>
<tr>
<td>$C_1$ ($I_{K_{v1.4}}$)</td>
<td>0.0522865</td>
<td>0.101971</td>
</tr>
<tr>
<td>$C_2$ ($I_{K_{v1.4}}$)</td>
<td>$0.00124396$</td>
<td>0.00539932</td>
</tr>
<tr>
<td>$C_3$ ($I_{K_{v1.4}}$)</td>
<td>$0.131359 \times 10^{-4}$</td>
<td>$0.127081 \times 10^{-3}$</td>
</tr>
<tr>
<td>$O$ ($I_{K_{v1.4}}$)</td>
<td>$0.522383 \times 10^{-7}$</td>
<td>$0.182742 \times 10^{-5}$</td>
</tr>
<tr>
<td>$C_{10}$ ($I_{K_{v1.4}}$)</td>
<td>0.118010</td>
<td>0.152769</td>
</tr>
<tr>
<td>$C_{11}$ ($I_{K_{v1.4}}$)</td>
<td>0.00334011</td>
<td>0.00962328</td>
</tr>
<tr>
<td>$C_{12}$ ($I_{K_{v1.4}}$)</td>
<td>$0.684631 \times 10^{-3}$</td>
<td>$0.00439043$</td>
</tr>
<tr>
<td>$C_{13}$ ($I_{K_{v1.4}}$)</td>
<td>$0.136717 \times 10^{-3}$</td>
<td>$0.00195348$</td>
</tr>
<tr>
<td>$O_{1}$ ($I_{K_{v1.4}}$)</td>
<td>$0.451249 \times 10^{-4}$</td>
<td>$0.00143629$</td>
</tr>
</tbody>
</table>
Table 3.7 (continued): State Variable Initial Conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial Value (Voltage Clamp)*</th>
<th>Initial Value (AP) #</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Ca}^{2+}]_{JSR, i}$</td>
<td>0.728921 mM (100%)</td>
<td>0.908408 mM (100%)</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{SS, i,j}$</td>
<td>$0.111074 \times 10^{-3}$ mM (100%)</td>
<td>$0.145370 \times 10^{-3}$ mM (100%)</td>
</tr>
<tr>
<td>LCC$_{i,j}$</td>
<td>state 1 (100%)</td>
<td>states 1, 2, 7 (95.8%, 3.8%, 0.4%)</td>
</tr>
<tr>
<td>$v_{i,j}$</td>
<td>state 1 (100%)</td>
<td>states 0, 1 (94.25%, 5.75%)</td>
</tr>
<tr>
<td>ClCh$_{i,j}$</td>
<td>state 0 (100%)</td>
<td>states 0, 1 (99.8%, 0.2%)</td>
</tr>
<tr>
<td>RyR$_{i,j,k}$</td>
<td>state 1 (100%)</td>
<td>states 1, 5-6 (60.9%, 39.1%)</td>
</tr>
</tbody>
</table>

For global state variable definitions, see text and references given in Table 6. For CaRU-compartment and CaRU-random state variable definitions, the initial distribution is given in parentheses for each state respectively.

*Initial values for voltage clamp simulations (Figs. 3.2-3.8) correspond to the state of the system after holding at –100 mV for ~ 3 seconds following a train of 200 ms voltage clamp pulses to 0 mV applied at 1 Hz (in order to load SR with $\text{Ca}^{2+}$).

#Initial values for APs (Figs. 3.9-3.11) are obtained at 1 Hz following 10 beats (i.e., at steady state).
Appendix II

Local Control Myocyte Simulation Algorithm

Each model state variable (SV) is a member of one of the following three groups: 1) global, 2) CaRU-compartment, or 3) CaRU-random. The JSR-NSR, subspace-cytosol, and subspace-sarcolemmal borders define the boundaries of a CaRU. Global SVs represent quantities external to the CaRU boundaries, including membrane potential ($V_m$), cytosolic ion concentrations ($Ca^{2+}$, $K^+$, and $Na^+$), and NSR $Ca^{2+}$ concentration. In addition, Hodgkin-Huxley-type gating variables (e.g., $m$, $h$, and $j$ for $I_{Na}$) and Markov model states (e.g., $C_1$, $C_2$, $C_3$, $I$, and $O$ for $I_{Kr}$) associated with currents that exist entirely outside of the CaRUs are global. The CaRU-compartment SVs are $Ca^{2+}$ concentrations in the JSR and in each SS of every CaRU, and are thus local states. CaRU-random SVs take on the value of the occupied states in single channel Monte-Carlo simulations (LCC, RyR, and ClCh), and are therefore also local states. Each group of SVs requires its own simulation sub-algorithm. First, each of these sub-algorithms is described, and then we show how they interact to form the complete simulation of the local control model.

Let global SVs be denoted by the vector $x(t)$. Let CaRU-compartment SVs and CaRU-random SVs within the $i^{th}$ CaRU be denoted by the vectors $y_i(t)$ and $z_i(t)$, respectively. $N_{CaRU}$ is the number of CaRUs in the myocyte and $N_s$ is the number of CaRUs actually simulated. The difference in $N_{CaRU}$ and $N_s$ arises from a useful approximation described below.
The coupled system of differential equations for the global SVs is represented by

$$\dot{x} = f^x(t, x, y_1, ..., y_{N_1}, z_1, ..., z_{N_2})$$  \hspace{1cm} (3.34)$$

where $f^x$ denotes the function defining the velocity field (time derivative) of $x$. Note that the right hand side of Eq. 3.34 depends upon SVs from all three groups. Integration of global SVs is performed with the Runge-Kutta-Merson 4th-order adaptive step method (RK4M) (Kubicek and Marek, 1983). The RK4M advances the global state vector in time from $x(t)$ to $x(t + h)$, where $h$ denotes a global time step, by subdividing $h$ into Euler-type intermediate steps (sub-steps). Fig. 3.15 shows the relationship among different time steps used throughout the simulation algorithm. Eqs. 3.35 through 3.39 comprise a single RK4M time-step for this system and make use of the compact notation $f^x([t \ x \ y \ z]^n) = f^x(t^n, x^n, y_1^n, ..., y_{N_1}^n, z_1^n, ..., z_{N_2}^n)$:

$$v^{n+1/3} = x^n + \frac{h^n}{3} f^x([t \ x \ y \ z]^n)$$  \hspace{1cm} (3.35)$$

$$\hat{v}^{n+1/3} = x^n + \frac{h^n}{3} \left( f^x([t \ x \ y \ z]^n) + f^x([t \ y \ z]^{n+1/3}) \right)$$  \hspace{1cm} (3.36)$$

$$v^{n+1/2} = x^n + \frac{h^n}{2} \left( f^x([t \ x \ y \ z]^n) + 3 f^x([t \ y \ z]^{n+1/3}) \right)$$  \hspace{1cm} (3.37)$$

$$v^{n+1} = x^n + h^n \left( f^x([t \ x \ y \ z]^n) - 3 f^x([t \ y \ z]^{n+1/3}) + 4 f^x([t \ y \ z]^{n+1/2}) \right)$$  \hspace{1cm} (3.38)$$

$$\hat{v}^{n+1} = x^n + h^n \left( f^x([t \ x \ y \ z]^n) + 4 f^x([t \ y \ z]^{n+1/3}) + f^x([t \ y \ z]^{n+1/2}) \right)$$  \hspace{1cm} (3.39)$$
where \( t^{n+1/p} = t^n + h^n \), \( p = 3, 2, 1 \) denote intermediate times within the \( n \)th global time step \( h^n \). Similarly, we have made use of the notation \( u^{n+1/p} = u(t^n + h^n / p) \) where \( u \) denotes any state vector. Eq. 3.39 yields the 4th-order estimate of the solution, \( x^{n+1} = \hat{x}^{n+1} \). The solution is accepted if the embedded truncation error estimate

\[
E = \|v^{n+1} - \hat{v}^{n+1}\|/5
\]  

(3.40)

is less than the tolerance (\( \varepsilon \)) for each global SV, otherwise Eqs. 3.35 through 3.39 are reevaluated with a smaller \( h^n \) determined by

\[
h_{x,new}^n = 0.85h_{x,old}^n \left( \varepsilon / |E| \right)^{0.2}
\]  

(3.41)

Eq. 3.41 is also used to increase the global time step if the solution over the previous step has been accepted. The errors for each SV are normalized to insure that each is equally weighted in the truncation error calculation. Global step-size is normally limited by the time-rate-of-change of the global SVs, but is not limited by fast dynamics within CaRUs. An important feature of this procedure is that every equation evaluation (of Eqs. 3.35 - 3.39) yields an estimated solution at the intermediate time-step (i.e., each \( v \) is an estimate of \( x \), e.g., \( x_{n+1/2} \approx v_{n+1/2} \)). We make use of this property when calculating fluxes that cross CaRU boundaries (see below). The left hand sides of Eqs. 3.36 and 3.39 are denoted with a carrot symbol (i.e., \( \hat{v} \)) to indicate that these are refinements of the estimates obtained in the immediately preceding equation (at the same time point).
The global state vector is advanced in time from $t_n$ to $t_{n+1}$ over the global time step $h^n$, where $n$ is an index to global time steps (i.e., $h^n$ is the $n^{th}$ global time step). Each global time step is divided into RK4M sub-steps, which are themselves divided into local time steps. CaRU-compartment and CaRU-random state vectors are simulated along local time steps, which are determined independently within each CaRU. The local time step in the $i^{th}$ CaRU between times $t^m_i$ and $t^{m+1}_i$ is denoted $h^{m}_{y,i}$, where $m$ is an index to local time steps. The index $M_i$ refers to the last local time step in the $i^{th}$ CaRU within the bounds of the encompassing global time step and $N_s$ is the number of CaRUs included in the Monte Carlo simulation.
Figure 3.15
The simulation of a CaRU does not require knowledge of events occurring in any other CaRU within the span of a single global time step (i.e., CaRUs are independent on the interval \([t, t + h_i]\)), which allows for parallelization of this part of the algorithm. The solution of both CaRU-compartment and CaRU-random SVs within the \(i^{th}\) CaRU is advanced in increments of the local time step, \(h_{yz,i}\). The coupled system of differential equations for the CaRU-compartment SVs of the \(i^{th}\) CaRU is represented by

\[
\dot{y}_i = f^y(t, x_i, y_i, z_i)
\]  

(3.42)

where \(f^y\) denotes the function defining the velocity field of \(y_i\) (for any \(i\)). Integration of CaRU-compartment SVs is performed with the 2nd-order trapezoidal method. The trapezoidal method is chosen because it provides higher order accuracy than the Euler method without the need to further subdivide the local time step. The solution is advanced in time as follows:

\[
w_i^{m+1} = y_i^m + h_{yz,i}^m f^y(t_i^m, x_i^m, y_i^m, z_i^m)
\]  

(3.43)

\[
y_i^{m+1} = y_i^m + h_{yz,i}^m \left( \frac{f^y(t_i^m, x_i^m, y_i^m, z_i^m) + f^y(t_{i}^{m+1}, x_i^{m+1}, w_i^{m+1}, z_i^m)}{2} \right)
\]  

(3.44)

where \(y_i^m = y_i(t_i^m)\), \(y_i^{m+1} = y_i(t_i^{m+1})\) and \(h_{yz,i}^m\) is the \(m^{th}\) local time step within the \(i^{th}\) CaRU. Note that while \(t\) and \(x\) are global, the notation \(t_i^m\) and \(x_i^m\) is used locally since local time steps are not synchronized across CaRUs, i.e., \(h_{yz,i}^m \neq h_{yz,j}^m\), which implies \(t_i^m \neq t_j^m\) and \(x_i^m \neq x_j^m\). The counter variable \(m\) restarts at the beginning of each RK4M sub-step (see Fig. 3.15). The CaRU-random SV vector, \(z_i^m\), is not updated to \(z_i^{m+1}\) within
Eq. 3.44, rather $z_{i}^{m}$ is assumed to remain constant during the local time step, and is updated immediately after the CaRU-compartment SVs have been updated to $y_{i}^{m+1}$ (see below for more details). Within Eqs. 3.43 and 3.44, $x_{i}^{m}$ is calculated by linearly interpolating from values previously calculated at the beginning and ending time of the current RK4M sub-step (Fig. 3.15). For example, if $t_{n+1/3}^{m} < t_{i}^{m} < t_{n+1/2}^{m}$ then $x_{i}^{m}$ is interpolated using the current estimates of the interval endpoints $x_{n+1/3}^{m} \approx v_{n+1/3}$ and $x_{n+1/2}^{m} \approx v_{n+1/2}$.

A Monte Carlo simulation is run to update $z_{i}^{m}$ to $z_{i}^{m+1}$ over the local time step $h_{yz,i}^{m}$ in the $i^{th}$ CaRU. It is assumed that CaRU-compartment SVs remain constant over the duration of a single local time step. Let $j=1,2,3,...$ index the group of CaRU-random variables for the $i^{th}$ CaRU (i.e., each $j$ is an index to a particular channel or Markov process). Let $r_{i,j}^{k}$, $k=1,2,3,...$ denote the transition rate for the $j^{th}$ channel in the $i^{th}$ CaRU from its currently occupied state into state $k$. Also let $v_{i,j} = \sum_{k} r_{i,j}^{k}$. Then the probability that the $j^{th}$ channel made a state transition within the time step $h_{yz,i}^{m}$ is

$$p_{i,j} \approx v_{i,j} h_{yz,i}^{m} \quad (3.45)$$

and the probability that no transition occurred is therefore $1 - v_{i,j} h_{yz,i}^{m}$. Then, by conditioning on whether a change of state has occurred, the probability that the $j^{th}$ channel entered state $k$ within the time-step $h_{yz,i}^{m}$ is

$$p_{i,j}^{k} \approx \left( v_{i,j} h_{yz,i}^{m} \right) \left( r_{i,j}^{k} / v_{i,j} \right) = h_{yz,i}^{m} r_{i,j}^{k} \quad (3.46)$$
For each $j$, a standard uniform random variable is generated and is used to determine into which state the process transits, if a transition occurs, based on Eqs. 3.45 and 3.46. This process is completed for each $i$ (each CaRU), and the newly occupied states are recorded as $z_i^{m+1}$.

The local time step $h_{yz,i}^m$ is determined based on two criteria: (1) to ensure that the approximate relation of Eq. 3.45 is valid, and (2) to ensure that dynamics of Ca$^{2+}$ within the CaRUs (CaRU-compartments SVs) are adequately captured. The first criterion is satisfied by imposing the condition

$$ h_{yz,i}^m \leq \frac{tol}{\max(v_{i,j})} \tag{3.47} $$

With $tol = 0.05$, $h_{yz,i}^m$ is no greater than 5% of the expected waiting time until the next transition of the channel with the fastest exit rate. Since $p_{i,j} = 1 - \exp(-v_{i,j}h_{yz,i}^m) \approx v_{i,j}h_{yz,i}^m$, the maximum transition probability is $p_{i,j} = 1 - \exp(-tol) = 0.04877 \approx tol = 0.05$. A bound on the error in $p_{i,j}$ due to the approximation of Eq. 3.45 is therefore 0.00133. The second criterion is satisfied by imposing the constraint: if Ca$^{2+}$ concentration in any subspace compartment of the $i$th CaRU is $> 1.5 \mu$M then $h_{yz,i}^m \leq 5\mu$s, else $h_{yz,i}^m \leq 10\mu$s. In addition to these criteria, local time steps are occasionally shortened in order to synchronize with the encompassing global time step, $h_x^n$.

Using the algorithmic components described above, the local control myocyte simulation advances the solution from the states defined by $x(t)$, $y(t),...,y_{N_y}(t)$,
$z_i(t),...,z_{N_i}(t)$ to $x(t+h^n_i)$, $y_i(t+h^n_i),...,y_{N_i}(t+h^n_i)$, $z_i(t+h^n_i),...,z_{N_i}(t+h^n_i)$ respectively, in the following manner:

1. Choose $h^n_i$ (initial guess or based on $E$ of previous RK4M step, Eq. 3.40).
2. Calculate $v^{n+1/3}$ (Eq. 3.35).
3. Loop over each $i$:
   a) While $t^n_i < t^{n+1/3}$ do:
      i. Choose $h^{m\times}_{xy,i}$ (Eq. 3.47 and other bounds).
      ii. Calculate $x^n_i$ and $x^{m+1}_i$ (interpolate between $x^n$ and $v^{n+1/3}$).
      iii. Calculate $y^{m+1}_i$ (Eqs. 3.43-3.44).
      iv. Calculate $z^{m+1}_i$ (Eqs. 3.45-3.46 and uniform random variables).
      v. Set $m = m + 1$.
   End While;
   b) Set $y^{n+1/3} = y^{m+1}_i$; Set $z^{n+1/3} = z^{m+1}_i$.
   End Loop over $i$;
4. Calculate $\hat{v}^{n+1/3}$ (Eq. 3.36) and $v^{n+1/2}$ (Eq. 3.37).
5. Repeat procedure as described in step 3 on the interval $[t^{n+1/3}, t^{n+1/2}]$.
6. Calculate $v^{n+1}$ (Eq. 3.38).
7. Repeat procedure as described in step 3 on the interval $[t^{n+1/2}, t^{n+1}]$.
8. Calculate $\hat{v}^{n+1}$ (Eq. 3.39).
9. If $E$ (Eq. 3.40) is acceptable then
a) \( \mathbf{x}^{n+1} = \hat{\mathbf{v}}^{n+1} \) (update global SVs).

b) Set \( n = n + 1 \).

c) Set new global time step \( h_x^{n+1} \) (Eq. 3.41).

d) Goto step 1 (to continue).

Else

a) revise \( h_x^n \), (Eq. 3.41).

b) Goto step 1 (to repeat).

The loops performed over all CaRUs \((i’s)\) can be parallelized (steps 3, 5, and 7). Sensible reductions in the number of necessary calculation can be made during many of these steps. For example, in step 3.a.ii of the CaRU simulation, the only global SVs that need be interpolated are NSR and cytosolic Ca\(^{2+}\) concentrations and \( V_m \).

Eqs. 3.45 and 3.46 give probabilities that a transition has occurred sometime during the local time step, \( h_{yz,i}^m \). It is always assumed that the state transition occurs exactly at time \( t_i^m + h_{yz,i}^m \) (i.e., \( z_i^m \) is updated to \( z_i^{m+1} \) after Eq. 3.44 is evaluated). Discretizing the Markov process in this manner introduces an additional source of approximation error. The expected waiting time, denoted \( E[T_{i,j}] \), until the \( j^{th} \) channel in the \( i^{th} \) CaRU exits its current state is \( 1/\nu_{i,j} \). When the process is discretized, the waiting time follows a geometric distribution, such that

\[
E[T_{i,j}] = \sum_{k=1}^{\infty} kh_{yz,i}^m (1 - p_{i,j})^{k-1} p_{i,j} = \frac{h_{yz,i}^m}{p_{i,j}}
\]  
(3.48)
In general, Eq. 3.48 would not yield an expected waiting time of exactly \(1/v_{i,j}\).

However, if the approximate expression for \(p_{i,j}\) defined by Eq. 3.45 and used in this algorithm is substituted into Eq. 3.48, the desired result is obtained, i.e., \(E[T_{i,j}]=1/v_{i,j}\).

When subspace \(\text{Ca}^{2+}\) level is high, the local time step for a CaRU is limited mainly by the fast transition rates of the RyRs. Computational time is reduced by about an order of magnitude by making use of the rapid equilibrium approximation for pairs of states when the transition rates between the pair are relatively fast compared to the rates exiting the pair (Keizer and Smith, 1998). The six-state RyR models are converted to lower order (five- or four-state) models dynamically under conditions of high local \(\text{Ca}^{2+}\) when the approximation is valid. In addition, some transition rates are bounded with a maximum value (in a manner that maintains microscopic reversibility) in order to prevent extremely fast and unrealistic transition rates at high local \(\text{Ca}^{2+}\) levels (Rice et al., 1999). For details, see the RyR model description in Appendix I.

An additional reduction in computation time can be achieved by simulating only a subset of CaRUs. Every evaluation of \(f^x\) (Eq. 3.34) requires that \(I_{\text{CaL}}, J_{\text{tr}}, J_{\text{xfer}},\) and \(I_{\text{to2}}\) are calculated by summing over all of the respective unitary currents/fluxes in each CaRU (see Eqs. 3.2, 3.5, 3.16, and 3.18). Simulation of only a subset of the full population of CaRUs dramatically reduces simulation time. A reduction of \(N_s\) will increase the stochastic gating noise in macroscopic \(I_{\text{CaL}}\) and hence in \(V_m\) and most global SVs. However, the level of noise in these signals remains acceptably low for values of \(N_s \ll N_{\text{CaRU}}\) making this a very useful and time-saving approximation. During the application of 1 Hz current stimuli, one second of model simulation time requires ~ 40
minutes of run time when implemented in parallel on 10 SGI Power Challenge R10000 processors with only 10% of the total population of CaRUs included in the Monte Carlo simulation (i.e., $N_{\text{CaRU}} = 12,500$ and $N_s = 1250$). Most simulations presented in this study were performed with 20% of the CaRUs included in the Monte Carlo simulation. At this approximation level, repeated runs showed minimal variation (e.g., error bars in Figs. 3.3B and 3.7A). L-type $\text{Ca}^{2+}$ currents shown in Fig. 3.3A were simulated without using this approximation (i.e., 100% of the CaRUs were simulated), and therefore, the noise in these signals is representative of channel gating noise for the entire population of LCCs.

The local control myocyte model requires ~ 20 minutes of run time for a one second simulation. All simulations presented in this study were performed with a minimum of 20% of the CaRUs included in the Monte Carlo simulation. The minimal approach to modeling the CaRU therefore represents a necessary compromise between biophysical detail and tractability.

Uniform random variables are obtained using the Numerical Recipes function $\text{ran2}$ (Press et al., 1992). This generator has a period $> 2 \times 10^{18}$ with added safeguards designed to eliminate possible serial correlations in the sequence of uniform deviates. An independent sequence is generated for each simulated channel and each sequence is initialized by generating seeds with the Numerical Recipes function $\text{ran1}$ (Press et al., 1992). At the beginning of each global time step, all random sequence seeds are stored. If a global time step is rejected in step 9 of the simulation algorithm, the random sequence seeds are reset to the stored values before repeating that step (with a smaller step size) to insure that the trajectory of the Monte Carlo simulation is not artificially
influenced or altered by the decision to accept or reject global time steps based on truncation error (Eq. 3.40).

The validity of the simulation algorithm was tested by applying a combined voltage- and Ca$^{2+}$-clamp protocol. From a –100 mV holding potential, voltage is stepped to 0 mV for 50 ms, and then returned to the holding potential. Concurrently, Ca$^{2+}$ in all subspace compartments is clamped at 0.1 mM, stepped to 20 mM for 50 ms, and then returned to 0.1 mM. As shown in Fig. 3.16A, RyR and LCC open probability determined using the local control model algorithm (gray solid lines) are nearly identical to those obtained by solving the associated set of ordinary differential equations (black dotted lines) for this protocol, with slight deviations due only to the presence of gating noise in the local control simulation. An additional test is performed to insure Ca$^{2+}$ flux between individual CaRUs and the whole cell are correctly handled. Total cell Ca$^{2+}$ (in all compartments, including both free and bound Ca$^{2+}$) is calculated at each point in time by: 1) tracking incremental changes in total Ca$^{2+}$ based on net sarcolemmal Ca$^{2+}$ flux, and 2) summing over all cellular compartments. As shown in Fig. 3.16B, total Ca$^{2+}$ calculated by method 1 and 2 (gray solid line and black dashed line, respectively) differ by < 0.05% at the end of a 10 second simulation of APs paced at 1 Hz (with only the final second of simulation shown in Fig. 3.16B).
Figure 3.16: Test of the local control model simulation algorithm. (A) Response to a combined voltage- and Ca\textsuperscript{2+}-clamp protocol (see text for details). RyR and LCC open probability determined using the local control model algorithm (gray solid lines) are nearly identical to those obtained by solving the associated set of ordinary differential equations (black dotted lines) for this protocol. (B) Total cell Ca\textsuperscript{2+} calculated by tracking incremental changes in total Ca\textsuperscript{2+} based on net sarcolemmal Ca\textsuperscript{2+} flux (gray solid line) and by summing over all cellular compartments (black dashed line) agree to within < 0.05\% at the end of a 10 second simulation of APs paced at 1 Hz.
CHAPTER 4:

Role of β-Adrenergic Stimulation and Heart Failure Related Alterations in Protein Expression on Cardiac Myocyte Properties
Introduction

During the process of cardiac excitation-contraction (EC) coupling, the dynamics of intracellular calcium (Ca\(^{2+}\)) play an important role in shaping the action potential (AP) and regulating contraction. Upon membrane depolarization, Ca\(^{2+}\) entry via voltage-gated L-type Ca\(^{2+}\) channels (LCCs) triggers the opening of sarcoplasmic reticulum (SR) Ca\(^{2+}\) release channels known as ryanodine receptors (RyRs) via Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) (Fabiato, 1985b). It has been demonstrated that SR Ca\(^{2+}\) release occurs as highly localized discrete events in the form of Ca\(^{2+}\) sparks (Cheng et al., 1993). Individual LCCs in the transverse (T) tubular membrane are situated in close apposition, immediately across the dyadic cleft space, to one or more RyRs in the junctional SR membrane. The opening of a single LCC has been demonstrated to trigger Ca\(^{2+}\) sparks from nearby RyRs (Cannell et al., 1995; Lopez-Lopez et al., 1995; Sham et al., 1995; Santana et al., 1996; Wang et al., 2001). While the collective summation of such Ca\(^{2+}\) release events yields a rise of global intracellular free Ca\(^{2+}\), leading to the activation of contractile proteins, tight regulation of CICR is made possible by the fact that LCCs and RyRs are sensitive to local rather than global Ca\(^{2+}\) levels. This is the basis of the local control theory of EC coupling (Stern, 1992), where graded control of SR Ca\(^{2+}\) release is achieved by the recruitment of independent, elementary Ca\(^{2+}\) release events. In addition to triggering SR Ca\(^{2+}\) release, rising local Ca\(^{2+}\) levels also feedback onto LCCs promoting Ca\(^{2+}\)-dependent inactivation (Peterson et al., 1999). Since L-type Ca\(^{2+}\) current (I\(_{\text{CaL}}\)) plays a primary role in determining AP shape and duration, local control theory implies
that the microscopic properties of Ca\(^{2+}\) cycling are likely to play a critical role in establishing the integrative electrophysiological properties of the cardiac myocyte.

The sympathetic neurotransmitter norepinephrine and the adrenal hormone epinephrine stimulate the heart via cell surface β-adrenergic receptors (β-ARs). Stimulation of β-ARs is an important mechanism by which heart function is modulated during stress or exercise. The β-AR is a member of the GTP-binding protein (G-protein)-coupled receptor family of membrane proteins. β-adrenergic signal transduction begins upon binding of the β-AR to agonist. The interaction of the β-AR with the stimulatory G-protein (Gs) activates adenylyl cyclase (AC), catalyzing the production of the signaling molecule cyclic AMP (cAMP). The cAMP subsequently activates cAMP-dependent protein kinase A (PKA), which leads to the phosphorylation of a number of proteins that are directly involved in the events of EC coupling (Xiao et al., 1999).

L-type Ca\(^{2+}\) channels, the SR membrane protein phospholamban (PLB), RyRs, and myofilament proteins are all targets of PKA-mediated phosphorylation. Ca\(^{2+}\) current is increased by PKA, which therefore increases the trigger signal for CICR, and the overall cellular Ca\(^{2+}\) content (Hussain and Orchard, 1997). Under normal conditions, PLB acts as an inhibitory regulator of the function of SR Ca\(^{2+}\) ATPase (SERCA2a pump). The phosphorylation of PLB relieves SERCA2a inhibition, thereby enhancing the SR Ca\(^{2+}\) uptake rate which increases SR Ca\(^{2+}\) content and accelerates the rate of decline of the cytosolic Ca\(^{2+}\) transient (Simmerman and Jones, 1998). The role PKA-mediated phosphorylation in modulation of RyR function is less clear. In single channel recording made from heavy SR-enriched microsomes, steady-state RyR open probability
has been shown to be decreased in the presence of PKA while the transient open probability in response to an abrupt increase in Ca$^{2+}$ concentration has been shown to be increased with PKA (Valdivia et al., 1995). In bilayer measurements, however, the steady-state RyR open probability was enhanced by PKA (Marx et al., 2000). In contrast, measurements obtained from intact cellular systems revealed no significant effect of PKA-dependent RyR phosphorylation on resting SR Ca$^{2+}$ leak under conditions where SR load was unchanged by knockout of PLB (Li et al., 2002). Recent measurements of altered EC coupling gain in response to β-AR stimulation have demonstrated both an increase (Viatchenko-Karpinski and Gyorke, 2001) and a decrease (Song et al., 2001) in gain. The mechanisms responsible for β-AR-mediated alterations in EC coupling and whole-cell electrophysiological properties, therefore, remain unclear.

While detailed studies of LCC availability and gating kinetics have demonstrated an increase in L-type Ca$^{2+}$ current in response to β-adrenergic stimulation (Yue et al., 1990; Chen-Izu et al., 2000), the ventricular AP becomes shorter under these conditions (Tomaselli and Marban, 1999). This likely occurs because an increase in β-adrenergic signaling results in a functional increase in repolarizing K$^+$ currents as well. It has recently been demonstrated that I$_{Kr}$ is enhanced by β-adrenoreceptor stimulation via a reduction in channel inactivation, and hence a reduction in rectification of the current (Heath and Terrar, 2000). In addition, isoproterenol (a β-AR agonist) has been shown to increase the current magnitude of I$_{Ks}$ 2- to 3-fold (Kathofer et al., 2000).

Understanding the role of β-AR signaling in the heart is further complicated by the fact that there are genetically and pharmacologically distinct receptor subtypes, which
display physiologically differing effects (reviewed in Xiao et al., 1999). The activation
of β1-ARs enhance \( I_{\text{CaL}} \), SERCA2a function, \( K^+ \) currents, and speed the dissociation of
\( \text{Ca}^{2+} \) from myofilaments, while the effect of β2-AR activation is targeted to regulation of
sarcolemmal proteins, but not cytosolic proteins (e.g., PLB). The co-assembly of the β2-
AR signaling cascade components, including PKA, with the LCC (Davare et al., 2001)
and the RyR (Marx et al., 2000) by way of regulatory subunits and anchoring proteins
form a structural basis for the local signaling associated exclusively with β2-ARs.

In failing canine myocytes, the repolarizing \( K^+ \) currents, \( I_{K1} \) and \( I_{\text{to1}} \), are reduced
by ~ 40% and ~ 70% respectively (Kääb et al., 1996). In addition, heart failure is
associated with reduced SERCA2a function and enhanced function of the \( \text{Na}^+\text{-Ca}^{2+} \)
(NCX) exchanger (reviewed in Tomaselli and Marban, 1999). Moreover, the expression
of β1-ARs is decreased while there is only little or no loss of β2-ARs in both human and
animal models of heart failure (reviewed in Xiao et al., 1999). Additional studies have
demonstrated that constitutive activation of PKA predisposes mice to arrhythmias and
SCD (Antos et al., 2001), RyRs from failing human myocytes have been found to be
hyperphosphorylated (Marx et al., 2000), and a defect in dephosphorylation of LCCs
consistent with decreased phosphatase activity has been identified in human heart failure
(Schroder et al., 1998). In order to understand the basis for changes in AP morphology
and \( \text{Ca}^{2+} \)-cycling that are associated with heart failure, it is important to elucidate the role
of heart failure related defects in protein phosphorylation associated with the β-
adrenergic signaling pathway.
In this study, we develop models of the \( \beta \)-AR stimulated, and failing ventricular myocytes. These models are formulated by modifying a recently developed control myocyte model that incorporates local control of EC coupling (see Chapter 3) (Greenstein and Winslow, 2002). The \( \beta \)-AR-stimulated model is formulated by incorporating PKA-mediated effects on LCCs, PLB, RyRs, \( I_{K,r} \), and \( I_{K,s} \) as described above. The model of the failing myocyte is developed by adjusting \( K^+ \) channel and \( \text{Ca}^{2+} \) cycling protein levels based on the functional up or down regulation measured experimentally (Kääb et al., 1996; O'Rourke et al., 1999b). We use these models to test the hypothesis that discrete effects of \( \beta \)-adrenergic inputs on the above mechanisms can explain alterations in EC coupling and macroscopic cellular responses to \( \beta \)-adrenergic inputs. In addition, the impact of heart failure related alterations of \( \beta \)-adrenergic activity on myocyte properties, such as increased RyR \( \text{Ca}^{2+} \)-sensitivity due to hyperphosphorylation (Marx et al., 2000), and reduced activity of type 1 protein phosphatase (Schroder and Herzig, 1999), is investigated. The model results indicate that heart failure associated impairment of the ability to load the SR with \( \text{Ca}^{2+} \) leads to dramatic prolongation of AP and severely blunted cytosolic \( \text{Ca}^{2+} \) transients, whereas altered LCC availability and open probability (Schroder et al., 1998), and a decreased number of LCCs (He et al., 2001) similar to that measured in failing myocytes have relatively modest effects on AP configuration and cytosolic \( \text{Ca}^{2+} \) transients.
Methods

Local Control Myocyte Model

We have previously developed a computational model of the canine ventricular midmyocardial cell which incorporates the following: (1) sarcolemmal ion currents of the Winslow et al. (1999) canine ventricular cell model based originally on those of the Luo-Rudy Phase II model (Luo and Rudy, 1994); (2) genetically based structural ion channel descriptions for the rapidly activating delayed rectifier current, \( I_{Kr} \) (Mazhari et al., 2001), and for the voltage-dependent Ca\(^{2+}\)-independent transient outward K\(^{+}\) current, \( I_{to1} \) (Greenstein et al., 2000); (3) a state-model of the L-type Ca\(^{2+}\) current in which Ca\(^{2+}\)-mediated inactivation occurs via the mechanism of mode-switching (Imredy and Yue, 1994); (4) a model of the Ca\(^{2+}\)-dependent transient outward Cl\(^{-}\) current, \( I_{to2} \) (Greenstein and Winslow, 2002; Collier et al., 1996); (5) an RyR channel model adapted from that of Keizer and Smith (1998); and (6) locally controlled CICR from SR via inclusion of LCCs, RyRs, Cl\(^{-}\) channels, local junctional SR compartments, and dyadic subspace compartments (into which local RyRs and LCCs empty) within Ca\(^{2+}\) release units (CaRUs) that are simulated independently of each other using Monte-Carlo methods (Greenstein and Winslow, 2002). The implementation of CaRUs within this model is based on the observation of Ca\(^{2+}\) sparks, and allows the model to capture the fundamental properties inherent in local control of CICR such as high EC coupling gain, graded Ca\(^{2+}\) release, and stable termination of release. The model used in this study is essentially that presented previously, with some modifications necessary in order to incorporate
modulation by β-adrenergic inputs and/or cellular electrophysiological remodeling associated with heart failure as described below.

**Incorporation of Sympathetic Inputs**

Whole cell L-type Ca\(^{2+}\) current can be described as a function of the total number of channels (\(N_{LCC}\)), the single channel current magnitude \(i\), the open probability \(p_o\), and the fraction of channels that are available for activation \(f_{active}\), i.e., in a phosphorylated mode), where \(I_{CaL} = N_{LCC} \times i \times p_o \times f_{active}\) (Handrock et al., 1998). Under conditions where \(f_{active}\) remains constant, \(I_{CaL} = N_{active} \times i \times p_o\), where \(N_{active} = N_{LCC} \times f_{active}\) is constant. Single L-type Ca\(^{2+}\) channel parameters are based on experimentally obtained constraints on both \(i\) and \(p_o\). \(N_{active}\) is therefore chosen such that the amplitude of the ensemble current summed over all LCCs corresponds to whole cell measurements in canine myocytes (Hobai and O'Rourke, 2001). This approach yields a value of 50,000 for \(N_{active}\), which agrees with experimental estimates of LCC density (Rose et al., 1992; McDonald et al., 1986), and which corresponds to 12,500 active CaRUs. Our previous description of the local control myocyte model (Greenstein and Winslow, 2002) excluded the process of slow cycling between active (phosphorylated) and inactive (unphosphorylated) channel states, and simulated only active LCCs. In order to study the role of β-adrenergic stimulation, the local control myocyte model is extended to include both active and inactive CaRUs. Based on experiments that indicate ~ 25% of LCCs are available (active) under control conditions (Herzig et al., 1993), \(N_{LCC}\) was set to 200,000, and \(f_{active}\) was set to 0.25 (corresponding to a total of CaRUs of 50,000, with 12,500
active CaRUs under control conditions). It was necessary to assume that RyRs residing within inactive CaRUs were less sensitive to Ca\(^{2+}\)-mediated activation (initial opening rate, \(k_{12}\), was reduced by a factor of 10, see Chapter 3 for RyR model description) in order to assure that the probability of RyR activation in the absence of local trigger Ca\(^{2+}\) remains small. Such an approach is consistent with a model of \(\beta\)-AR mediated CaRU recruitment in which raised local levels of diffusible cAMP lead to phosphorylation (and the absence of cAMP leads to dephosphorylation) of both LCCs and RyRs which are co-located in the junctional complex, and is supported by evidence that PKA-mediated phosphorylation enhances RyR open probability (Marx et al., 2000). After incorporating the changes necessary to include both active and inactive CaRUs, some membrane currents and ionic fluxes were slightly adjusted to preserve cytosolic ion concentrations and AP shape. These parameter adjustments are given in Table 4.1.

In addition to modulating functional LCC availability, an increasing degree of PKA-mediated phosphorylation of LCCs has also been shown to shift the distribution of channels into high-activity gating modes (Yue et al., 1990; Chen-Izu et al., 2000; Herzig et al., 1993). Mode 0a gating is characterized by infrequent brief openings (least active, low \(p_o\)), whereas mode 1 is characterized by bursts of brief openings, and mode 2 by long lasting openings (most active, high \(p_o\)). In this study it has been assumed that mode 0a openings do not significantly contribute to whole-cell \(I_{\text{CaL}}\) (e.g. see Herzig et al., 1993), and mode 0a is therefore lumped into the inactive population of LCCs. Mode 1 gating of the LCC corresponds to the control parameter set defined for this model (see Chapter 3, Appendix I). Mode 2 gating is defined as a modification of mode 1 parameters based on
the data of Yue et al., 1990, where mean open time is increased from 0.5 ms to 5 ms. This is implemented by reducing the exit rate from the Mode Normal open state \((g)\) by a factor of 10 (Jafri et al., 1998).

Experiments have shown that in the presence of \(\beta\)-adrenergic agonists, peak L-type \(Ca^{2+}\) currents are increased 2- to 3-fold both in canine (Kääb et al., 1996) and in rat (Song et al., 2001) compared to controls. Underlying these observations are both an increase in availability, and a shift to high-activity gating mode behavior (Herzig et al., 1993; Yue et al., 1990; Chen-Izu et al., 2000). On the basis of these data, PKA-mediated phosphorylation of LCCs is modeled by increasing the fraction of active LCCs \((f_{\text{active}})\) and by redistributing the active LCCs into populations of mode 1 and mode 2 channels. Under control conditions, all active LCCs are assumed to operate in mode 1, while in response to \(\beta\)-adrenergic inputs, 15% of the active population of LCCs are assumed to operate in mode 2, while 85% remain in model 1 (Yue et al., 1990). In the model, the fraction of active CaRUs is equal to the fraction of active LCCs (i.e., active CaRUs contain active LCCs). Therefore, this implementation implicitly assumes that the CaRUs that are recruited by \(\beta\)-AR stimulation contain RyRs with increased \(Ca^{2+}\) sensitivity (relative to those within inactive CaRUs, see above). In addition, \(\beta\)-adrenergic stimulation has been shown to enhance SERCA2a function (Simmerman and Jones, 1998), reduce inactivation/rectification of \(I_{Kr}\) (Heath and Terrar, 2000), and increase current amplitude of \(I_{Ks}\) (Kathofer et al., 2000). Functional increase in SERCA2a availability is modeled by simultaneous scaling of both its forward and reverse maximum pump rates \(V_{\max f}\) and \(V_{\max r}\) by a scale factor, \(K_{SR}\) (Shannon et al., 2000). An increase in
SERCA2a function is assumed to occur in response to $\beta_1$-AR, but not $\beta_2$-AR stimulation (Kuschel et al., 1999). While $\beta_2$-AR stimulated cardiac relaxation has been observed in canine myocytes, it is not associated with PKA-dependent phosphorylation of PLB or myofilament proteins (Kuschel et al., 1999). Since the mechanism of $\beta_2$-AR stimulated relaxation remains unknown, this phenomenon is not implemented and does not occur in this model. Reduction in the degree of steady-state inactivation of $I_{Kr}$ is modeled by downscaling rates entering the inactivation state ($\alpha_i$ and $\alpha_{i3}$) and upscaling, by an equal factor, the rates exiting the inactivation state ($\beta_i$ and $\Psi$) (Mazhari et al., 2001). Functional upregulation of $I_{Ks}$ is modeled by scaling maximal conductance. Parameter changes associated with the $\beta$-AR stimulation cell model are given in Table 4.2.

**Models of Heart Failure**

Experiments have demonstrated a reduction of $I_{K1}$ and $I_{to1}$ current density, but no change in channel gating kinetics, in the canine tachycardia pacing-induced model of heart failure (Kääb et al., 1996). In addition, human and canine heart failure is associated with reduced SERCA2a function and enhanced function of NCX (Limas et al., 1987; Hobai and O'Rourke, 2000; O'Rourke et al., 1999b). As described by Winslow et al. (1999), effects of terminal heart failure are modeled by altering the maximal conductance of $I_{K1}$ and $I_{to1}$ ($I_{Kv4.3}$), and by scaling the maximal pump and exchanger rates for SERCA2a and NCX respectively, by the degree measured in these experiments. For the case of $I_{to1}$, the maximal conductance of the Kv4.3 component of this current is reduced in the failing model by 84% with no change in the Kv1.4 component, which yields a net
Table 4.1: Adjusted Control Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{\text{CaRU}}$</td>
<td>Number of Ca$^{2+}$ release units</td>
<td>50,000</td>
</tr>
<tr>
<td>FracAct</td>
<td>Fraction of CaRUs that are active</td>
<td>0.25</td>
</tr>
<tr>
<td>$V_{\text{NSR}}$</td>
<td>NSR volume</td>
<td>0.913 pL</td>
</tr>
<tr>
<td>$V_{\text{JSR}}$</td>
<td>JSR volume (single compartment)</td>
<td>$8.236 \times 10^6$ pL</td>
</tr>
<tr>
<td>$r_{\text{RyR}}$</td>
<td>Rate of Ca$^{2+}$ flux through open RyR</td>
<td>6.586 ms$^{-1}$</td>
</tr>
<tr>
<td>$V_{\text{maxf}}$</td>
<td>SR Ca$^{2+}$ pump forward maximum rate</td>
<td>251.5 µM s$^{-1}$</td>
</tr>
<tr>
<td>$V_{\text{maxr}}$</td>
<td>SR Ca$^{2+}$ pump reverse maximum rate</td>
<td>251.5 µM s$^{-1}$</td>
</tr>
<tr>
<td>$G_{\text{Kv4.3}}$</td>
<td>Kv4.3 channel maximal conductance</td>
<td>0.1458 mS µF$^{-1}$</td>
</tr>
<tr>
<td>$P_{\text{Kv1.4}}$</td>
<td>Kv1.4 channel permeability</td>
<td>$2.088 \times 10^{-7}$ cm s$^{-1}$</td>
</tr>
<tr>
<td>$G_{\text{Kr}}$</td>
<td>$I_{\text{Kr}}$ maximal conductance</td>
<td>0.0203 mS µF$^{-1}$</td>
</tr>
<tr>
<td>$G_{\text{K1}}$</td>
<td>$I_{\text{K1}}$ maximal conductance</td>
<td>2.8 mS µF$^{-1}$</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>RyR rate constant: state 1 to state 2</td>
<td>877.5 mM$^{-2}$ ms$^{-1}$ (active CaRU)</td>
</tr>
</tbody>
</table>

Parameters given here are adjusted from those in Tables 3.1 – 3.7 in order to preserve cytosolic ion concentrations and AP shape. Only parameters that are different than those in Tables 3.1 – 3.7 are given here.
Table 4.2: β-AR Stimulation Parameters

<table>
<thead>
<tr>
<th>Mechanism/Parameter</th>
<th>β-AR Parameter Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available LCC Fraction = 0.25</td>
<td>Available LCC Fraction = 0.60</td>
</tr>
<tr>
<td>All active LCCs in Mode 1</td>
<td>85% LCCs in Mode 1</td>
</tr>
<tr>
<td></td>
<td>15% LCCs in Mode 2</td>
</tr>
<tr>
<td></td>
<td>( g(\text{Mode 2}) = 0.1 \times g(\text{Mode 1}) )</td>
</tr>
<tr>
<td>SERCA2a pump scale factor KSR = 1</td>
<td>KSR = 3.3</td>
</tr>
<tr>
<td>( I_{Kr} ) (parameters ( \alpha_i, \alpha_{i3}, \beta_i ) and ( \Psi ))</td>
<td>( \alpha_i = 0.25 \times \alpha_i(\text{control}) )</td>
</tr>
<tr>
<td></td>
<td>( \alpha_{i3} = 0.25 \times \alpha_{i3}(\text{control}) )</td>
</tr>
<tr>
<td></td>
<td>( \beta_i = 4 \times \beta_i(\text{control}) )</td>
</tr>
<tr>
<td></td>
<td>( \Psi = 4 \times \Psi(\text{control}) )</td>
</tr>
<tr>
<td>( I_{Ks} ) (maximal conductance ( G_{Ks} ))</td>
<td>( G_{Ks} = 2 \times G_{Ks}(\text{control}) )</td>
</tr>
</tbody>
</table>
Table 4.3: Heart Failure Model Parameters

<table>
<thead>
<tr>
<th>Mechanism/Parameter</th>
<th>Heart Failure Parameter Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Kv4.3}$ (maximal conductance $G_{Kv4.3}$)</td>
<td>$G_{Kv4.3} = 0.16 \times G_{Kv4.3}(\text{control})$</td>
</tr>
<tr>
<td>$I_{K1}$ (maximal conductance $G_{K1}$)</td>
<td>$G_{K1} = 0.65 \times G_{K1}(\text{control})$</td>
</tr>
<tr>
<td>$V_{maxf}$ (SERCA2a forward max pump rate)</td>
<td>$V_{maxf} = 0.38 \times V_{maxf}(\text{control})$</td>
</tr>
<tr>
<td>$k_{NaCa}$ (NCX maximum rate)</td>
<td>$k_{NaCa} = 2 \times k_{NaCa}(\text{control})$</td>
</tr>
</tbody>
</table>
65% reduction in $I_{\text{sol}}$ (Kääb et al., 1996; Kääb et al., 1998). This approach forms a minimal model of heart failure. Parameter changes associated with the minimal model of heart failure are given in Table 4.3. In addition to these changes, the model of LCC voltage-dependent inactivation has been modified to insure that the steady-state open probability approaches zero at positive potentials (i.e., the sustained component of current is replaced with a slowly inactivating component of current). This modification is consistent with experimental observations (unpublished data, David T. Yue) and is required to avoid nonrepolarizing model APs when Ca$^{2+}$-mediated inactivation is weak or absent. The revised voltage-dependence of LCC inactivation is described in the Appendix.

Additional experiments have suggested that defective regulation of RyR in failing myocytes is associated with PKA-mediated hyperphosphorylation of the channel leading to increased sensitivity to Ca$^{2+}$ dependent activation as a result of the dissociation of FKBP12.6, a protein which is normally bound to the RyR (Marx et al., 2000). Pathological consequences of RyR over stimulation may include systolic hypersensitivity to trigger Ca$^{2+}$ and/or diastolic leak of Ca$^{2+}$ contributing to SR depletion (Marks, 2001). Increased RyR Ca$^{2+}$ sensitivity is modeled by scaling the initial rate of opening ($k_{12}$) in the RyR model (Keizer and Smith, 1998). Experiments have also indicated a decrease in the level of protein phosphatase 1 (PP1) (Marx et al., 2000) and a functional defect in dephosphorylation of LCCs from failing human ventricular myocytes (Schroder et al., 1998). While a number of studies have indicated that there is little or no change in macroscopic $I_{\text{CaL}}$ in heart failure (reviewed in Tomaselli and Marban, 1999), the study of
Schroder et al. (1998) suggests that LCC availability is increased, $p_o$ is increased (due predominantly to shorter closed times), and that the total number of LCCs is decreased, leading to no apparent change in macroscopic current properties. Similarly He et al. (2001) have found a decrease in intramembrane gating charge movement in canine heart failure, but no change in macroscopic $I_{Ca,L}$. The altered single channel current properties are modeled by adjusting LCC availability, the Mode Normal transition rate into the open state ($f$), and the total number of LCCs ($N_{LCC}$) based on these findings. The local control myocyte model is extended to include these phosphorylation defects in order to determine the role of heart failure related alterations of $\beta$-adrenergic activity on EC coupling and AP properties.

**Local Control Model Simulation Algorithm**

The simulation algorithm for the local control myocyte model has been described previously (Greenstein and Winslow, 2002) (Chapter 3, Appendix II). Briefly, each CaRU contains 20 RyRs, 4 LCCs, and 4 Cl$^-$ channels ($I_{Cl^-}$). Transition rates for each channel are determined by their gating schemes and their dependence on local Ca$^{2+}$ level. Gating of each channel within a CaRU is simulated using Monte Carlo techniques, and simulation of each CaRU is independent of any other CaRU. The CaRUs are embedded within the global description of the myocyte (defined by a set of ordinary differential equations) that includes all currents, pumps, exchangers, and intracellular ionic fluxes and compartment concentrations that do not reside within a CaRU. The summation of all Ca$^{2+}$ fluxes crossing the CaRU boundaries are taken as inputs to the global model. The
majority of computational time is spent in stochastic simulation of the large number of independent CaRUs, and this allows for efficient parallelization of the simulation. The dynamical equations defining the global model are solved using the Merson modified Runge-Kutta $4^{th}$-order adaptive time step algorithm (Kubicek and Marek, 1983), further modified to embed the CaRU simulations within each time step. Action potentials are initiated with a $-100 \text{ pA} \text{ pF}^{-1}$ current injection stimulus with a duration of 0.5 ms.

Results

Model of $\beta$-Adrenergic Stimulation

Fig. 4.1 demonstrates the ability of the model to reconstruct action potentials and Ca$^{2+}$ transients of canine midmyocardial ventricular myocytes for both control and $\beta$-AR stimulated conditions. Panels A and B of Fig. 4.1 show simultaneous experimental measurements of APs and Ca$^{2+}$ transients, respectively, under control conditions (black lines) and following application of isoproterenol (ISO), a $\beta$-AR agonist, to the bath solution (gray lines) (Brian O’Rourke, unpublished data). Measured Ca$^{2+}$ transient signals are shown in Fig. 4.1B as the Indo-1 fluorescence ratio. In response to ISO, action potential duration (APD) is shortened by $\sim 30\%$, the plateau becomes $\sim 10-15 \text{ mV}$ more depolarized, and phase 1 notch depth and duration are reduced. In addition, ISO produces a substantial increase in Ca$^{2+}$ transient amplitude ($\sim 2-3$ fold) and speeds the relaxation rate of the Ca$^{2+}$ transient ($\sim 3$ fold). This rapid rate of decay produces a “crossover” effect when the normal and ISO Ca$^{2+}$ transients are overlaid in Fig. 4.1B.
Figure 4.1: Model and experimental action potentials, $\text{Ca}^{2+}$ transients, and membrane currents. Each AP and $\text{Ca}^{2+}$ transient is in response to 1 Hz stimuli measured at steady state. In all panels, control conditions (black) are compared with $\beta$-AR stimulated conditions (gray). (A) Experimentally measured membrane potential in isolated canine myocytes as a function of time in normal physiological bath solution (black) and after application of isoproterenol (gray). (B) Experimentally measured Indo-1 fluorescence ratio (a $\text{Ca}^{2+}$ indicator) measured simultaneously with the APs in panel A. (C) Membrane potential as a function of time simulated using the control myocyte model (black) and the $\beta$-AR stimulated model (gray). See text for details regarding model parameter changes associated with $\beta$-AR stimulation. (D) Simulated cytosolic $\text{Ca}^{2+}$ concentration ($\mu\text{M}$) as a function of time corresponding to the APs in panel C. (E) L-type $\text{Ca}^{2+}$ current (pA pF$^{-1}$) as a function of time corresponding to the APs shown in panel C. (F) Rapid component of the delayed rectifier current, $I_{\text{Kr}}$ (pA pF$^{-1}$), as a function of time corresponding to the APs shown in panel C.
In the β-AR stimulated model simulations, LCC availability and occupation of high-activity gating modes are increased, SERCA2a function is increased, $I_{Kr}$ inactivation is decreased, and $I_{Ks}$ current density is increased (see Methods and Table 4.2). The enhanced function of these currents (and the SERCA2a pump) produce the changes in AP and Ca$^{2+}$ transient properties that are shown in panels C and D of Fig. 4.1, respectively. The model AP exhibits shortening in duration of ~ 30%, similar to that observed in experiments. In addition, depolarization of the model AP plateau, as well as a reduction in phase 1 notch depth and duration, are similar to that observed in Fig. 4.1A. The amplitude of the model Ca$^{2+}$ transient is increased ~ 3-fold and the rate of decay of the transient is similarly increased (shortening its overall duration), in agreement with the data of Fig. 4.1B.

Fig. 4.1E shows $I_{CaL}$, under control (black) and β-AR stimulated (gray) conditions, corresponding to the APs of panel C. The magnitude of $I_{CaL}$ is increased in response to β-AR stimulation for the entire duration of the AP, and is responsible for the β-AR associated depolarization of the phase 1 notch and early plateau. The reduction in notch depth occurs as a result of the increase in amplitude of $I_{CaL}$ relative to that of $I_{bol}$, which is unchanged from control. Peak $I_{CaL}$ is increased ~ 2-fold in response to β-AR stimulation. This increase in current magnitude is less than that expected based on the increase in model LCC availability (25% to 60%, an ~ 2.5-fold increase) due to a reduction in driving force for $I_{CaL}$ associated with the depolarization of the phase 1 notch and early plateau. The late phase of $I_{CaL}$ is increased by only ~ 40% during β-AR stimulation. The increase in late L-type current occurs to a lesser extent than that of peak
current because the enhanced operation of SERCA2a under β-AR stimulated conditions results in an increase in steady-state SR Ca\(^{2+}\) load, producing stronger Ca\(^{2+}\)-mediated inactivation of \(I_{\text{CaL}}\) compared to control (data not shown). Fig. 4.1F shows \(I_{\text{Kr}}\), under control (black) and β-AR stimulated (gray) conditions, corresponding to the APs of panel C. The reduction of \(I_{\text{Kr}}\) inactivation (with no change in current density) has a dramatic impact on its waveform, allowing the current to operate in a nearly fully activated state during the entire duration of the AP. This results in an ~ 6 to 7-fold increase in current amplitude, and plays the primary role in shortening APD. The increase in \(I_{\text{Ks}}\) density has only a slight impact on APD because of its small relative magnitude (data not shown).

The β-adrenergic stimulation produced in Fig. 4.1 represents the global stimulatory effects of mixed β-AR (i.e. both β\(_1\)-AR and β\(_2\)-AR) stimulation by a nonselective β-agonist such as ISO. Both nonselective and β\(_1\)-AR stimulation activate the G\(_s\)-AC-cAMP signaling cascade (Xiao, 2001). The highly similar effects of nonselective and β\(_1\)-AR agonists are due in part to the fact that ~ 85% of all β-ARs in the healthy canine heart are of the β\(_1\)-AR subtype (Murphree and Saffitz, 1988). Dual coupling of β\(_2\)-ARs to both G\(_s\), and the inhibitory G-protein, G\(_i\), results in functional compartmentalization of β\(_2\)-AR signaling to the sarcolemma, uncoupling this signaling pathway from phosphorylation of cytoplasmic proteins, such as PLB, in canine heart (Kuschel et al., 1999). Fig. 4.2 demonstrates model simulations of β\(_1\)-AR vs. β\(_2\)-AR stimulation. It has been assumed that β\(_1\)-AR stimulation effects \(I_{\text{CaL}}\), PLB/SERCA2a, \(I_{\text{Kr}}\), and \(I_{\text{Ks}}\) in the same manner as described for the baseline β-stimulated model in Fig. 4.1 and that β\(_2\)-AR has the same effects on sarcolemmal proteins, but no functional effect on SERCA2a. In all panels of
Fig. 4.2, the black solid lines show $\beta_1$-AR stimulation, the gray solid lines show $\beta_2$-AR stimulation, and the dotted lines show the control case. While model action potentials are shortened in response to $\beta_1$-AR stimulation (as described previously in Fig. 4.1A), APD remains essentially unchanged in response to exclusive $\beta_2$-AR stimulation (Fig. 4.2A). These effects are consistent with experimental data obtained from canine ventricular myocytes that exhibit no change in APD in the presence of the $\beta_2$-agonist zinterol, or in the presence of ISO and the $\beta_1$-blocker CGP20712A (Sosunov et al., 2000). While APD remains unchanged, the amplitude of AP plateau is increased by $\sim 20$ mV. At higher concentrations (1 $\mu$M), zinterol has been shown to raise the amplitude of the AP plateau potential by $\sim 5$-8 mV (Sosunov et al., 2000). The higher increase in the plateau potential of the model may be attributed to the assumption that $\beta_2$-AR stimulation increases LCC availability to the same degree as $\beta_1$-AR stimulation. While $\beta_1$-AR membrane density is 5- to 10-fold greater than that of $\beta_2$-AR in canine (Murphree and Saffitz, 1988), it is unclear how this difference in receptor density translates to quantitative differences in downstream protein phosphorylation. Thus, the model simulations presented here may overestimate the quantitative effects of $\beta_2$-AR stimulation. Panels B through E of Fig. 4.2 demonstrate the cytosolic Ca$^{2+}$ transients, $I_{Ca,L}$, SR Ca$^{2+}$ release flux, and network SR Ca$^{2+}$ concentration, respectively, corresponding to the APs of panel A. In response to $\beta_2$-AR stimulation, the cytosolic Ca$^{2+}$ transient (Fig. 4.2B) displays an initial rapid rising phase which reaches an amplitude similar to control, then displays a slower rising phase for the duration of the AP, and finally declines at a rate similar to control ($\tau_{Ca} \sim 180$ ms). In contrast, the Ca$^{2+}$ transient in response to $\beta_1$-stimulation peaks after $\sim 100$ ms, with an
Figure 4.2: Effects of $\beta_1$-AR vs. $\beta_2$-AR stimulation on the AP and Ca$^{2+}$ cycling. The data in each panel are simulated at 1 Hz and measured at steady state. In all panels, $\beta_1$-AR stimulation is shown in black, $\beta_2$-AR stimulation is shown in gray, and control is shown as dotted lines. (A) Membrane potential as a function of time showing APs under $\beta_1$-stimulated, $\beta_2$-stimulated, and control conditions. (B) Simulated cytosolic Ca$^{2+}$ concentration (µM) as a function of time corresponding to the APs in panel A. (C) L-type Ca$^{2+}$ current (pA pF$^{-1}$) as a function of time corresponding to the APs shown in panel A. (D) SR Ca$^{2+}$ release flux (µM s$^{-1}$) via RyRs as a function of time corresponding to the APs shown in panel A. (E) Network SR Ca$^{2+}$ concentration (mM) as a function of time corresponding to the APs shown in panel A.
Figure 4.2
amplitude nearly twice that for $\beta_2$-stimulation, and then declines rapidly ($\tau_{Ca} \sim 110$ ms). The difference in the relaxation rate of the $Ca^{2+}$ transient for $\beta_1$-AR vs. $\beta_2$-AR stimulation is a direct result of the functional enhancement of SERCA2a that is present during $\beta_1$-AR, but not $\beta_2$-AR stimulation. Peak $I_{CaL}$ (Fig. 4.2C) is increased similarly (~2-fold) in response to stimulation of either $\beta$-receptor subtype. The late phase of $I_{CaL}$ displays a greater increase in response to $\beta_2$-AR vs. $\beta_1$-AR stimulation. The difference in magnitude of late $I_{CaL}$ occurs due to differences in SR $Ca^{2+}$ release and its effect on $Ca^{2+}$-mediated inactivation of the LCCs. $Ca^{2+}$-mediated inactivation of $I_{CaL}$ is stronger during $\beta_1$-receptor activation compared with $\beta_2$-receptor activation as a direct result of increased SR $Ca^{2+}$ release (Fig. 4.2D), which results from enhanced SERCA2a function and increased SR loading (Fig. 4.2E). The difference in APD (Fig. 4.2A) in response to $\beta_1$-AR vs. $\beta_2$-AR stimulation is a direct consequence of differing degrees of $Ca^{2+}$-mediated inactivation of $I_{CaL}$, and are not due to the effects of delayed rectifier currents which are identical under these conditions. The results of Fig. 4.2E demonstrate that steady-state SR $Ca^{2+}$ load increases by ~30% in response to $\beta_1$-AR stimulation. This is a direct consequence of the $\beta_1$-associated enhancement of SERCA2a function and leads to increased fractional SR $Ca^{2+}$ release during the AP. The response to $\beta_2$-AR stimulation, however, demonstrates ~20% decrease in SR $Ca^{2+}$ load. This occurs because $\beta$-adrenergic stimulation recruits both LCCs and their nearby RyRs (i.e., CaRUs are recruited, see Methods). A greater number of RyRs become active due to the greater number of LCCs which provide trigger $Ca^{2+}$ to the these RyRs. SR $Ca^{2+}$ release is particularly enhanced during the late phase of the AP compared to control in response to
β2-AR stimulation (Fig. 4.2D). The decrease in steady-state Ca\(^{2+}\) load is therefore a consequence of the increase in RyR activity in the absence of SERCA2a functional enhancement.

The local control myocyte model used in this study provides direct insight into properties of EC coupling associated with β-adrenergic stimulation as shown in Fig. 4.3. Peak LCC Ca\(^{2+}\) influx (\(F_{\text{LCC(max)}}\), Fig. 4.3A) and peak RyR Ca\(^{2+}\) release flux (\(F_{\text{RyR(max)}}\), Fig. 4.3B) are shown in response to a 200 ms voltage clamp step as a function of the test voltage. EC coupling gain is defined as the ratio of peak SR release flux to peak LCC Ca\(^{2+}\) influx (\(F_{\text{RyR(max)}}/F_{\text{LCC(max)}}\)) (Wier et al., 1994), and is shown in panel C of Fig. 4.3. Under control conditions, the voltage dependence of \(F_{\text{LCC(max)}}\) (Fig. 4.3A, black circles) is bell shaped and peaks at +10 mV with amplitude of 0.118 mM s\(^{-1}\) which is equivalent to \(I_{\text{CaL}}\) of \(~3.85\) pA pF\(^{-1}\). The control voltage dependence of \(F_{\text{RyR(max)}}\) (Fig. 4.3B, black circles) is also bell shaped and peaks at 0 mV with amplitude of 1.28 mM s\(^{-1}\) corresponding to a gain of \(~12\) at this potential (Fig. 4.3C, black circles). As described previously in Chapter 3, the monotonically decreasing gain function arises as a result of the fact that \(F_{\text{LCC}}\) and \(F_{\text{RyR}}\) do not share the same voltage dependent properties (i.e., peak \(F_{\text{RyR(max)}}\) occurs at a more negative potential than peak \(F_{\text{LCC(max)}}\)). These data agree well with experimentally obtained measurements of whole cell Ca\(^{2+}\) fluxes (Wier et al., 1994; Santana et al., 1996; Song et al., 2001). As a result of β-AR stimulation, \(F_{\text{LCC(max)}}\) is increased at all potentials by \(~2.5\)-fold (Fig. 4.3A, black triangles), consistent with the increase in LCC availability associated with β-AR stimulation in the model. In Fig. 4.3B, \(F_{\text{RyR(max)}}\) shows an \(~5\)-fold increase in response to β-AR stimulation (black triangles).
Figure 4.3: Effects of β-adrenergic stimulation on macroscopic LCC Ca\textsuperscript{2+} influx, SR Ca\textsuperscript{2+} release, and EC coupling gain. All panels show control (black circles), β-stimulated model (black triangles), and β-stimulated model using control initial conditions for SR Ca\textsuperscript{2+} load (gray triangles). (A) Peak LCC Ca\textsuperscript{2+} influx, $F_{\text{LCC}(\text{max})}$, as a function of membrane voltage. (B) Peak RyR Ca\textsuperscript{2+} release flux, $F_{\text{RyR}(\text{max})}$, as a function of membrane voltage. (C) EC coupling gain as a function of membrane potential defined as $F_{\text{RyR}(\text{max})}/F_{\text{LCC}(\text{max})}$. 
Figure 4.3
SR Ca\(^{2+}\) release flux increases to an extent that is disproportionately greater than the increase in LCC Ca\(^{2+}\) influx due the β-stimulation associated increase in SR Ca\(^{2+}\) (i.e., this simulation was performed using initial conditions based on 1 Hz pacing, which results in an increased SR Ca\(^{2+}\) load resulting from enhanced SERCA2a function as described in Fig. 4.2E). The result of the enhanced SR Ca\(^{2+}\) release is an increase in gain at all potentials (Fig. 4.3C, black triangles). In order to separate the effects of β-AR stimulation associated alterations in I\(_{CaL}\) and SR Ca\(^{2+}\) load on EC coupling gain, the simulations were repeated using the β-stimulated model in conjunction with resting SR load initial conditions. With normal SR Ca\(^{2+}\) load, \(F_\text{LCC(max)}\) (Fig. 4.3A, gray triangles) displays nearly identical voltage dependence to that for highly loaded SR. The amplitude of \(F_\text{RyR(max)}\) (Fig. 4.3B, gray triangles) is reduced by ~½ under conditions of control SR load, and this effect reduces the gain back to control levels at all potentials (Fig. 4.3C, gray triangles). The increased EC coupling gain arising from β-AR stimulation is therefore entirely attributed to the increase in SR Ca\(^{2+}\) load associated with the effects of PLB phosphorylation in this model.

The results of Fig. 4.3A indicate that peak I\(_{CaL}\) is essentially unaffected by SR Ca\(^{2+}\) load. This behavior arises in the local control model because Ca\(^{2+}\)-mediated inactivation of an LCC is sensitive to local Ca\(^{2+}\) concentration in its adjacent microdomain of the CaRU, and will generally occur only after the channel has opened and triggered SR Ca\(^{2+}\) release. Therefore, Ca\(^{2+}\)-mediated inactivation of LCCs manifests itself by modulating the late component of macroscopic I\(_{CaL}\), without dramatically affecting the amplitude of the peak current. This effect was demonstrated in Fig. 4.2C,
where the difference in SR load associated with $\beta_1$-AR vs. $\beta_2$-AR stimulation had little effect on peak $I_{Ca,L}$, but produced a rather prominent difference in the amplitude of the late sustained component of current.

Recent measurements of altered EC coupling gain in response to $\beta$-AR stimulation have demonstrated an increase (Viatchenko-Karpinski and Gyorke, 2001), a decrease (Song et al., 2001), and no change (Ginsburg and Bers, 2001) in EC coupling gain under conditions where SR Ca$^{2+}$ load remained unchanged. Model simulations of Fig. 4.3 demonstrated an increase in EC coupling gain associated with enhancement of SERCA2a function and SR Ca$^{2+}$ load, but showed no change in gain when SR Ca$^{2+}$ load was unchanged. The inconsistency in experimental measurements of the role of $\beta$-AR stimulation on EC coupling gain may be related to variations in the degree of phosphorylation of discrete proteins that are sensitive to experimental conditions and/or specific $\beta$-AR agonists. The model described thus far has assumed that $\beta$-AR stimulation redistributes active LCCs among modes 1 and 2, each of which are assumed to be stationary (see Methods) (Yue et al., 1990; Herzig et al., 1993). Chen-Izu et al. (2000) have found that increased open probability of LCCs in response to both $\beta_1$- and $\beta_2$-adrenergic stimulation is attributable to increased channel availability (by 20% - 30%) and an ~ 3- to 4-fold increase in mode 1 open frequency (i.e., number of open events per sweep), with no significant change in mode 1 mean open time. Fig. 4.4 demonstrates the effects of $\beta$-adrenergic stimulation in the model when it is assumed that mode 1 open frequency is increased 4-fold by scaling the LCC opening rate, $f$, by a factor of four within the $\beta$-AR stimulated model described above (see Chapter 3, Appendix I for LCC
model details). Panels A, B, and C of Fig. 4.4 show model $F_{LCC(max)}$, $F_{RyR(max)}$, and EC coupling gain, respectively, in response to the same protocol described in Fig. 4.3. Control simulations are repeated from Fig. 4.3 (circles). In the presence of β-AR stimulation with increased mode 1 open frequency (triangles), both LCC Ca$^{2+}$ influx and SR Ca$^{2+}$ release flux are increased dramatically. The increase in LCC Ca$^{2+}$ influx is ~3-fold greater than that observed without increased mode 1 open frequency in Fig. 4.3. In addition, $F_{LCC(max)}$ peaks at 0 mV, compared to 10 mV in control. The shift of the peak of this current-voltage relation to more negative potentials is a feature that is often observed in experiments in response to β-agonists (Song et al., 2001; Kääb et al., 1996). While $F_{RyR(max)}$ is increased as a result of increased trigger Ca$^{2+}$ influx, the gain of EC coupling is decreased by up to 50% at potentials more negative than 10 mV. A similar reduction in gain has been observed by Song et al. (2001) in response to β-AR stimulation by norepinephrine in rat myocytes, and may be attributed to local saturation of the trigger Ca$^{2+}$ signal.

The role PKA-mediated phosphorylation in modulation of RyR is controversial with reports of increased open probability due to increased Ca$^{2+}$ sensitivity (Marx et al., 2000) and reports showing no effect of phosphorylation on resting SR Ca$^{2+}$ release (Li et al., 2002). In Fig 4.4, the role of increasing RyR Ca$^{2+}$ sensitivity on EC coupling gain is demonstrated (squares). In this simulation, the rate exiting the RyR resting state, $k_{12}$, is increased 10-fold (see Chapter 3, Appendix I for RyR model details) within the β-AR stimulation model described in Fig. 4.3. Mode 1 open frequency is not altered in this simulation. $F_{LCC(max)}$ (Fig. 4.4A, squares) displays an increase in magnitude that is
Figure 4.4: Effects of LCC open frequency and RyR Ca\(^{2+}\)-sensitivity associated with \(\beta\)-adrenergic stimulation on macroscopic LCC Ca\(^{2+}\) influx, SR Ca\(^{2+}\) release, and EC coupling gain. All panels show control (circles), increased LCC open frequency (4 \(\times\) \(f\), triangles), and heightened RyR Ca\(^{2+}\) sensitivity (10 \(\times\) \(k_{12}\), squares). (A) Peak LCC Ca\(^{2+}\) influx, \(F_{\text{LCC(max)}}\), as a function of membrane voltage. (B) Peak RyR Ca\(^{2+}\) release flux, \(F_{\text{RyR(max)}}\), as a function of membrane voltage. (C) EC coupling gain as a function of membrane potential defined as \(F_{\text{RyR(max)}}/F_{\text{LCC(max)}}\).
Figure 4.4
similar to that shown in Fig 4.3A, and occurs predominantly as a result of increased LCC availability, indicating that the alteration in RyR Ca\(^{2+}\) has no effect on the amplitude of \(F_{\text{LCC(max)}}\). \(F_{\text{RyR(max)}}\) (Fig 4.4B, squares) is however greatly increased at all potentials compared to control, displaying a shift in its peak toward more positive potentials. The dramatic increase in SR Ca\(^{2+}\) release flux at the more positive potentials is indicative of the loss of tight control of EC coupling. This phenomenon is evident in the EC coupling gain curve (Fig. 4.4C, squares). EC coupling gain is increased at all potentials, however, whereas the control case (circles) is monotonically decreasing with increasing voltage, the increased RyR Ca\(^{2+}\) sensitivity results in a U-shaped gain function, which is increasing at voltages greater than 0 mV. A similar U-shaped gain function has been observed by Viatchenko-Karpinski and Györke (2001) in response to β-AR stimulation by ISO in rat myocytes. In their study, both an increase in the ability of individual sparks to ignite adjacent release sites and an increase in the efficiency of I\(_{\text{CaL}}\) to trigger Ca\(^{2+}\) release (i.e., increased fidelity) were reported. Both of these observations are consistent with an increased Ca\(^{2+}\) sensitivity of RyR activation.

**Model of Heart Failure**

Fig. 4.5 demonstrates the ability of the model to reproduce failing action potentials and Ca\(^{2+}\) transients. The solid and dotted lines of Fig. 4.5A show experimental measurements of normal and failing action potentials, respectively (B. O’Rourke, unpublished data). Model APs are demonstrated in panel C of Fig. 4.5. In panel C, a normal AP is shown by the solid line and has duration of ~ 300 ms. The long dashed line
shows an AP when the density of the Kv4.3 component of $I_{to1}$ has been reduced by 84%, which corresponds to a 65% net reduction in $I_{to1}$ from its normal value. The short dashed line shows an AP with this reduction in $I_{to1}$ as well as a 35% reduction in density of $I_{K1}$. The degrees to which $I_{to1}$ and $I_{K1}$ are reduced are based on the average percent reductions observed in heart failure for each of these currents (Kääb et al., 1996). The dot-dashed line correspond to these same reductions in $I_{to1}$ and $I_{K1}$, in addition to a 62% reduction in forward rate of the SERCA2a pump. The dotted line corresponds these same changes in $I_{to1}$, $I_{K1}$, and SERCA2a, in addition to a 100% increase in NCX function, and represents the AP of the failing myocyte. The degree to which SERCA2a and NCX are altered are consistent with previously determined model-based estimates of the average percentage change in their activity based on experimentally derived limits on their function (O'Rourke et al., 1999b; Winslow et al., 1999).

The model simulations shown in Fig. 4.5C demonstrate that downregulation of $I_{to1}$ reduces the depth of the phase 1 notch and shortens APD to ~210 ms (long dashed line). The mechanism of $I_{to1}$-related shortening of the AP involves an interaction of this current with $I_{CaL}$ and has been described in detail previously (see Chapter 2) (Greenstein et al., 2000). The added reduction of $I_{K1}$ (short dashed line) prolongs the AP, however the net effect of combined $I_{to1}$ and $I_{K1}$ reduction is a moderate reduction (by ~20 ms) in APD. The most dramatic change in AP configuration and duration occurs with downregulation of SERCA2a (dot-dashed line). This change results in an AP with a heightened plateau (producing a reappearance of the phase 1 notch) and a greater than 100% increase in APD compared to control to ~650 ms. Finally, with the addition of upregulation of NCX
Figure 4.5: Model vs. experimental action potentials and Ca\textsuperscript{2+} transients. Each AP and Ca\textsuperscript{2+} transient is in response to a 1-Hz pulse train measured in the steady-state. In the case of model heart failure, APs are shown from a 0.5-Hz pulse train because steady-state could not be defined at 1-Hz due to the presence early after depolarizations (not shown). (A) Experimentally measured membrane potential as a function of time in normal (solid line) and failing (dotted line) canine ventricular myocytes. (B) Experimentally measured cytosolic Ca\textsuperscript{2+} concentration for normal (solid line) and failing (dotted line) myocytes (panels A and B obtained from B. O’Rourke, unpublished data). (C) Simulated APs using the normal canine myocyte model (solid line), the myocyte model with I\textsubscript{to1} downregulation (long dashed line), with both I\textsubscript{to1} and I\textsubscript{K1} downregulation (short dashed line), with downregulation of I\textsubscript{to1}, I\textsubscript{K1}, and the SERCA2a pump (dot-dashed line), and the heart failure myocyte model (dotted line, downregulation of I\textsubscript{to1}, I\textsubscript{K1}, SERCA2a, and upregulation of NCX). (D) Model cytosolic Ca\textsuperscript{2+} transients for normal (solid line) and failing (dotted line) conditions. (E) L-type Ca\textsuperscript{2+} current as a function of time for normal (solid line) and failing (dotted line) cell models. The arrow indicates the amplitude of peak current for the failing model. (F) Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger current as a function of time for normal (solid line) and failing (dotted line) cell models.
Figure 4.5
to form the model of heart failure, APD becomes only slightly shorter than that simulated with no change in NCX.

The model normal (solid line) and failing (dotted line) Ca\(^{2+}\) transients are shown in Fig 4.5D. The amplitude of the heart failure model Ca\(^{2+}\) is significantly reduced compared to normal and displays a slowly increasing phase during the AP, compared to the rapid rise and slow decline displayed during the control AP. In addition, Ca\(^{2+}\) transient duration is prolonged and relaxation is slowed in the failing model. The altered properties of the Ca\(^{2+}\) transient are qualitatively similar to those shown in the experimental data of Fig. 4.5B.

Panels E and F of Fig. 4.5 show L-type Ca\(^{2+}\) current and Na\(^{+}\)-Ca\(^{2+}\) exchanger currents (I\(_{\text{NaCa}}\)) for normal (solid lines) and failing (dotted lines) model cells corresponding to the APs shown in panel A. The reduction in peak I\(_{\text{CaL}}\) shown in Fig. 4.5E (indicated by the arrow) is a consequence if I\(_{\text{to1}}\) downregulation. The reduction in I\(_{\text{to1}}\) results in a more depolarized membrane potential during the phase 1 notch, and consequently reduces the driving force for Ca\(^{2+}\) flux through LCCs. During the later plateau phase of the AP, however, I\(_{\text{CaL}}\) magnitude is greater than that in a normal cell. The increase in late I\(_{\text{CaL}}\) provides the inward current which prolongs the AP in the failing model, and is associated with the reduction in SERCA2a pumping capacity (Fig 4.5A). In the model, the inability of the cell to adequately sequester Ca\(^{2+}\) in the SR leads to a reduction in steady-state SR Ca\(^{2+}\) load, which reduces the SR Ca\(^{2+}\) release flux during CICR, and hence, relieves Ca\(^{2+}\)-mediated inactivation of I\(_{\text{CaL}}\), enhancing the late phase of this current. The details of voltage- and Ca\(^{2+}\)-mediated inactivation, and their
dependence on SR Ca$^{2+}$ load were shown previously in Chapter 3, Fig. 3.10. Panel F of Fig. 4.5 demonstrates that NCX operates in reverse mode, generating an outward current during most of the plateau phase of the AP in both normal (solid lines) and failing (dotted lines) model cells. Modest shortening of the AP as a result of NCX upregulation (Fig. 4.5A, dotted line vs. dot-dashed line) occurs due to enhancement of NCX reverse mode operation during the AP plateau. The amplitude of outward $I_{\text{NaCa}}$ is, however, less than that of inward $I_{\text{CaL}}$ during the AP plateau. Fig 4.5 demonstrates that reduction in Ca$^{2+}$-mediated inactivation of $I_{\text{CaL}}$ may be the primary mechanism by which AP prolongation occurs in heart failure.

**Role of Ca$^{2+}$-mediated Inactivation of $I_{\text{CaL}}$ on Action Potential Duration**

The data of Fig. 4.5 suggest the hypothesis that in heart failure, alterations in the expression of Ca$^{2+}$ handling proteins that decrease SR Ca$^{2+}$ load and reduce the amplitude of the Ca$^{2+}$ transient may contribute substantially to prolongation of APD by reducing Ca$^{2+}$-mediated inactivation of the L-type current. An alternative way to test this hypothesis is to subject a normal cell to a pacing protocol in which SR load varies, and measure the consequent effects on APD. One such protocol is post-rest pacing, in which a cell is allowed to rest for ~ 3 min and then, following the rest period, 1-Hz current pulses are applied to the cell. During the resting period, SR Ca$^{2+}$ in canine myocytes partially unloads (O'Rourke et al., 1999a). SR Ca$^{2+}$ will therefore be depleted on the first post-rest pulse, and will gradually reload in a pulse dependent manner. Panels A through C of Fig. 4.6 demonstrate this protocol using the local control myocyte model. Fig. 4.6A
Figure 4.6: Model and experimental post-rest action potentials and Ca\textsuperscript{2+} transients. Action potentials and corresponding Ca\textsuperscript{2+} transients are in response to a 1-Hz pulse train (initiated at time = 0 sec) following an ~ 3 min resting period. (A) Membrane potential as a function of time during the first 20 stimuli in the model cell. (B) Model cytosolic Ca\textsuperscript{2+} concentration as a function of time corresponding to the APs shown in panel A. (C) Summary of pulse dependent action potential duration and Ca\textsuperscript{2+} transient amplitude for the model cell based on the simulations shown in panels A and B. (D) Summary of pulse dependent action potential duration and Ca\textsuperscript{2+} transient amplitude for post-rest pacing experiments performed in canine ventricular myocytes (O'Rourke et al., 1999a).
Figure 4.6
shows APs during the first 20 seconds of 1-Hz pacing starting from resting initial conditions of the model. Both the height of the AP plateau and APD decrease gradually over the initial 10 beats. Fig. 4.6B shows the cytosolic Ca$^{2+}$ transients corresponding to the APs in panel A. The pulse dependent increase in peak magnitude of the Ca$^{2+}$ transient is a direct result of pulse dependent loading of the SR. In Fig. 4.6C, the pulse dependent APD is compared to the Ca$^{2+}$ transient amplitude for the model cell. The simulation demonstrates a correlation between the rise in Ca$^{2+}$ transient amplitude and the reduction in APD. The behavior of the model is in close agreement with the experimental data shown in Fig. 4.6D (O'Rourke et al., 1999a). The relatively short model APD in response to the first pulse compared to that of the second pulse occurs due to properties of RyR inactivation/adaptation. At the time of the first pulse, all RyRs are fully recovered from inactivation, while during all subsequent pulses ~ 20% - 30% of the population of RyRs will be inactive due to their failure to recover from inactivation within one second. Due to this effect, the mean subspace Ca$^{2+}$ transient during the first pulse is greater than that of the second pulse (data not shown), producing the sharper peak of the cytosolic Ca$^{2+}$ transient on pulse one (Fig 4.6B) and a shorter AP on pulse one compared to pulse two (Fig 4.6, A and C).

A more direct approach to demonstrating the prominent role of LCC Ca$^{2+}$-mediated inactivation on the duration of the cardiac action potential is to completely remove this mechanism. Under normal conditions, calmodulin (CaM) has been demonstrated to be the Ca$^{2+}$ sensor for LCC Ca$^{2+}$-mediated inactivation, and has been shown to be constitutively tethered to the LCC (Peterson et al., 1999). Ablation of Ca$^{2+}$-
mediated inactivation of the L-type current has been accomplished recently by viral expression of mutant Ca\(^{2+}\)-insensitive CaM in adult guinea pig ventricular myocytes (Alseikhan et al., 2002). Fig. 4.7A (reprinted from Alseikhan et al., 2002) demonstrates the effect of various mutant CaMs on experimentally measured guinea pig APs. Control myocyte APs lasted ~ 500 ms (labeled NV, no virus), while expression of a mutant CaM in which all Ca\(^{2+}\) binding sites are disabled (labeled 1234) resulted in dramatic elongation of the plateau phase producing an AP with duration of ~ 2.4 sec. In addition, the experiments of Alseikhan et al. (2002) demonstrated that mutations disabling Ca\(^{2+}\) sensing in the C-lobe of CaM (labeled 34) removed Ca\(^{2+}\)-mediated inactivation and prolonged APD while mutations disabling Ca\(^{2+}\) sensing in the N-lobe of CaM (labeled 12) did not disable Ca\(^{2+}\)-mediated inactivation. Fig. 4.7B demonstrates the effect of complete removal of Ca\(^{2+}\)-mediated inactivation on the AP generated using the local control myocyte model. Ca\(^{2+}\)-mediated inactivation is removed by setting to zero all LCC model rates that govern transitions from Mode Normal to Mode Ca (see Chapter 3, Appendix I for LCC model details). In the model cell, the AP is dramatically prolonged from ~ 300 ms in control to ~ 2.8 sec in the absence of the Ca\(^{2+}\)-mediated inactivation mechanism. In a qualitatively similar manner to that displayed in the experimental data, the model AP displays both depolarization and elongation of the plateau phase of the AP. The gradual decline in the height of the plateau potential occurs as a result of the relatively slow process of LCC voltage-dependent inactivation.
Figure 4.7: Experimental and model action potentials in the presence and absence of \( \text{Ca}^{2+} \)-mediated inactivation. (A) Action potentials recorded in guinea pig ventricular myocytes in a non-infected cell (NV, no virus), in a cell expressing wildtype CaM (WT), in a cell expressing a mutant CaM in which the N-lobe \( \text{Ca}^{2+} \) binding sites have been disabled (12), in a cell expressing a mutant CaM in which the C-lobe \( \text{Ca}^{2+} \) binding sites have been disabled (34), and in a cell expressing a mutant CaM in which all \( \text{Ca}^{2+} \) binding sites have been disabled (1234). Loss of \( \text{Ca}^{2+} \) binding to the C-lobe produces prominent prolongation of AP with duration > 2 sec. (Panel A reproduced from Alseikhan et al., 2002) (B) Action potentials simulated using the local control canine myocyte model under normal conditions and in the absence of \( \text{Ca}^{2+} \)-mediated inactivation. Model AP prolongation is qualitatively similar to that observed in experiments.
Figure 4.7
Altered LCC Phosphorylation in Heart Failure

Figs. 4.5 through 4.7 demonstrate the clear and prominent relationship between the amplitude of SR Ca\(^{2+}\) release flux and APD. As shown by Fig. 4.5, this mechanism appears to be sufficient to explain AP prolongation associated with heart failure, and does not involve an intrinsic loss of EC coupling gain or I\(_{\text{CaL}}\) (i.e., LCCs and RyRs are functionally unaltered) consistent with experimental findings that demonstrated recovery of normal EC coupling upon normalization of SR load (via application of increased extracellular Ca\(^{2+}\) concentration) in myocytes from pacing-induced canine heart failure (Hobai and O'Rourke, 2001). In contrast, some researchers have demonstrated a loss of EC coupling gain without prominent loss of SR Ca\(^{2+}\) load in heart failure (Gomez et al., 1997). In addition, altered LCC single channel gating kinetics (Schroder et al., 1998) and a reduced number of LCCs (He et al., 2001) have both been observed in association with human and canine heart failure, but in the absence of significant changes in macroscopic I\(_{\text{CaL}}\). In accordance with the findings of Schröder et al. (1998) and He et al. (2001), the total number of LCCs was reduced to 28.75% of the control value (corresponding to a reduction in number of CaRUs to 14,375 vs. 50,000 in control), the fraction of LCCs assumed to be available was increased from 25% to 50%, and the LCC opening rate, \(f_o\), was doubled (from 0.85 ms\(^{-1}\) to 1.7 ms\(^{-1}\)) in order to increase open probability. The alterations in LCC availability and open probability that have been reported in heart failure are consistent with those observed as a result of PKA-mediated phosphorylation of LCCs, suggesting that heart failure may be associated with a defect in dephosphorylation and/or phosphatase activity (Schroder and Herzig, 1999; Marx et al., 2000). The results
of Fig. 4.8 demonstrate the consequences of modifying LCC density and kinetics in this manner on EC coupling and AP properties. This modified model will be referred to as the HOP (“high open probability”) model and is compared to the minimal heart failure model described in Fig. 4.5. Panel A of Fig. 4.8 shows $F_{\text{LCC(max)}}$ for control (circles, solid line), for the HOP model (triangles, dashed line), and for the heart failure model (squares, dotted line) based on the same voltage clamp protocol described in Fig. 4.3. The bell-shaped curve for $F_{\text{LCC(max)}}$ of the HOP model is similar to that of control at most clamp potentials. While experimental findings indicate no significant changes in macroscopic properties of peak $I_{\text{CaL}}$ (Schroder et al., 1998; He et al., 2001), it was not possible to achieve a current-voltage relation for the HOP model that matches control at all potentials due to the 10 mV hyperpolarizing shift in the peak of the HOP model current-voltage relation. The heart failure model shows little difference in $F_{\text{LCC(max)}}$ compared to control. This is expected because intrinsic properties of LCCs are unchanged vs. the control model and (as demonstrated in Figs. 4.2A, 4.3A, and 4.8E) SR load dependent modulation of $I_{\text{CaL}}$ effects the late phase of the current with little effect on its peak amplitude. Fig 4.8B shows $F_{\text{RyR(max)}}$ for control (circles, solid line), the HOP model (triangles, dashed line), and the heart failure model (squares, dotted line). The altered LCC properties associated with the HOP model leads to a modest reduction (up to ~30%) in peak SR $\text{Ca}^{2+}$ release compared to control at all test potentials. In the heart failure model, however, SR $\text{Ca}^{2+}$ release is dramatically reduced by no less than 80% of control values. EC coupling gain is therefore reduced far more prominently in the heart failure model than in the HOP model, as shown in Fig. 4.8C. In fact, EC coupling gain in
Figure 4.8: Comparison of EC coupling and whole cell properties of the “high open probability” (HOP) model and the minimal heart failure model. All panels show control (circles and/or solid line), HOP model (triangles and/or dashed line), and heart failure model (squares and/or dotted line) simulations. (A) Peak LCC Ca\(^{2+}\) influx, \(F_{\text{LCC(max)}}\), as a function of membrane voltage. (B) Peak RyR Ca\(^{2+}\) release flux, \(F_{\text{RyR(max)}}\), as a function of membrane voltage. (C) EC coupling gain as a function of membrane potential \((F_{\text{RyR(max)}}/ F_{\text{LCC(max)}})\). (D) L-type Ca\(^{2+}\) channel open probability shown as a function of time in response to a 200 ms voltage clamp test pulse to 0 mV from a holding potential of –100 mV (inset, voltage protocol). (E) L-type Ca\(^{2+}\) current as a function of time in response to the protocol described in panel D. (F) Action potentials: Membrane potential as a function of time in response to a depolarizing current stimulus. (G) Cytosolic Ca\(^{2+}\) concentration as a function of time corresponding to the action potentials in panel F.
Figure 4.8
the heart failure model is less than 4 at all test potentials. The reduction in EC coupling gain in the HOP model arises due to a net reduction in the number of active LCCs, and hence a reduction in the number of RyRs that are triggered by LCC Ca\(^{2+}\) influx in combination with increased LCC \(p_o\) (similar to that demonstrated in Fig. 4.4), whereas the reduction of gain in the heart failure model is due to a decrease in SR Ca\(^{2+}\) load as a result of impaired SERCA2a function.

Panels D and E of Fig. 4.8 show the time progression of L-type Ca\(^{2+}\) channel open probability and \(I_{CaL}\), respectively, in response to a 200 ms voltage clamp to 0 mV from a holding potential of –100 mV (Fig. 4.8D, inset). Under control conditions (solid line), LCC \(p_o\) reaches a peak value of ~ 9% which corresponds to peak L-type current density of ~ 3.6 pA pF\(^{-1}\). The altered kinetics of the HOP model (dashed line) results in a nearly 2-fold increase in peak LCC \(p_o\) to ~ 16.5%, however, with the concomitant reduction in number of LCCs, peak \(I_{CaL}\) for the HOP model is essentially unchanged from that of control. In fact, control vs. HOP model \(I_{CaL}\) are nearly identical for the entire duration of the voltage clamp pulse. A small deviation between control and HOP model \(I_{CaL}\) currents occurs early in the inactivation process (Fig. 4.8E, time range of 20 to 50 ms) and is due to the modestly faster progression of Ca\(^{2+}\)-mediated inactivation in the HOP model vs. control (data not shown). The intrinsic kinetics of Ca\(^{2+}\)-mediated inactivation are unaltered in the HOP model, however, the faster progression of macroscopic Ca\(^{2+}\)-mediated inactivation occurs as a result of increased synchronization of Ca\(^{2+}\) release events, due to the increased rate of LCC channel opening \((f)\). Both peak LCC \(p_o\) and \(I_{CaL}\) of the heart failure model (dotted line) exhibit similar peak values to that of control,
whereas the rate of inactivation is slowed resulting in a late component of $I_{CaL}$ with amplitude as much as 2-fold greater than that of control. The profound alteration in waveform of $I_{CaL}$ in the heart failure model, compared to the modest changes that occur in the HOP model, suggests that a loss in SR Ca$^{2+}$ in heart failure (Hobai and O'Rourke, 2001) may have more severe effects on the AP than the measured changes in LCC gating kinetics and availability (Schroder et al., 1998; He et al., 2001).

Panels F and G of Fig. 4.8 demonstrate how the altered $I_{CaL}$ within the HOP model affects the AP and cytosolic Ca$^{2+}$ transient, respectively. Simulations of the HOP model are in response to 1-Hz steady-state stimuli (dashed lines) and are compared to control (solid lines) and heart failure models (dotted lines, replotted from Fig. 4.5). The AP simulated with the HOP model exhibits modest prolongation by ~30 ms compared to control. The HOP model exhibits a slowed progression from the phase 1 notch into the plateau phase vs. that of control. As described in detail in Chapter 2, outward $I_{to1}$ produces the phase 1 notch, which leads to a partial, but temporary, deactivation of $I_{CaL}$. The slowed exit from the notch occurs in the HOP model vs. control because a greater fraction of LCCs become inactivated (as described in Fig. 4.8E), and are therefore no longer available during the latter part of phase 1 (i.e., during the ascending portion of the notch). The net result is that the phase 1 notch is modestly prolonged in duration, producing modest prolongation of APD. The key observation, however, is that the altered kinetics and availability of the LCCs in the HOP model produce only modest changes in AP configuration, and do not lead to dramatic AP prolongation as demonstrated by the heart failure model. The peak amplitude of the cytosolic Ca$^{2+}$
transient generated by the HOP model (Fig. 4.8, dashed line) is reduced to ~ 20% of the control value (~ 0.55 µM HOP vs. ~ 0.7 µM control). The reduction in amplitude of the Ca^{2+} transient reflects the reduction in SR Ca^{2+} release flux vs. control (Fig. 4.8B). With decreased SR Ca^{2+} release due to there being fewer RyRs exposed to trigger Ca^{2+}, NSR Ca^{2+} content increases from ~ 0.83 mM in control to ~ 1.15 mM in the HOP model (pre-release free Ca^{2+} level, data not shown). A rise in SR Ca^{2+} is predicted by the mechanism of autoregulation (Eisner et al., 2000), based on the necessity of Ca^{2+} flux balance across the sarcolemma at steady state, however it is not sufficient to fully restore the amplitude of the Ca^{2+} transient to normal levels. The increase in SR, and total cell Ca^{2+} is reflected in the rise of the diastolic Ca^{2+} concentration in Fig 4.8G from ~ 130 nM in control to ~ 160 nM in the HOP model. While the alterations in I_{CaL} associated with the HOP model do result in a reduction of the Ca^{2+} transient amplitude, its initial rate of rise (d[Ca^{2+}]/dt) appears to be unchanged compared to that of control. In contrast, the cytosolic Ca^{2+} transient of the heart failure model (Fig. 4.8G, dotted line) displays a prolonged rising phase consistent with the lack of substantial SR Ca^{2+} release. It is likely that the slow prolonged Ca^{2+} transient produced by the heart failure model would produce a greater impairment of cardiac contraction, than that of the HOP model. In summary, the model simulations of Fig. 4.8 indicate that an impaired ability to load the SR, a finding consistent with both human (Lindner et al., 1998) and canine (Hobai and O'Rourke, 2001) heart failure, may lead to: (1) a predisposition to cellular arrhythmias associated with AP prolongation, such as early after depolarizations; and (2) loss of contractility due to severely altered Ca^{2+} transients. In addition, increased LCC availability and open
probability (Schroder et al., 1998), and a decreased number of LCCs (He et al., 2001) associated with measurements of single L-type channel properties in failing myocytes have relatively modest effects on AP configuration and cytosolic Ca$^{2+}$ transients.

**Discussion**

In this study, we have extended the local control canine myocyte model (Greenstein and Winslow, 2002) to study the role of altered membrane protein expression level and/or function associated with β-AR stimulation and heart failure. The local control model includes a detailed description of the tight regulation of CICR by incorporation of CaRUs, within which individual LCCs interact in a stochastic manner with nearby RyRs in localized regions where junctional SR membrane and T-tubular membrane are in close proximity. The CaRUs are embedded within and interact with the global systems of the myocyte describing ionic and membrane pump/exchanger currents, SR Ca$^{2+}$ uptake, and time-varying cytosolic concentrations. A local control model encompasses mechanisms of both microscopic and macroscopic cellular phenomena, and therefore is a powerful tool for predicting the impact of alterations in protein function, such as those associated with β-AR stimulation and/or heart failure, on integrative cellular behavior.

The first goal of this study was to develop a model of β-AR stimulation based on experimentally determined targets of the β-AR signaling cascade. The APs and Ca$^{2+}$ transients shown in Fig. 4.1 demonstrate that the model is in close agreement with experiments. Model APs exhibit a raised plateau potential and shortened duration, and
model Ca\textsuperscript{2+} transients exhibit increased peak amplitude and rapid relaxation. The rise in the AP plateau can be attributed to increased I\textsubscript{CaL} (Fig. 4.1E), mainly due to the increase in LCC availability, whereas the shortening of the AP can be attributed to the increased functional magnitude of I\textsubscript{Kr}. The increase in Ca\textsuperscript{2+} transient amplitude, as well as the increased rate of cytosolic Ca\textsuperscript{2+} removal can be attributed to enhanced SERCA2a function.

In addition to the increase in availability, 15% of the LCCs were assumed to be operating with mode 2 gating kinetics (long open time, see Methods for details). The contribution of altered active gating modes (mode 1 vs. mode 2) to macroscopic I\textsubscript{CaL} was not significant relative to the role of increased availability due to the relatively small fraction of channels that actually occupy mode 2 upon \( \beta \)-AR stimulation (data not shown). The model for I\textsubscript{CaL} implemented in the local control myocyte model is based on that originally developed by Jafri et al. (1998), and does not allow for inactivation mode switching while the open state is occupied (i.e., transitions between Mode Normal and Mode Ca cannot occur while the channel is open, see Fig. 3.13). The implication of this model structure is that LCCs cannot inactivate by the Ca\textsuperscript{2+}-mediated mechanism while they are open. The net result is that mode 2 channels, in which the rate \((g)\) exiting the open state has been slowed in order to prolong channel openings, exhibit slowed macroscopic inactivation kinetics. Experiments which have characterized phosphorylation dependent modal gating used Ba\textsuperscript{2+} as the LCC charge carrier (Chen-Izu et al., 2000; Yue et al., 1990), and therefore no conclusion can be made as to whether the processes of Ca\textsuperscript{2+}-mediated inactivation is influenced by occupation of different active
gating modes (e.g., mode 1 vs. mode 2) in these experiments. A possible limitation of the LCC model, therefore, is that mode 2 gating, in addition to prolonging open duration, slows Ca\(^{2+}\)-dependent inactivation. The impact of this model property to the simulations presented in this study, however, is minimized by the fact that only a small fraction of the population of LCCs are assumed to occupy mode 2 during β-AR stimulation.

A number of studies have indicated that delayed rectifier K\(^+\) currents are increased in response to β-AR stimulation (Hartzell and Duchatelle-Gourdon, 1993; Kathofer et al., 2000; Heath and Terrar, 2000; Cui et al., 2000). Recently, Heath and Terrar (2000) reported that β-AR stimulation reduces C-type inactivation of I\(_{Kr}\) via a PKC-dependent mechanism. Based on this report, model I\(_{Kr}\) inactivation was reduced in the β-AR stimulated state (see Methods). Other mechanisms of I\(_{Kr}\) regulation have been reported as well. The channel that underlies I\(_{Kr}\), HERG, has been shown to be inhibited by PKA-dependent phosphorylation and to be enhanced by direct binding of cAMP to HERG. The net result was a stimulatory effect on the current when HERG is complexed with the K\(^+\) channel accessory protein MiRP1 (Cui et al., 2000). While more study is necessary to determine the signaling pathways involved, most findings indicate that I\(_{Kr}\) is functionally increased in the presence of β-AR agonists. AP shortening in the model is attributed to enhanced I\(_{Kr}\). Experiments indicate that I\(_{Ks}\) is also increased in response to β-AR stimulation, however the small magnitude of I\(_{Ks}\) in canine ventricular myocytes minimizes its impact on AP properties relative to those of I\(_{Kr}\) (Varro et al., 2000). Finally, it is possible that currents other than delayed rectifier K\(^+\) currents contribute to AP shortening upon β-AR stimulation. A PKA-dependent Cl\(^-\) current, I\(_{Cl\text{PKA}}\), which is
carried by the channel encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene, has been identified in ventricular myocytes of some species (reviewed in Hume et al., 2000). No evidence, however, has been found for $I_{C_{L_{PKA}}}$ in adult canine myocytes (Sorota et al., 1991), and it is therefore not included in the local control model.

A key difference between $\beta_1$-AR and $\beta_2$-AR signaling in canine myocytes is that $\beta_2$-AR mediated cAMP/PKA signaling modulates only sarcolemmal targets such as LCCs, but does not regulate cytoplasmic targets such as PLB and myofilament proteins, whereas $\beta_1$-AR mediated cAMP/PKA signaling modulates both sarcolemmal and cytoplasmic targets (Kuschel et al., 1999; Xiao et al., 1999). Based on these findings, it was assumed that $\beta_1$-AR, but not $\beta_2$-AR, stimulation enhances SERCA2a function in the model. It has been shown that $\beta_2$-ARs are coupled to adenylyl cyclase more efficiently than are $\beta_1$-ARs (Bristow et al., 1989), however there are far fewer $\beta_2$-ARs than $\beta_1$-ARs in the cell membrane (Murphree and Saffitz, 1988). Since the net efficiency of $\beta_1$-AR vs. $\beta_2$-AR coupling to key effector proteins remains unclear, the approach taken in Fig. 4.2 was to assume that PKA-dependent regulation of sarcolemmal proteins occurs similarly regardless of $\beta$-AR subtype. The results demonstrated that exclusive $\beta_2$-AR stimulation produces a rise in AP plateau, without significant change in APD, qualitatively similar to that observed in experiments (Sosunov et al., 2000). A somewhat nonintuitive result is that steady-state SR Ca$^{2+}$ load falls in response to $\beta_2$-AR stimulation. This result follows from the theory of “autoregulation” (Eisner et al., 2000), and is due to the fact that increased LCC trigger leads to increased RyR activity and Ca$^{2+}$ release, which over a
series of repeated beats, leads to net loss of SR Ca\(^{2+}\) vs. control because the number of SERCA2a pumps remains unchanged.

Figs. 4.3 and 4.4 demonstrate how the local control model is used to interpret experimental results. Recent measurements of altered EC coupling gain in response to β-AR stimulation have demonstrated an increase (Viatchenko-Karpinski and Györke, 2001) a decrease (Song et al., 2001), and no change (Ginsburg and Bers, 2001) in gain. The reasons for the discrepancies among these experimental results is not clear, however, the local control model indicates that characteristic changes in the voltage dependent EC coupling gain function may be attributed to alterations of specific subcellular mechanisms. The model of β-AR stimulation, as described in Fig 4.1, displayed no change in gain under conditions where SR Ca\(^{2+}\) was unaltered (Fig 4.3) consistent with the findings of Ginsburg and Bers (2001). Increasing LCC open frequency in the β-AR stimulation model produced a decrease in gain similar to that measured by Song et al. (2001), while increasing RyR Ca\(^{2+}\) sensitivity produced an increase in gain similar in shape to that measured by Viatchenko-Karpinski and Györke (2001) (Fig 4.4). Whether or not β-AR stimulation produces these specific changes in LCC and RyR kinetics remains controversial. The model results suggest that the particular way in which EC coupling gain is altered in the presence of β-AR agonists may yield clues as to which of the underlying mechanisms are under regulation of downstream signaling in any particular experiment. More experimental study is required in order to understand if there is a consistent role of β-AR stimulation on EC coupling gain.
In the latter part of this study, we formulated a model of heart failure using the local control myocyte model. Membrane K\(^+\) currents and Ca\(^{2+}\) transport mechanisms were adjusted based on experiments that have demonstrated a reduction of I\(_{K1}\) and I\(_{to1}\) current density (Kääb et al., 1996), reduced SERCA2a function and enhanced function of NCX (Limas et al., 1987; Hobai and O'Rourke, 2000; O'Rourke et al., 1999b) in the human and canine heart failure. This approach is similar to that described by Winslow et al. (1999), and forms a minimal model of heart failure. The data of Fig. 4.5 demonstrate that downregulation of I\(_{to1}\), I\(_{K1}\), and the SERCA2a pump, combined with upregulation of NCX can account for differences in both the AP and the cytosolic Ca\(^{2+}\) transient in heart failure. Among these changes, Fig. 4.5C demonstrates that AP prolongation results primarily from the reduction in SERCA2a function, suggesting that altered expression of Ca\(^{2+}\) proteins plays a more significant role in prolongation of APD than does downregulation of K\(^+\) currents. In fact, the reduction of I\(_{to1}\) by the degree measure in heart failure shortens APD (based on the mechanism described in Chapter 2).

The heart failure model suggests that AP prolongation occurs upon loss of the ability to load SR with Ca\(^{2+}\). The suggested correlation between SR Ca\(^{2+}\) load and APD has been observed in nonfailing canine myocytes during a post-rest pacing protocol (O'Rourke et al., 1999a), and is reproduced well by the model (Fig. 4.6). Based on these phenomena, the prediction of the model is that under conditions of reduced SR Ca\(^{2+}\) load, less Ca\(^{2+}\) is released, thereby relieving Ca\(^{2+}\)-mediated inactivation of I\(_{CaL}\). The resulting increase of inward L-type current (Fig. 4.5E), maintains and prolongs the plateau phase of the action potential. The SR Ca\(^{2+}\) load dependent effect on APD is also readily
apparent upon comparison of β₁-AR and β₂-AR stimulation (Fig 4.2A), where SR Ca\(^{2+}\) load is greatly enhanced during β₁-AR vs. β₂-AR stimulation (Fig. 4.2E). The critical role of Ca\(^{2+}\)-mediated inactivation of LCCs in determining APD has been verified by complete removal of this mechanism, which yields dramatically prolonged APs of greater than 2 secs in duration both in experiments (Alseikhan et al., 2002) and in the local control model (Fig 4.7).

The heart failure model Ca\(^{2+}\) transient in Fig. 4.5D exhibits a slowly rising phase that is typical of Ca\(^{2+}\) transients observed in myocytes with severely impaired SR Ca\(^{2+}\) load (O'Rourke et al., 1999b). During the prolonged AP, the Ca\(^{2+}\) transient amplitude slowly rises to a level of \(~ 0.5 \mu M\), which is notably higher than that measured in isolated canine myocytes (e.g., Fig. 4.5B, \(~ 0.35 \mu M\)). The slow rising phase of the model Ca\(^{2+}\) transient occurs due to influx of Ca\(^{2+}\) via LCCs and reverse-mode NCX operation during the AP (in the absence of normal SR Ca\(^{2+}\) sequestration by SERCA2a). Due to this net influx of Ca\(^{2+}\), the heart failure model does not exhibit flat Ca\(^{2+}\) transients during an AP. The discrepancy between model and experiment may be related to the details of NCX function. Recently it has been demonstrated that NCX operates as if it were sensing a submembrane Ca\(^{2+}\) concentration (with peak value \(~ 3 \mu M\)) that is greater than cytosolic Ca\(^{2+}\) concentration in rabbit myocytes (Weber et al., 2002). This result implies that reverse-mode operation of NCX may occur to a lesser degree than described by the current model. The crucial role of NCX in the regulation of intracellular Ca\(^{2+}\), and altered Ca\(^{2+}\) cycling during heart failure indicate that investigation into the details of
NCX Ca\(^{2+}\)-dependence and regulation is an important area for future experimental and modeling studies.

It has been suggested that reduction in Ca\(^{2+}\) transient amplitude in heart failure may be associated with altered coupling of LCCs and RyRs without prominent loss of SR Ca\(^{2+}\) load (Gomez et al., 1997). Moreover, Schroder et al. (1998) observed increased LCC availability and open probability in failing human ventricular myocytes which closely resembled effects of cAMP-dependent stimulation. Analysis of slow cycling between active and silent modes suggested a defect in LCC dephosphorylation (Schroder et al., 1998), consistent with the finding that protein phosphatase 1 is found to be down regulated in failing myocytes (Marx et al., 2000). The model simulations of Fig. 4.8 were designed to compare the role of a defect in LCC dephosphorylation (the HOP model) with that of impaired SR loading (the heart failure model). While the altered LCC availability and kinetics associated with failing myocytes (Schroder et al., 1998; He et al., 2001) do lead to reduced EC coupling gain, the loss of gain is modest (Fig. 4.8C) and there is only a slight effect on AP configuration (Fig. 4.8G). An impaired ability of the SR to sequester Ca\(^{2+}\) due to downregulation of the SERCA2a pump dramatically reduced EC coupling gain, blunted and prolonged the Ca\(^{2+}\) transient, and dramatically prolonged APD (Fig. 4.8). These results indicate that, while a defect in LCC dephosphorylation may contribute to the cellular phenotype of heart failure by reducing coupling between LCCs and RyRs, it is not likely to be the primary factor. The simulations of this study indicate that the altered expression of Ca\(^{2+}\) handling proteins which impair SR Ca\(^{2+}\) loading can account for changes in Ca\(^{2+}\) transient amplitude and AP shape in heart
failure. It follows that restoration of SR Ca\(^{2+}\) by overexpression of SERCA2a via gene therapy (Hajjar et al., 2000; del Monte et al., 1999) may prove to be beneficial for restoration of EC coupling and cell contractility in patients with heart failure.
Appendix

L-Type channels are modeled as described previously in Chapter 3, Appendix I with the following modification. Voltage dependent inactivation of an LCC is modeled by the three state gating scheme shown in Fig. 4.9 with rates determined by

\[
y_{1\infty} = 0.4 / \left(1 + e^{(V_m + 12.5)/5}\right) + 0.6 \tag{4.1}
\]

\[
\tau_{1y} = 340 / \left(1 + e^{(V_m + 30)/12}\right) + 60 \tag{4.2}
\]

\[
y_{2\infty} = 1 / \left(1 + e^{(V_m + 40)/4}\right) \tag{4.3}
\]

\[
\tau_{2y} = 800 / \left(1 + e^{(V_m + 40)/4}\right) \tag{4.4}
\]

\[
k_{1f} = (1 - y_{1\infty}) / \tau_{1y} \tag{4.5}
\]

\[
k_{1b} = y_{1\infty} / \tau_{1y} \tag{4.6}
\]

\[
k_{2f} = (1 - y_{2\infty}) / \tau_{2y} \tag{4.7}
\]

\[
k_{2b} = y_{2\infty} / \tau_{2y} \tag{4.8}
\]

where \(V_m\) is membrane potential in mV, with \(\tau_{1y}\) and \(\tau_{2y}\) given in ms, and \(k_{1f}\), \(k_{1b}\), \(k_{2f}\) and \(k_{2b}\) given in ms\(^{-1}\).
Depolarization promotes transitions from the conducting state (state 1) into the inactive states (states 2 and 3). See Eqs. 4.1 through 4.8 for definitions of the transition rates.
CHAPTER 5:

General Discussion
Summary and Conclusions

The studies presented in this dissertation use the approach of integrative modeling in order to better understand the dynamic processes that govern excitation and contraction of a cardiac myocyte. In these studies, experimentally based computational models which span multiple levels of biological analysis are developed. These levels include single ion channel function, macroscopic membrane current and transporter function, and integrative behavior of the myocyte. These models are used to better understand how the interaction of these various subcellular components determine integrative cellular properties, such as action potential configuration, in both normal and diseased states.

In Chapter 2, a new model of canine $I_{to1}$ was developed. The model was built as a combination of two current components, the Kv4.3 and the Kv1.4 encoded currents, each with distinct kinetic properties. A ten-state Markov model with voltage dependent rate constants was formulated to represent each current component. The models accurately reproduce a wide range of data under various voltage protocols (e.g., peak current-voltage relation, steady-state inactivation, voltage dependent kinetics of recovery from inactivation). In order to investigate the role of $I_{to1}$ on action potential profile and duration, the model of $I_{to1}$ was incorporated into the Winslow et al. (1999) canine ventricular myocyte model. This was motivated by recent experimental findings which suggest that reductions in magnitude of $I_{to1}$, as a consequence of reduced Kv4.3 expression, may be important in modulating APD in normal vs. failing canine and human cardiac myocytes (Kääb et al., 1996; Beuckelmann et al., 1993). Incorporation of the canine $I_{to1}$ model into the canine ventricular cell model (Winslow et al., 1999) reveals a
complex interaction between $I_{Kv4.3}$ density and $I_{CaL}$ magnitude which in turn modulates APD. At relatively low levels of $I_{Kv4.3}$, increasing $I_{Kv4.3}$ augments the driving force for $I_{CaL}$ and produces a delay in activation of the late phase of $I_{CaL}$. Both effects contribute to the modest prolongation of APD. Further increasing $I_{Kv4.3}$ density reveals a threshold phenomenon, whereby the early outward current overcomes $I_{CaL}$, thus eliminating phase 2 producing a short AP with triangular shape. The major finding of this study is that as a consequence of this bimodal phenomenon, increasing $I_{Kv4.3}$ density shortens APD at high baseline densities, while at lower levels, increasing $I_{Kv4.3}$ density produces modest prolongation of APD. Thus, the effect of perturbing $I_{Kv4.3}$ density is dependent on the underlying current level against which the changes are imposed.

The study of Chapter 2 indicates the relationship between APD and $I_{to1}$ and/or $I_{Kv4.3}$ density is not a simple monotonic correlation. Rather, this relationship exhibits a bifurcation separating two distinct modes of behavior. $I_{to1}$ density is significantly greater in mouse than in human or canine cells (Barry et al., 1998; Kääb et al., 1996; Näbauer et al., 1996). Model predictions for the APD vs. $I_{to1}$ relationship at high baseline levels of $I_{to1}$ indicate APD will shorten with increasing $I_{to1}$, consistent with mouse data. However, the complex nature of the relationship between APD and $I_{to1}$ over a wider range of $I_{to1}$ density suggests that extrapolation of the consequences of altering expression levels of $I_{to1}$ in mouse to other species may not be valid. In fact, the Winslow et al. (1999) canine model predicts that reduction of $I_{to1}$ from normal levels will lead to modest shortening of APD (Fig. 2.6A). Via this mechanism, a rate dependent decrease in the availability of $I_{to1}$ is expected to contribute to both a shallower phase 1 notch potential and a shorter APD. Such rate dependent changes in AP morphology have been observed in canine (Liu et al.,
1993; Lukas and Antzelevitch, 1993; Litovsky and Antzelevitch, 1989; Zygmunt et al., 1997) and human cardiac myocytes (Näbauer et al., 1996; Li et al., 1998). Moreover, recent experimental evidence for this mechanism has been demonstrated in canine midmyocardial cells (Fig 2.7, Brian O’Rourke, unpublished data) where AP prolongation occurred following a 3 sec pause in the pacing protocol. These findings have led to the suggestion that the main impact of $I_{to1}$ on APD is secondary to its effects on $I_{CaL}$ (Litovsky and Antzelevitch, 1989; Zygmunt et al., 1997), consistent with the mechanism described in this study.

The influence of $I_{to1}$ on the trajectory of $I_{CaL}$ and on the profile and duration of the AP demonstrates the complex interaction of currents that are active during phase 1. A reduction of $I_{to1}$ from normal levels tends to produce modest shortening of APD, contrary to the belief that loss of $I_{to1}$ may be responsible for the extreme APD prolongation observed in heart failure. Implicit in this finding is that $I_{to1}$ may not play a critical role in APD prolongation-induced arrhythmias such as early after depolarizations.

In the study of Chapter 3, a biophysically detailed model of the normal canine ventricular myocyte that conforms to the theory of local control of EC coupling in cardiac muscle was presented. Local control theory asserts that L-type $Ca^{2+}$ current tightly controls SR $Ca^{2+}$ release because elementary, independent SR $Ca^{2+}$ release events occur in response to highly localized $Ca^{2+}$ transients which are produced by the opening of single L-type $Ca^{2+}$ channels in the vicinity of a small cluster of RyRs (Stern, 1992; Bers, 1993; Wier et al., 1994; Sham et al., 1995; Sham, 1997). Tight regulation of CICR is made possible by the fact that LCCs and RyRs are sensitive to local submembrane $Ca^{2+}$, rather than global $Ca^{2+}$ levels, allowing for graded control of $Ca^{2+}$ release while
maintaining high EC coupling gain (Fabiato, 1985b; Beuckelamnn and Wier, 1988). In order to capture these properties of local control, the myocyte model incorporates EC coupling in the form of Ca$^{2+}$ release units in which individual sarcolemmal L-type Ca$^{2+}$ channels interact in a stochastic manner with nearby RyRs in localized regions where junctional SR membrane and T-tubular membrane are in close proximity. The CaRUs are embedded within and interact with the global systems of the myocyte describing ionic and membrane pump/exchanger currents, SR Ca$^{2+}$ uptake, and time-varying cytosolic ion concentrations. The results demonstrate that a single comprehensive model of the cardiac myocyte can reproduce both detailed properties of EC coupling predicted by local control theory, such as variable gain and graded SR Ca$^{2+}$ release, while at the same time reproducing whole-cell phenomena, such as modulation of AP duration by SR Ca$^{2+}$ release. The ability of this model to encompass mechanisms of both microscopic and macroscopic phenomena afford it the unique ability to provide insight into the integrative properties of the myocyte that arise from the interaction among these subcellular processes.

The main goal of this study was to develop a myocyte model incorporating mechanistic descriptions of the processes that underlie local control of SR Ca$^{2+}$ release. This requires the simulation of a large number of individual stochastically gating channels, which poses a substantial computational task. In order to maintain tractability, the CaRU was designed as a “minimal model”, including biophysically detailed descriptions of individual LCCs and RyRs (Jafri et al., 1998; Rice et al., 1999; Keizer and Smith, 1998). Each CaRU contains four subspace compartments in which elementary Ca$^{2+}$ release events may occur, allowing for passive flow of Ca$^{2+}$ to neighboring
subspaces providing for the possibility that an initial release event may trigger an additional release event in the neighboring cluster of RyRs (Parker et al., 1996; Song et al., 2001). This geometry allows the summation of release events within a CaRU to serve as a minimal model of a Ca\textsuperscript{2+} spike, while reproducing fundamental properties of EC coupling measured at the whole cell level, such as graded Ca\textsuperscript{2+} release.

As a consequence of the detailed accounting of local Ca\textsuperscript{2+} mediated events, the local control model exhibits emergent whole cell properties. The model steady-state inactivation curve for I\textsubscript{CaL} (Fig. 3.3D) is U-shaped under normal conditions, but monotonically decreases and is incomplete with increasing membrane potential when Ca\textsuperscript{2+} is not the charge carrier. This feature agrees well with experiments (Hadley and Hume, 1987; Linz and Meyer, 1998) and emerges in this model because the local Ca\textsuperscript{2+} transient is graded (bell-shaped) as a function of LCC Ca\textsuperscript{2+} influx, and therefore the strength of Ca\textsuperscript{2+}-mediated inactivation of I\textsubscript{CaL} is similarly graded (and hence weaker at more positive potentials). Another emergent property of the local control model is the EC coupling gain function. Gain decreases with increasing depolarization due to the decrease in local triggering efficiency that occurs as unitary current magnitude decreases. The shape of the gain function arises as a result of the relationship between local Ca\textsuperscript{2+} influx and RyR Ca\textsuperscript{2+} sensitivity. Finally, an important emergent property is the stability of the Ca\textsuperscript{2+}-mediated inactivation process during the AP. While experimental evidence indicates that the Ca\textsuperscript{2+}-mediated inactivation process is dominant and the voltage-dependent process is far slower (Linz and Meyer, 1998; Sipido et al., 1995; Sham, 1997), mathematical models of I\textsubscript{CaL} have consistently been formulated with strong voltage-dependent inactivation and relatively weaker Ca\textsuperscript{2+}-mediated inactivation (Winslow et al.,
The all-or-none nature of SR Ca\textsuperscript{2+} release in a common pool model (where released Ca\textsuperscript{2+} is sensed by the RyRs) leads to instability when constrained such that Ca\textsuperscript{2+}-mediated inactivation process is dominant and the voltage-dependent process is weak. The local control model, however, displays robust graded SR Ca\textsuperscript{2+} release and strong Ca\textsuperscript{2+}-mediated inactivation of I\textsubscript{CaL}, processes that are central to properties of CICR, and must be properly described in order to enhance the ability of the model to predict the consequences of defects in EC coupling, such as those associated with heart failure (Lindner et al., 1998; Hobai and O'Rourke, 2001; Ahmmed et al., 2000; Marx et al., 2000; Schroder et al., 1998; He et al., 2001; Gomez et al., 1997; O'Rourke et al., 1999b).

In the study of Chapter 4, the local control canine myocyte model was extended to study the role of altered membrane protein expression level and/or function associated with β-AR stimulation and heart failure. The model of β-AR stimulation was based on experimentally determined targets of the β-AR signaling cascade. The APs and Ca\textsuperscript{2+} transients shown in Fig. 4.1 demonstrate that the model is in close agreement with experiments. The main factors that altered the shape of the AP and Ca\textsuperscript{2+} transient were the enhanced function of I\textsubscript{CaL}, I\textsubscript{Kr}, and the SERCA2a pump.

Recent measurements of altered EC coupling gain in response to β-AR stimulation have demonstrated an increase (Viatchenko-Karpinski and Gyorke, 2001) a decrease (Song et al., 2001), and no change (Ginsburg and Bers, 2001) in gain. The reasons for the discrepancies among these experimental results is not clear. However, the local control model has proven a useful tool to help interpret these experiments.
model indicates that characteristic changes in the voltage dependent EC coupling gain function may be attributed to alterations of specific subcellular mechanisms. The model of β-AR stimulation, as described in Fig 4.1, displayed no change in gain under conditions where SR Ca\textsuperscript{2+} was unaltered (Fig 4.3) consistent with the findings of Ginsburg and Bers (2001). Increasing LCC open frequency in the β-AR stimulation model produced a decrease in gain similar to that measured by Song et al. (2001), while increasing RyR Ca\textsuperscript{2+} sensitivity produced an increase in gain similar in shape to that measured by Viatchenko-Karpinski and Györke (2001) (Fig 4.4). Whether or not β-AR stimulation produces these specific changes in LCC and RyR kinetics remains controversial, however, the modeling results suggest that the particular way in which EC coupling gain is altered in the presence of β-AR agonists may yield clues as to which of the underlying mechanisms are under regulation of downstream signaling in any particular experiment.

In the latter part of this study, a model of heart failure was formulated by adjusting membrane K\textsuperscript{+} currents and Ca\textsuperscript{2+} transport mechanisms based on experiments that have demonstrated a reduction of I\textsubscript{K1} and I\textsubscript{I01} current density (Kääb et al., 1996), reduced SERCA2a function and enhanced function of NCX (Limas et al., 1987; Hobai and O'Rourke, 2000; O'Rourke et al., 1999b) in the human and canine heart failure. The model demonstrated that altered expression of these four proteins could account for differences in both the AP and the cytosolic Ca\textsuperscript{2+} transient in heart failure. The primary factor leading to AP prolongation and reduced Ca\textsuperscript{2+} transient amplitude was the reduction in SERCA2a function, and subsequent impaired ability to load SR with Ca\textsuperscript{2+}. The
suggested correlation between SR Ca\(^{2+}\) load and APD has been observed in nonfailing canine myocytes during a post-rest pacing protocol (O'Rourke et al., 1999a), and is reproduced well by the model (Fig. 4.6). Based on these phenomena, the prediction of the model is that under conditions of reduced SR Ca\(^{2+}\) load, less Ca\(^{2+}\) is released, thereby relieving Ca\(^{2+}\)-mediated inactivation of I\(_{\text{CaL}}\). The resulting increase of inward L-type current (Fig. 4.5E), maintains and prolongs the plateau phase of the action potential.

It has been suggested that reduction in Ca\(^{2+}\) transient amplitude in heart failure may be associated with altered coupling of LCCs and RyRs without prominent loss of SR Ca\(^{2+}\) load (Gomez et al., 1997). Moreover, Schroder et al. (1998) observed increased LCC availability and open probability in failing human ventricular myocytes which closely resembled effects of cAMP-dependent stimulation, and a possible defect in LCC dephosphorylation. The local control model was used to compare the role of a defect in LCC dephosphorylation (the HOP model) with that of impaired SR loading (the heart failure model). While the altered LCC availability and kinetics associated with failing myocytes (Schroder et al., 1998; He et al., 2001) do lead to reduced EC coupling gain, the loss of gain is modest (Fig. 4.8C) and there is only a slight effect on AP configuration (Fig. 4.8G). In contrast, impaired ability of the SR to sequester Ca\(^{2+}\) due to downregulation of the SERCA2a pump dramatically reduced EC coupling gain, blunted and prolonged the Ca\(^{2+}\) transient, and dramatically prolonged APD suggesting that while a defect in LCC dephosphorylation may contribute to the cellular phenotype of heart failure by reducing coupling between LCCs and RyRs, it is not likely to be the primary factor. The simulations of this study indicate that the altered expression of Ca\(^{2+}\) handling proteins which impair SR Ca\(^{2+}\) loading are mainly responsible for changes in Ca\(^{2+}\)
transient amplitude and AP shape in heart failure. It follows that restoration of SR Ca$^{2+}$ by overexpression of SERCA2a via gene therapy (Hajjar et al., 2000; del Monte et al., 1999) may prove to be beneficial for restoration of EC coupling and cell contractility in patients with heart failure.

**Future Directions**

Any computational model of a biological system can be classified as a “work in progress” and will never be complete. Ongoing experimental work continues to provide a stream of data which can be used to upgrade models and improve our understanding of biological systems. The local control model of the myocyte developed in this dissertation encompasses a greater degree of detail in its mathematical descriptions of subcellular systems than any preceding myocyte model. Despite this achievement, however, recent studies have elucidated new details regarding processes such as Na$^{+}$-Ca$^{2+}$ exchange, RyR gating, and Ca$^{2+}$-mediated inactivation of I$_{CaL}$ (described below) that have not been implemented in the model presented in this work. Future model refinements, which incorporate these new data, would further strengthen the predictive capability of the local control model.

The transporter model for NCX used in the local control myocyte model is that employed in the guinea pig myocyte model of Luo and Rudy (1994). A number of more recent studies have demonstrated that NCX current is allosterically activated by intracellular Ca$^{2+}$ (Weber et al., 2001; Hobai and O'Rourke, 2000), a property that is not included in the NCX formulation used in the local control model. In addition,
measurements in rabbit myocytes have suggested that NCX senses submembrane $\text{Ca}^{2+}$, which is $\sim 3$-fold greater than that in the bulk cytosol, leading to an NCX current which is inward during the majority of the action potential (Weber et al., 2002), but this may be highly species dependent. Data obtained in canine myocytes suggest that NCX is outward during the majority of the action potential (unpublished data, Brian O’Rourke). The function of NCX in its role of regulating cellular $\text{Ca}^{2+}$ levels is also likely to be influenced by its distribution in the T-tubules and the peripheral sarcolemma (Scriven et al., 2000). While certain aspects of NCX function remain controversial, the local control model would be a useful tool for interpreting recent data regarding the role of NCX in EC coupling and $\text{Ca}^{2+}$-cycling.

The RyR channels in the local control myocyte model are each represented by the model developed by Keizer and Smith (1998) and later modified by Rice et al. (1999). In this model, gating is governed by rates which are sensitive to $\text{Ca}^{2+}$ levels on the cytosolic face of the channel. Recently, evidence has been found indicating that cardiac RyRs can be regulated by $\text{Ca}^{2+}$ on the lumenal side of the channel (Ching et al., 2000; Gyorke and Gyorke, 1998), and it has been suggested that the a lumenal $\text{Ca}^{2+}$-regulatory mechanism may be an important contributing factor in the potentiation of SR $\text{Ca}^{2+}$ release that is observed in response to increases in intra-SR $\text{Ca}^{2+}$. Moreover, spontaneous SR $\text{Ca}^{2+}$ release is believed to transiently activate NCX current, leading to delayed after depolarizations (DADs), which can potentially trigger arrhythmias (Pogwizd et al., 2001). In order to better understand the role of spontaneous SR $\text{Ca}^{2+}$ release and $\text{Ca}^{2+}$ oscillations, an RyR model with lumenal $\text{Ca}^{2+}$ dependence has recently been developed (White et al., 2002). Incorporation of this type of RyR model into the local control
myocyte model would be necessary in order to better understand the underlying mechanisms of DADs and Ca\(^{2+}\) oscillations in this context.

Ca\(^{2+}\)-mediated inactivation of the L-type Ca\(^{2+}\) channel is implemented using a mode switching model (Jafri et al., 1998) in which transition rates from Model Normal into Model Ca (see Fig. 3.13) have a linear dependence on Ca\(^{2+}\) concentration in the adjacent dyadic subspace. It is likely that the Ca\(^{2+}\)-dependence of the inactivation process is more complex. Recent experiments have elucidated mechanistic details of the Ca\(^{2+}\)-mediated inactivation process, indicating that calmodulin (CaM) is tethered to the LCC and acts as a critical Ca\(^{2+}\) sensor, and that targeted mutations which prevent CaM binding to the LCC (Zuhlke et al., 1999) or prevent Ca\(^{2+}\) binding to CaM (Peterson et al., 1999; Peterson et al., 2000), dramatically reduce the degree of \(I_{CaL}\) inactivation observed during a depolarizing voltage clamp pulse. A mechanism for Ca\(^{2+}\)/CaM-dependent inactivation has been proposed (Peterson et al., 2000) in which Ca\(^{2+}\) first binds tethered CaM, which in turn binds to the IQ motif of the \(\alpha_{1C}\) subunit of the LCC. In this state, the channel still conducts current, and a conformational change of the EF hand is required to stabilize the closed inactivation state. This multi-step inactivation process implies that the kinetic properties of Ca\(^{2+}\)/CaM-mediated inactivation are likely more complex than those represented by the model used in this study. An improved description of Ca\(^{2+}\)/CaM-mediated inactivation may be important in understanding the relationship between SR Ca\(^{2+}\) load, AP configuration, and Ca\(^{2+}\) transient properties in both normal and failing myocytes.

It is likely that the role of local stochastic interactions in determining macroscopic properties applies to physiological systems other than CICR in cardiac myocytes.
model of phage λ-infection (Arkin et al., 1998), molecular level fluctuations in the rates of gene expression can arise due to stochastic local actions of regulatory proteins. These fluctuations lead to highly erratic time patterns of protein production in individual cells, which determine the phenotypic outcome for the phage λ lysis-lysogeny decision circuit. Recordings from rat hippocampal neurons have demonstrated the existence of a preassembled macromolecular signaling complex, which associates the β-AR with the L-type Ca\(^{2+}\) channel (Davare et al., 2001). The complex also contains a G protein, adenylyl cyclase, PKA, and a phosphatase. Similarly, the RyR has been shown to act as a scaffolding protein, with PKA and protein phosphatases bound to it via anchoring proteins (Marx et al., 2001b). The physical association of the molecular components of these signaling pathway elements suggests that the chain of signaling events will be determined by interactions between a small number of molecular entities within their local environment. Similarly, it has recently been demonstrated that spatial confinement of specific nitric oxide synthase isoforms in cardiac myocytes plays a critical role in modulating contractility, allowing both inhibition of LCCs and stimulation of RyRs (Barouch et al., 2002). Modeling studies of systems such as these may benefit from an approach, such as the one presented in this study, which combines stochastic and deterministic methods in order to maintain detailed descriptions of local molecular interactions. The model design and algorithms implemented in the local control myocyte model can therefore serve as a template for future work of this nature.

With the emerging theme of local signaling in biology comes the need for user-friendly computational tools that can be utilized to study these systems. The
implementation of local molecular interactions in model systems poses a unique challenge to the scientific community. The approach taken in this dissertation can be considered a “brute force” technique, in which the gating of every LCC and RyR is explicitly simulated. While the success of this approach hinged on the implementation of numerous simplifying approximations, more mathematically efficient ways of describing local signaling processes, such as local control of SR Ca$^{2+}$ release, are necessary in order to build models that can be used to rapidly explore hypotheses and/or can be incorporated into larger scale multicellular tissue or whole heart models.
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