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WASHINGTON UNIVERSITY IN ST. LOUIS

School of Engineering and Applied Science

Department of Biomedical Engineering

Dissertation Examination Committee:

Yoram Rudy, chair

Jianmin Cui

Igor R. Efimov

Sándor J. Kovács

Colin G. Nichols

Richard B. Schuessler

IONIC MECHANISMS OF  
ACTION POTENTIAL RATE DEPENDENCE,  
CONDUCTION AND BLOCK IN NORMAL EPICARDIUM  
AND IN REMODELED EPICARDIUM POST-INFARCTION

by

Keith Forest Decker

A dissertation presented to the  
Graduate School of Arts and sciences  
of Washington University in  
partial fulfillment of the  
requirements for the degree  
of Doctor of Philosophy

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Saint Louis, Missouri

## Abstract

In this work, detailed computational models are used to study the electrophysiology of normal epicardium and the arrhythmogenic effects of epicardial cell remodeling post-infarction. The canine epicardial myocyte model described here reproduces a wide range of experimentally observed rate dependent phenomena in cell and tissue. Model behavior depends on updated formulations for the 4-AP sensitive transient outward current ( $I_{to1}$ ), the slow component of the delayed rectifier potassium current ( $I_{Ks}$ ), the L-type  $Ca^{2+}$  channel ( $I_{Ca,L}$ ) and the sodium-potassium pump ( $I_{NaK}$ ) fit to data from canine ventricular myocytes. The model shows that  $I_{to1}$  plays a limited role in potentiating peak  $I_{Ca,L}$  and  $Ca^{2+}$  release for propagated action potentials (APs), but modulates the time course of action potential duration (APD) restitution.  $I_{Ks}$  plays an important role in APD shortening at short diastolic intervals but a limited role in AP repolarization at longer cycle lengths. In addition, simulations demonstrate that  $I_{Ca,L}$ ,  $I_{NaK}$  and  $[Na^+]_i$  play critical roles in APD accommodation and the rate dependence of APD restitution. Starting from the ionic model of a normal epicardial cell described above, an epicardial border zone (EBZ) model was developed based on available remodeling data. Ionic models of normal zone (NZ) and EBZ myocytes were incorporated into one-dimensional models of propagation to gain mechanistic insight into how ion channel remodeling affects APD and refractoriness, vulnerability to conduction block and conduction safety post-infarction. Simulations of EBZ APD restitution show that remodeled  $I_{Na}$  and  $I_{Ca,L}$  promote increased effective refractory period (ERP) and prolonged APD at short diastolic interval (DI). Heterogeneous tissue simulations show that increased post-repolarization refractoriness and altered restitution lead to a large rate dependent vulnerable window for conduction block. In simulations of conduction post-infarction, EBZ  $I_{K1}$  remodeling partially offsets the reduction in conduction safety due to altered

$I_{Na}$ , while  $I_{to1}$  and  $I_{CaL}$  have a negligible effect on conduction. Further simulations show that injection of skeletal muscle sodium channel SkM1- $I_{Na}$ , a recently proposed anti-arrhythmic therapy, has several desirable effects including normalization of EBZ ERP and APD restitution, elimination of vulnerability to conduction block and normalization of conduction in uncoupled tissue.

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# Table of Contents

<b>Abstract</b>	-ii-
<b>Acknowledgements</b>	-iv-
<b>List of Figures</b>	-vi-
<b>Abbreviations</b>	-vii-
<b>Epigraph</b>	-1-
<b>Chapter 1. Background</b>	-2-
<b>1.1 The Epicardial Border Zone and Arrhythmogenesis Post-Infarction</b>	-2-
<b>1.2 Computational models and the study of cardiac arrhythmias</b>	-6-
<b>1.3 Electrophysiological and Structural Determinants of Arrhythmia</b>	-7-
<b>1.4 Objectives</b>	-9-
<b>Chapter 2. Action Potential Rate Dependence in a Computational Model of Canine Epicardium</b>	-11-
<b>2.1 Introduction</b>	-11-
<b>2.2 Methods</b>	-12-
<b>2.3 Model Development</b>	-14-
<b>2.3.1 <math>I_{CaL}</math></b>	-14-
<b>2.3.2 <math>I_{Ks}</math></b>	-16-
<b>2.3.3 <math>I_{to1}</math></b>	-18-
<b>2.3.4 <math>I_{to2}</math></b>	-19-
<b>2.3.5 <math>I_{NaK}</math></b>	-19-
<b>2.3.6 <math>I_{NaL}</math></b>	-20-
<b>2.4 Ionic Mechanisms of Action Potential Duration (APD) Adaptation, Restitution and Accommodation</b>	-22-
<b>2.4.1 APD rate dependence in cell and strand</b>	-22-
<b>2.4.2 Differences in cell and strand behavior</b>	-23-
<b>2.4.3 Ionic Mechanisms of APD Restitution</b>	-25-
<b>2.4.4 Ionic Mechanisms of APD accommodation</b>	-30-
<b>2.4.5 Ionic Mechanisms of APD adaptation</b>	-35-
<b>2.4.6 Ionic Mechanism of S1 Dependent APD restitution</b>	-36-
<b>2.5 Discussion</b>	-38-
<b>Chapter 3. Ionic Mechanisms of Electrophysiological Heterogeneity and Conduction Block in the Infarct Border Zone</b>	-43-
<b>3.1 Introduction</b>	-43-
<b>3.2 Methods</b>	-44-
<b>3.3 Results</b>	-46-
<b>3.3.1 An Ionic Model of the Epicardial Border Zone myocyte</b>	-46-
<b>3.3.2 APD Rate Dependence in the Epicardial Border Zone</b>	-48-
<b>3.3.3 Ionic Mechanisms of AP Heterogeneity and Conduction Block</b>	-50-
<b>3.3.4 Anti-arrhythmic effects of SkM1-<math>I_{Na}</math> Post-Infarction</b>	-57-
<b>3.4 Discussion</b>	-59-
<b>Chapter 4. Conclusions and Future Directions</b>	-64-
<b>Appendix A. Canine Epicardial Cell Model Equations.</b>	-66-
<b>Appendix B. NZ and EBZ Model Equations</b>	-92-
<b>References</b>	-104-

# List of Figures

## Chapter 1

## Chapter 2

<b>Figure 2-1</b>	Canine Epicardial Cell Model	-13-
<b>Figure 2-2</b>	$I_{CaL}$ Model and Experimental Fits	-15-
<b>Figure 2-3</b>	$I_{Ks}$ Model and Experimental Fits	-17-
<b>Figure 2-4</b>	$I_{to1}$ and $I_{to2}$ Experimental Fits	-18-
<b>Figure 2-5</b>	$I_{NaK}$ and $[Na^+]_i$ Experimental Fits	-20-
<b>Figure 2-6</b>	APD Rate Dependence in Cell and Strand	-22-
<b>Figure 2-7</b>	Differences in Rate Dependence in Cell and Strand	-23-
<b>Figure 2-8</b>	$I_{to1}$ Block in Cell and Strand	-24-
<b>Figure 2-9</b>	$I_{to1}$ and APD Restitution	-26-
<b>Figure 2-10</b>	$I_{Na}$ and APD Restitution	-28-
<b>Figure 2-11</b>	$I_{Ks}$ and APD Restitution	-30-
<b>Figure 2-12</b>	Linking Accommodation, Restitution and Adaptation	-31-
<b>Figure 2-13</b>	$[Na^+]_i$ , $I_{NaK}$ and APD Accommodation	-33-
<b>Figure 2-14</b>	$I_{CaL}$ and APD Accommodation	-34-
<b>Figure 2-15</b>	Mechanisms of APD Adaptation	-35-
<b>Figure 2-16</b>	Mechanism of S1 Dependent APD Restitution	-37-
<b>Figure 2-17</b>	Rate Dependence of APD Restitution Kinetics	-38-

## Chapter 3

<b>Figure 3-1</b>	Canine Epicardial Border Zone (EBZ) Cell Model	-46-
<b>Figure 3-2</b>	Model fits to NZ and EBZ Experimental Data	-47-
<b>Figure 3-3</b>	EBZ Action Potentials in Cell and Strand	-48-
<b>Figure 3-4</b>	APD Restitution in the EBZ	-50-
<b>Figure 3-5</b>	APD Adaptation and Block in Heterogeneous Strands	-51-
<b>Figure 3-6</b>	Conduction Block at fast $CL_{S1}$ in Heterogeneous Strands	-53-
<b>Figure 3-7</b>	Role of $I_{Na}$ and $I_{K1}$ in EBZ conduction	-54-
<b>Figure 3-8</b>	Role of $I_{CaL}$ and $I_{to1}$ in NZ and EBZ conduction	-56-
<b>Figure 3-9</b>	Anti-arrhythmic effects of SkM1 $I_{Na}$	-58-

## Abbreviations

Commonly used abbreviations are below. Additional abbreviations accompany equations in Appendix A.

NZ	Normal Zone
EBZ	Epicardial Border Zone
VW	vulnerable window for formation of the initial line of block
AP	action potential, (ms)
APD	action potential duration, (ms)
PRR	post-repolarization refractoriness, (ms)
ERP	effective refractory period, (ms)
DI	diastolic interval, (ms)
SF	safety factor
LAD	left-anterior descending coronary artery
CL <sub>S1</sub>	S1 pacing cycle length, (ms)
CI <sub>S2</sub>	S2 coupling interval, (ms)
SR	sarcoplasmic reticulum
SS	subspace
$g_j$	gap junction conductance, $\mu\text{S}$
$dV/dt_{\text{max}}$	maximum AP upstroke velocity, (V/s)
$\tau_{\text{recovery}}$	time constant of recovery of AP upstroke velocity (ms)
$\tau_{\text{VDI}}$	time constant of $I_{\text{CaL}}$ voltage dependent inactivation (ms)
$V_m$	membrane potential (mV)
$V_{\text{rest}}$	resting membrane potential (mV)
$V_{\text{hold}}$	holding potential (mV)
CT <sub>KC</sub>	$\text{K}^+$ - $\text{Cl}^-$ cotransporter
CT <sub>NaCl</sub>	$\text{Na}^+$ - $\text{Cl}^-$ cotransporter
$I_{\text{NaL}}$	late $\text{Na}^+$ current
$I_{\text{Na}}$	fast $\text{Na}^+$ current
SkM1- $I_{\text{Na}}$	Skeletal muscle $\text{Na}^+$ current isoform 1
$I_{\text{Nab}}$	background $\text{Na}^+$ current
$I_{\text{NaCa,i}}$	$\text{Na}^+$ / $\text{Ca}^{2+}$ exchanger (localized to myoplasm)
$I_{\text{Cab}}$	background $\text{Ca}^{2+}$ current
$I_{\text{pca}}$	sarcolemmal $\text{Ca}^{2+}$ pump
$I_{\text{to1}}$	4-aminopyridine-sensitive transient outward $\text{K}^+$ current
$I_{\text{Kr}}$	fast delayed rectifier $\text{K}^+$ current
$I_{\text{Ks}}$	slow delayed rectifier $\text{K}^+$ current
$I_{\text{K1}}$	time-independent $\text{K}^+$ current
$I_{\text{NaK}}$	$\text{Na}^+$ - $\text{K}^+$ pump current
$I_{\text{leak}}$	NSR leak
$I_{\text{diff}}$	$\text{Ca}^{2+}$ diffusion, SR subspace to myoplasm
$I_{\text{tr}}$	$\text{Ca}^{2+}$ transfer, NSR to JSR
$I_{\text{rel}}$	$\text{Ca}^{2+}$ release from JSR
$I_{\text{diff,ss}}$	ion diffusion, subspace to local $I_{\text{CaL}}$ subspace
$I_{\text{NaCa,ss}}$	$\text{Na}^+$ / $\text{Ca}^{2+}$ exchanger (localized to SR subspace)
$I_{\text{Ca,L}}$	L-type $\text{Ca}^{2+}$ current



$I_{to2}$	$Ca^{2+}$ -dependent transient outward $Cl^-$ current
SS(CaL)	$I_{CaL}$ subspace
SS(SR)	SR subspace
PLB	phospholamban
SERCA	SR $Ca^{2+}$ -ATPase;
CSQN	calsequestrin
CaMKII	$Ca^{2+}$ /calmodulin-dependent kinase
NSR	network SR
JSR	junctional SR.

## Epigraph

*“When first I engaged in this work, I resolved to leave neither words nor things unexamined...I soon found that...whatever abilities I had brought to my task, with those I must finally perform it. To deliberate whenever I doubted, to enquire whenever I was ignorant, would have protracted the undertaking without end, and perhaps without much improvement... I saw that one enquiry only gave occasion to another, that book referred to book, that to search was not always to find, and to find was not always to be informed; and that thus to pursue perfection was, like the first inhabitants of Arcadia, to chase the Sun, which, when they had reached the hill where he seemed to rest, was still beheld at the same distance from them. I then contracted my design, determining to confide in myself...by this I obtained at least one advantage, that I set limits to my work, which would in time be ended, though not completed.”*

- Preface to A Dictionary of the English Language, Samuel Johnson

## **Chapter 1. Background**

### **1.1 The Epicardial Border Zone and Arrhythmogenesis Post-Infarction**

Survivors of myocardial infarction are at an increased risk of ventricular arrhythmia and sudden death in the days and weeks following the initial ischemic event. Years of experiments have been devoted to increasing our understanding of the pathophysiological basis of this enhanced risk (Janse and Wit, 1989; Wit and Janse, 1992). The initiation of reentrant ventricular tachycardia is thought to depend on the presence of a substrate that has undergone structural and electrophysiological remodeling in response to the initial myocardial insult (Pinto and Boyden, 1999). This remodeling is thought to increase vulnerability to initiation of an arrhythmia in response to a triggering event. While ventricular tachycardia and its degeneration into ventricular fibrillation following myocardial infarction are leading causes of sudden cardiac death, the connection between structural and electrophysiological remodeling and increased vulnerability to arrhythmia remains poorly understood.

A canine model in which a transmural infarct is created by left anterior descending (LAD) coronary artery ligation is thought to provide an excellent model of arrhythmogenesis in humans, and as such has been the focus of years of experiments (Janse and Wit, 1989). Three to five days post-infarction, programmed stimulation protocols can be used to initiate tachycardias similar to those that are observed clinically (Wit and Janse, 1992). These programmed stimulation protocols are thought to mimic the spontaneous premature depolarizations that trigger arrhythmias in vivo. Arrhythmias in this model are reentrant in nature and have been mapped to a thin rim of surviving epicardial tissue that borders the infarct (the epicardial border zone or EBZ) (El-Sherif et al., 1977c)

The nature of reentry in the EBZ was first described in a series of experiments by El-Sherif and coworkers (El-Sherif et al., 1977a; El-Sherif et al., 1977c; El-Sherif et al., 1981;

Gough et al., 1985; Mehra et al., 1983; Restivo et al., 1990). This work showed that reentry can be initiated when a properly timed premature beat results in a line of conduction block. Wavefronts propagate around and then coalesce distal to this initial line of block. Reentry results when the coalesced wavefront breaks through and excites recovered tissue. This pattern of initiation often results in the figure-of-eight reentry characteristic of the EBZ preparation, although other patterns are observed. The initial line of block tends to form perpendicular to the myocardial fiber direction, while the long axis of the circuit during sustained reentry tends to align parallel to the fiber axis (Cardinal et al., 1988; Ciaccio et al., 2001a; Dillon et al., 1988). Reentry in this preparation is usually functional, in that gross anatomical obstacles are not often present within the reentrant circuit. When inducible, episodes of monomorphic tachycardia are often very stable, persisting for more than 100 cycles (Ciaccio et al., 2001b).

In whole heart and isolated tissue preparations, investigators have used epicardial mapping of paced beats and reentrant circuits to gain insight into the mechanisms underlying the initiation and maintenance of reentry. El-Sherif and coworkers have identified a gradient of increasing effective refractory period (ERP) from the periphery to the interior of the EBZ, and have argued that the formation of the initial line of block depends on propagation of a premature beat into EBZ tissue with abnormally long ERP (Gough et al., 1985; Restivo et al., 1990). Wit and coworkers have emphasized the importance of anisotropic tissue structure in the vulnerability to sustained reentry (Dillon et al., 1988). Early experiments (Dillon et al., 1988) and more recent high resolution mapping studies (Cabo et al., 2006) have reported regions of localized conduction slowing, especially for conduction transverse to the fiber axis and within the outer regions of the reentrant circuit. Tissue anisotropy may result in lowered safety factor for longitudinal conduction (Spach and Dolber, 1986; Spach et al., 1982; Spach et al., 1981),

contributing to the formation of initial lines of block, while preferential side-to-side uncoupling may contribute to formation of lines of apparent block during sustained reentry. A recent series of experiments by Ciaccio and coworkers (Ciaccio, 2000, 2001, 2002; Ciaccio et al., 2001a; Ciaccio et al., 2001b) has provided additional valuable information on the spatial and temporal properties of reentrant circuits in the EBZ. Reentrant circuits are characterized by a slow conduction zone (SCZ) in the region where the reentrant wavefront exits the central common pathway. The inducibility and cycle length (CL) of reentry are correlated with the length of the initial line of block and the geometry of the central common pathway. This work suggests that the spatial properties of the infarct are critical determinants of vulnerability to arrhythmia.

Studies in myocytes isolated from the EBZ have characterized the electrophysiological and structural remodeling processes that occur after infarction (Pinto and Boyden, 1999). Cells isolated from the EBZ have lowered upstroke velocity, slowed recovery of excitability and exhibit increased post-repolarization refractoriness (Lue and Boyden, 1992). These abnormalities are caused by altered  $\text{Na}^+$  channel ( $I_{\text{Na}}$ ) kinetics (Pu and Boyden, 1997), and the severity of  $I_{\text{Na}}$  remodeling is increased near the center of the reentrant circuit (Baba et al., 2005). EBZ cells lack a phase 1 notch due to a reduction in expression of both components of the transient outward current  $I_{\text{to1}}$  (Lue and Boyden, 1992) and  $I_{\text{to2}}$  (Aggarwal et al., 1997)). EBZ cells have a triangularized AP morphology linked to a reduction in  $I_{\text{K1}}$  density (Lue and Boyden, 1992). In addition to the aforementioned remodeling processes, altered density and kinetics of the L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca(L)}}$ ) (Aggarwal and Boyden, 1995) and delayed rectifier potassium currents ( $I_{\text{Kr}}$  and  $I_{\text{Ks}}$ ) (Jiang et al., 2000) may play critical roles in altered rate dependence of action potential duration and morphology in the EBZ. Cells from the EBZ also show alterations in  $\text{Ca}^{2+}$  handling, including increased diastolic  $[\text{Ca}^{2+}]_i$ , decreased systolic  $[\text{Ca}^{2+}]_i$ , decreased rate

dependence of  $\text{Ca}^{2+}$ -transient (CaT) relaxation and a decrease in CaT amplitude with increased pacing frequency (Licata et al., 1997; Pu et al., 2000). These alterations in  $\text{Ca}^{2+}$  handling may be caused by a combination of ion channel remodeling (especially  $I_{\text{Ca(L)}}$ ) and alterations in  $\text{Ca}^{2+}$  handling proteins, in particular calcium/calmodulin-dependent protein kinase II (CaMKII) (Hund and Rudy, 2004, 2006). Recent studies have shown increased levels of auto-phosphorylated (Hund et al., 2008a) and oxidized CaMKII (Christensen et al., 2009) in the EBZ. Increased CaMKII activation may contribute to altered  $I_{\text{Na}}$  and  $I_{\text{NaL}}$  activity in the EBZ (Christensen et al., 2009). Additional studies have characterized structural remodeling processes in the EBZ. In cells isolated from the outer pathway of the reentrant circuit, gap junction distribution is altered, with increased disarray and side-to-side localization (Cabo et al., 2006; Peters et al., 1997; Yao et al., 2003). This altered distribution is accompanied by a reduction in side-to-side coupling in isolated cell pairs (Cabo et al., 2006; Yao et al., 2003). The fact that the reentrant circuit is localized to regions of gap junction disarray that span the entire thickness of the EBZ (Peters et al., 1997) suggests an important role for structural remodeling in arrhythmogenesis.

Several computational studies of EBZ cell and tissue have provided important insights into mechanisms of arrhythmogenesis after infarction. In single cell and strand simulations, Cabo and Boyden studied the ionic determinants of EBZ ERP and repolarization (Cabo and Boyden, 2003). This study confirmed that  $I_{\text{Na}}$  remodeling plays a key role in post-repolarization refractoriness, and suggested that  $I_{\text{CaL}}$  remodeling plays a key role in APD heterogeneity between NZ and EBZ. A major limitation of this study was the failure to validate the ion channel properties and rate dependence of APD in the control model against available experimental data. Using this single cell model, Cabo and Boyden have developed two-dimensional models of the

EBZ and studied the stability of reentry after initiation. One study found that heterogeneity in gap junction coupling within the EBZ stabilizes reentry (Cabo and Boyden, 2006). A second study showed that heterogeneity in  $I_{Na}$  and  $I_{CaL}$  remodeling within the reentrant circuit also stabilizes reentry (Baba et al., 2005). These studies provide important hypotheses about how remodeling heterogeneity within the EBZ effects the stability of reentry, which should be tested using models of the NZ and EBZ where ion channel formulations and APD rate dependence have been more thoroughly validated. Additional studies by Hund and coworkers have emphasized the role of CaMKII remodeling in the EBZ post-infarction. CaMKII remodeling may alter  $Ca^{2+}$  homeostasis and contribute to altered  $I_{Na}$  and  $I_{NaL}$  function in the EBZ (Hund et al., 2008a). A recent study has linked CaMKII to reduced conduction velocity, increase effective refractory period and increased vulnerability to conduction block in the EBZ (Christensen et al., 2009). Importantly, neither the studies of Cabo et al. or Hund and coworkers have characterize the full range of rate dependent APD dynamics in NZ or EBZ models. In particular, the effect of EBZ remodeling on APD restitution was not explored. Considering the wide range of experimental and computational studies suggesting that the complex dependence of APD on rate is a critical determinant of the dynamics of reentry, this is an important limitation of previous computational studies of arrhythmia and remodeling in the EBZ.

## **1.2 Computational Models and the Study of Cardiac Arrhythmias**

The initiation and maintenance of arrhythmia depends on the heterogeneous response of normal and remodeled cells to complex patterns of excitation. Studies of cardiac electrophysiology and arrhythmogenesis using detailed computational models often focus on results for standing action potentials or for steady state pacing, however. Experimentally, arrhythmia is often initiated by a single premature beat after sustained pacing at a slower cycle

length. Rate dependent heterogeneity of action potential duration and refractoriness will be a key determinant of whether that premature beat will lead to conduction block. The spatial distribution of APD after the initial line of block forms will help determine whether reentry is initiated. The spatial and temporal response of APD to changes in rate will govern the transition from the initiating premature beat to the fast cycle lengths of reentry and the chaotic activation patterns of fibrillation. Thus, while important insights can be gained by the study of APD at one or a few pacing rates, understanding of arrhythmogenesis will depend on the study of a wider range of pacing protocols. The study of arrhythmia in physiologically detailed models has been limited by their computational complexity and the failure of available models to reproduce the range of rate dependent phenomena observed experimentally (Cherry and Fenton, 2006). This has led to the development of simplified computational models that reproduce a desired set of rate dependent phenomena observed experimentally, but do not ground APD dynamics in physiological representations of underlying ionic currents and ion concentrations. This approach has led to important contributions to our understanding of the link between APD rate dependence and arrhythmia, but questions arise as to whether models that do not contain accurate representations of underlying physiological processes will lead to clinically relevant findings. Clearly, there is a critical need for computational models that both accurately represent underlying physiological processes and reproduce a wide range of rate dependent phenomena relevant to arrhythmogenesis.

### **1.3 Electrophysiological and Structural Determinants of Arrhythmia**

The rate dependence of APD has been extensively characterized in experiment and linked to the initiation and maintenance of arrhythmogenesis through a large body of computational research. APD depends on both the time since repolarization of the previous action potential



(diastolic interval or DI) and the history of stimulation prior to the previous action potential (memory). APD adaptation (also referred to as dynamic restitution) is defined as the dependence of APD on steady state pacing cycle length ( $CL_{S1}$ ). APD restitution is defined as the dependence of APD on the diastolic interval (DI) or coupling interval (CI) of an additional (S2) stimulus after pacing to steady state at  $CL_{S1}$ . After pacing to steady state at  $CL_{S1}$ , APD accommodation (Watanabe and Koller, 2002) (also referred to as short term memory (Franz et al., 1988)) is defined as the time course of adjustment of APD during sustained pacing at a new CL ( $CL_{S2}$ ). APD adaptation, restitution and accommodation are aspects of action potential rate dependence ubiquitously observed in the ventricular myocardium of many species including human, despite wide variation in action potential duration morphology and ion channel expression across species and cell types. Experimentally characterized restitution curves typically show increasing APD with increasing DI, with the steepest slope at the shortest DI. During accommodation after a change from a fast to a slow CL, APD adjusts monotonically to a new steady state over a time course of minutes. As with APD restitution, APD adaptation typically shows an increasing APD as a function of increasing DI or CL. However, APD adaptation shows a shallower slope than APD restitution at the intersection of the two curves. A large body of computational studies have emphasized the importance of APD rate dependence in the initiation and maintenance of arrhythmias. The steepness of APD restitution is thought to promote APD alternans and govern the transition from stable to chaotic arrhythmias, the so-called 'Restitution Hypothesis' (Karma, 1994). APD accommodation and memory are thought to play a role in the stability of fibrillation (Baher et al., 2006; Toal et al., 2009). Heterogeneity in APD adaptation is a key determinant of the dispersion of repolarization and susceptibility to the initiation of arrhythmias. Despite the ubiquitously observed properties of APD adaptation, restitution and accommodation and their

likely role in arrhythmia, many currently available models fail to reproduce some or all of these phenomena (Cherry and Fenton, 2006). In addition, the underlying mechanisms of these phenomena remain incompletely understood.

Additional electrophysiological and structural properties of tissue beyond the rate dependence of APD will determine susceptibility to arrhythmia. Conduction safety and vulnerability to conduction block will depend on the complex interplay of electrophysiological and structural remodeling processes. Currents contributing to depolarization and phase 1 action potential (AP) amplitude, including  $I_{Na}$ ,  $I_{CaL}$ ,  $I_{to1}$  and  $I_{to2}$  are remodeled in the EBZ and may play a role in abnormal conduction (Huelsing et al., 2001; Joyner et al., 1996; Rohr and Kucera, 1997; Shaw and Rudy, 1997). Structural factors including gap junction coupling (Shaw and Rudy, 1997), tissue anisotropy (Spach and Dolber, 1986; Spach et al., 1982; Spach et al., 1981) and wave-front curvature (Cabo et al., 1994; Fast and Kleber, 1995, 1997) are also critical determinants of conduction safety. Other electrophysiological and structural determinants of the stability of reentry after induction include cell excitability (Beaumont et al., 1998), conduction velocity restitution (Cherry and Fenton, 2004; Fenton et al., 2002), AP morphology (Cherry and Fenton, 2004) and  $Ca^{2+}$ -dependent voltage alternans (Sato et al., 2006; Shiferaw et al., 2005).

### **1.3 Objectives**

The development of rational approaches to the prevention and treatment of arrhythmia depends on our understanding of underlying ionic mechanisms. A large body of literature has characterized the electrophysiological and structural remodeling processes that may increase vulnerability to arrhythmia after infarction. Computational studies are critical for underlying how these remodeling processes lead to heterogeneity in the electrophysiological properties of normal and diseased cells, and how this heterogeneity creates a substrate vulnerable to the

initiation and maintenance of arrhythmia. Given this critical need, the research presented here was undertaken: 1) To develop an ionic model of the normal canine epicardial myocyte that accurately reproduces a wide range of rate dependent phenomena, and to link these rate dependent phenomena to underlying ionic processes. 2) To develop an ionic model of the remodeled epicardial border zone myocyte, and link ion channel remodeling to abnormal APD rate dependence. 3) To study, in multicellular tissue models, how heterogeneity in normal and EBZ cell behavior leads to a substrate with increased vulnerability to arrhythmia due to abnormal APD rate dependence, altered conduction and increased susceptibility to conduction block.

## **Chapter 2. Action Potential Rate Dependence in a Computational Model of Canine**

### **Epicardium**

#### **2.1 Introduction**

Cardiac arrhythmias and sudden death involve complex myocardial activation patterns including conduction block, reentry and fibrillation. In order to understand the relations and transitions between these patterns, the ionic determinants of the response of healthy and diseased cardiac myocytes to complex patterns of excitation must be understood. The single cell response to such excitation patterns depends on the complex interaction between ionic currents, intracellular ion concentrations and membrane voltage. Computational cell models provide critical tools for exploring these interactions, allowing the development and testing of hypotheses about underlying ionic mechanisms based on careful integration of available experimental data (Rudy and Silva, 2006). The canine is a common animal model for studying cell electrophysiology in a range of disease states. Our group and others have developed detailed mathematical models of the canine action potential (Fox et al., 2002; Hund and Rudy, 2004; Winslow et al., 1999). While these models have been used to study arrhythmia mechanisms after heart failure (Winslow et al., 1999) and myocardial infarction (Cabo and Boyden, 2003; Hund et al., 2008b) as well as ionic mechanisms of alternans (Fox et al., 2002; Livshitz and Rudy, 2007), they are limited in their ability to simulate important rate-dependent phenomena (Cherry and Fenton, 2007), including the dependence of steady-state action potential duration on pacing cycle length (CL) (APD adaptation), the dependence of APD on diastolic interval (DI) (APD restitution) and the time course of the adjustment of APD to changes in rate (short-term memory (Franz et al., 1988) or APD accommodation (Watanabe and Koller, 2002)). These limitations extend to ionic models of other species, including the human (Cherry and Fenton,

2007). We hypothesized that canine epicardial APD adaptation, restitution and accommodation could be simulated and understood based on available descriptions of subcellular ionic processes. We incorporated updated and validated formulations of the 4-AP sensitive transient outward current ( $I_{to1}$ ), the slow component of the delayed rectifier potassium current ( $I_{Ks}$ ), the L-type  $Ca^{2+}$  channel ( $I_{Ca,L}$ ) and the sodium-potassium pump ( $I_{NaK}$ ) into a previously published model of the canine epicardial myocyte (Hund et al., 2008b; Hund and Rudy, 2004; Livshitz and Rudy, 2007). Model behavior was examined in both single cell and multicellular (strand) simulations. Our work provides new insight into molecular mechanisms underlying important rate-dependent action potential properties, including APD restitution, adaptation and accommodation. Specifically, our studies highlight the importance of  $I_{to1}$  and  $I_{Ks}$  in APD restitution, and the role of  $I_{Ca,L}$  and  $I_{NaK}$  in APD accommodation and rate dependent APD restitution.

## **2.2 Methods**

### **Cell Simulations**

Steady-state results are shown after pacing for 1800 seconds at a given CL with a conservative  $K^+$  stimulus (Hund et al., 2001). This simulation time resulted in a beat-to-beat change in APD, maximum intracellular sodium ( $[Na^+]_{i,max}$ ) and calcium ( $[Ca^{2+}]_{i,max}$ ) of less than 0.1%. Restitution results were obtained for an additional beat after pacing to steady-state as described. Accommodation simulations involved pacing to steady-state at a given CL ( $CL_{S1}$ ), followed by pacing to steady-state at a different CL ( $CL_{S2}$ ).

### **Strand Simulations**

One-dimensional simulations of propagation were performed in a strand of 96 cells, following previous work from our laboratory (Shaw and Rudy, 1997). Cell 1 was directly stimulated, and simulation results are shown for a central cell (cell 48). Stimulation protocols

were similar to those used for single cells (duration of pacing = 1800 s), resulting in beat-to-beat changes in APD,  $[Na^+]_{i,max}$  and  $[Ca^{2+}]_{i,max}$  of less than 0.1%. Gap junction conductance of  $2.5 \mu S$  gave a maximum upstroke velocity (Di Diego et al., 1996) and conduction velocity (Spach et al., 2000) consistent with experimental results in canine epicardial tissue. Additional parameters used in multicellular simulations are included in Appendix A.

### 2.3 Model Development

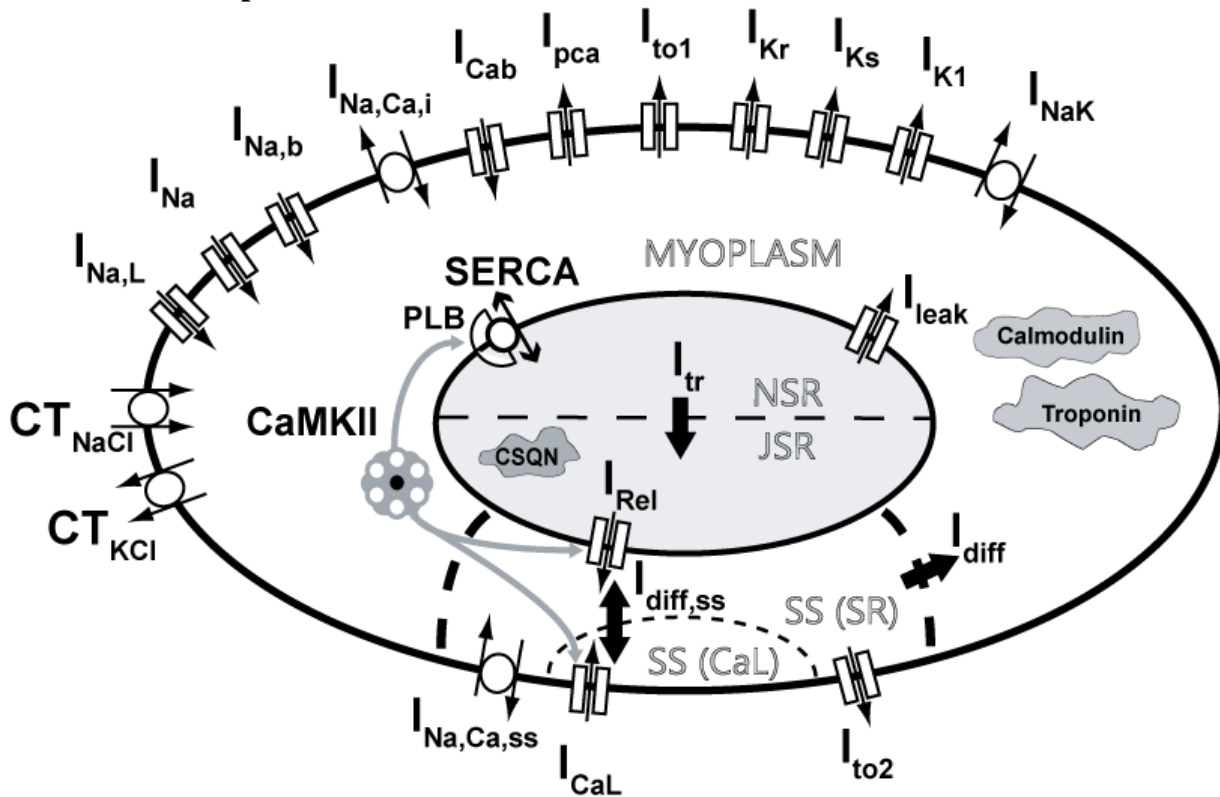


Figure 2-1 Canine Epicardial Cell Model . See page vii and Appendix A for abbreviations and equations.

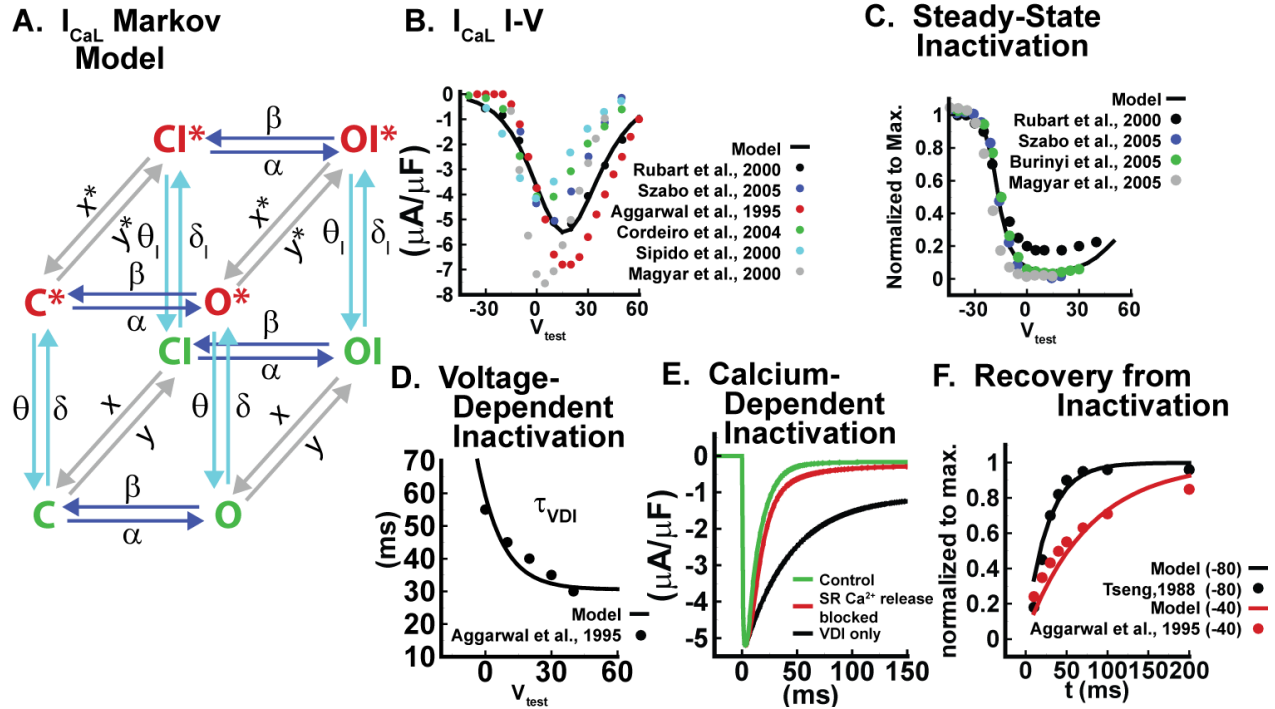
The Hund-Rudy dynamic (HRd) model (Fig. 2-1) (Hund et al., 2008b; Hund and Rudy, 2004; Livshitz and Rudy, 2007) served as a starting point for development of an updated model of the canine epicardial myocyte. Major reformulations were made to the L-type  $Ca^{2+}$  current ( $I_{Ca,L}$ ), the potassium component of the transient outward current ( $I_{to1}$ ), the delayed rectifier potassium current ( $I_{Ks}$ ), the  $Na^+-K^+$  pump ( $I_{NaK}$ ), the chloride component of the transient outward current

( $I_{to2}$ ) and the late sodium current ( $I_{NaL}$ ). Parameters for reformulated currents were fit to experimental data from canine epicardial myocytes whenever possible. Experimental data was chosen that represented either the consensus of a wide range of experimental results or came from the most complete available studies. Special attention was given to the role of each of these reformulated currents in the rate dependence of action potential duration in canine epicardial model and experiment. Updates made to intracellular calcium ( $[Ca^{2+}]_i$ ) handling in a recent study of APD and  $[Ca^{2+}]_i$ -transient (CaT) alternans were also included (Livshitz and Rudy, 2007). Appendix A includes a complete set of model equations and descriptions of adjustments to model parameters not included in this section

### 2.3.1 $I_{CaL}$

Figure 2.2A shows the state structure of a novel Markov model of  $I_{CaL}$ . This model was formulated with the goals of 1) reproducing a wide range of experimental data 2) maintaining computational tractability in order to facilitate long-term multicellular simulations. Activation is voltage dependent ( $\alpha$  and  $\beta$  transitions). In  $Ca^{2+}$  free conditions, inactivation and recovery from inactivation are voltage dependent ( $x$  and  $y$  transitions). Elevation of  $Ca^{2+}$  in a local subspace ( $[Ca^{2+}]_{SS,CaL}$ ) moves channels from the  $Ca^{2+}$ -free gating tier to an upper tier ( $\theta$  and  $\delta$  transitions) characterized by accelerated inactivation ( $x^*$  and  $y^*$  transitions). This structure reflects the hypothesis that  $Ca^{2+}$  binding to calmodulin removes a “brake” and speeds up  $I_{CaL}$  voltage dependent inactivation (Mahajan et al., 2008; Pitt, 2007).  $[Ca^{2+}]_{SS,CaL}$  elevation depends on  $I_{CaL}$   $Ca^{2+}$  entry and diffusion of  $Ca^{2+}$  ( $I_{diff,SS}$ ) after SR  $Ca^{2+}$ -induced  $Ca^{2+}$  release. This phenomenological approach allows reproduction of slowed  $I_{CaL}$   $Ca^{2+}$  dependent inactivation when SR function is blocked or intracellular  $Ca^{2+}$  buffering is high.

The  $I_{CaL}$  Markov model was fit to data from canine ventricular myocytes wherever possible. The  $I_{CaL}$  current-voltage (I-V) relationship was fit to data from left ventricular



**Figure 2-2**  $I_{CaL}$  Model and Experimental Fits. (A)  $I_{CaL}$  Markov Model diagram, (B)  $I_{CaL}$  current-voltage (I-V) relationship and (C) steady-state inactivation, (D) voltage-dependent inactivation, (E) calcium-dependent inactivation and (F) recovery from inactivation .

myocytes, (Rubart et al., 2000) (Fig. 2-2B). The Rubart I-V curve data (Rubart et al., 2000) was chosen because it falls in the middle range of canine ventricular data recorded at body temperature (Aggarwal and Boyden, 1995; Cordeiro et al., 2004; Magyar et al., 2002; Rubart et al., 2000; Sipido et al., 2000; Szabo et al., 2005). Model  $I_{CaL}$  steady state inactivation is compared to multiple experimental data sets in Figure 2-2C. The Szabo steady state inactivation data (Szabo et al., 2005) chosen for model fitting has a slope and  $V_{1/2}$  consistent with multiple experiments from canine ventricle at body temperature (Birinyi et al., 2005; Magyar et al., 2002; Rubart et al., 2000; Szabo et al., 2005).  $I_{CaL}$  steady state inactivation data with lesser degrees of

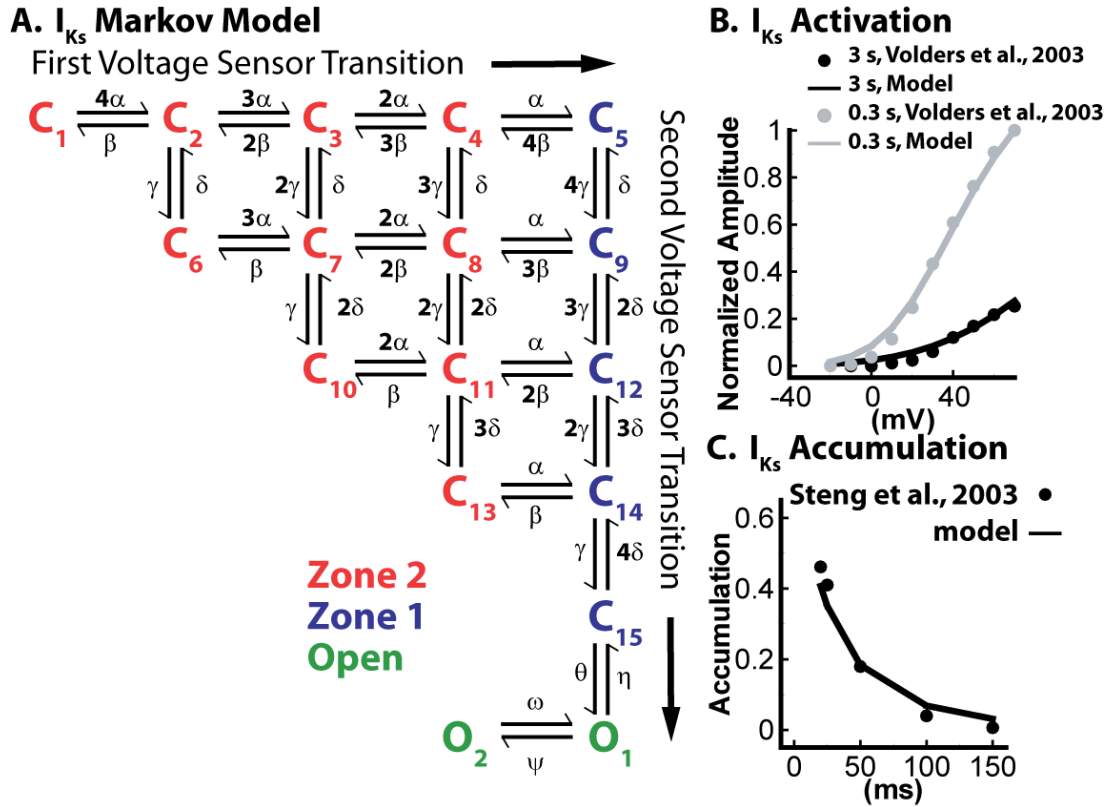


inactivation at steady state (Rubart et al., 2000) resulted in model APD outside the range observed in canine experiments. Experimental studies have shown that  $I_{CaL}$  inactivation is  $Ca^{2+}$  dependent, and is significantly slowed in  $Ca^{2+}$ -free solutions. The time constant of voltage dependent inactivation ( $\tau_{VDI}$ ) in  $Ca^{2+}$ -free solutions is fit to data from canine epicardial myocytes (Aggarwal and Boyden, 1995) (Fig. 2-2D). Voltage dependent inactivation was simulated in the model by eliminating  $Ca^{2+}$  dependent transitions to upper tier states. Figure 2-2E compares the relative contribution of voltage,  $Ca^{2+}$  entry via  $I_{CaL}$  and SR  $Ca^{2+}$  release to inactivation in the model.  $I_{CaL}$  inactivation is fastest in control, is modestly slowed by block of SR  $Ca^{2+}$  release and is substantially slowed by complete elimination of  $Ca^{2+}$  dependent inactivation (VDI only). The quantitative dependence of  $I_{CaL}$  inactivation on  $I_{CaL}$   $Ca^{2+}$  entry, SR  $Ca^{2+}$ -release and voltage is difficult to characterize experimentally, but model behavior is qualitatively consistent with available experimental evidence (Findlay, 2002a, b; Sun et al., 1997). Model voltage dependence of the time constant of recovery from inactivation (Fig. 2-2F) is consistent with canine ventricular data (Aggarwal and Boyden, 1995; Tseng, 1988).

### 2.3.2 $I_{Ks}$

The Hodgkin-Huxley based formulation for  $I_{Ks}$  used in the Hund-Rudy model of the canine epicardial myocyte (Hund and Rudy, 2004) was replaced with the 17 state Markov model proposed by Silva and Rudy (Silva and Rudy, 2005). The structure of the  $I_{Ks}$  model is shown in Figure 2-3A. The original Silva-Rudy model accurately reproduces the kinetics of  $I_{Ks}$  in guinea pig and human based on underlying voltage sensor transitions and has provided insight into the role of  $I_{Ks}$  in APD adaptation in these species (Silva and Rudy, 2005). The  $I_{Ks}$  model can be divided into deep closed states (Zone 2), closed states near the open state (Zone 1) and open states. Transition from rest (Zone 2) to the open state (Open) requires a slow Zone 2 to Zone 1

transition (the first voltage sensor transition), followed by a fast Zone 1 to Open state transition (the second voltage sensor transition). Transition rate parameters were adjusted to fit the following data from canine epicardial and midmyocardial myocytes: i) tail current amplitudes at holding potentials from -20 to +70 mV after 300 ms and 3000 ms of activation (Fig. 2-3B) (Volders et al., 2003); ii) tail currents at holding potentials from -10 to 60 mV after 5000 ms of



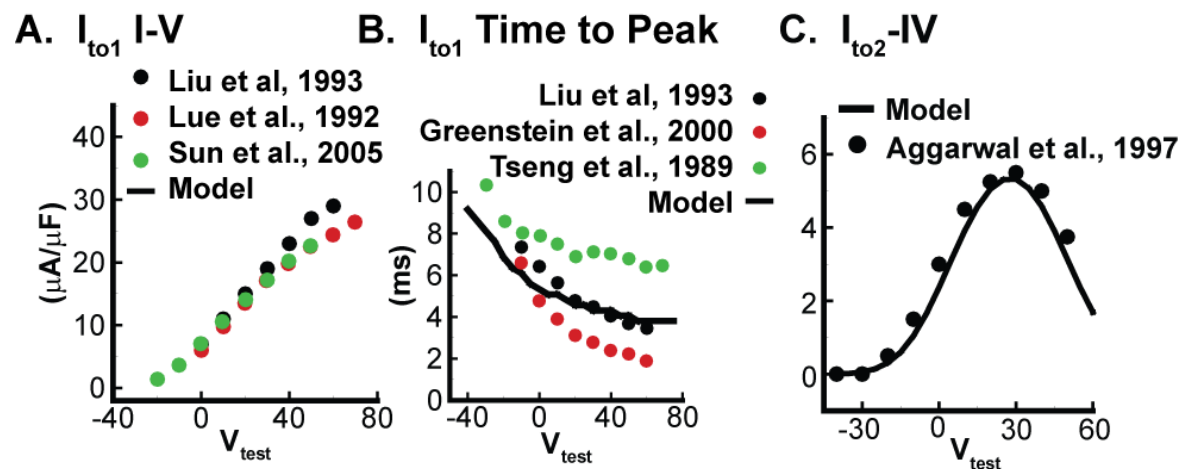
**Figure 2-3  $I_{Ks}$  Model and Experimental Fits.** (A)  $I_{Ks}$  Markov Model state diagram and model fits to (B) activation and (C) accumulation.

activation (Liu and Antzelevitch, 1995; Stengl et al., 2003); iii) activation half-time on depolarization to +20 mV (Liu and Antzelevitch, 1995; Stengl et al., 2003); iv) deactivation half-times for tail currents to -25, -50 and -80 mV (Liu and Antzelevitch, 1995; Stengl et al., 2003) and v) accumulation characteristics for inter-pulse intervals between 20 and 150 ms after 200 ms

depolarizing steps (Fig. 2-3C) (Stengl et al., 2003). Current density was scaled to that of canine epicardial myocytes (Liu and Antzelevitch, 1995; Stengl et al., 2003).

### 2.3.3 $I_{to1}$

Model  $I_{to1}$  is derived from a previous formulation (Dong et al., 2006). Modifications were made to better fit experimental measurements from the canine. Model  $I_{to1}$  density is consistent with multiple experiments from canine left ventricular epicardial cells (Fig. 2-4A) (Liu et al., 1993; Lue and Boyden, 1992; Sun and Wang, 2005). Activation and inactivation



**Figure 2-4  $I_{to1}$  and  $I_{to2}$  Experimental Fits.** Model fits to (A)  $I_{to1}$  I-V Curve, (B)  $I_{to1}$  time-to-peak and (C)  $I_{to1}$  I-V curve.

kinetics were adjusted to reproduce experimentally measured time to peak (Greenstein et al., 2000; Lue and Boyden, 1992; Tseng and Hoffman, 1989). Model time to peak falls in the middle range of published data from canine epicardial cells (Fig. 2-4B) and is a significant improvement on other published Hodgkin-Huxley formulations (Dumaine et al., 1999; Sun and Wang, 2005), in which time to peak is much faster ( $<1$  ms) than that observed experimentally. The time constant of inactivation of  $I_{to1}$  at positive voltages in canine epicardial myocytes is typically around 10 ms (Cordeiro et al., 2009; Lue and Boyden, 1992). Consistent with a

previous modeling study (Flaim et al., 2006), this time constant of inactivation resulted in a non-physiological notch and dome shape for the action potential (Simulations not shown). Following Flaim et al.,  $I_{to1}$  inactivation was accelerated ( $\tau = 5$  ms) to achieve a realistic action potential shape. The required acceleration of  $I_{to1}$  inactivation in the model may reflect in vivo acceleration of  $I_{to1}$  inactivation by intracellular  $Ca^{2+}$  (Flaim et al., 2006; Patel et al., 2004). A slow inactivation gate was included to reproduce the slow recovery from inactivation observed in canine (Liu et al., 1993).

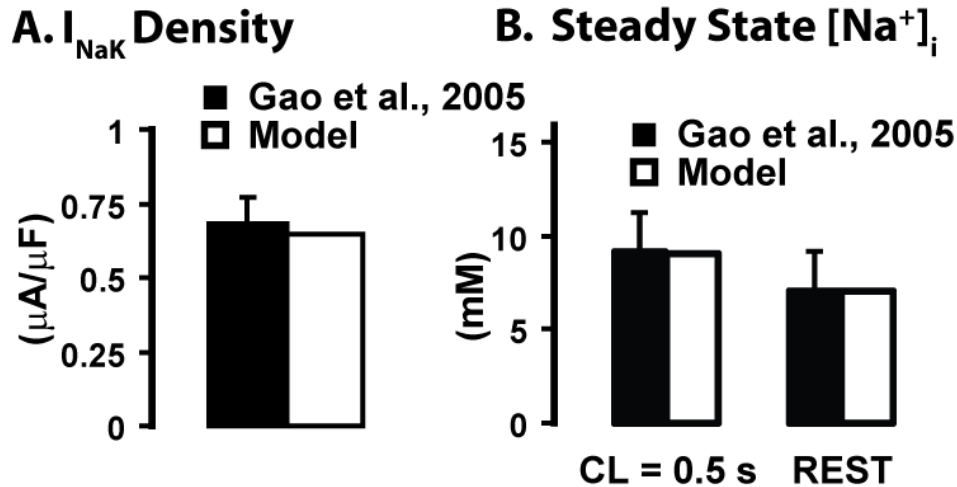
#### **2.3.4 $I_{to2}$**

Although  $I_{to2}$  remains a poorly characterized current, a novel formulation was developed to reflect the best available information in canine left ventricular myocytes (Aggarwal et al., 1997; Tseng and Hoffman, 1989; Zygmunt, 1994). Model  $I_{to2}$  is a  $Cl^-$  carrying current, activates and deactivates rapidly (Aggarwal et al., 1997; Zygmunt, 1994) and can be completely eliminated by block of SR  $Ca^{2+}$  release, consistent with experiment.  $I_{to2}$  current voltage relationship was fit to data from canine epicardial myocytes (Fig. 2-4C) (Aggarwal et al., 1997).

#### **2.3.5 $I_{NaK}$**

A recently developed  $I_{NaK}$  formulation based on data from canine left ventricular epicardial myocytes (Gao et al., 1995; Gao et al., 2005) was incorporated into the model. Model  $I_{NaK}$  density was fit to experimental data (Fig. 2-5A) (Gao et al., 2005) adjusted to 37°C using  $Q_{10} = 2.1$  (Sakai et al., 1996).  $I_{NaK}$  is a critical determinant of  $[Na^+]_i$  homeostasis in the model. Fig. 2-5B demonstrates that the model accurately reproduces  $[Na^+]_i$  at rest and after sustained pacing at  $CL = 500$  ms. Model fit to the pacing dependence of  $[Na^+]_i$  also depends on the density of 'background'  $Na^+$  fluxes ( $I_{Nab}$  and  $CT_{NaCL}$ ). While information on the magnitude and

dependence of these background fluxes on  $V_m$ ,  $[Na^+]_i$  and other factors remains limited, it is



**Figure 2-5  $I_{NaK}$  and  $[Na^+]_i$  Experimental Fits.** Model fits to experimental (A)  $I_{NaK}$  density and (B) steady-state  $[Na^+]_i$

clear that both electrogenic and non-electrogenic background  $Na^+$  fluxes exist and are important determinants of  $Na^+$  homeostasis in model and experiment (Despa et al., 2002; Gao et al., 2005; Shivkumar et al., 1997).

### 2.3.6 $I_{NaL}$

A novel  $I_{NaL}$  formulation was developed based on recent data from human and canine ventricular myocytes and reflects present understanding of the current (Zaza et al., 2008). At 24°C, human (Maltsev and Undrovinas, 2006) and canine (Maltsev et al., 2008a) ventricular  $I_{NaL}$  inactivates with fast ( $\tau \sim 50$  ms) and slow ( $\tau \sim 500$  ms) time constants during a pulse to -30 mV. These fast and slow time constants are thought to represent burst and late activating modes of the cardiac  $Na^+$  channel isoform ( $Na_v1.5$ ), respectively. Total  $I_{NaL}$  in the model is calculated as the sum of channels in the late bursting ( $LB$ ) and late scattered ( $LS$ ) modes. Time and voltage dependent inactivation of the burst and late activating modes was implemented using Hodgkin-Huxley type gates  $h_{LB}$  and  $h_{LS}$ . Considering the similarities in reported behavior in human

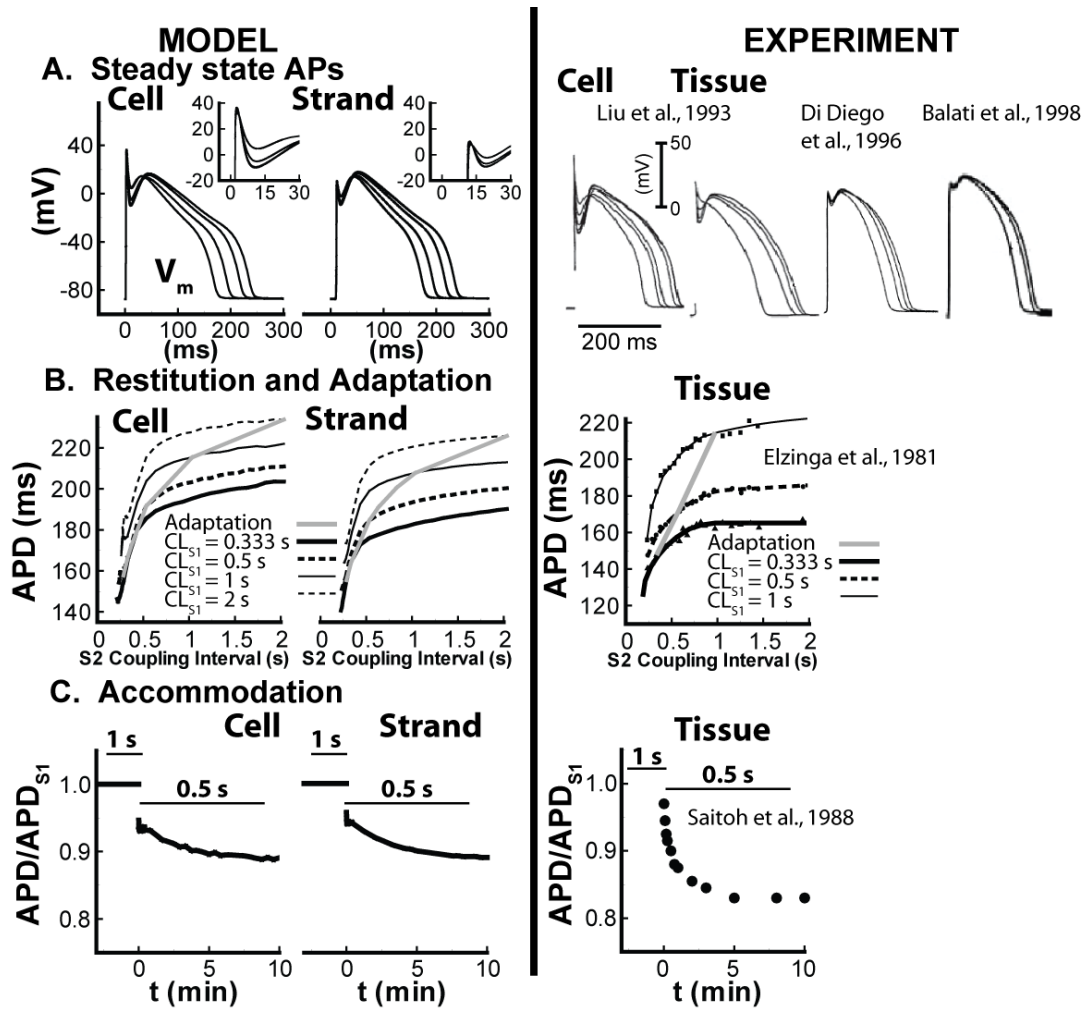
(Maltsev and Undrovinas, 2006) and canine ventricle (Maltsev et al., 2008a),  $I_{NaL}$  onset of inactivation is based on characterization of the time course of inactivation in human ventricular myocytes (Maltsev and Undrovinas, 2006), as adjusted to 37°C based on Q10 data (Maltsev and Undrovinas, 2006). The relative contribution of the late scattered mode ( $f_{LS} = 0.27$ ) and steady state voltage dependence of inactivation is based on canine ventricular myocyte data (Maltsev et al., 2008a). The time constant of recovery from inactivation was adapted from data in human ventricular myocytes (Maltsev et al., 1998), also adjusted to 37°C based on Q10 of 2.2 (Maltsev and Undrovinas, 2006). Current density was adjusted to reproduce the experimentally measured APD prolongation of approximately 40 ms after application of low concentrations of TTX in canine epicardial myocytes (Zygmunt et al., 2001). No experimental data are available on  $I_{NaL}$  in the EBZ.

## **2.4 Ionic Mechanisms of Action Potential Duration Adaptation, Restitution and Accommodation**

### **2.4.1 APD Rate Dependence in Cell and Strand**

Figure 2-6 compares the rate dependence of APD in model and experiment. The rate dependence of simulated steady-state APs is shown in Fig. 2-6A (*left*). Both cell and strand simulations exhibit the spike and dome morphology characteristic of canine epicardium (Fig. 2-6A *right*). (Liu et al., 1993). Steady-state APs in the strand show a reduced upstroke amplitude and velocity compared to the single cell due to electronic load during propagation. Maximum upstroke voltage ( $V_{max}$ ) and velocity ( $dV/dt_{max}$ ) match experiments in canine ventricular epicardium at CL = 0.8 s (Di Diego et al., 1996) (experiment  $V_{max} = 13.1 \pm 4.7$  mV,  $dV/dt_{max} = 151.8 \pm 39.8$  V/s; model  $V_{max} = 9.24$  mV,  $dV/dt_{max} = 162.6$  V/s). In addition, phase 1 notch depth is less rate dependent in the strand than in the single cell, also consistent with experiments (Fig.

2-6A) (Liu et al., 1993). We next determined APD restitution in our single cell and strand models. After pacing to steady-state at a given CL ( $CL_{S1}$ ), additional stimuli (S2) scanning the

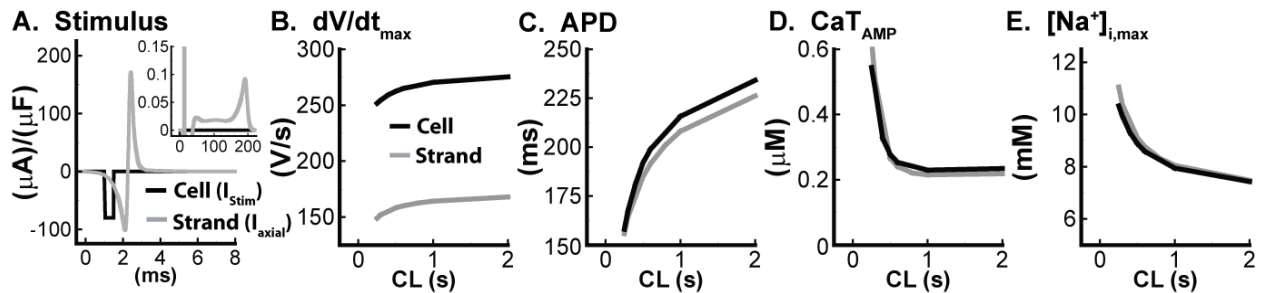


**Figure 2-6 APD Rate Dependence in Cell and Strand.** (A, left) Model steady-state APs at  $CL = 0.3, 0.5, 1$  and  $2$  s in cell and strand. Peak upstroke voltage and rate dependence of notch depth are shown in the inset. (A, right) Experimental steady state APs in single cell and three tissue preparations are included for comparison. (B, left) Model  $CL_{S1}$  dependence of APD restitution in cell and strand. Gray traces denote adaptation ( $S2$  coupling interval =  $CL_{S1}$ ). (B, right)  $CL_{S1}$  dependence of APD restitution in experiment (right ventricle, open chest dogs) (Elzinga et al., 1981). (C, left) Model accommodation of APD after a change in  $CL$  from  $1$  s to  $0.5$  s in cell and strand. (C, right) APD accommodation in canine ventricular muscle fiber experiments (Saitoh et al., 1988).

diastolic interval were applied to generate restitution curves (Fig. 2-6B, left). As the basic pacing cycle length ( $CL_{S1}$ ) decreases, restitution curves in the single cell and strand models shift towards shorter APD, consistent with experimental measurements (Fig. 2-6B, right) (Boyett and Jewell, 1978; Cherry and Fenton, 2007; Elzinga et al., 1981; Han and Moe, 1969). Fig 2-6C (left) shows the time course of APD in model cell and strand after pacing to steady-state at  $CL_{S1} = 1$  s, followed by sustained pacing at  $CL_{S2} = 0.5$  s starting at  $t = 0$ . APD at the new CL approaches steady-state after several minutes of pacing, consistent with experiments in canine ventricle (Fig. 2-6C, right) (Saitoh et al., 1988).

#### 2.4.2 Differences in cell and strand behavior

Cardiac myocyte electrophysiology and AP dynamics are often studied and simulated in



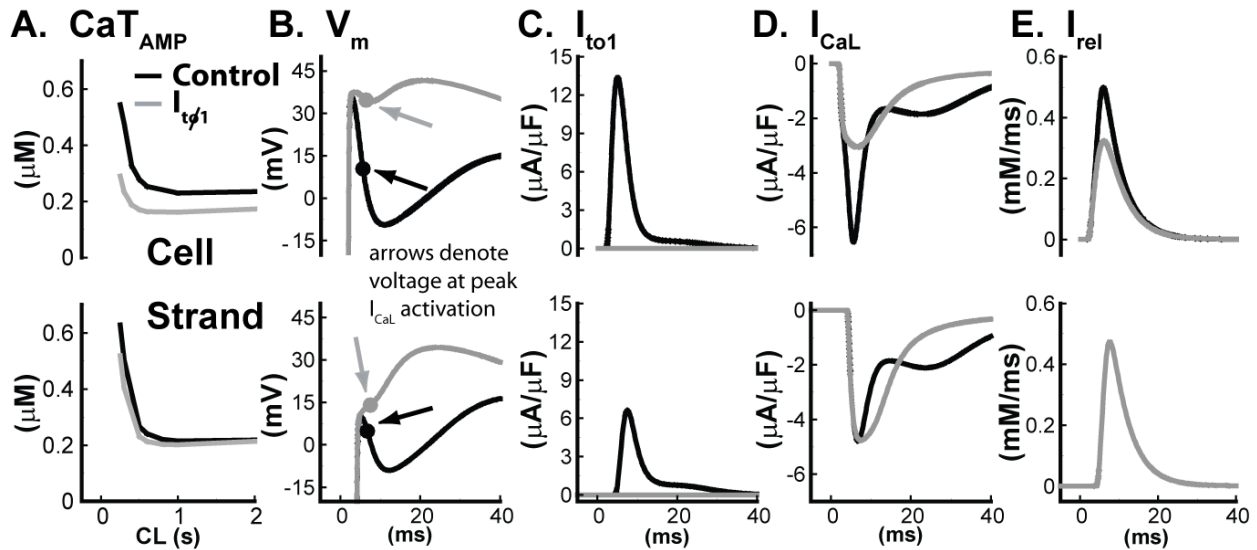
**Figure 2-7 Differences in Rate Dependence in Cell and Strand.** (A) Comparison of isolated cell stimulus (black) and strand axial current (gray). Inset shows sustained repolarizing axial current.  $CL_{S1}$  dependence of (B)  $dV/dt_{max}$  (C) APD (D)  $CaT_{AMP}$  and (E)  $[Na^+]_{i,max}$  in cell and strand simulations.

isolated cells. We hypothesized that AP dynamics would differ between isolated cell and tissue due to differences in applied stimulus and electrotonic loading. A  $-80 \mu A/\mu F$ ,  $0.5$  ms stimulus was used in single cell simulations (Fig. 2-7A). In the strand, excitatory axial current in well-coupled tissue (Fig. 2-7A) consists of i.) an initial transient depolarizing current ii.) a transient repolarizing current as the cell supplies charge to depolarizing downstream cells and iii.) a small sustained repolarizing current (Fig. 2-7A, inset). The biphasic axial current in the tissue reduces



AP upstroke velocity relative to its value in the single cell (Fig. 2-7B). In addition, steady-state tissue APD is reduced slightly (Fig. 2-7C) due to the sustained repolarizing axial current (Fig. 2-7A, inset). APD adaptation in cell and strand simulations (Fig. 2-7) is consistent with experiments (Liu et al., 1993). Both cell and strand simulations show an increase in  $\text{Ca}^{2+}$  transient amplitude ( $\text{CaT}_{\text{AMP}}$ ) (Fig. 2-7D) (Sipido et al., 2000) and maximum intracellular  $\text{Na}^+$  ( $[\text{Na}^+]_{\text{i,max}}$ ) (Fig. 2-7E) (Gao et al., 2005) with pacing rate, consistent with experiment. Differences in  $\text{CaT}_{\text{AMP}}$  and  $[\text{Na}^+]_{\text{i,max}}$  in cell and strand simulations are minimal. Subsequent results are from strand simulations unless otherwise specified.

Experimental (Saitoh et al., 1988) and computational (Greenstein et al., 2000) studies have suggested that  $I_{\text{to1}}$ , by controlling phase 1 repolarization, is an important modulator of  $I_{\text{Ca,L}}$  activation and intracellular  $[\text{Ca}^{2+}]_{\text{i}}$  release. We hypothesized that the effect of  $I_{\text{to1}}$  on  $I_{\text{Ca,L}}$



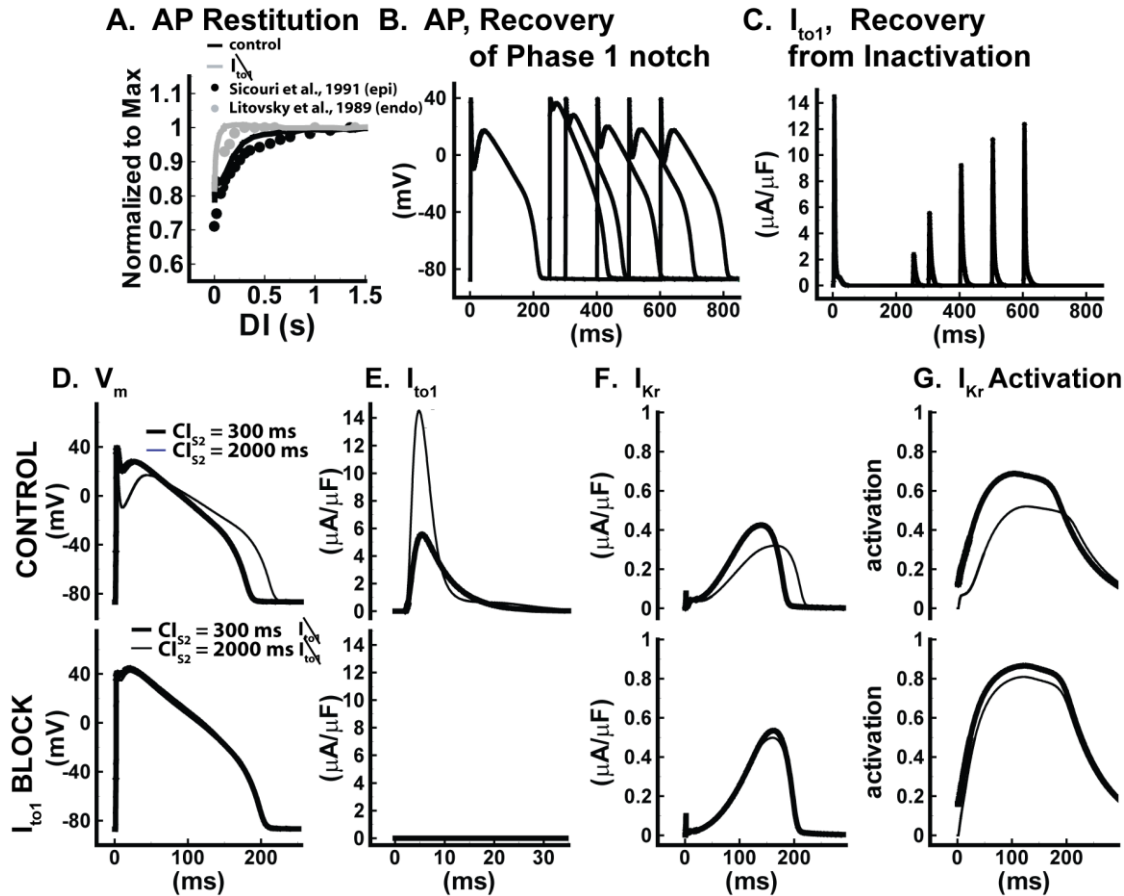
**Figure 2-8  $I_{\text{to1}}$  Block in Cell and Strand.** (A)  $\text{CaT}_{\text{AMP}}$  (B)  $V_m$  (C)  $I_{\text{to1}}$  (D)  $I_{\text{Ca,L}}$  and (E)  $I_{\text{rel}}$ , in control and after 100%  $I_{\text{to1}}$  block in cell (top) and strand (bottom) simulations. B-E are for  $\text{CL}_{\text{S1}} = 1$  s. Arrows in (B) denote time and  $V_m$  at peak  $I_{\text{Ca,L}}$ .

activation and  $[\text{Ca}^{2+}]_{\text{i}}$  release would differ between isolated cells and multi-cellular tissue due to differences in upstroke velocity and AP amplitude. Fig. 2-8A shows that, while block of  $I_{\text{to1}}$

leads to a significant reduction in  $\text{CaT}_{\text{AMP}}$  in cell simulations, reduction in  $\text{CaT}_{\text{AMP}}$  after  $I_{\text{to1}}$  block in tissue simulations is minimal. The interplay between  $V_m$ ,  $I_{\text{to1}}$ ,  $I_{\text{Ca,L}}$  and  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) in cell and strand is examined in panels B-E. We note several important differences between simulated APs in the strand and in the isolated cell. These unique features of the strand AP include 1) reduced peak upstroke voltage (Fig. 2-8B); 2) reduced  $I_{\text{to1}}$  activation (Fig. 2-8C); and 3) minimal dependence of peak  $I_{\text{Ca,L}}$  on  $I_{\text{to1}}$  activation (Fig. 2-8D). Reduced peak upstroke voltage in the strand leads to a reduction in peak  $I_{\text{to1}}$  due to the approximately linear dependence of  $I_{\text{to1}}$  activation on  $V_m$ . This reduction in  $I_{\text{to1}}$  activation leads to a reduction in phase 0 repolarization. Arrows in Fig. 2-8B denote  $V_m$  at the peak of  $I_{\text{Ca,L}}$  activation in cell and strand, respectively. In cell simulations, a large  $I_{\text{to1}}$  repolarizes  $V_m$  (Fig. 2-8C) towards the peak of the  $I_{\text{Ca,L}}$  I-V relationship (Fig. 2-8B), promoting  $I_{\text{Ca,L}}$  activation (Fig. 2-8D) and SR  $\text{Ca}^{2+}$  release (Fig. 2-8E). When  $I_{\text{to1}}$  is blocked in the cell,  $I_{\text{Ca,L}}$  activation occurs at a  $V_m$  that is far from the peak of the I-V curve (Fig. 2-8B), decreasing peak  $I_{\text{Ca,L}}$  (Fig. 2-8D) and SR  $\text{Ca}^{2+}$  release (Fig. 2-8E). For a propagating action potential, however, increased load decreases peak upstroke voltage so that it is near the peak of the  $I_{\text{Ca,L}}$  I-V curve (Fig. 2-8B, approximately 10 mV). Maximal  $I_{\text{Ca,L}}$  activation occurs shortly after the peak upstroke  $V_m$  such that the depth of the phase 1 notch plays little role in determining  $V_m$  as  $I_{\text{Ca,L}}$  is peaking. Consequently, block of  $I_{\text{to1}}$  in the strand has little effect on peak  $I_{\text{Ca,L}}$  (Fig. 2-8D) and SR  $\text{Ca}^{2+}$  release (Fig. 2-8E). Furthermore, reduced phase 1 repolarization (Fig. 2-8B) due to reduced  $I_{\text{to1}}$  activation (Fig. 2-8C) also suggests a diminished importance of  $I_{\text{to1}}$  in the strand relative to the cell.

### 2.4.3 Ionic Mechanisms of APD restitution

APD restitution will determine the response of cells to the premature stimuli that trigger arrhythmia, and is thought to be a critical determinant of the stability of reentry after initiation. The ionic mechanisms underlying the kinetics of APD restitution remain incompletely understood, however. A series of simulations was performed to characterize the most important determinants of restitution kinetics in canine epicardial myocytes. Fig. 2-9A shows that the

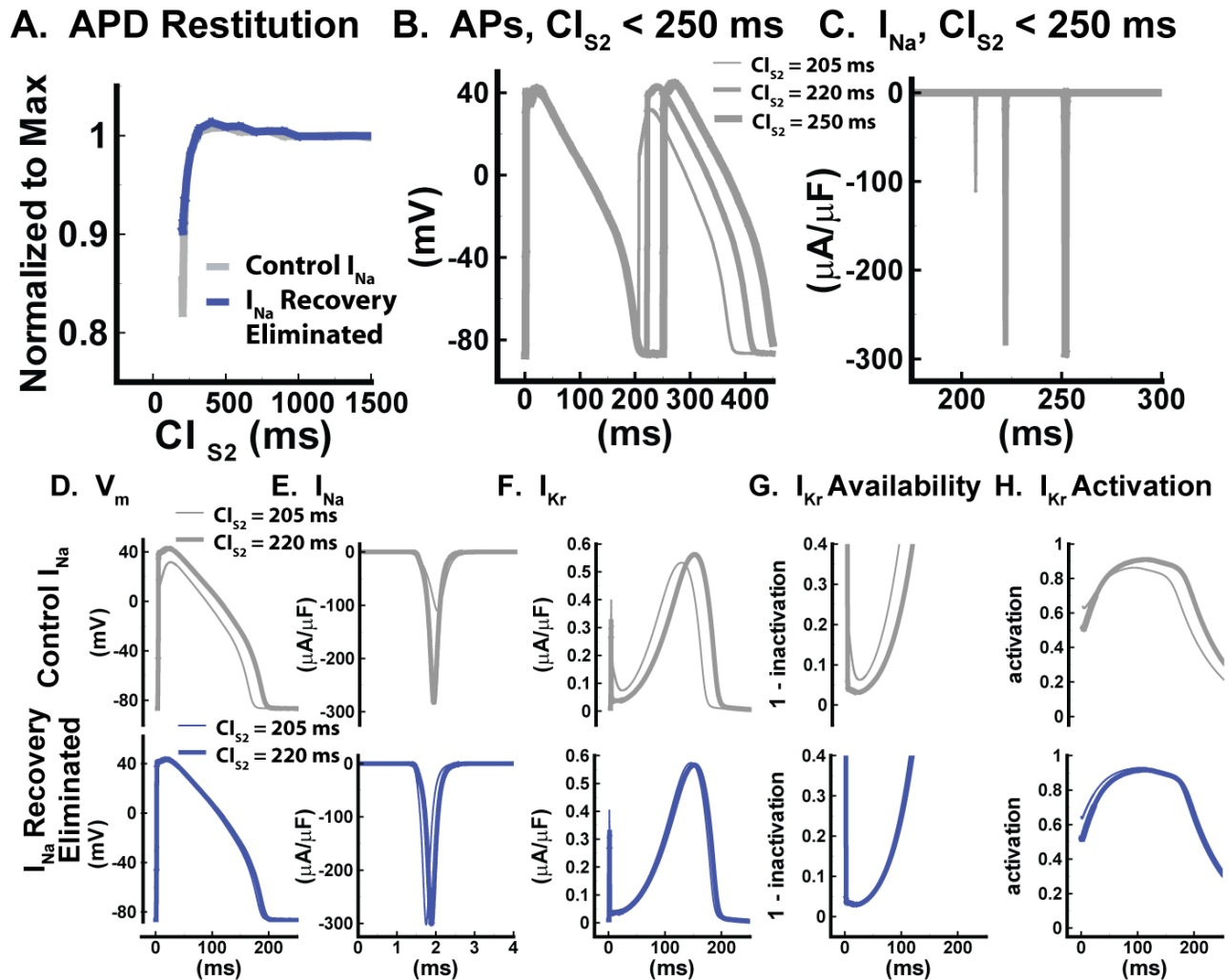


**Figure 2-9  $I_{to1}$  and APD Restitution.** (A) APD (normalized to maximum) as a function of diastolic interval (DI), during restitution at  $CL_{S1} = 2$  s in control and after 100%  $I_{to1}$  block in strand simulations. Results are compared to data from canine epicardial (Sicouri and Antzelevitch, 1991) and endocardial (Litovsky and Antzelevitch, 1989) tissue experiments. Recovery of (B) action potential phase 1 notch and (C)  $I_{to1}$  during restitution. (D)  $V_m$ , (E)  $I_{to1}$ , (F)  $I_{Kr}$  current and (G)  $I_{Kr}$  activation during APD restitution at S2 coupling intervals ( $CI_{S2}$ ) of 0.3 and 2 s in control and after  $I_{to1}$  block in the strand. Action potential after  $I_{to1}$  block at short  $CI_{S2}$  (thick, gray) and long  $CI_{S2}$  (thin, gray) have nearly identical APD.

model time course of APD restitution is consistent with experiments in epicardial tissue (Sicouri and Antzelevitch, 1991). In canine epicardium, APD gradually increases as S2 coupling interval ( $CI_{S2}$ ) increases beyond 0.3 s. When  $I_{to1}$  is blocked, the increase in APD with increasing DI is more abrupt, as observed in canine endocardium (Litovsky and Antzelevitch, 1989), consistent with the absence of  $I_{to1}$  in this tissue. Fig. 2-9B and Fig. 2-9C shows action potentials and  $I_{to1}$  during restitution in the model. Phase 1 notch depth is shallow at short DI, and gradually increases to its maximum depth at long DI (Fig. 2-9B). This increase in notch depth is the result of slow recovery of canine epicardial  $I_{to1}$  from inactivation (Fig. 2-9C), consistent with experiment (Liu et al., 1993). Figs. 2-9D-G examine the role that  $I_{to1}$  plays in APD restitution time course. At short  $CI_{S2}$ ,  $I_{to1}$  has not fully recovered from inactivation (Fig. 2-9E, top) resulting in minimal phase 1 repolarization (Fig. 2-9D, top). As  $CI_{S2}$  increases, notch depth increases as  $I_{to1}$  recovers from inactivation. Increasing notch depth slows  $I_{Kr}$  activation (Fig. 2-9G, top), since  $I_{Kr}$  activation is slower at negative voltages, leading to decreased  $I_{Kr}$  current at long  $CI_{S2}$  (Fig. 2-9F, top). The gradual increase in APD during restitution follows the slow time course of  $I_{to1}$  recovery and the suppression of  $I_{Kr}$  activation due to increasing notch depth. When  $I_{to1}$  is absent, phase 1 voltage does not change as  $CI_{S2}$  increases beyond 0.3 s (Fig. 2-9D, bottom),  $I_{Kr}$  activation is constant at  $CI_{S2}$  greater than 0.3 s (Fig. 2-9F-G, bottom) and the gradual change in APD observed in epicardial tissue is absent (Fig. 2-9A).

While block of  $I_{to1}$  eliminates the DI dependence of APD at  $CI_{S2} > 0.3$  s, a substantial degree of APD shortening still occurs at  $CI_{S2} < 0.3$  s. Additional simulations were performed to identify the ionic mechanisms responsible for APD shortening at  $CI_{S2} < 0.3$  s. Fig. 2-10A demonstrates that with normal  $I_{Na}$ , APD shortens by 20% at the shortest DI, but with  $I_{Na}$  recovery kinetics eliminated (by resetting h and j gates to 1 to remove inactivation when the S2 is applied)

APD shortens by only 10%. Fig. 2-10B-C characterize normal  $I_{Na}$  recovery and its effect on membrane  $V_m$  during restitution. In control, incomplete  $I_{Na}$  recovery at short DI (Fig. 2-10C)

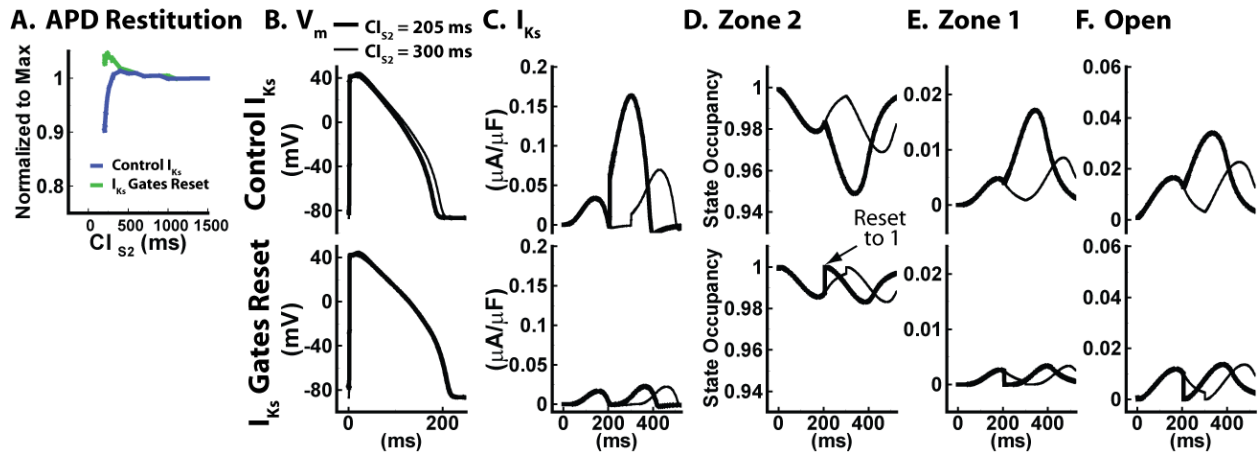


**Figure 2-10  $I_{Na}$  and APD Restitution.** (A) APD (normalized to maximum) as a function of diastolic interval ( $CI_{S2}$ ), during restitution at  $CL_{S1} = 2$  s with normal  $I_{Na}$  and after elimination of recovery of  $I_{Na}$ . Recovery of  $I_{Na}$  was eliminated by resetting  $I_{Na}$   $h$  and  $j$  gates to 0 upon application of the S2 stimulus. Simulations were performed with  $I_{to1}$  blocked to isolate the role of  $I_{Na}$ . Recovery of (B)  $V_m$  and (C)  $I_{Na}$  during restitution. (D)  $V_m$ , (E)  $I_{Na}$ , (F)  $I_{Kr}$  current, (G)  $I_{Kr}$  availability and (H)  $I_{Kr}$  activation during APD restitution at S2 coupling intervals ( $CI_{S2}$ ) of 0.3 and 2 s with normal  $I_{Na}$  and after elimination of  $I_{Na}$  recovery. Action potential after  $I_{to1}$  block at short  $CI_{S2}$  (thick, gray) and long  $CI_{S2}$  (thin, gray) have nearly identical APD.

results in reduced early AP  $V_m$  (Fig. 2-10B). Maximum  $I_{Na}$  magnitude and upstroke  $V_m$  are reached within 50 ms after repolarization of the previous AP. The time constant of  $I_{Na}$  recovery from inactivation reflects model fit to data on the recovery of  $dV/dt_{max}$  in canine epicardial myocytes. Ionic mechanisms underlying the effects of  $I_{Na}$  recovery on APD are examined in Panels D-H. At short  $CI_{S2}$ , ( $CI_{S2} = 205$  ms) incomplete recovery of  $I_{Na}$  (Fig. 2-10E, top) decreases early AP  $V_m$  (Fig. 2-10D, top). Decreased early AP  $V_m$  leads to increased  $I_{Kr}$  availability (Fig. 2-10E G, top) and  $I_{Kr}$  current (Fig. 2-10F, top), promoting APD shortening. Although decreased membrane  $V_m$  also leads to slower  $I_{Kr}$  activation, the increase in  $I_{Kr}$  availability dominates and leads to net enhancement of  $I_{Kr}$ . The bottom panels in Figs. 2-10D-H confirm that elimination of  $I_{Na}$  recovery (Fig. 2-10E, bottom) reduces the  $CI_{S2}$  dependence of AP  $V_m$ ,  $I_{Kr}$  availability,  $I_{Kr}$  current and APD.

The role of  $I_{Ks}$  in canine APD restitution is examined in Figure 2-11. Fig. 2-11A demonstrates that elimination of the  $CI_{S2}$  dependence of  $I_{Ks}$  activation (by resetting channels to the Zone 2 closed state  $CI$  when the S2 stimulus was applied) decreases APD shortening at premature  $CI_{S2}$  by 10%. Note that  $I_{to1}$  and  $I_{Na}$  recovery were eliminated in order to isolate the effect of  $I_{Ks}$  on restitution. Figs. 2-11 B-F examine the ionic mechanisms underlying the contribution of  $I_{Ks}$  to restitution. The 17 state  $I_{Ks}$  model can be divided into two open states, five *Zone 1* closed states that can activate rapidly and ten ‘deep’ *Zone 2* closed states which require slow transitions to *Zone 1* before activation is possible. At  $CI_{S2} = 205$  ms, a significant fraction of channels activated during the S1 AP remain in *Open* states (Fig. 2-11 D), while an additional fraction occupy *Zone 1* (Fig. 2-11F) states. When a premature stimulus is applied, channels that are already in *Open* states and those moving rapidly from *Zone 1* to *Open* states combine to give prominent  $I_{Ks}$  activation (Fig. 2-11 C). Peak activation of  $I_{Ks}$  at  $CI_{S2} = 260$  ms is markedly

increased relative to peak density at  $CI_{S2} = 300$  ms. As DI increases, channels activated during the S1 stimulus move from *Zone 1* and *Open* states to *Zone 2*, resulting in less prominent  $I_{Ks}$

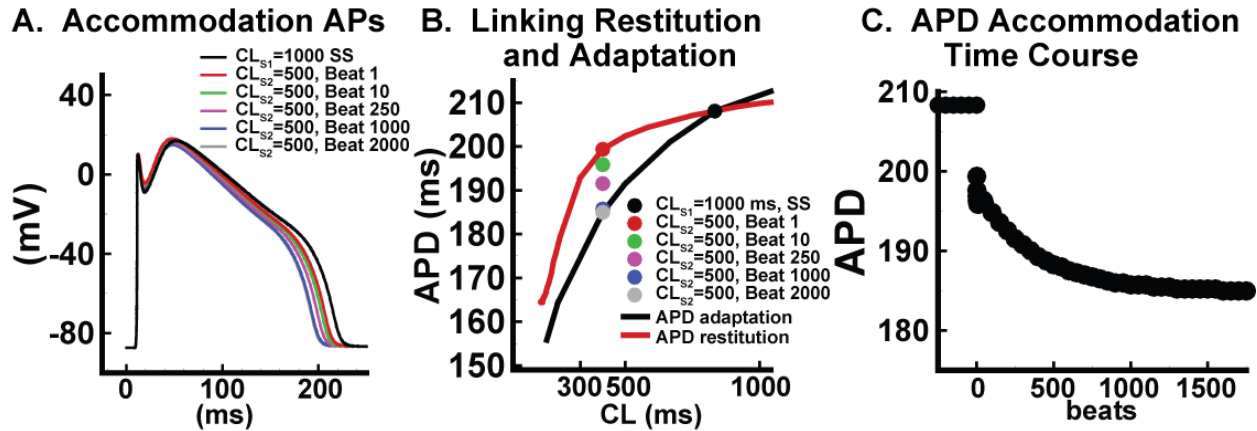


**Figure 2-11  $I_{Ks}$  and APD Restitution.** (A) APD (normalized to maximum) as a function of diastolic interval ( $CI_{S2}$ ), during restitution at  $CL_{S1} = 2$  s with normal  $I_{Ks}$  and after elimination of recovery of  $I_{Ks}$ . Recovery of  $I_{Ks}$  was eliminated by resetting  $I_{Ks}$  states to *Zone 2* upon application of the S2 stimulus. Simulations were performed with  $I_{to1}$  blocked and  $I_{Na}$  recovery eliminated to isolate the role of  $I_{Ks}$ . (B)  $V_m$ , (C)  $I_{Ks}$ , and state occupancy of (D) *Zone 2*, (E) *Zone 1* and (F) *Open State* during APD restitution at S2 coupling intervals ( $CI_{S2}$ ) of 205 and 300 ms with normal  $I_{Ks}$  (top) and after elimination of  $I_{Ks}$  recovery (bottom).

activation and reduced APD prolongation for more mature stimuli. The bottom panels of Figs. 2-11 B-E confirm the proposed role of  $I_{Ks}$  in APD shortening. If  $I_{Ks}$  channels are reset to *Zone 2* when the S2 stimulus is applied, the rapid movement to *Zone 1* and *Open* states during the S2 AP is eliminated.  $I_{Ks}$  activation is weak at  $CI_{S2} = 205$  and 300 ms and the  $CI_{S2}$  dependence of APD is dramatically decreased. Note that when  $I_{to1}$  and the  $CI_{S2}$  dependence of  $I_{Na}$  and  $I_{Ks}$  are eliminated, APD no longer shortens at premature  $CI_{S2}$ . This result suggests that these three currents are the most critical determinants of APD shortening during restitution in canine epicardium.

#### 2.4.4 Ionic Mechanisms of APD accommodation

Figure 2-12 describes important characteristics of APD accommodation in the canine epicardial model. The black trace in Fig. 2-12 A shows a steady state AP at  $CL_{S1} = 1000$  ms. This APD also can be represented by the black circle on the APD adaptation curve in Fig. 2-12 B. The red trace in Fig. 2-12 A shows the 1st beat after pacing rate is changed to  $CL_{S2} = 500$  ms. This APD can also be represented by the red circle on the restitution curve in Fig. 2-12 B.



**Figure 2-12 Linking Accommodation, Restitution and Adaptation.** (A) Steady state APD at  $CL_{S1} = 1000$ , and selected action potentials after the transition from  $CL_{S1} = 1000$  ms to  $CL_{S2} = 500$  ms. (B) APD adaptation, APD restitution at  $CL_{S1} = 1000$  ms, and APDs for selected beats after the transition from  $CL_{S1} = 1000$  ms to  $CL_{S2} = 500$  ms. (C) The time course of APD accommodation after a the transition from  $CL_{S1} = 1000$  ms to  $CL_{S2} = 500$  ms.

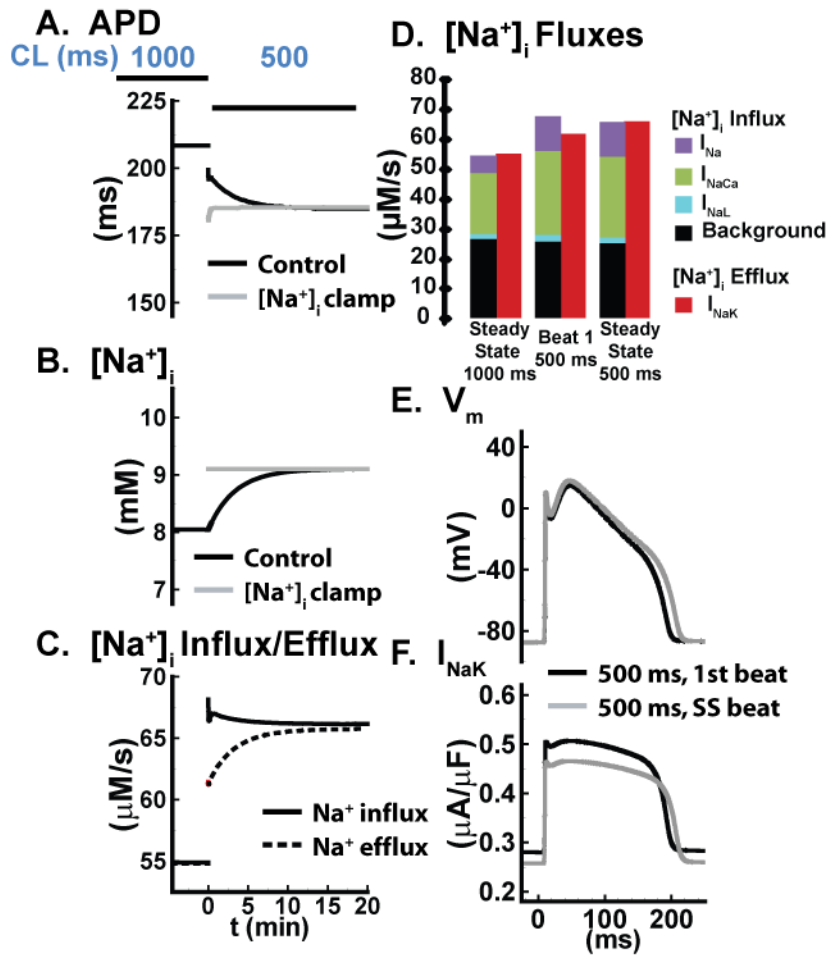
Green, blue, and magenta traces in Fig. 2-12 A and points in Fig. 2-12B show APs and APDs for the transition from steady state at  $CL_{S1} = 1000$  ms to steady state at  $CL_{S2} = 500$  ms (gray trace or circle). The transition from steady state at  $CL_{S1} = 1000$  ms to steady state at  $CL_{S2} = 500$  ms can therefore be thought of as consisting of two processes; 1) restitution, the APD shortening that occurs for the 1st beat at the new  $CL_{S2}$  and 2) accommodation, which describes further APD shortening after the 1st beat. Fig. 2-12C shows that approximately 2000 beats (1000 seconds) of pacing are required to reach steady state at  $CL_{S2}$ . The qualitative features relating APD adaptation and restitution via APD accommodation described in the model are consistent with



observations in a large range of tissue and preparations. As shown in Fig. 2-12B, the slope of the APD adaptation curve is steeper than that of the APD restitution curve at their intersection, since accommodation leads to APD shortening after a change to a shorter  $CL_{S2}$ . This relationship between the slope of restitution and adaptation has been observed in, for example, canine endocardium (Cherry and Fenton, 2006; Elzinga et al., 1981), canine ventricular epicardial tissue (Elzinga et al., 1981; Han and Moe, 1969), human ventricular epicardium (Franz et al., 1988) and bullfrog myocardium (Kalb et al., 2004). The time required for APD to reach steady state after a change in CL ranges from 1-3 (Franz et al., 1988; Lux and Ershler, 2003; Saitoh et al., 1988; Williams et al., 1999) to as many as 5-10 (Saitoh et al., 1988) minutes. While accommodation is a widely observed phenomenon and may play a role in the dynamics of fibrillation (Baher et al., 2006; Toal et al., 2009), the phenomena is poorly understood and is not reproduced by many available models (Baher et al., 2006).

The ionic mechanisms of APD accommodation are explored in Figure 2-13. When the pacing CL is changed from 1000 to 500 ms, APD decreases abruptly at the 1st beat, then decreases over a time course of minutes until reaching steady state at the new CL (Fig. 2-13A). Figure 2-13B demonstrates that  $[Na^+]_i$  approaches steady state at the new CL with a similar time course. We therefore hypothesized that this  $[Na^+]_i$  increase was the primary cause of APD decrease during accommodation. Consistent with this hypothesis, clamp of  $[Na^+]_i$  to its steady state value at CL = 500 ms eliminates accommodation during the transition from CL = 1000 ms to CL = 500 ms. Further analysis was performed to investigate the ionic determinants of  $[Na^+]_i$  increase and equilibration at the new CL. At steady state at CL = 1000 ms,  $Na^+$  influx and efflux are matched (Fig. 2-13 C). Influx and efflux were calculated for each cycle in terms of the change in  $[Na^+]_i$  divided by the cycle length. Figure 2-13 D shows that  $I_{NaCa}$ ,  $I_{Na}$ ,  $I_{NaL}$  and

background fluxes ( $I_{NaB}$  and  $I_{NaL}$ ) are the sources of  $Na^+$  influx in canine ventricular myocytes, while  $I_{NaK}$  is the only source of  $Na^+$  efflux. After CL changes to 500 ms,  $Na^+$  influx exceeds

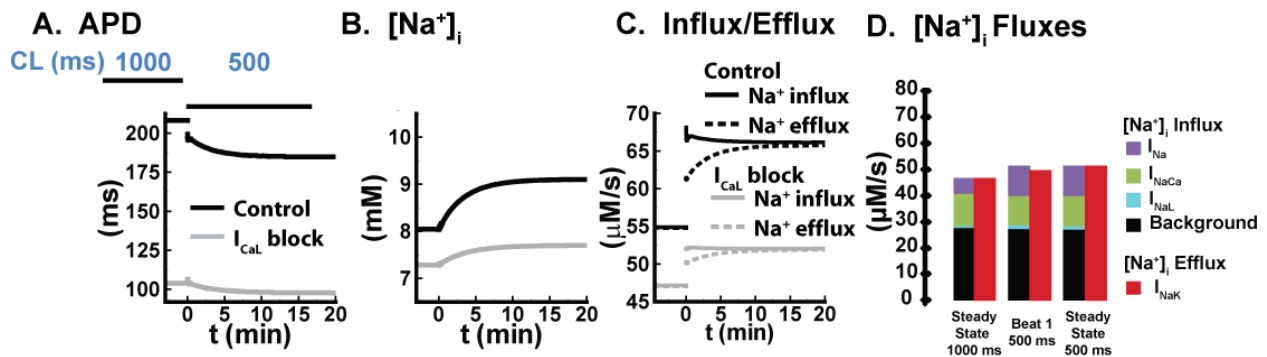


**Figure 2-13  $[Na^+]_i$ ,  $I_{NaK}$  and APD Accommodation.** Accommodation of (A) APD and (B)  $[Na^+]_i$  after a change in CL from 1 s to 0.5 s in control and with  $[Na^+]_i$  clamped to its steady-state value at CL = 0.5 s. (C) Integrated  $Na^+$  fluxes calculated for each beat during the transition from steady-state at CL = 1 s to steady-state at CL = 0.5 s. Integrated  $Na^+$  influx was calculated as  $I_{NaCa} + I_{Na} + I_{NaL} + I_{NaB} + CT_{NaCl}$  and  $Na^+$  efflux as  $I_{NaK}$ . (D) Components of integrated  $Na^+$  influx and efflux at steady state  $CL_{S1} = 1000$  ms, beat 1 at  $CL_{S2} = 500$  ms and steady state at  $CL_{S2} = 500$  ms. (E)  $V_m$  and (F)  $I_{NaK}$  for the 1<sup>st</sup> beat and a steady-state (SS) beat after a change in CL from 1 s to 0.5 s.

efflux until  $Na^+$  influx increases to match efflux and  $[Na^+]_i$  reaches equilibrium. After a change from CL = 1000 ms to CL = 500 ms,  $Na^+$  flux via  $I_{NaCa}$  and  $I_{Na}$  increase, but this increase is not

matched by the increase in  $I_{NaK}$ . The shorter DI at the faster CL prevents  $I_{NaK}$  from extruding the  $[Na^+]_i$  that enters the cell during the action potential, primarily via  $I_{Na}$  and  $I_{NaCa}$ . The net  $Na^+$  influx drives an increase in  $[Na^+]_i$ , which promotes increased  $I_{NaK}$  activity until influx and efflux match. The increase in  $I_{NaK}$  activity increases the net outward current from the 1st to the SS beat at CL = 500 ms (Fig. 2-13 F), providing the primary mechanisms for APD accommodation.

A recent experimental study has shown that block of  $I_{CaL}$  dramatically attenuates APD accommodation in rabbit myocytes (Tolkacheva et al., 2006). Fig. 2-14A demonstrates that block of  $I_{CaL}$  also attenuates APD accommodation in the canine epicardial model during a CL change from 1000 to 500 ms. This attenuation of APD accommodation is accompanied by a decrease in the degree of  $[Na^+]_i$  accumulation (Fig. 2-14B). Fig. 2-12C demonstrates that this



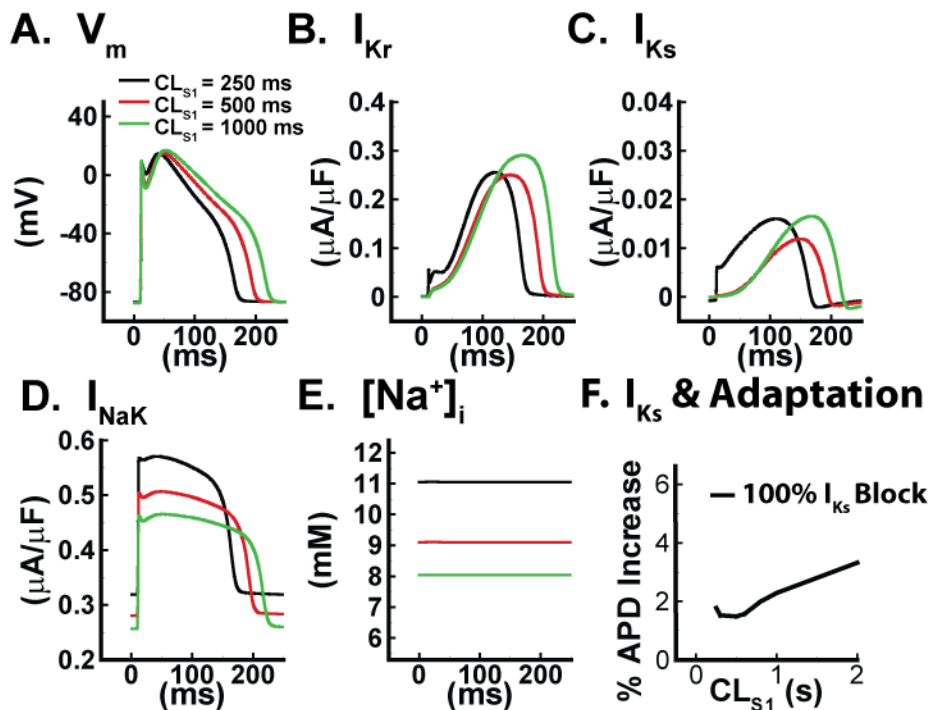
**Figure 2-14  $I_{CaL}$  and APD Accommodation.** Accommodation of (A) APD and (B)  $[Na^+]_i$  after a change in CL from 1 s to 0.5 s in control and  $I_{CaL}$  blocked. (C) Integrated  $Na^+$  fluxes calculated for each beat during the transition from steady-state at CL = 1 s to steady-state at CL = 0.5 s in control and with  $I_{CaL}$  blocked. Integrated  $Na^+$  influx was calculated as  $I_{NaCa} + I_{Na} + I_{NaL} + I_{Nab} + CT_{NaCl}$  and  $Na^+$  efflux as  $I_{NaK}$ . (D) Components of integrated  $Na^+$  influx and efflux at steady state  $CL_{S1} = 1000$  ms, beat 1 at  $CL_{S2} = 500$  ms and steady state at  $CL_{S2} = 500$  ms after  $I_{CaL}$  block.

decrease in  $[Na^+]_i$  accumulation is caused by a decreased imbalance between  $Na^+$  influx and efflux after the transition to CL = 500 ms. Fig. 2-14D examines the contributions of ion channels and exchangers to the decreased imbalance in  $Na^+$  fluxes after a change to CL = 500

ms. In control (Fig. 2-14D), transition to a faster CL results in a large increase in  $I_{NaCa}$   $Na^+$  influx. This increase in  $I_{NaCa}$  forward mode activity ( $Na^+$  entry/ $Ca^{2+}$  efflux) is driven by increased  $Ca^{2+}$  influx via  $I_{CaL}$  at the faster rate.  $Ca^{2+}$  influx increases because a similar degree of  $I_{CaL}$   $Ca^{2+}$  entry occurs during the AP at the faster CL, but this similar amount of  $Ca^{2+}$  entry occurs during a shorter CL. When  $I_{CaL}$  is blocked,  $Ca^{2+}$  efflux does not increase at the faster rate and the increase in  $Na^+$  influx due to increased  $I_{NaCa}$   $Na^+$  entry at the faster rate is attenuated, leading to decreased  $Na^+$  accumulation and APD accommodation.

#### 2.4.5 Ionic Mechanisms of APD adaptation

The analysis of APD restitution and accommodation provided above leads to a clearer understanding of the ionic mechanisms underlying APD adaptation as demonstrated in Figure 2-15. The DI dependence of  $I_{Kr}$  (Fig. 2-15 B) due to mechanisms described in the above section



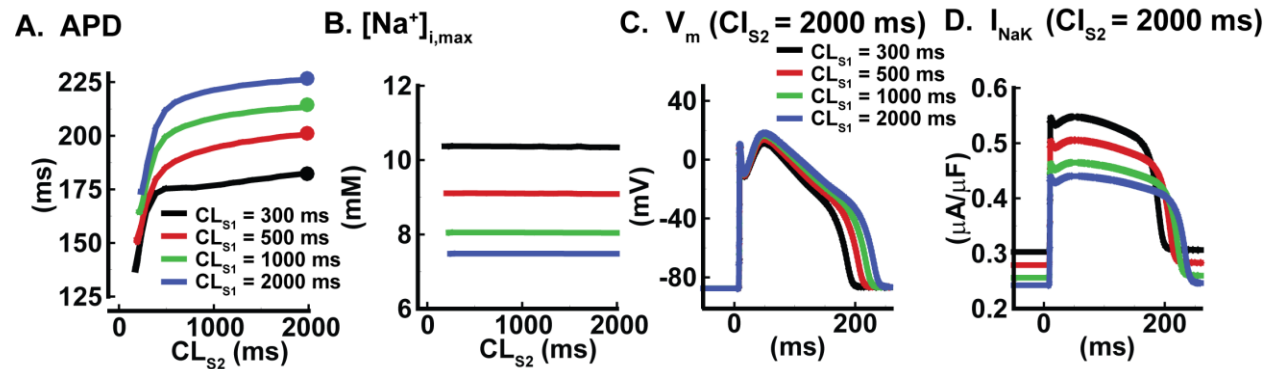
**Figure 2-15 Mechanism of APD adaptation.**  $V_m$ ,  $I_{Kr}$ ,  $I_{Ks}$ ,  $I_{NaK}$  and  $[Na^+]_i$  at steady state  $CL_{S1} = 250, 500$  and  $1000$  ms. (F) APD prolongation after  $I_{Ks}$  block as a function of  $CL_{S1}$ .

on restitution leads to increased  $I_{Kr}$  magnitude and promotes APD shortening at  $CL_{S1} = 250$  ms. The accumulation of  $[Na^+]_i$  that occurs with sustained pacing at fast  $CL_{S1}$  (Fig. 2-15E) described in the above section on accommodation leads to increased  $I_{NaK}$  (Fig. 2-15D), which also promotes APD shortening at  $CL_{S1} = 250$  ms. Although accumulation of  $I_{Ks}$  in Open and Zone 1 states at short DI does increase  $I_{Ks}$  activation at  $CL_{S1} = 250$  ms, block of  $I_{Ks}$  reveals that this current plays a limited role in adaptation. While elimination of  $I_{Ks}$  DI dependence can lead to an approximately 10% increase in APD at the shortest DI during restitution (Fig. 2-15A), APD increases by  $< 2\%$  after  $I_{Ks}$  block at  $CL_{S1} = 250$  ms. It is important to note that the processes of restitution and accommodation are not independent. For instance, sustained pacing at fast  $CL_{S2}$  shortens APD via accommodation, which in turn tends to prolong DI ( $DI = CL_{S2} - APD$ ) and lengthen APD via restitution. However, in spite of their interdependence, studying the two processes separately provides important insight into ionic mechanisms of APD rate dependence.

#### **2.4.6 Ionic Mechanisms of S1 Dependent APD Restitution**

Several experimental studies have shown that the APD restitution curve shifts to shorter APD as basic pacing CL decreases (Boyett and Jewell, 1978; Cherry and Fenton, 2007; Elzinga et al., 1981; Han and Moe, 1969). The S1 dependence of restitution may play a role in the stability of fibrillation (Baher et al., 2006), but many recently published models fail to reproduce this phenomena (Cherry and Fenton, 2006). Consistent with experimental observations, our model reproduces the dependence of restitution on the rate of S1 pacing (Fig. 2-16). The level of  $[Na^+]_i$  during restitution depends on  $CL_{S1}$ , with pacing at fast  $CL_{S1}$  leading to  $[Na^+]_i$  accumulation during accommodation (Fig. 2-16) as described above APD. Fig. 2-16C and Fig. 2-16D show APs and  $I_{NaK}$  for a range of  $CL_{S1}$ , but with  $CL_{S2}$  held constant, in order to eliminate restitution dependent effects on APD. The shortening of APD with decreasing  $CL_{S1}$  in Fig. 2-

16C results from the increase in  $I_{NaK}$  activity in Fig. 2-16D, which is in turn due to the  $CL_{S1}$

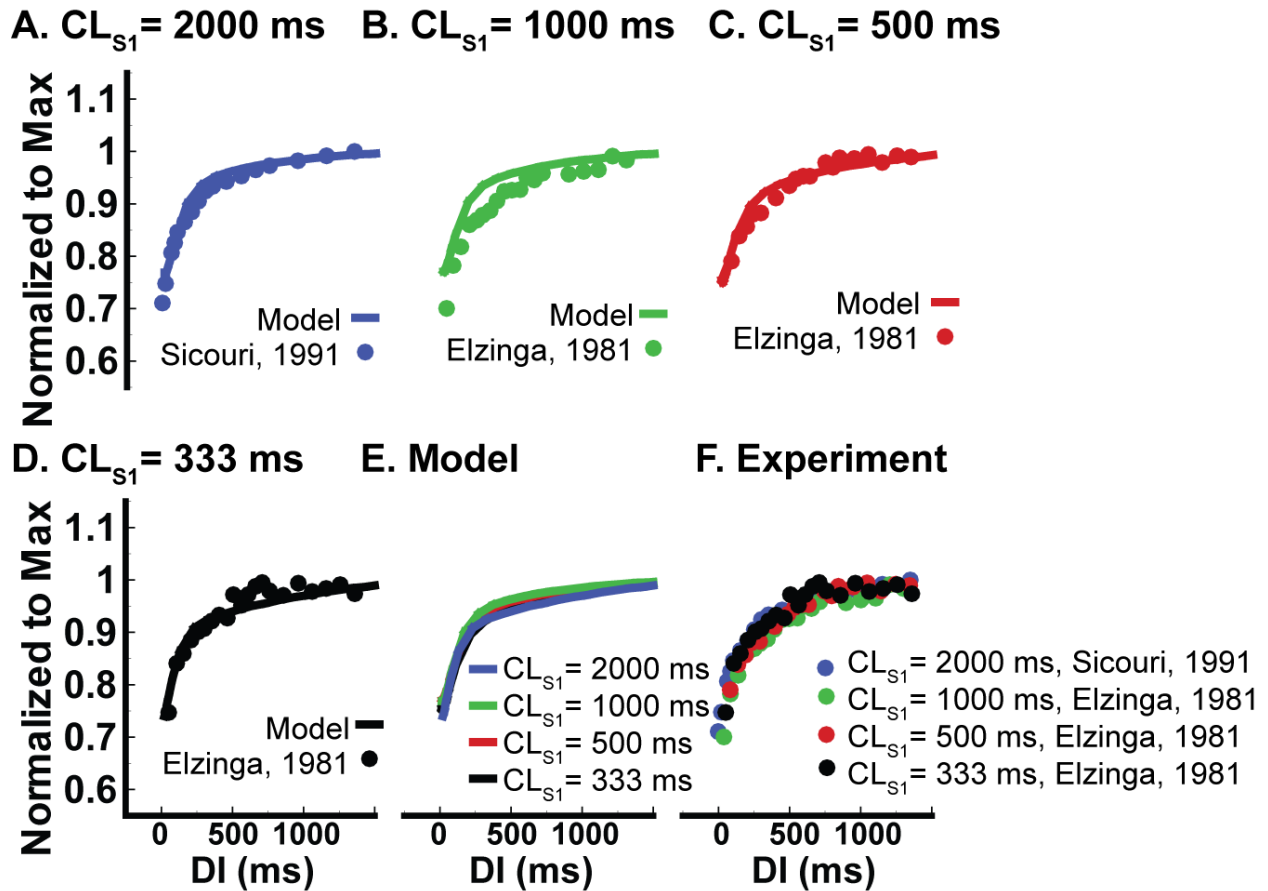


**Figure 2-16 Mechanism of S1 Dependent APD Restitution.** S1 dependence of (A) APD restitution and (B)  $[Na^+]_{i,max}$ . (C)  $V_m$  and (D)  $I_{NaK}$  at  $CL_{S2} = 2000$  ms and  $CL_{S1} = 300, 500, 1000$  and  $2000$  ms.

dependence of  $V_m$  to  $[Na^+]_i$  shown in Fig. 2-16B.

Fig. 2-17 examines the S1 dependence of APD restitution kinetics in model and experiment. Panels 2-17A-D demonstrate that the model reproduces the kinetics of APD restitution at  $CL_{S1} = 2000$ ,  $CL_{S1} = 1000$ ,  $CL_{S1} = 500$  and  $CL_{S1} = 333$  ms. Comparison at  $CL_{S1} = 2$  s (Fig. 2-17A) is to left ventricular (LV) epicardial tissue experiment (Elzinga et al., 1981; Sicouri and Antzelevitch, 1991), while  $CL_{S1} = 1$  s (Fig. 2-17B),  $0.5$  s (Fig. 2-17C) and  $0.333$  s (Fig. 2-17D) is to right ventricular (RV) epicardial tissue experiment (Elzinga et al., 1981; Sicouri and Antzelevitch, 1991) because left ventricular data at these rates were not available. Fig. 2-17E and Fig. 2-17F demonstrate that model and experimental restitution kinetics show a small dependence on  $CL_{S1}$ . Differences in restitution kinetics between the model and the RV experiments are relatively small and may be due to differences in LV and RV  $I_{to1}$  expression and kinetics (Di Diego et al., 1996),  $Ca^{2+}$  handling or other heterogeneities. Additional simulations (not shown) suggest that rate dependent SR  $Ca^{2+}$  loading and  $Ca^{2+}$  release can have significant effects on APD restitution. S1 dependent SR  $Ca^{2+}$  loading and  $Ca^{2+}$  release and the effects of

this S1 dependent  $\text{Ca}^{2+}$  release on APD are underexplored and poorly understood phenomena and should be explored in future modeling and experimental studies.



**Figure 2-17 Rate Dependence of APD Restitution Kinetics.** APD restitution time course in model and experiment at (A)  $CL_{S1} = 2000$  ms, (B)  $CL_{S1} = 1000$  ms, (C)  $CL_{S1} = 500$  ms and  $CL_{S1} = 333$  ms. Comparison of APD restitution kinetics in model (E) and experiment (F)

## 2.5 Discussion

The rate dependent phenomena examined in this study are thought to play an important role in the dynamics of arrhythmia (Baher et al., 2007; Karma, 1994; Mironov et al., 2008). The model presented here reproduces experimentally observed APD adaptation, restitution and accommodation in both cell and tissue. While the rate dependence of model APD, CaT and  $[\text{Na}^+]_i$  accumulation are similar in cell and strand, our simulation results show that the effects of  $I_{to1}$  block on ion accumulation differ. Other simulation results (Qu, 2004) have suggested that

APD restitution kinetics and the transition to APD alternans and more complex excitation patterns in cell and tissue also differ. Taken together, these results suggest that experimental results in isolated myocytes should be extrapolated to the multicellular tissue with caution.

Recent studies have emphasized limitations in our mechanistic understanding of the rate dependence of action potential duration (Cherry and Fenton, 2007). The detailed, physiologically based mathematical descriptions of critical ionic currents, pumps and exchangers incorporated into the model lead to novel insight into underlying ionic mechanisms. Specific mechanistic insights generated by our study include: i.)  $I_{to1}$  potentiates  $I_{Ca,L}$  and SR  $Ca^{2+}$  release during early AP repolarization in isolated cells but not in multicellular tissue. ii.)  $I_{to1}$  plays an important role in APD restitution due to its slow recovery kinetics. As DI increases,  $I_{to1}$  recovery and phase 1 notch depth increase, suppressing  $I_{Kr}$  activation and lengthening APD. iii.)  $I_{Ks}$  plays a limited role in repolarization of paced APs, but plays an important role in APD shortening for premature stimuli. iv.)  $I_{Ca,L}$  plays a critical role in APD accommodation and memory. As a response to  $Ca^{2+}$  entry via  $I_{Ca,L}$  at fast rates, forward mode  $I_{NaCa}$  increases, leading to increased  $Ca^{2+}$  efflux and  $Na^+$  influx. Sodium accumulation increases repolarizing  $I_{NaK}$ , thereby shortening APD during accommodation. v.)  $I_{Ca,L}$  dependent increase in  $[Na^+]_i$  and  $I_{NaK}$  at fast rates is responsible for the shift in APD restitution curves towards shorter APD.

Previous experiments and simulation studies have suggested that  $I_{to1}$ , by increasing phase 1 repolarization, increases peak  $I_{Ca,L}$  (the trigger for SR release) and consequently SR  $Ca^{2+}$  release (Greenstein et al., 2000; Sah et al., 2002). These studies showed enhanced  $I_{Ca,L}$  and SR  $Ca^{2+}$  release when phase 1 repolarization increases the  $I_{Ca,L}$  driving force. While this scenario is relevant to an isolated, directly stimulated myocyte, model simulations indicate that it does not apply to the situation *in vivo*, where electrotonic loading from neighboring cells weakens the AP



upstroke. Our simulations suggest that, for propagating APs,  $I_{to1}$ -dependent phase 1 repolarization has little effect on peak  $I_{CaL}$  and SR  $Ca^{2+}$  release. In addition, simulations show that the weakened AP upstroke in the tissue decreases  $I_{to1}$  activation and phase 1 repolarization.  $I_{to1}$  has been accorded a role in conduction (Huelsing et al., 2001; Wang et al., 2000) and in electrophysiological remodeling in various diseased states (Kaab et al., 1996; Lue and Boyden, 1992; Yu et al., 1999). Our results suggest that predictions of the effect of  $I_{to1}$  and  $I_{to1}$  block based on single cell simulations and experiments may not apply to the *in vivo* situation. In contrast, model simulations predict that  $I_{to1}$  plays a significant role in APD restitution. The interplay between  $I_{Kr}$  activation and the slow recovery kinetics of  $I_{to1}$  leads to a gradual increase in APD during restitution, while  $I_{to1}$  block results in a more abrupt APD increase. This result provides a mechanistic explanation for the correlation between notch depth and APD observed in canine ventricular tissue (Litovsky and Antzelevitch, 1989). The time course of APD restitution is thought to play an important role in the stability of ventricular arrhythmias (Karma, 1994). Our results suggest that experimentally observed heterogeneity in  $I_{to1}$  density and recovery kinetics (Oudit et al., 2001; Patel and Campbell, 2005) may play a role in the stability of arrhythmias in different species, regions of tissue or pathophysiological states. Canine experiments have shown transmural heterogeneity in  $I_{NaL}$  (Zygmunt et al., 2000),  $I_{to1}$  (Liu et al., 1993),  $I_{Ks}$  (Liu and Antzelevitch, 1995) and  $I_{NaCa}$  (Zygmunt et al., 2001). The epicardial model accurately reproduces endocardial restitution kinetics after blocking only  $I_{to1}$ , suggesting that  $I_{to1}$  is the dominant determinant of heterogeneity in restitution between the two cell types.

Experimental measurements of the effect of  $I_{Ks}$  block on APD in canine ventricle have ranged from near 0 (Stengl et al., 2003) to as much as 30% (Shimizu and Antzelevitch, 1998). Varro et al. (Varro et al., 2001) report a modest prolongation (3-7%) and biphasic CL

dependence after chromanol block, consistent with our simulation results. Although the role of  $I_{Ks}$  in repolarization remains controversial, LQT mutations linked to genes for  $I_{Ks}$   $\alpha$ - and  $\beta$ -subunits (Splawski et al., 1997) argue for an important role in repolarization. Our simulations demonstrate that slow activation and relatively low density limit the role of  $I_{Ks}$  in AP repolarization at physiological heart rates in the absence of  $\beta$ -adrenergic stimulation, consistent with our previous modeling studies (Hund and Rudy, 2004). Simulations with a detailed Markov model of  $I_{Ks}$  predict an important role for  $I_{Ks}$  in APD restitution. During deactivation, channels accumulate in closed states (Zone 1) where rapid activation in response to a premature stimulus is possible, leading to an important role for  $I_{Ks}$  in APD restitution at short DI. A role for  $I_{Ks}$  in APD shortening at fast  $CL_{S1}$  via accumulation in closed states has been previously shown in the guinea pig and human (Silva and Rudy, 2005). While major differences exist between  $I_{Ks}$  density and kinetics in guinea pig and canine, the mechanism for enhanced participation of  $I_{Ks}$  at fast  $CL_{S1}$  during adaptation and at short DI during restitution are similar in the two species. Importantly, while large density in the guinea pig ensures an important role for  $I_{Ks}$  at all rates, we find that  $I_{Ks}$  plays a major role in canine restitution at short DI, despite a limited role at longer physiological CL. While the effect of  $I_{Ks}$  block on paced APs has been studied experimentally, no studies on the role of  $I_{Ks}$  in restitution have been reported.  $I_{Ks}$  is also likely to play an important role in repolarization in the presence of  $\beta$ -adrenergic stimulation (Volders et al., 2003) or for abnormally prolonged APD (Varro et al., 2001).

Recent simulations have addressed the role of APD accommodation and short-term memory in the dynamics of arrhythmia (Baher et al., 2007; Boyett and Jewell, 1978; Mironov et al., 2008). Both simulations (Baher et al., 2007) and experiments (Tolkacheva et al., 2006) have emphasized uncertainty in the ionic mechanisms underlying this phenomenon. The results

presented here predict a major role for  $I_{Ca,L}$  driven  $[Na^+]_i$  accumulation in this phenomenon. A role for  $[Na^+]_i$  and  $I_{NaK}$  in gradual APD changes during long-term pacing has been previously proposed (Boyett and Jewell, 1978; Faber and Rudy, 2000; Hund and Rudy, 2000). Similarities between the effect of block of  $I_{Ca,L}$  and SR function in simulation and experiment (Tolkacheva et al., 2006) provide support for this hypothesis. While a similar time course of APD accommodation after a CL change has been observed in a range of preparations, APD accommodation in other species and preparations is often more rapid (Franz et al., 1988; Tolkacheva et al., 2006; Williams et al., 1999). This suggests that other mechanisms may play a role or, alternatively, that additional experiments are required to improve our understanding of equilibration of  $[Na^+]_i$  and  $[Ca^{2+}]_i$  during long term pacing. A role for  $I_{Ca,L}$  in the dynamics of arrhythmia has been proposed by many investigators, usually through effects on APD restitution (Boyett and Jewell, 1978; Qu et al., 1999). Our simulations suggest that the effect of  $I_{Ca,L}$  on  $[Na^+]_i$  and APD accommodation should also be considered. The importance of  $[Na^+]_i$  in determining the rate dependence of APD also points to an important limitation of simplified computational models, where intracellular ion concentrations are often held constant.

The predictive value of multicellular simulations of arrhythmia depends on the ability of cell models to reproduce experimental observation. Despite variability across species, cell type and preparation, APD adaptation, restitution and accommodation are qualitatively similar in many species. Our model examines the ionic basis of these rate dependent phenomena in the canine, but may provide insight into ionic mechanisms in other species including human. In addition, our model will provide a valuable tool for linking initiation and maintenance of arrhythmia to underlying cellular processes and ionic currents through multicellular simulations.

## **Chapter 3. Ionic Mechanisms of Electrophysiological Heterogeneity and Block in the Infarct Border Zone**

### **3.1 Introduction**

Survivors of myocardial infarction are at an increased risk of ventricular arrhythmia in the days and weeks that follow the initial ischemic event. Years of experiments have been devoted to increasing our understanding of the pathophysiological basis for this enhanced risk (Janse and Wit, 1989; Wit and Janse, 1992). A large body of research has focused on a canine model in which a transmural infarct is created by ligation of the left anterior descending coronary artery (LAD) (Dillon et al., 1988; El-Sherif et al., 1977b; El-Sherif et al., 1977c; Gough et al., 1985; Ursell et al., 1985). Arrhythmias in this model are reentrant in nature and have been mapped to a thin rim of surviving epicardial tissue that borders the infarct (the epicardial border zone or EBZ) (El-Sherif et al., 1977c). Initiation and maintenance of arrhythmia are thought to depend on the presence of a structurally and electrophysiologically remodeled substrate with enhanced vulnerability to a triggering event (Pinto and Boyden, 1999). Numerous studies have characterized remodeling of ion channels (Aggarwal and Boyden, 1995; Aggarwal et al., 1997; Baba et al., 2005; Dun et al., 2004; Dun and Boyden, 2005; Jiang et al., 2000; Lue and Boyden, 1992; Pu and Boyden, 1997),  $\text{Ca}^{2+}$  handling (Licata et al., 1997; Pu et al., 2000) and gap junctions (Cabo et al., 2006; Peters et al., 1997; Yao et al., 2003) in the EBZ 3–5 days post-infarction. The clinical importance of arrhythmia and the wealth of experimental data characterizing arrhythmias and remodeling make the EBZ an excellent paradigm for computational modeling studies aimed at elucidating the underlying ionic mechanisms.

Transitions between successful conduction, formation of the initial line of block, reentry and fibrillation depend on the heterogeneous response of healthy and diseased cells to complex

myocardial activation patterns. Our recently published computational model (Decker et al., 2009) accurately reproduces the response of the canine ventricular epicardial cell to a range of relevant pacing protocols. Starting from this control model, an EBZ myocyte model was developed based on experimental remodeling data. We tested the hypothesis that ion channel remodeling results in heterogeneity in the rate dependence of normal zone (NZ) and EBZ APD and refractoriness. Simulations using a multi-cellular model of heterogeneous myocardium were used to study the link between EBZ remodeling and the VW. In addition, we tested the hypothesis that ion channel remodeling combined with gap junction uncoupling leads to abnormal, unsafe conduction in the EBZ. The potential of gene therapy using a skeletal muscle sodium channel isoform (SkM1- $I_{Na}$ ) in the prevention of arrhythmia post-infarction (Lau et al., 2009) was also explored.

### **3.2 Methods**

NZ and EBZ cell models were paced for 1800 s at a given S1 cycle length ( $CL_{S1}$ ) with a conservative  $K^+$  stimulus (Hund et al., 2001) (magnitude =  $-80 \mu A/\mu F$ , duration = 0.5 ms) to achieve steady state. Previous studies from our laboratory have used strand models to gain insight into the ionic mechanisms of propagation and conduction block (Shaw and Rudy, 1995, 1997). In this study, strands were composed of 96 serially arranged cells connected by gap junctions, with direct stimulation of cell 1 (magnitude =  $-200 \mu A/\mu F$ , duration = 1 ms). Parameter values used in the present study and detailed validation of the strand model of propagation are found in (Shaw and Rudy, 1997). Simulations were performed in homogeneous (96 NZ or 96 EBZ cells) or heterogeneous (48 NZ followed by 48 EBZ cells) strands. To achieve steady state, strands were paced for 1800 s at a given  $CL_{S1}$ . Restitution was studied with a single additional beat at the S2 coupling interval ( $CI_{S2}$ ) after pacing to steady state at a given

$CL_{S1}$ . In simulations where SkM1- $I_{Na}$  was added to the EBZ region, SkM1- $I_{Na}$  density (1.65 mS/ $\mu$ F) was adjusted to reproduce the experimentally observed 80% increase in EBZ  $dV/dt_{max}$  after SkM1- $I_{Na}$  injection (Lau et al., 2009).

The effective refractory period (ERP) was defined as the smallest  $CI_{S2}$  for which AP propagation was successful. Post-repolarization refractoriness (PRR) was defined as ERP – APD. The vulnerable window (VW) for conduction block at the NZ to EBZ transition was used as a quantitative measure of vulnerability to formation of the initial line of conduction block, a prerequisite for initiation of reentry in infarcted myocardium. For pacing at a constant  $CL_{S1}$ , the VW was defined as the range of  $CL_{S1}$  for which conduction block occurred at the NZ to EBZ transition. For restitution protocols, VW was defined as the range of  $CI_{S2}$  for which conduction block occurred at the NZ to EBZ margin.

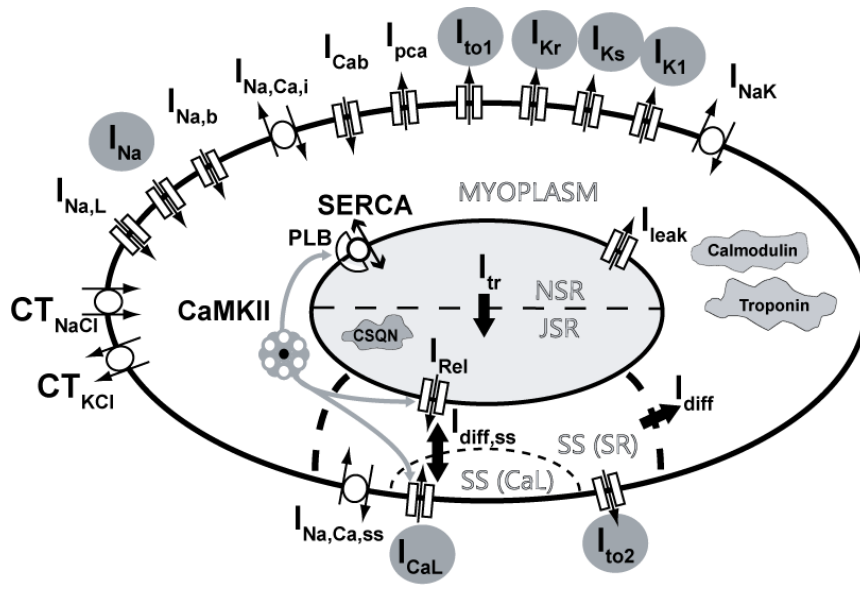
Safety factor (SF) indicates the margin of safety of action potential (AP) propagation, and is a useful quantitative measure of conduction safety (Shaw and Rudy, 1997). A detailed description and discussion of the method for calculating SF is included in (Shaw and Rudy, 1997). Briefly, SF for a cell during propagation is defined as:

$$SF = \frac{\text{Charge generated by cell excitation}}{\text{Charge received for excitation}} = \frac{\int_A I_C \cdot dt + \int_A I_{out} \cdot dt}{\int_A I_{in} \cdot dt} \quad A|Q_m > 0$$

where  $I_C$  is the capacitive current of the cell of interest,  $I_{out}$  is the axial current between the cell of interest and its downstream neighbor,  $I_{in}$  is the axial current received by the cell of interest from its upstream neighbor and  $Q_m$  is the time integral of transmembrane current. The integration limit  $A$  ( $Q_m > 0$ ) used to calculate SF denotes the time period during which the cell has completed its sink-source cycle. The fraction of  $SF > 1$  indicates the relative safety of conduction, while conduction fails for  $SF < 1$  (Shaw and Rudy, 1997).

### 3.3 Results

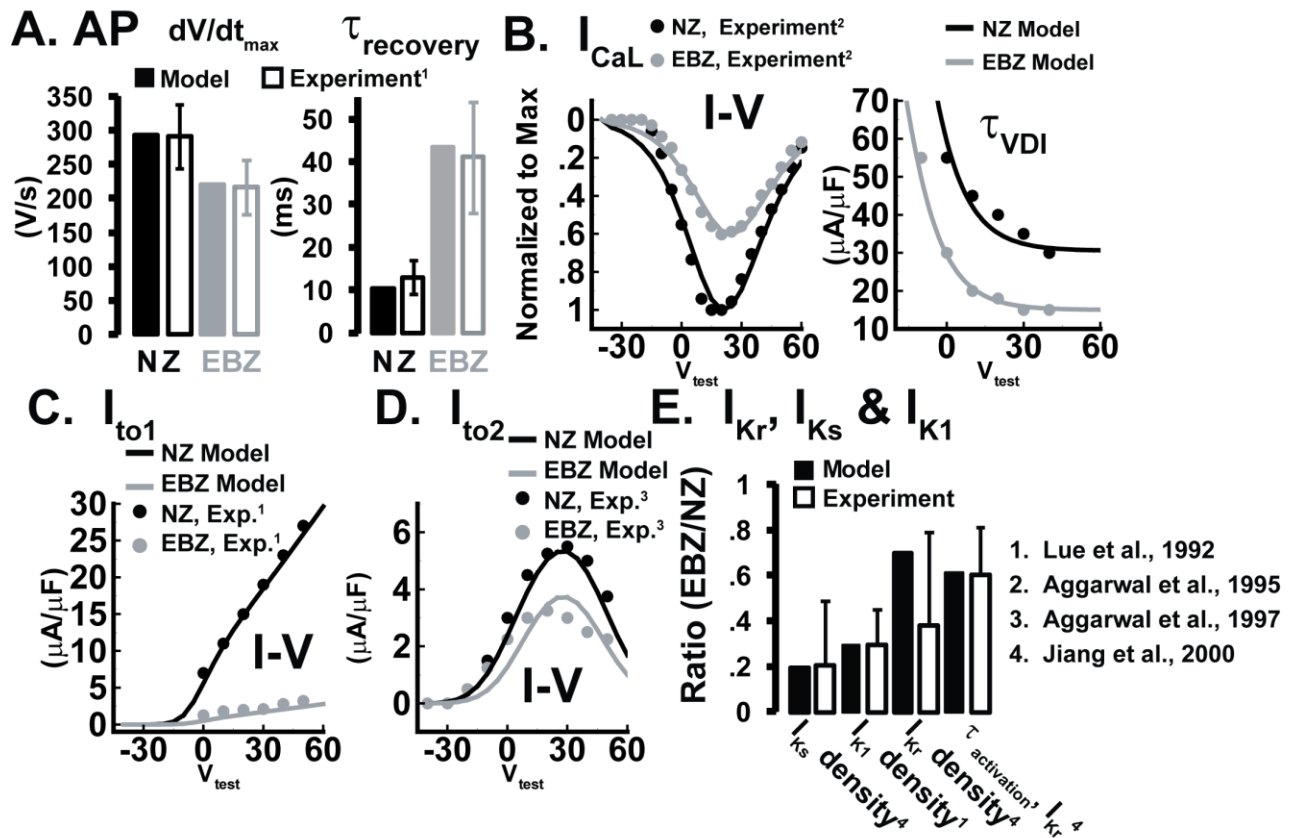
#### 3.3.1 An Ionic Model of the Epicardial Border Zone myocyte



**Figure 3-1 Canine Epicardial Border Zone (EBZ) cell model.** Remodeled Epicardial Border Zone currents are circled in gray.

A model of the epicardial border zone myocyte was derived from the control (normal zone or NZ) model by incorporating myocyte remodeling data from 3-5 day old canine infarct preparations. Remodeled currents incorporated into the EBZ model are circled in gray in Figure 3-1. Modifications to the original epicardial cell model are described in Appendix B. Figure 3-2 shows a comparison between model-generated and experimentally recorded EBZ remodeling data. Reduction of  $I_{Na}$  density and leftward shift in  $I_{Na}$  steady state inactivation result in a reduced maximum AP upstroke velocity (Fig. 3-2A, *left*) and increased time constant of recovery of AP upstroke velocity ( $\tau_{recovery}$ , Fig. 3-2A, *right*), quantitatively consistent with experiment (Lue and Boyden, 1992). Figure 3-2B shows that the model reproduces the reduced L-type  $Ca^{2+}$  channel current ( $I_{CaL}$ ) (Fig. 3-2B, *left*) and faster  $I_{CaL}$  voltage dependent inactivation ( $\tau_{VDI}$ ) (Fig. 3-2B, *right*) recorded in the EBZ (Aggarwal and Boyden, 1995). Figure 3-2C and Figure 3-2D

show reductions in the magnitude of  $K^+$  (Lue and Boyden, 1992) and



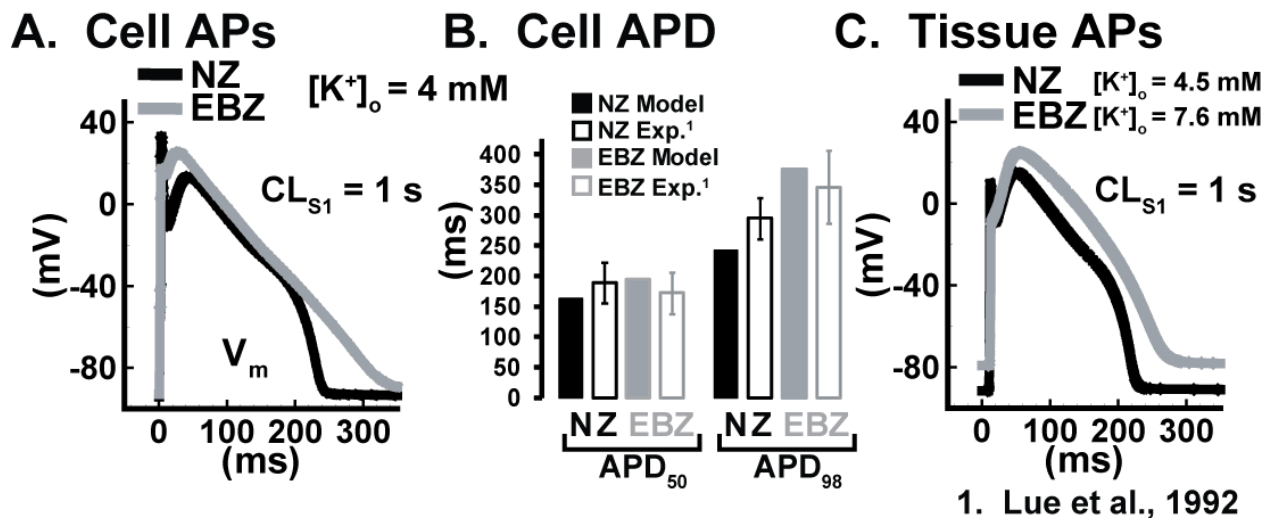
**Figure 3-2 Model fits to NZ and EBZ Experimental Data.** (A) Normal Zone (NZ) and (EBZ) maximum AP upstroke velocity ( $dV/dt_{max}$ ) and time constant of recovery of AP  $dV/dt_{max}$  ( $\tau_{recovery}$ ) in model and experiment (Lue and Boyden, 1992).  $dV/dt_{max}$  properties were determined as described in (Lue and Boyden, 1992).  $V_m$  was held at 0 mV for 500 ms, followed by varying time intervals at the holding potential ( $V_{hold}$ ). APs were elicited by application of a stimulus after release of voltage clamp at  $V_{hold}$ . Fully recovered  $dV/dt_{max}$  was determined for an AP elicited after 500 ms at  $V_{hold} = -110$  mV.  $\tau_{recovery}$  was determined by an exponential fit to the time course of recovery of AP  $dV/dt_{max}$  at  $V_{hold} = -80$  mV. (B)  $I_{CaL}$  current-voltage (I-V) relationship and time constant of voltage dependent inactivation (VDI) in model and experiment (Aggarwal and Boyden, 1995). NZ and EBZ I-V relationship of (C)  $I_{to1}$  (Lue and Boyden, 1992) and (D)  $I_{to2}$  (Aggarwal et al., 1997) in model and experiment. (E) Ratio (EBZ:NZ) of  $I_{Ks}$  density (Jiang et al., 2000),  $I_{K1}$  density (Lue and Boyden, 1992),  $I_{Kr}$  density (Jiang et al., 2000) and  $I_{Kr}$  (Jiang et al., 2000) time constant of activation in model and experiment.



Cl<sup>-</sup> (Aggarwal et al., 1997) components of the transient outward current ( $I_{to1}$  and  $I_{to2}$ ) in the EBZ, respectively. Delayed and inward rectifier potassium channel data are shown in Figure 3-2E. Reduced fast and slow components of the delayed rectifier potassium current ( $I_{Kr}$  and  $I_{Ks}$ ) (Jiang et al., 2000), reduced inward rectifier current ( $I_{K1}$ ) (Lue and Boyden, 1992) and more rapid  $I_{Kr}$  activation (Jiang et al., 2000) are reproduced by the EBZ model.

### 3.3.2 APD Rate Dependence in the Epicardial Border Zone

Figure 3-3A compares steady state APs ( $CL_{S1} = 1$  s,  $[K^+]_o = 4$  mM) in NZ (black) and



**Figure 3-3 EBZ Action Potentials in Cell and Strand.** A. Model NZ and EBZ cell APs ( $CL_{S1} = 1$  s,  $[K^+]_o = 4$  mM). B. NZ and EBZ APD ( $CL_{S1} = 1$  s,  $[K^+]_o = 4$  mM) at 50% and full (98%) repolarization in model and experiment (Janse and Wit, 1989; Lue and Boyden, 1992; Wit and Janse, 1992). C. Model NZ ( $[K^+]_o = 4.5$  mM) and EBZ ( $[K^+]_o = 7.6$  mM) APs ( $CL_{S1} = 1$  s) for the central cell in homogeneous strand simulations.

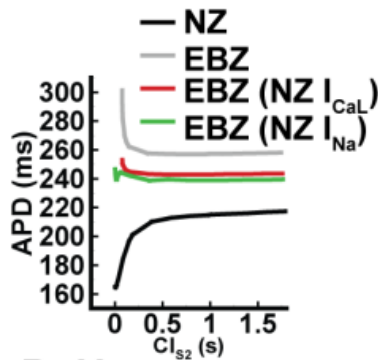
EBZ (gray) single cell simulations. The EBZ model AP reproduces the absence of a phase 1 notch (due to loss of  $I_{to1}$ ) and AP triangulation (due to reduction in  $I_{K1}$ ) observed experimentally (Lue and Boyden, 1992). Both model and experiment show similar  $APD_{50}$  in NZ and EBZ (Fig. 3-3B, left). In addition, the model reproduces the significant prolongation of EBZ APD at full repolarization observed in cell experiments with  $[K^+]_o = 4$  mM (Fig. 3-3B, right). AP

triangulation, loss of the notch and prolonged EBZ cell APD at  $[K^+]_o = 4 \text{ mM}$  are consistent with previous single-cell modeling results (Cabo and Boyden, 2003; Hund et al., 2008a). Figure 3-3C compares simulated APs from the middle cell of homogeneous NZ and EBZ strands. Elevation of EBZ  $[K^+]_o$  was required to reproduce the marked elevation of EBZ resting  $V_m$  observed in tissue experiments (Ursell et al., 1985) (model NZ  $[K^+]_o = 4.5 \text{ mM}$ ,  $V_m = -91.5 \text{ mV}$ ; EBZ  $[K^+]_o = 7.6 \text{ mM}$ ,  $V_m = -78.6 \text{ mV}$ ; experiment NZ  $V_m = -90 \text{ mV}$ , EBZ  $V_m = -79 \text{ mV}$ ). The degree of elevation of  $[K^+]_o$  required to reproduce the elevation of resting membrane potential ( $V_{rest}$ ) observed experimentally is a critical determinant of EBZ repolarization and refractoriness. An EBZ strand paced at  $CL_{S1} = 1000 \text{ ms}$  with  $[K^+]_o = 4.5 \text{ mM}$  has APD = 305 ms, PRR = 12 ms and ERP = 317 ms, while at elevated  $[K^+]_o = 7.6 \text{ mM}$  EBZ APD = 257 ms, PRR = 76 ms and ERP = 334 ms. Increasing  $[K^+]_o$  substantially increases PRR due to the markedly slower recovery of EBZ  $dV/dt_{max}$  at elevated resting potentials (Fig. 3-2A, right). The elevation of EBZ  $[K^+]_o$  thus leads to a net increase in ERP of 17 ms, with slowing of  $I_{Na}$  recovery due to resting  $V_m$  elevation causing an increase in PRR and offsetting ERP abbreviation due to APD shortening.

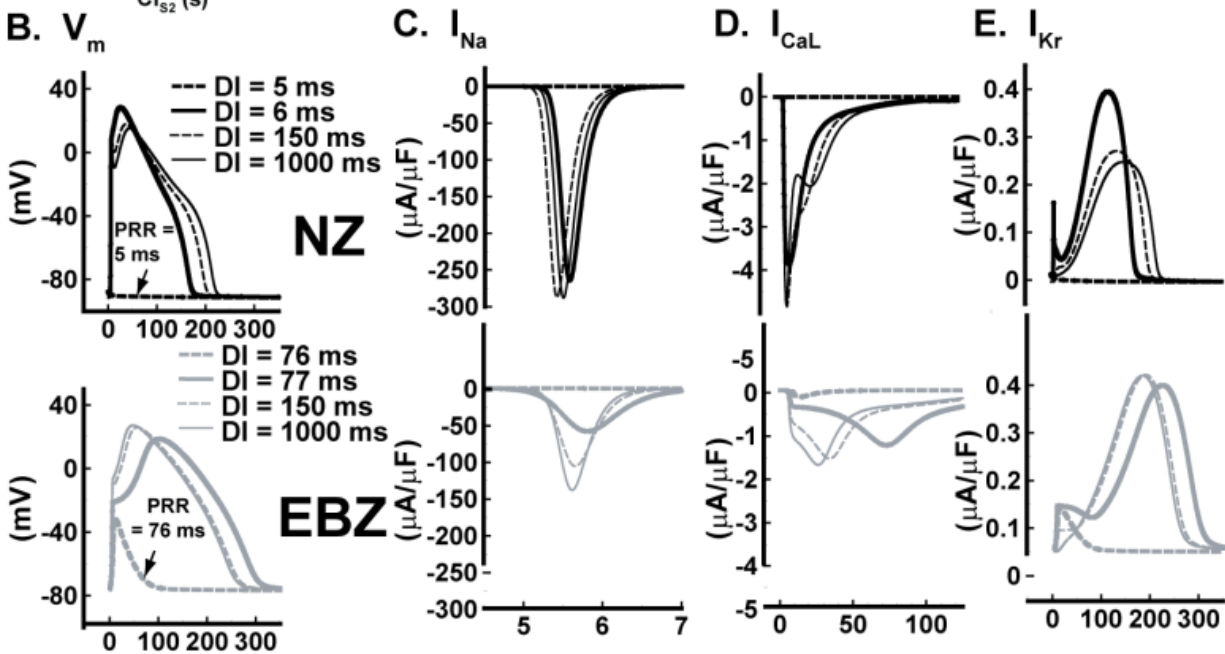
In order to understand ionic mechanisms underlying heterogeneity in the response of NZ and EBZ tissue to premature stimuli, APD restitution in NZ and EBZ strands was characterized. Figure 3-4 examines ionic mechanisms underlying altered refractoriness and restitution in the EBZ. Restitution in the EBZ is characterized by a  $CI_{S2}$  range with abnormally prolonged APD at short DI (Fig. 3-4A, *gray curve*). In the NZ, successful conduction occurs at a DI of 6 ms (Fig. 3-4B *left*), due to near complete recovery of  $I_{Na}$  availability (Fig. 3-4C, *left*). In the EBZ successful conduction does not occur until DI = 77 ms (Fig. 3-4B *right*) due to slow recovery of  $I_{Na}$  from inactivation (Fig. 3-4C *right*). Weak  $I_{Na}$  and diminished upstroke  $V_m$  amplitude at DI = 77 ms are associated with abnormally prolonged EBZ APD. Peak  $V_m$  during the upstroke is

reduced to approximately  $-20$  mV, resulting in slow  $I_{CaL}$  activation (Fig. 3-4D, right) and slow

### A. APD Restitution



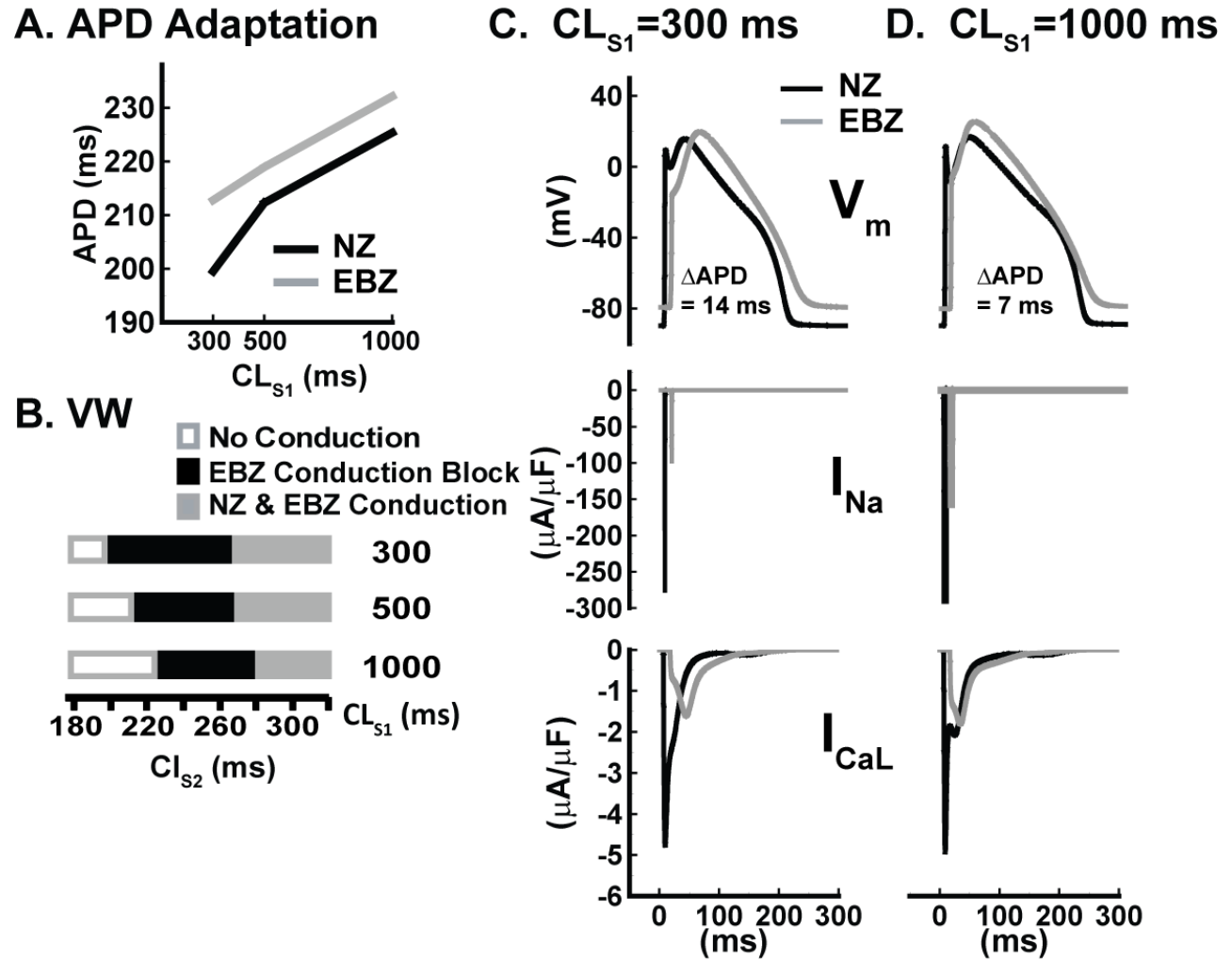
**Figure 3-4. APD restitution in the EBZ.** A. APD restitution measured in the central cell of homogeneous NZ and EBZ strands ( $CL_{S1} = 1$  s). NZ and EBZ B.  $V_m$ , C.  $I_{Na}$ , D.  $I_{CaL}$  and E.  $I_{Kr}$  as a function of DI in NZ (left) and EBZ (right) strands. APs were time shifted to align  $dV/dt_{max}$  for each DI. APD was calculated at 95% repolarization.



$I_{CaL}$  dependent depolarization (Fig. 3-4B, right). In the NZ, positive  $V_m$  promotes robust  $I_{CaL}$  activation at short DI (Fig. 3-4D, left), in contrast to the slow, weak activation of  $I_{CaL}$  observed in the EBZ. In addition, positive  $V_m$  leads to rapid  $I_{Kr}$  activation (Fig. 3-4E, left) and promotes short APD at short DI in the NZ. In contrast, suppression of EBZ  $I_{Kr}$  (Fig. 3-4E, right) due to negative  $V_m$  promotes APD lengthening at short DI. The critical role of slow  $I_{Na}$  recovery from inactivation and weak  $I_{CaL}$  activation in leading to abnormal EBZ restitution was confirmed via additional simulations. Restoration of normal  $I_{Na}$  or normal  $I_{CaL}$  dramatically reduces the region of prolonged APD at short DI in the EBZ strand (Fig. 3-4A).

### 3.3.3 Ionic Mechanisms of AP Heterogeneity and Conduction Block

We hypothesized that altered APD restitution in the EBZ leads to rate dependent dispersion of APD and conduction block in heterogeneous myocardium. Fig. 3-5A shows the



**Figure 3-5 APD Adaptation and Block in heterogeneous strands.** A. Rate dependence of NZ and EBZ APD in heterogeneous strand simulations. Strands were composed of 48 NZ and 48 EBZ cells, with stimulation at the NZ end. APD is shown for the central NZ (cell 24) and central EBZ (cell 72) cells. B. VW as a function of S2 coupling interval ( $CI_{S2}$ ) for a range of  $CL_{S1}$ .  $V_m$ ,  $I_{Na}$  and  $I_{CaL}$  from NZ (cell 24) and EBZ (cell 72) cells are shown at C.  $CL_{S1} = 300$  and D.  $CL_{S1} = 1000$  ms. APD was calculated at 95% repolarization.

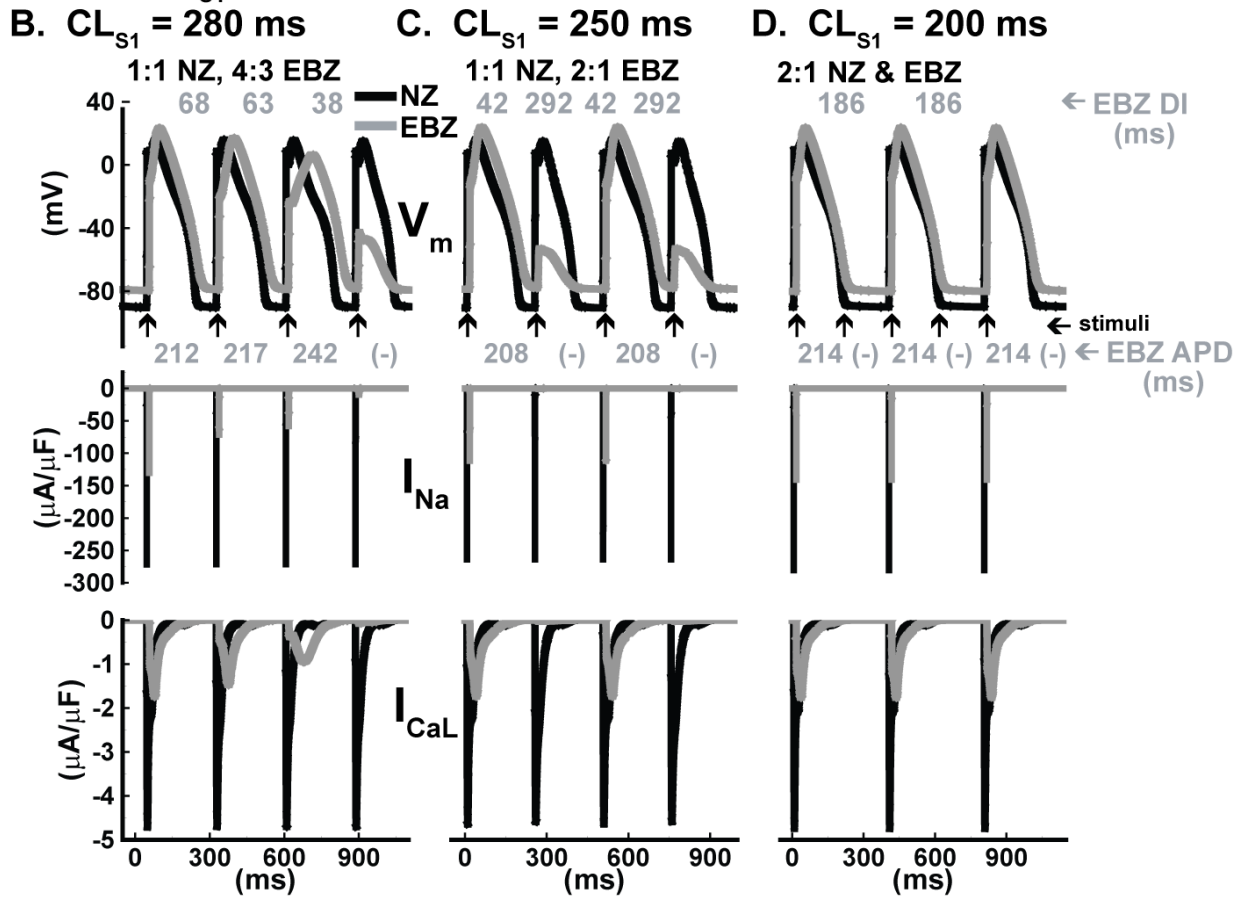
rate dependence of APD in a heterogeneous strand simulation. The strand was composed of 48 EBZ (cell 72) cells. APD heterogeneity between NZ and EBZ increases at  $CL_{S1} = 300$  ms. The

VW in heterogeneous strand simulations was characterized via S1-S2 stimulation protocols to test the hypothesis that heterogeneity in APD and refractoriness would lead to a large, rate-dependent VW. Fig. 3-5B shows the VW in a heterogeneous strand in response to S2 stimuli as a function of  $CL_{S1}$ . The width of the VW was 70 ms at  $CL_{S1} = 300$  ms, 56 ms at  $CL_{S2} = 500$  and 55 ms at  $CL_{S1} = 1000$  ms. The width of the VW at all  $CL_{S1}$  is much greater than the APD dispersion, demonstrating that post-repolarization refractoriness is the primary determinant of the width of the VW. However, increased APD dispersion at  $CL_{S1} = 300$  ms does lead to an increase in the VW relative to that at slower rates. Figs. 3-5C-D explore the ionic mechanism of increased APD dispersion at  $CL_{S1} = 300$  ms. Conduction at  $CL_{S1} = 300$  ms is characterized by reduced  $I_{Na}$  activation (Fig. 3-5C, *middle*), a weakened AP upstroke (Fig. 3-5C, *top*), delayed  $I_{CaL}$  activation (Fig. 3-5C, *bottom*) and prolonged APD relative to the NZ (Fig. 3-5C, *top*). At  $CL_{S1} = 1000$  ms  $I_{Na}$  is more fully recovered (Fig. 3-5D, *middle*),  $I_{CaL}$  activation is less delayed (Fig. 3-5D, *bottom*), and APD heterogeneity between NZ and EBZ is decreased (Fig. 3-5D, *top*).

The effects of heterogeneity in NZ and EBZ APD and refractoriness were also explored during sustained pacing at the rapid S1 pacing rates that characterize tachycardia and are often required to induce arrhythmia experimentally. Fig. 3-6A characterizes the VW in a heterogeneous strand for  $CL_{S1} < 300$  ms. Pacing over a  $CL_{S1}$  range from 190 to 270 ms results in 2:1 block at the transition from NZ to EBZ, while pacing at  $CL_{S1} = 280$  ms results in 4:3 block at the NZ to EBZ transition. In order to explore the ionic mechanisms underlying this large VW and abnormal block patterns, Figure 3-6B-D shows  $V_m$ ,  $I_{Na}$  and  $I_{CaL}$  from the central NZ cell (cell 24) and central EBZ cell (cell 72) at  $CL_{S1} = 280, 250$  and  $200$  ms respectively. At  $CL_{S1} = 280$  ms, 4:3 block at the NZ to EBZ transition occurs (Fig. 3-6B). Block is preceded by a series of 3 successful EBZ APs in which APD progressively increases (212 to 217 to 242 ms) and DI

shortens (68 to 63 to 38 ms) until the EBZ ERP is exceeded and block occurs. Increasing APD is

### A. VW, Fast $CL_{S1}$

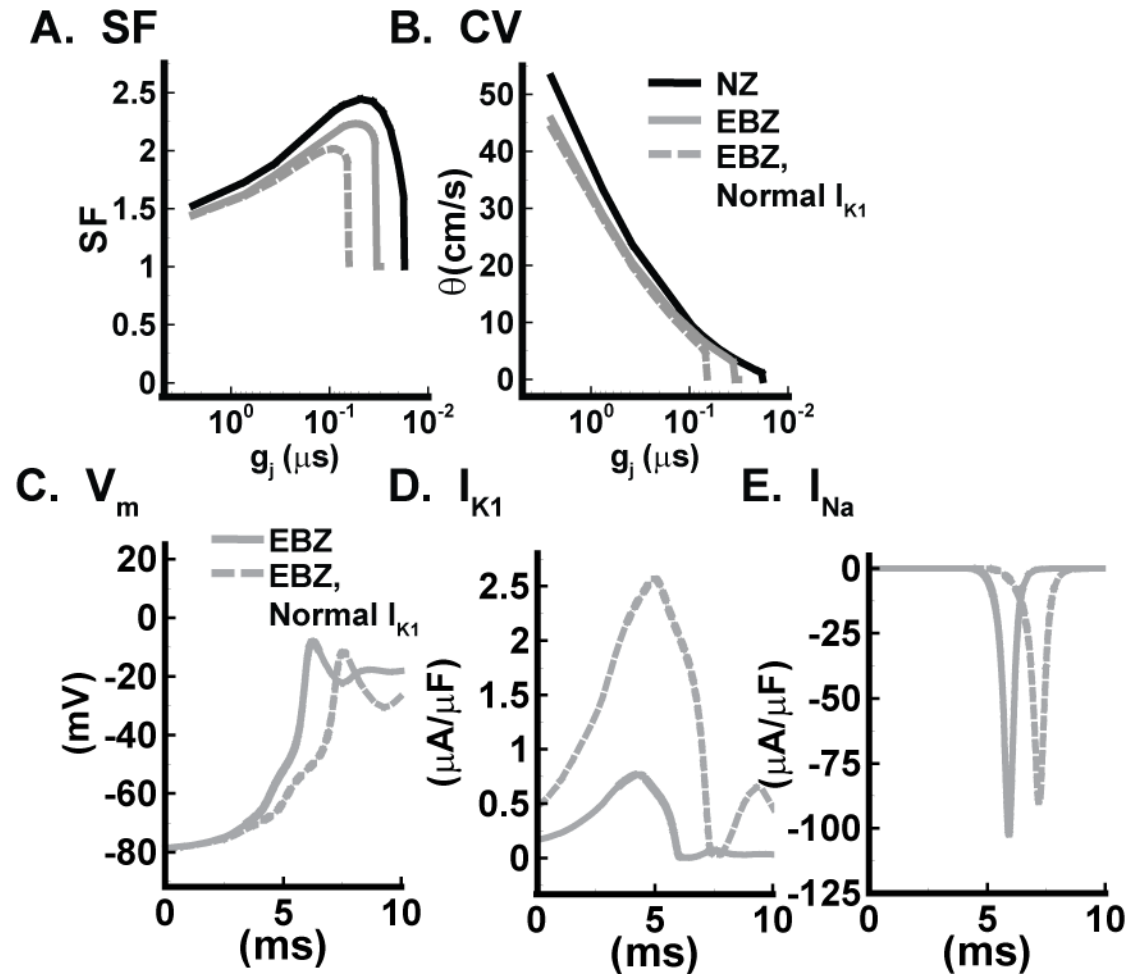


**Figure 3-6 Conduction Block at fast  $CL_{S1}$  in heterogeneous strand.** A. VW in heterogeneous strands during pacing at fast  $CL_{S1}$ . Strands were composed of 48 NZ and 48 EBZ cells. Stimuli were applied at the NZ end. B-D. NZ (cell 24) and EBZ (cell 72)  $V_m$ ,  $I_{Na}$  and  $I_{CaL}$  in heterogeneous strands during pacing at fast  $CL_{S1}$  illustrating representative patterns of conduction and block; B. 4:3 EBZ conduction block ( $CL_{S1} = 280$  ms). C. 2:1 EBZ conduction block ( $CL_{S1} = 250$  ms). D. 2:1 NZ and EBZ conduction block ( $CL_{S1} = 200$  ms). APD was calculated at 95% repolarization.

accompanied by decreasing  $I_{Na}$  activation (Fig. 3-6B, middle) and increasingly slow and delayed activation of  $I_{CaL}$  (Fig. 3-6B, bottom). The abnormal increase in APD with decreasing DI during APD restitution is therefore responsible for the region of 4:3 block. At  $CL_{S1} = 250$  ms, 2:1 block at the NZ to EBZ transition occurs (Fig. 3-6C) since the ERP in the EBZ exceeds the pacing CL. At  $CL_{S1} = 200$  ms, (Fig. 3-6D) the pacing rate exceeds the ERP of both the NZ and EBZ and 2:1 block occurs across the entire strand.

### 3.3.4 Ionic Mechanisms of Conduction Post-Infarction

We hypothesized that remodeling of key determinants of conduction velocity and safety,



**Figure 3-7 Role of  $I_{Na}$  and  $I_{K1}$  in EBZ conduction.** A. Safety factor and B. conduction velocity as a function of gap junction conductance ( $g_j$ ) in homogeneous strands of NZ cells, EBZ cells and EBZ cells with normal  $I_{K1}$

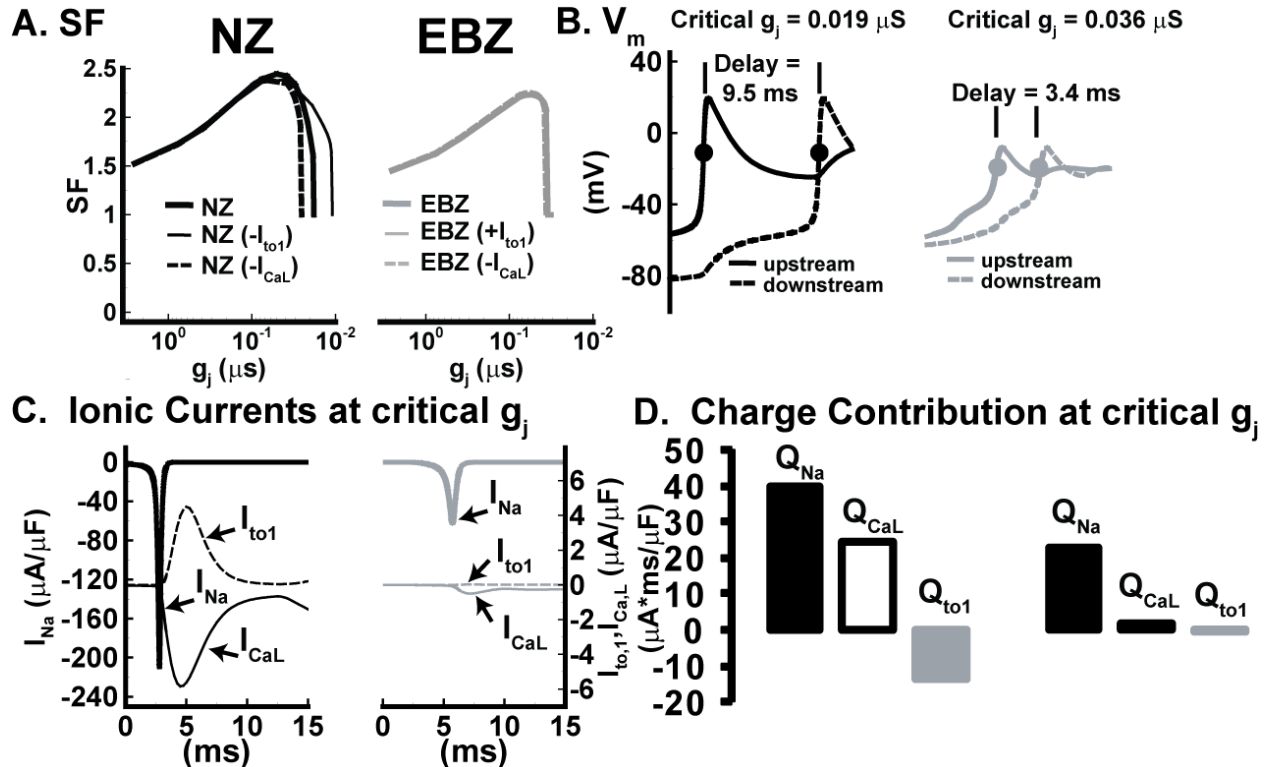
restored. *C.*  $V_m$ , *D.*  $I_{K1}$  and *E.*  $I_{Na}$  for propagating APs in EBZ strands with remodeled and normal  $I_{K1}$  (greatly reduced gap junction coupling,  $g_j = 0.069 \mu S$ ).

including gap junction conductance,  $I_{Na}$ ,  $I_{CaL}$  and  $I_{to1}$ , would lead to abnormal conduction in the EBZ. NZ and EBZ conduction safety and velocity as a function of gap junction conductance ( $g_j$ ) are compared in Figures 3-7A and 3-7B. Despite the range of remodeling processes that occur in the EBZ, conduction safety and velocity remain near normal except at the most severe degrees of gap junction uncoupling. The ionic mechanisms promoting near-normal conduction in the EBZ were unclear, however, considering the key role of  $I_{Na}$  in (Shaw and Rudy, 1997) conduction and the severe remodeling of this current in the EBZ. It was therefore hypothesized that additional remodeling processes in the EBZ counteract the effect of reduced  $I_{Na}$  and help maintain near normal conduction. Additional simulations revealed a key role for  $I_{K1}$  remodeling in maintaining near normal conduction in the EBZ. When EBZ  $I_{K1}$  is restored to the larger magnitude that occurs in the NZ, SF is more severely reduced (Fig. 3-7A) and conduction block occurs at a lesser degree of uncoupling (Fig. 3-7B). Figures 3-7C-E compare propagating APs,  $I_{K1}$  and  $I_{Na}$  in uncoupled EBZ strands ( $g_j = 0.069 \mu S$ ) and EBZ strands with  $I_{K1}$  restored to its normal density. Reduction of  $I_{K1}$  (Fig. 3-7D) in the EBZ increases cell excitability, leading to a faster AP upstroke (Fig. 3-7C), increased  $I_{Na}$  activation (Fig. 3-7E) and thus increased SF.

Experiments and simulations have emphasized the importance of  $I_{to1}$  (Huelsing et al., 2001) and  $I_{CaL}$  (Joyner et al., 1996; Rohr and Kucera, 1997; Shaw and Rudy, 1997) as determinants of conduction safety in the context of gap junction uncoupling. It was therefore hypothesized that remodeling of EBZ  $I_{to1}$  and  $I_{CaL}$  would have a significant effect on conduction in the EBZ. Simulations using the NZ strand model are consistent with the expected role of  $I_{CaL}$  and  $I_{to1}$  in conduction (Fig. 3-8A, *left*). In uncoupled tissue, block of  $I_{CaL}$  reduces NZ SF while block of  $I_{to1}$  facilitates NZ conduction and increases SF (Fig. 3-8A, *left*). In contrast, EBZ strand



simulations show that block of  $I_{CaL}$  and restoration of normal  $I_{to1}$  both have a negligible effect on



**Fig. 3-8 Role of  $I_{CaL}$  and  $I_{to1}$  in NZ and EBZ conduction.** *A.* Safety factor for propagation as a function of gap junction coupling in homogeneous NZ (*left*) and EBZ (*right*) strands. *B.*  $V_m$  of upstream and downstream cells during propagation in homogeneous NZ (*left*) and EBZ (*right*) strands at the minimum gap junction conductance (critical  $g_j$ ) for which propagation occurs. *C.* Activation of  $I_{Na}$ ,  $I_{to1}$  and  $I_{CaL}$  for a propagating AP at the critical  $g_j$  in homogeneous NZ (*left*) and EBZ (*right*) strands. Normal  $I_{to1}$  was included in the EBZ strand simulation to assess its potential role in propagation. *D.* Charge contribution ( $Q$ ) of  $I_{Na}$ ,  $I_{CaL}$  and  $I_{to1}$  to propagation at the critical  $g_j$  in NZ (*left*) and EBZ strands with normal  $I_{to1}$  restored (*right*).

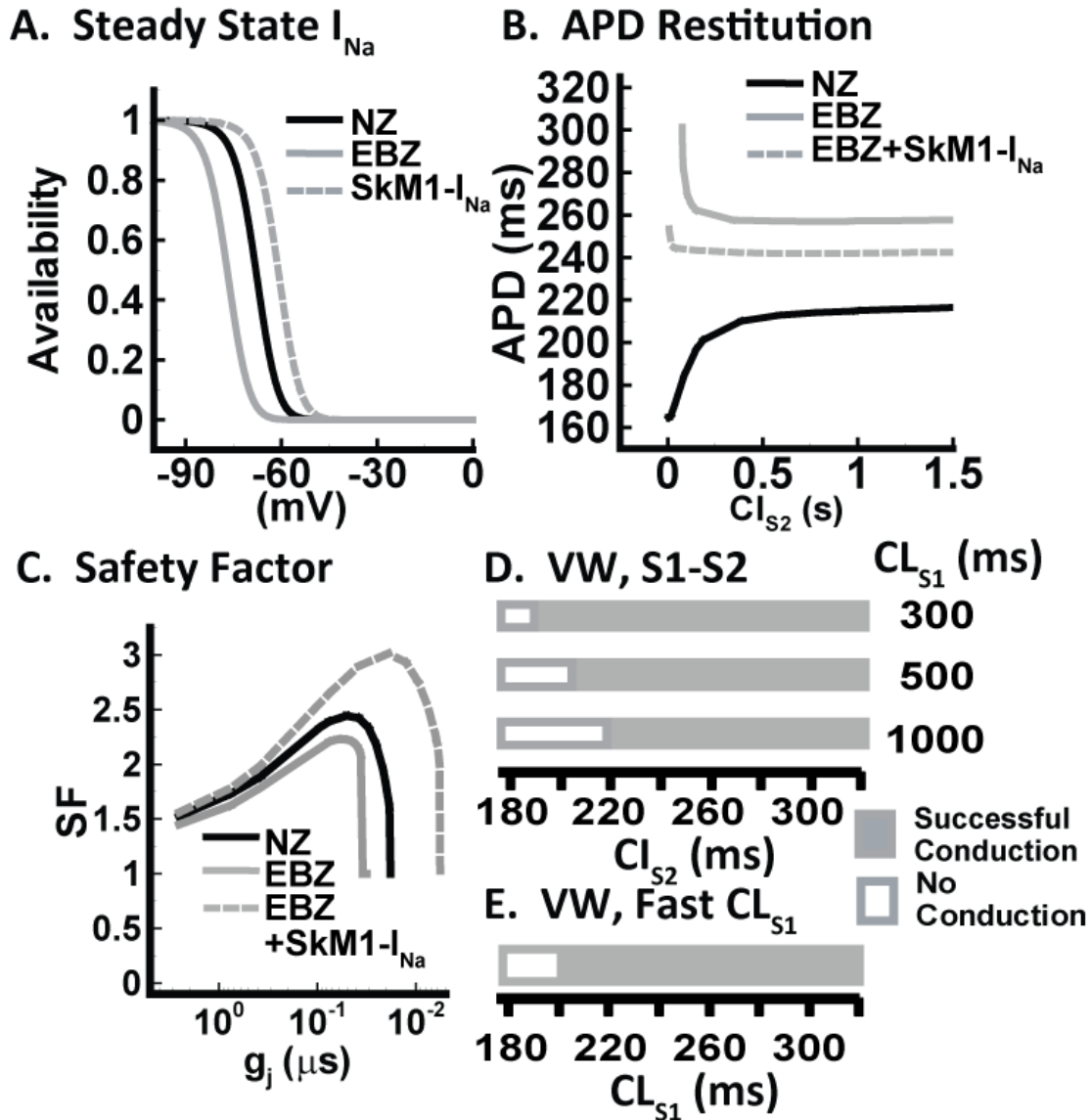
conduction safety (Fig. 3-8A, *right*). Figure 3-8B-D investigate the role of  $I_{to1}$  and  $I_{CaL}$  in conduction in NZ and EBZ strands, at the smallest gap junction conductance for which conduction occurs (NZ critical  $g_j = 0.019 \mu S$ , EBZ critical  $g_j = 0.036 \mu S$ ). Simulations are

shown with normal  $I_{to1}$  restored in the EBZ, to determine the potential importance of this current in propagation. In the NZ strand, robust  $I_{Na}$  leads to a strong AP upstroke (maximum upstroke  $V_m = +18$  mV) and a long delay (9.5 ms) between the excitation of the neighboring upstream and downstream cells (Fig. 3-8B, *left*). During this delay,  $I_{CaL}$  and  $I_{to1}$  activation are both large (Fig. 3-8C, *left*). Integration of  $I_{Na}$ ,  $I_{CaL}$  and  $I_{to1}$  during the delay between activation of adjacent cells was used to characterize their relative contribution of excitatory charge (Q) during propagation (Fig. 3-8D, *left*). In the NZ,  $I_{CaL}$  contributes excitatory charge on the order of that contributed by  $I_{Na}$ . Since  $I_{to1}$  is a repolarizing current it makes a negative contribution to excitation. In the NZ, this negative contribution is comparable to the positive contributions made by  $I_{Na}$  and  $I_{CaL}$ . In the EBZ, the reduction in  $I_{Na}$  due to decreased density and slowed recovery from inactivation leads to a very weak AP upstroke (maximum upstroke  $V_m = -13$  mV) (Fig. 3-8B, *right*). Intercellular conduction delay (3.4 ms) at the larger critical EBZ  $g_j$  is much shorter than in the NZ (Fig. 3-8C). The decreased AP upstroke  $V_m$  leads to much weaker activation of  $I_{CaL}$  and  $I_{to1}$  (restored to NZ density) in the EBZ (Fig. 3-8C, *right*), and the shorter intercellular delay limits the time period within which these currents can contribute charge to propagation. The charge contributions of  $I_{CaL}$  and  $I_{to1}$  to EBZ conduction are consequently negligible relative to the contribution of  $I_{Na}$  (Fig. 3-8D, *right*).

### 3.3.5 Anti-arrhythmic Effects of SkM1- $I_{Na}$ Post-Infarction

A recent study has shown that injection of the skeletal sodium channel SkM1- $I_{Na}$  into the EBZ decreases the inducibility of sustained tachycardia and fibrillation (Lau et al., 2009). Strand simulations were used to seek insight into the ionic mechanism underlying the anti-arrhythmic action of SkM1- $I_{Na}$ . SkM1- $I_{Na}$  was modeled via a 6 mV rightward shift in steady state availability relative to NZ  $I_{Na}$  (Fig. 3-9A) and the density of injected EBZ SkM1- $I_{Na}$  was adjusted

to reproduce the 80% increase in EBZ  $dV/dt_{max}$  recorded in experiments (Lau et al., 2009). In homogeneous strand simulations, the increase in APD at short DI observed during EBZ restitution is attenuated (Fig. 3-9B), and PRR is dramatically reduced (EBZ PRR = 76 ms,



**Fig. 3-9 Anti-arrhythmic effects of SkM1  $I_{Na}$ .** A. Safety factor for propagation as a function of gap junction coupling in homogeneous NZ (left) and EBZ (right) strands. B.  $V_m$  of upstream and downstream cells during propagation in homogeneous NZ (left) and EBZ (right) strands at the minimum gap junction conductance (critical  $g_j$ ) for which propagation occurs. C. Activation of  $I_{Na}$ ,  $I_{to1}$  and  $I_{CaL}$  for a propagating AP at the critical  $g_j$  in homogeneous NZ (left) and EBZ (right) strands. Normal  $I_{to1}$  was included in the EBZ strand simulation to assess its

potential role in propagation. *D.* Charge contribution ( $Q$ ) of  $I_{Na}$ ,  $I_{CaL}$  and  $I_{to1}$  to propagation at the critical  $g_j$  in NZ (*left*) and EBZ strands with normal  $I_{to1}$  restored (*right*).

EBZ+SkM1- $I_{Na}$  PRR = 2 ms). Figure 3-9C demonstrates that the addition of SkM1- $I_{Na}$  dramatically increases the SF for conduction in the EBZ, especially in regions of severe uncoupling. In order to explore the potential effect of SkM1- $I_{Na}$  on arrhythmia inducibility, the VW in heterogeneous strands with SkM1- $I_{Na}$  added to the EBZ region was assessed. The dramatic reduction in ERP in the SkM1- $I_{Na}$  treated EBZ region completely eliminates the VW for premature S2 stimuli at all  $CL_{S1}$  (Fig. 3-9D, compare to Fig. 5B). During pacing at fast  $CL_{S1}$  (without S2 stimulation), the VW is also completely eliminated after addition of SkM1- $I_{Na}$  (Fig. 3-9E, compare to Fig. 6A). These results suggest that elimination of abnormal EBZ refractoriness and restitution are critical determinants of the anti-arrhythmic action of SkM1- $I_{Na}$ .

### 3.4 Discussion

An extensively studied experimental canine model of the 3-5 day old infarct has provided critical insights into the nature of the arrhythmias that originate in the EBZ. Additional studies have characterized the ionic remodeling processes that accompany these arrhythmias. The mechanistic link between EBZ remodeling and increased susceptibility to arrhythmia remains incompletely understood, however. The present work yields several novel mechanistic insights into ionic mechanisms of arrhythmia after infarction. 1) EBZ tissue exhibits abnormal APD restitution, with a region of increasing APD at short DI. At short DI slow recovery of  $I_{Na}$  severely weakens the AP upstroke, causing abnormally slow and delayed  $I_{CaL}$  activation and suppression of  $I_{Kr}$  activation, prolonging APD. 2) Prolonged refractoriness is the primary cause of the large VW in response to premature S2 stimuli. Abnormal EBZ restitution also promotes APD dispersion between NZ and EBZ at fast  $CL_{S1}$  and increases the VW in response to S2 stimuli. 3) Prolonged refractoriness in the EBZ also promotes a large VW during pacing at

sufficiently fast pacing rates ( $CL_{S1} < 300$  ms). Abnormal APD restitution increases the width of the VW by promoting 4:3 EBZ conduction block. 4) Despite severely reduced  $I_{Na}$ , EBZ safety factor for conduction and conduction velocity are near normal, even in severely uncoupled tissue. The detrimental effect of reduced  $I_{Na}$  on conduction is partially offset by the reduction in EBZ  $I_{K1}$ , which facilitates conduction. 5) Although  $I_{CaL}$  and  $I_{to1}$  play a role in conduction in the NZ, remodeling of these currents does not significantly affect SF in the EBZ. Reduced EBZ  $I_{Na}$  decreases upstroke  $V_m$  during propagation, suppressing  $I_{CaL}$  and  $I_{to1}$  activation and precluding any role for these currents in conduction safety. 6) The anti-arrhythmic effects of SkM1- $I_{Na}$  injection are likely attributable to a range of desirable actions including; a. Elimination of abnormal APD restitution. b. Shortening of EBZ ERP and c. increased safety factor for conduction in uncoupled tissue. Elimination of abnormal restitution and shortening of ERP in the EBZ by SkM1- $I_{Na}$  addition eliminates the VW in heterogeneous strand simulations.

Previous computational studies have provided important insights into the ionic determinants of EBZ ERP and repolarization (Cabo and Boyden, 2003), the role of heterogeneous gap junction coupling in stabilizing reentrant circuits (Cabo and Boyden, 2006), and the role of elevated CaMKII in increased ERP and susceptibility to conduction block in the EBZ (Christensen et al., 2009; Hund et al., 2008a). The present work compliments these previously published studies in several ways. APD restitution is thought to be a critical determinant of reentry initiation and maintenance, but has not been explored in detail in previous EBZ modeling studies. Slow conduction and localized gap junction uncoupling are thought to be important in arrhythmogenesis post-infarction, but the contribution of the range of EBZ remodeling processes to conduction in this setting has not been explored. Previous simulations studies have emphasized the importance of  $I_{Na}$  remodeling in ERP prolongation (Cabo and

Boyden, 2003; Christensen et al., 2009), slowed conduction (Cabo and Boyden 2003; Christensen, Dun et al. 2009) and formation of the VW (Christensen, Dun et al. 2009). The present study demonstrates that remodeling of  $I_{Na}$  has several additional important consequences, including reduction in SF, alteration of the role of  $I_{to1}$  and  $I_{CaL}$  in conduction and alteration of restitution kinetics.

The present study demonstrates that abnormal EBZ restitution promotes dispersion of refractoriness and increases the VW. The period of increasing APD at short DI in the EBZ is caused by slow  $I_{Na}$  recovery (due to  $I_{Na}$  remodeling and elevated  $V_{rest}$ ) and decreased  $I_{CaL}$  density. EBZ APs where a weak upstroke ( $V_m \sim -15$  mV) results in slow and delayed  $I_{CaL}$  activation and AP prolongation are analogous to those observed in epicardium in response to  $I_{Na}$  block (Krishnan and Antzelevitch, 1991) or increased  $I_{to1}$  (Calloe et al., 2010). The present study suggests that a sufficiently negative peak AP upstroke can lead to delayed  $I_{CaL}$  activation and AP prolongation, even in the absence of large  $I_{to1}$ .

The ionic mechanisms of APD restitution and the response to rapid pacing have not been studied in detail in previous EBZ experimental and modeling studies. Heterogeneity in the response of NZ and EBZ tissue at fast  $CL_{S1}$  and short DI is no doubt critical to the initiation and maintenance of reentry, and is an important area of further experimental and modeling studies. Resting membrane potential (Ursell et al., 1985),  $I_{Na}$  remodeling (Baba et al., 2005) and  $I_{CaL}$  remodeling (Baba et al., 2005) vary regionally, and this spatial variation may be an additional determinant of APD dispersion and arrhythmogenesis through effects on EBZ APD and ERP at fast  $CL_{S1}$ .

The healing infarct is characterized by compromised  $I_{Na}$  (Pu and Boyden, 1997), localized gap junction uncoupling (Cabo et al., 2006; Yao et al., 2003) and depolarized resting

membrane potential (Ursell et al., 1985). The inducibility of sustained reentry will depend on the safety and velocity of conduction in the context of this remodeling. It is well established that reduced  $I_{Na}$  slows conduction and decreases conduction safety.  $I_{to1}$  (Huelsing et al., 2001) and  $I_{CaL}$  (Joyner et al., 1996; Rohr and Kucera, 1997; Shaw and Rudy, 1997) have also been accorded a role in conduction safety in uncoupled tissue. The present study demonstrates that  $I_{CaL}$  facilitates and  $I_{to1}$  inhibits conduction in poorly coupled NZ strands, but remodeling of these currents has no effect on conduction in the EBZ. Participation of  $I_{CaL}$  and  $I_{to1}$  in conduction requires; 1) prominent  $I_{Na}$ , so that a robust AP upstroke strongly activates  $I_{CaL}$  and  $I_{to1}$  and 2) highly uncoupled gap junctions, so that long intercellular delays allow time for  $I_{CaL}$  and  $I_{to1}$  to make large charge contributions during propagation. The model suggests that conduction in pathophysiological conditions in which  $I_{Na}$  is compromised, such as the healing infarct, will not be directly affected by  $I_{CaL}$  and  $I_{to1}$  expression. This result also suggests that cell pair experiments (Huelsing et al., 2001; Joyner et al., 1996), in which direct stimulation leads to a more robust AP upstroke than that observed during multi-cellular propagation, are likely to overestimate the role of these currents in propagation. In addition, the anti-arrhythmic effects of enhanced  $I_{CaL}$  on the healing infarct (Pu et al., 1999) are likely attributable to effects other than direct facilitation of conduction by  $I_{CaL}$ . Our simulation results do, however, find that  $I_{K1}$  reduction in the EBZ increases conduction safety and allows conduction at more severe degrees of gap junction uncoupling than would otherwise be possible.

The reduction in inducibility of tachycardia and fibrillation by SkM1- $I_{Na}$  injection post-infarction reported experimentally (Lau et al., 2009) suggests an exciting new anti-arrhythmic approach. The authors attribute this anti-arrhythmic effect of SkM1- $I_{Na}$  to promotion of propagation at normal conduction velocity in depolarized tissue. Our simulations suggest several

desirable effects of SkM1- $I_{Na}$  in the EBZ. In regions of normal gap junction coupling, EBZ conduction safety and velocity are near normal, despite reduced  $I_{Na}$  and increased  $V_{rest}$ . SkM1- $I_{Na}$  is likely to play its most important role in regions of uncoupled tissue, where its effects on conduction safety and velocity are most pronounced. In addition, the simulations suggest that SkM1- $I_{Na}$  normalizes EBZ APD restitution and refractoriness and eliminates the VW. Potentially undesirable side effects, such as  $[Na^+]_i$  overload, were not observed. Taken together, these results suggest that SkM1- $I_{Na}$  treatment of EBZ tissue is a promising anti-arrhythmic approach.



## Chapter 4. Conclusions, Limitations and Future Directions

APD restitution, accommodation and adaptation have been observed in a range of species and cell types, and are thought to play an important role in arrhythmogenesis. The control epicardial myocyte presented in Chapter 2 accurately reproduces a wider range of these rate dependent phenomena than previously published models, and the accompanying simulations provide important insight into their underlying ionic mechanisms. This model is a valuable control model for the study of the effects of the ion channel remodeling that occurs in a wide range of disease states, as demonstrated in Chapter 3. Future studies could study the effects of alterations in ion channels shown to play an important role in restitution, accommodation and adaptation including  $I_{CaL}$ ,  $I_{Ks}$  and  $I_{to1}$  on the stability of reentry, and compare these results against the predictions made in multi-dimensional simulations using simplified ionic models.

Additional simulations and refinements to the model should focus on increasing the physiological accuracy of the model response to block of the major ionic currents. Additional work in collaboration with Jordi Heijman has shown that the availability of  $I_{CaL}$  at negative voltages is an important determinant of the response of the model to ion channel block, and these results should be incorporated into future studies using the model. Cell signaling pathways, especially the adrenergic and CaMKII pathways, are important determinants of cardiac cell electrophysiology and it is important that they be incorporated into future studies using the model. Future work by Jordi Heijman, building on the work of Hund and coworkers, is likely to provide important insight into the role of these signaling pathways in cardiac electrophysiology. APD alternans due to intracellular  $Ca^{2+}$  dynamics are also important determinants of cardiac electrophysiology and health and disease, and should be further studied in the model. Dr. Leonid

Livshitz has provided an important contribution to the ionic mechanisms of  $\text{Ca}^{2+}$  dependent alternans in a previous version of the Hund-Rudy canine model.

Although the present work provides several novel mechanistic insights into ionic mechanisms of remodeling and arrhythmogenesis post-infarction, the EBZ is a complex substrate where many additional factors may promote initiation and maintenance of arrhythmia. Our study examines vulnerability to and underlying mechanisms of formation of the initial line of block, a necessary prerequisite for initiation of reentry. Studies of reentry dynamics will require simulations in two or three-dimensional models of the EBZ. Tissue anisotropy may contribute to the formation of lines of block and lines of apparent block during reentry (Dillon et al., 1988) in the EBZ and requires a detailed description of cell orientation (Spach et al., 2000) and gap junction distribution (Peters et al., 1997; Yao et al., 2003) for accurate modeling. The variable thickness of the EBZ (Peters et al., 1997) may also contribute to the formation of stable reentrant circuits, a phenomenon best studied in three-dimensional models of the EBZ. Arrhythmogenesis in the EBZ may also depend on spatial heterogeneity of remodeling (Baba et al., 2005; Cabo and Boyden, 2006; Cabo et al., 2006). A recent modeling study has shown that increased levels of oxidized CaMKII (Christensen et al., 2009) may contribute to slowed conduction and prolonged ERP in the EBZ through effects of CaMKII on the steady state availability of  $I_{\text{Na}}$  (Wagner et al., 2006). The arrhythmogenic effects of  $I_{\text{Na}}$  remodeling characterized here may depend on CaMKII hyperactivity and oxidation state, although this dependence is not explicitly included in the present model. The role of CaMKII in the EBZ is an important subject for future experimental and modeling studies and will depend on regulation of CaMKII activation by intracellular  $\text{Ca}^{2+}$  and oxidation state, and the effect of CaMKII activation level on  $\text{Ca}^{2+}$  handling proteins (Maier, 2009),  $\text{Na}^+$  (Wagner et al., 2006),  $\text{Ca}^{2+}$  and  $\text{K}^+$  currents (Wagner et al., 2009).

## Appendix A. Control Epicardial Cell Formulation

The equations below are for the original control model (Decker et al., 2009). Note that  $I_{Na}$  and  $I_{CaL}$  formulations were modified and a new model of  $I_{NaL}$  was used to construct the NZ and BZ models (See Appendix B).

### Propagation in a Strand

A detailed description of computational methods for strand simulations is included in previous work from our laboratory (23). Briefly, propagation in a strand of serially connected HRd model cells was simulated via a finite difference approximation of the cable equation.

$$\frac{1}{C_M} \left( \frac{(V_{t-1}^t - V_i^t) - (V_i^t - V_{t+1}^t)}{\Delta x^2} \right) \frac{a}{2R_i} = R_{CG} \left( \left( \frac{V_i^{t+1} - V_i^t}{\Delta t} \right) + \frac{\bar{I}_{ion}}{C_M} \right)$$

$V_i^t$	membrane potential in cell $i$ at time $t$
$R_{CG}$	ratio between capacitative and geometric areas, 2
$a$	fiber radius, $11 \mu M$
$C_m$	specific membrane capacitance, $1 \mu F / cm^2$
$\bar{I}_{ion}$	specific ionic current, $\mu A / cm^2$
$I_{ion}$	ionic current, $\mu A / \mu F \left( \frac{\bar{I}_{ion}}{C_M} \right)$
$\Delta x$	cell length, $100 \mu M$
$R_{myo}$	myoplasmic resistance, $150 \Omega cm$
$R_g$	gap junction conductance, $1.5 \Omega cm^2$
$R_i = R_{myo} + \frac{R_g}{\Delta x}$	axial resistance per unit length
$\Delta t$	time step, $0.005 ms$

No flux boundary conditions ( $\delta V/\delta x = 0$ ) were set at the beginning and end of the strand. A 1 ms stimulus ( $-200 \mu\text{A}/\mu\text{F}$ ) was applied to cell 1 and cell behavior was examined in a central cell (cell 48) where end effects were negligible.

### General Parameters

$V_m$	membrane voltage
$E_x$	Reversal potential of current x, mV
$\bar{I}_x$	Maximum current carried through channel x, $\mu\text{A}/\mu\text{F}$
$\bar{G}_x$	Maximum conductance of channel x, $\text{ms}/\mu\text{F}$
$X_\infty$	Steady state value of variable X
$X_\tau$	Time constant of variable
$\alpha_y, \beta_y$	Opening and closing rate constants of gate y, respectively
$P_S$	Membrane permeability to ion S, cm/s
$P_{S,A}$	Permeability ratio of ion S to ion A
$\gamma_S$	Activity coefficient of ion S
$z_s$	Valence of ion S
$V_X$	Volume of Compartment X
R	Gas constant, 8314 J/kmol/K
T	Temperature, 310°K
F	Faraday constant, 96,487 C/mol
$A_{\text{Cap}}$	Capacitative membrane area, $\text{cm}^2$
$A_{\text{Geo}}$	Geometric membrane area, $\text{cm}^2$

### Currents, Pumps and Exchangers

$I_{\text{Na}}$	Fast $\text{Na}^+$ current, $\mu\text{A}/\mu\text{F}$
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$I_{Na,L}$	Slowly inactivating late $Na^+$ current, $\mu A/\mu F$
$I_{Na,b}$	Background $Na^+$ current, $\mu A/\mu F$
$I_{Ca,L}$	$Ca^{2+}$ current through the L-type $Ca^{2+}$ channel, $\mu A/\mu F$
$I_{Ca,b}$	Background $Ca^{2+}$ current, $\mu A/\mu F$
$I_{p,Ca}$	Sarcolemmal $Ca^{2+}$ pump, $\mu A/\mu F$
$I_{Kr}$	Rapid delayed rectifier $K^+$ current, $\mu A/\mu F$
$I_{Ks}$	Slow delayed rectifier $K^+$ current, $\mu A/\mu F$
$I_{K1}$	Time-independent $K^+$ current, $\mu A/\mu F$
$I_{Kp}$	Plateau $K^+$ current, $\mu A/\mu F$
$I_{to1}$	4AP-sensitive transient outward $K^+$ current, $\mu A/\mu F$
$I_{to2}$	$Ca^{2+}$ dependent transient outward $Cl^-$ current, $\mu A/\mu F$
$I_{Cl,b}$	Background $Cl^-$ current, $\mu A/\mu F$
$I_{NaK}$	$Na^+-K^+$ pump, $\mu A/\mu F$
$I_{NaCa}$	$Na^+-Ca^{2+}$ exchanger, $\mu A/\mu F$
$CT_{NaCl}$	$Na^+-Cl^-$ cotransporter, mmol/L per ms
$CT_{KCl}$	$K^+-Cl^-$ cotransporter, mmol/L per ms
$I_{Ca,t}$	Total transmembrane $Ca^{2+}$ current $I_{Ca,t} = I_{Ca,L} + I_{Ca,b} + I_{p,Ca} - 2I_{Na,Ca}$
$I_{Na,t}$	Total transmembrane $Na^+$ current $I_{Na,t} = I_{Na} + 3I_{NaK} + 3I_{NaCa} + I_{NaL} + I_{Nab}$
$I_{K,t}$	Total transmembrane $K^+$ current $I_{K,t} = I_{Ks} + I_{Kr} + I_{K1} + I_{to1} + I_{Kp} - 2I_{NaK}$
$I_{Cl,t}$	Total transmembrane $Cl^-$ current $I_{Cl,t} = I_{to2} + I_{Clb}$
$I_{tot}$	Total transmembrane current $I_{tot} = I_{Ca,t} + I_{Na,t} + I_{K,t} + I_{Cl,t}$
$I_{stim}$	Stimulus current, $\mu A/\mu F$

ACT	$I_{CaL}$ activation parameter
$\alpha$	$I_{CaL}$ Rate Constant, activation
$\beta$	$I_{CaL}$ Rate Constant, deactivation
C	$I_{CaL}$ State, closed ( $Ca^{2+}$ free)
O	$I_{CaL}$ State, open ( $Ca^{2+}$ free)
$I_v$	$I_{CaL}$ inactivation parameter ( $Ca^{2+}$ free)
$x$	$I_{CaL}$ Rate Constant, recovery from inactivation ( $Ca^{2+}$ free)
$y$	$I_{CaL}$ Rate Constant, inactivation ( $Ca^{2+}$ free)
CI	$I_{CaL}$ State, closed state inactivation ( $Ca^{2+}$ free)
OI	$I_{CaL}$ State, open state inactivation ( $Ca^{2+}$ free)
$I_{Ca}^*$	$I_{CaL}$ inactivation parameter, ( $Ca^{2+}$ present)
$I_v^*$	$I_{CaL}$ inactivation parameter, ( $Ca^{2+}$ present)
$x^*$	$I_{CaL}$ Rate Constant, recovery from inactivation ( $Ca^{2+}$ free)
$y^*$	$I_{CaL}$ Rate Constant, inactivation ( $Ca^{2+}$ free)
$C^*$	$I_{CaL}$ State, closed ( $Ca^{2+}$ present)
$O^*$	$I_{CaL}$ State, open ( $Ca^{2+}$ present)
$\delta$	$I_{CaL}$ Rate Constant, transition to $Ca^{2+}$ gating tier
$\theta$	$I_{CaL}$ Rate Constant, transition from $Ca^{2+}$ gating tier
$\delta_I$	$I_{CaL}$ Rate Constant, from inactivated state to $Ca^{2+}$ gating tier
$\theta_I$	$I_{CaL}$ Rate Constant, to inactivated state from $Ca^{2+}$ gating tier
CI*	$I_{CaL}$ State, closed state inactivation ( $Ca^{2+}$ present)
OI*	$I_{CaL}$ State, open state inactivation $Ca^{2+}$ present)
$V_{1/2}$	$I_{NaK}$ , half maximal voltage dependent activation, mV
$\sigma$	$I_{NaK}$ , slope factor for voltage dependence at $V_{1/2}$

$P_{Na}$	$I_{NaK}$ , $[Na^+]_i$ dependent activation parameter
$P_K$	$I_{NaK}$ , $[K^+]_o$ dependent activation parameter
$K_{Nai}$	$I_{NaK}$ , $[Na^+]_i$ dependent activation parameter
$K_{Ko}$	$I_{NaK}$ , $[K^+]_o$ dependent activation parameter
$f_V$	$I_{NaK}$ , $V_m$ dependent activation parameter
$\bar{G}_{NaK}$	$I_{NaK}$ , maximally activated pump density, $\mu A/\mu F$
$a, i_{1f}, i_{1s}$	$I_{to1}$ , Activation gate, fast inactivation gate, and slow inactivation gate, respectively
$R_{to1}$	$I_{to1}$ , Time-independent rectification gate
$P_{Na/K}$	$I_{Ks}$ , $Na^+/K^+$ permeability ratio, 0.01833
$\alpha, \beta, \gamma, \delta, \eta, \theta, \omega, \psi$	$I_{Ks}$ , rate constants
$C_1 - C_{15}$	$I_{Ks}$ , closed states
$O_1, O_2$	$I_{Ks}$ , open states
$O_{Ks}$	$I_{Ks}$ , open probability
$\bar{G}_{Ks}$	$I_{Ks}$ , $Ca^{2+}$ dependent maximum conductance
$i_2$	$I_{to2}$ , Inactivation gate for $I_{to2}$
$k_{Ca,ito2}$	$I_{to2}$ , half-maximal $Ca^{2+}$ release activation constant
$K_{Ca,ito2}$	$I_{to2}$ , $Ca^{2+}$ dependent activation parameter
$v_{max}$	$I_{NaCa}$ , Maximal flux, $4.5 \mu A/\mu F$
$k_{sat}$	$I_{NaCa}$ , saturation factor at negative potentials, 0.32
$\eta$	$I_{NaCa}$ , Position of energy barrier 0.27

$K_{m,Na_i}$	$I_{NaCa}$ , $[Na^+]_i$ dissociation constant, 12.3 mmol/L
$K_{m,Na_o}$	$I_{NaCa}$ , $[Na^+]_o$ dissociation constant, 87.5 mmol/L
$K_{m,Ca_i}$	$I_{NaCa}$ , $[Ca^{2+}]_i$ dissociation constant, 0.0036 mmol/L
$K_{m,Ca_o}$	$I_{NaCa}$ , $[Ca^{2+}]_o$ dissociation constant, 1.3 mmol/L
$K_{mCa,act}$	$I_{NaCa}$ , 1.25e-4 mmol/L
$m, h, j$	$I_{Na}$ , Activation gate, fast inactivation gate, and slow inactivation gate, respectively
$m_L, h_L$	$I_{Na,L}$ , Activation gate and inactivation gate, respectively
$X_r$	$I_{Kr}$ , activation gate
$R_{Kr}$	$I_{Kr}$ , time independent rectification gate
$K_1$	$I_{K1}$ , inactivation gate of $I_{K1}$

### Ionic fluxes and Diffusion

$I_{rel}$	$Ca^{2+}$ release from JSR to myoplasm, mmol/L per ms
$I_{up}$	$Ca^{2+}$ uptake from myoplasm to NSR, mmol/L per ms
$I_{leak}$	$Ca^{2+}$ leak from JSR to myoplasm, mmol/L per ms
$I_{tr}$	$Ca^{2+}$ transfer from NSR to JSR, mmol/L per ms
$I_{diff,x}$	Ionic diffusion of $Ca^{2+}$ , $Cl^-$ or $Na^+$ from SR subspace to myoplasm
$I_{diff,SS}$	$Ca^{2+}$ diffusion from L-type subspace to SR subspace
$CaMK_{bound}$	Fraction of CaMKII binding sites bound to $Ca^{2+}$ /calmodulin
$CaMK_{active}$	Fraction of active CaMKII binding sites
$CaMK_{trap}$	Fraction of autonomous CaMKII binding sites with trapped calmodulin
$CaMK_o$	Equilibrium fraction of active CaMKII binding sites, 0.05



$\alpha_{\text{CaMK}}, \beta_{\text{CaMK}}$	Phosphorylation and dephosphorylation rates of CaMKII, respectively, $\alpha_{\text{CaMK}} = 0.05 \text{ ms}^{-1}$ $\beta_{\text{CaMK}} = 0.00068 \text{ ms}^{-1}$
$\Delta P_{\text{CaMK}}$	CaMKII-dependent factor of substrate parameter P
$\bar{\Delta P}$	Maximal CaMKII-dependent change in parameter P
$K_{m, \text{CaM}}$	CaMK half-saturation coefficient, 0.0015 mmol/L
$K_{m, \text{CaMK}}$	Half-saturation coefficient of substrate parameter, 0.15
$\beta_0$	$I_{\text{rel}}$ , minimal value of $\beta_\tau$ , 4.75 ms
$K_\beta$	$I_{\text{rel}}$ , half saturation coefficient for $\Delta\beta_{\tau, \text{CaMK}}$ 0.28 mmol/L
$\Delta\beta_0$	$I_{\text{rel}}$ , Maximal CaMK dependent change for $\beta_\tau$ , 1 mmol/L
$K_{\text{Rel}, \infty}$	$I_{\text{rel}}$ , half saturation coefficient for $I_{\text{rel}, \infty}$ , 1 mmol/L
$h_{\text{Rel}}$	$I_{\text{rel}}$ , Hill coefficient for $I_{\text{rel}, \infty}$ , 8
$h_\beta$	$I_{\text{rel}}$ , Hill coefficient for $\Delta\beta_{\tau, \text{CaMK}}$ , 10
$\kappa$	$I_{\text{rel}}$ , Amplitude coefficient for $\alpha_{\text{Rel}}$ , 0.1125 $\mu\text{A}/\mu\text{F}$
$K_{\text{Rel}, \tau}$	$I_{\text{rel}}$ , Half saturation coefficient for $I_{\text{Rel}, \tau}$ 0.0123 mmol/L
$\bar{\Delta K}_{m, \text{PLB}}$	$I_{\text{up}}$ , Phospholamban Half-saturation factor, 1.7e-4 mmol/L
$\bar{\Delta I}_{\text{up}, \text{CaMK}}$	$I_{\text{up}}$ , Maximal CaMK dependent change in $I_{\text{up}}$ , 0.75
$\bar{I}_{\text{up}}$	$I_{\text{up}}$ , Maximal value of $I_{\text{up}}$ , 0.004375 mmol/L per ms
$K_{m, \text{up}}$	$I_{\text{up}}$ , Half saturation of $I_{\text{up}}$ , 0.00092 mmol/L
$\tau_{\text{tr}}$	$I_{\text{tr}}$ , Time constant for NSR/JSR $\text{Ca}^{2+}$ transfer, 25 ms

## Compartments

i	intracellular space
o	extracellular space
SR	sarcoplasmic reticulum

JSR	junctional sarcoplasmic reticulum
NSR	network sarcoplasmic reticulum
SS,SR	SR Ca <sup>2+</sup> release subspace
SS,CaL	L-type Ca <sup>2+</sup> entry subspace

### Concentrations and Buffering

[Ca <sup>2+</sup> ] <sub>x</sub>	Calcium concentration in compartment X, mmol/L
[Na <sup>+</sup> ] <sub>x</sub>	Sodium concentration in compartment X, mmol/L
[K <sup>+</sup> ] <sub>x</sub>	Potassium concentration in compartment X, mmol/L
[Cl <sup>-</sup> ] <sub>x</sub>	Chloride concentration in compartment X, mmol/L
csqn	Calsequestrin, Ca <sup>2+</sup> buffer in JSR
trpn	Troponin, Ca <sup>2+</sup> buffer in JSR
BSR <sub>x</sub>	Anionic SR binding sites for Ca <sup>2+</sup> (SS or SS,CaL)
BSL <sub>x</sub>	Anionic sarcolemmal binding sites for Ca <sup>2+</sup> (SS or SS,CaL)
$\overline{[trpn]}$	Maximum troponin concentration, 0.07 mmol/L
$\overline{[cmdn]}$	Maximum calmodulin concentration, 0.05 mmol/L
$K_{m,cmdn}$	Calmodulin half saturation, 0.00238 mmol/L
$K_{m,trpn}$	Troponin half saturation, 0.0005 mmol/L
$\overline{[csqn]}$	Calsequestrin concentration, 10.0 mmol/L
$K_{m,csqn}$	Troponin half saturation, 0.8 mmol/L

### Cell geometry

Length (L) = 0.01 cm; radius (r) = 0.0011 cm

Cell volume:	$V_{cell} = \pi \cdot r^2 \cdot L = 38e-6 \mu L$
Geometric membrane area:	$A_{geo} = 2\pi \cdot r^2 + 2\pi \cdot r \cdot L = 0.767e-4 cm^2$
Capacitative membrane area:	$A_{cap} = R_{CG} \cdot A_{geo} = 1.534e-4 cm^2$
Myoplasmic volume:	$V_{myo} = V_{cell} \cdot 68\% = 25.84e-6 \mu L$
Mitochondrial volume:	$V_{mito} = V_{cell} \cdot 26\% = 9.88e-6 \mu L$
SR volume:	$V_{SR} = V_{cell} \cdot 6\% = 2.28e-6 \mu L$
NSR volume:	$V_{NSR} = V_{cell} \cdot 5.52\% = 2.098e-6 \mu L$
JSR volume:	$V_{JSR} = V_{cell} \cdot 0.48\% = 0.182e-6 \mu L$
SS,SR volume:	$V_{SS,SR} = V_{cell} \cdot 2.0\% = 0.76e-6 \mu L$
SS,CaL volume:	$V_{SS,CaL} = V_{cell} \cdot 0.2\% = 0.076e-6 \mu L$

### External Concentrations

$[Ca^{2+}]_o$	1.8 mmol/L
$[Cl^-]_o$	100 mmol/L
$[K^+]_o$	5.4 mmol/L
$[Na^+]_o$	140 mmol/L

### Resting voltage and concentrations (Single cell, unpaced for 5400 seconds)

$V_m$	-87.472528 mV
$[Ca^{2+}]_i$	0.000096833 mmol/L
$[Ca^{2+}]_{SS,SR}$	0.000138151 mmol/L
$[Ca^{2+}]_{SS,CaL}$	0.000143898 mmol/L
$[Ca^{2+}]_{JSR}$	1.133109 mmol/L
$[Ca^{2+}]_{NSR}$	1.159942 mmol/L

[Cl <sup>-</sup> ] <sub>i</sub>	20.26905mmol/L
[K <sup>+</sup> ] <sub>i</sub>	145.5689 mmol/L
[Na <sup>+</sup> ] <sub>i</sub>	6.935575 mmol/L
[Na <sup>+</sup> ] <sub>ss</sub>	6.935607 mmol/L
[Cl <sup>-</sup> ] <sub>ss</sub>	20.26905 mmol/L

## Model Equations

### 1. I<sub>CaL</sub>

See Chapter 2 for description of model and experimental fits. Note that this formulation was modified in the NZ model (see Appendix B).

$$ACT_{\tau} = 0.59 + 0.8 \cdot \frac{\exp(0.052 \cdot (V_m + 13))}{1 + \exp(0.132 \cdot (V_m + 13))}$$

$$ACT_{\infty} = \frac{1}{1.0 + \exp(-(V_m - 13.56)/9.45)}$$

$$\alpha = \frac{ACT_{\infty}}{ACT_{\tau}}$$

$$\beta = \frac{1 - ACT_{\infty}}{ACT_{\tau}}$$

$$I_{V,\infty} = \frac{1/(1 + \exp((V_m + 17.5)/3)) + 0.25}{1.25}$$

$$I_{V,\tau} = \frac{1}{\frac{1}{24.828 \cdot (1 + \exp(((V_m + 49.10)/10.349)))} + \frac{1}{30.553 \cdot (1 + \exp((-V_m + 0.213)/10.807))}}$$

$$x = \frac{I_{V,\infty}}{I_{V,\tau}}$$

$$y = \frac{1 - I_{V,\infty}}{I_{V,\tau}}$$

$$I_{Ca}^* = 25 - \frac{17.5}{1 + \left( \frac{0.003}{[Ca^{2+}]_{SS, CaL}} \right)^4}$$

$$I_{V,\tau}^* = \frac{1}{\frac{1}{24.828 \cdot (1 + \exp(((V_m + 49.10)/10.349)))} + \frac{1}{I_{Ca}^* \cdot (1 + \exp((-V_m + 0.213)/10.807))}}$$

$$I_{V,\infty}^* = \frac{1/(1 + \exp((V_m + 17.5)/3)) + 0.0001}{1.0001}$$

$$x^* = \frac{I_{V,\infty}^*}{I_{V,\tau}^*}$$

$$y^* = \frac{1 - I_{V,\infty}^*}{I_{V,\tau}^*}$$

$$\delta = \frac{3}{1 + \left( \frac{0.003}{[Ca^{2+}]_{SS, CaL}} \right)^4}$$

$$\theta = 1$$

$$\theta_I = 0.000001$$

$$\delta_I = \theta_I \cdot \left( \frac{x \cdot y^* \cdot \delta}{y \cdot x^* \cdot \theta} \right)$$

### State transitions:

$$\frac{dC}{dt} = -(\alpha + \delta + y) \cdot C + \beta \cdot O + \theta \cdot C^* + x \cdot CI$$

$$\frac{dO}{dt} = -(\beta + \delta + y) \cdot O + \alpha \cdot C + \theta \cdot O^* + x \cdot OI$$

$$\frac{dC^*}{dt} = -(\alpha + \theta + y^*) \cdot C^* + \delta \cdot C + \beta \cdot O^* + x^* \cdot CI^*$$

$$\frac{dO^*}{dt} = -(\beta + \theta + y^*) \cdot O^* + \delta \cdot O + \alpha \cdot C^* + x^* \cdot OI^*$$

$$\frac{dCI}{dt} = -(\alpha + \delta_I + x) \cdot CI + y \cdot C + \theta_I \cdot CI^* + \beta \cdot OI$$

$$\frac{dOI}{dt} = -(\beta + \delta_I + x) \cdot OI + y \cdot O + \theta_I \cdot OI^* + \alpha \cdot CI$$

$$\frac{dCI^*}{dt} = -(\alpha + \theta_I + x^*) \cdot CI^* + \delta_I \cdot CI + y^* \cdot C^* + \beta \cdot OI^*$$

$$\frac{dOI^*}{dt} = -(\beta + \theta_I + x^*) \cdot OI^* + \delta_I \cdot OI + y^* \cdot O^* + \alpha \cdot CI^*$$

### Current:

$$P_{Ca} = 1.5552 \times 10^{-4} \text{ cm/s}; \gamma_{Cai} = 1; \gamma_{Cao} = 0.341$$

$$\bar{I}_{CaL} = P_{Ca} \cdot z_{Ca}^2 \cdot \frac{V \cdot F^2}{RT} \cdot \frac{\gamma_{Cai} \cdot [Ca^{2+}]_{SS, CaL} \cdot \exp(z_{Ca} \cdot V_m \cdot F / (RT)) - \gamma_{Cao} \cdot [Ca^{2+}]_0}{\exp(z_{Ca} \cdot V_m \cdot F / (RT)) - 1}$$

$$I_{CaL} = \bar{I}_{CaL} \cdot (O + O^*)$$

## 2. Slow component of the delayed rectifier potassium channel ( $I_{Ks}$ )

See Chapter 2 for description of model and experimental fits.

### Kinetic parameters:

$$\alpha = 1.4864 \cdot 10^{-2} \cdot \exp(2.9877 \cdot 10^{-2} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\beta = 8.3986 \cdot 10^{-2} \cdot \exp(-5.5461 \cdot 10^{-2} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\gamma = 1.4601 \cdot 10^{-2} \cdot \exp(2.4465 \cdot 10^{-1} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\delta = 3.1173 \cdot 10^{-3} \cdot \exp(-4.2625 \cdot 10^{-1} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\eta = 7.7320 \cdot 10^{-2} \cdot \exp(-6.4726 \cdot 10^{-2} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\theta = 8.9538 \cdot 10^{-2}$$

$$\omega = 7.9405 \cdot 10^{-1} \cdot \exp(-8.0174 \cdot 10^{-2} \cdot V_m \cdot (F / (R \cdot T)))$$

$$\psi = 5.8638 \cdot 10^{-1} \cdot \exp(2.8206 \cdot 10^{-1} \cdot V_m \cdot (F / (R \cdot T)))$$

### State transitions:

$$\frac{dC_1}{dt} = -(4\alpha) \cdot C_1 + \beta \cdot C_2$$

$$\frac{dC_2}{dt} = -(3\alpha + \beta + \gamma) \cdot C_2 + 4\alpha \cdot C_1 + 2\beta \cdot C_3 + \delta \cdot C_6$$

$$\frac{dC_3}{dt} = -(2\alpha + 2\beta + 2\gamma) \cdot C_3 + 3\alpha \cdot C_2 + 3\beta \cdot C_4 + \delta \cdot C_7$$

$$\frac{dC_4}{dt} = -(\alpha + 3\beta + 3\gamma) \cdot C_4 + 2\alpha \cdot C_3 + 4\beta \cdot C_5 + \delta \cdot C_8$$

$$\frac{dC_5}{dt} = -(4\beta + 4\gamma) \cdot C_5 + \alpha \cdot C_4 + \delta \cdot C_9$$

$$\frac{dC_6}{dt} = -(3\alpha + \delta) \cdot C_6 + \beta \cdot C_7 + \gamma \cdot C_2$$

$$\frac{dC_7}{dt} = -(2\alpha + \beta + \gamma + \delta) \cdot C_7 + 3\alpha \cdot C_6 + 2\beta \cdot C_8 + 2\gamma \cdot C_3 + 2\delta \cdot C_{10}$$

$$\frac{dC_8}{dt} = -(\alpha + 2\beta + 2\gamma + \delta) \cdot C_8 + 2\alpha \cdot C_7 + 3\beta \cdot C_9 + 3\gamma \cdot C_4 + 2\delta \cdot C_{11}$$

$$\frac{dC_9}{dt} = -(3\beta + 3\gamma + \delta) \cdot C_9 + \alpha \cdot C_8 + 4\gamma \cdot C_5 + 2\delta \cdot C_{12}$$

$$\frac{dC_{10}}{dt} = -(2\alpha + 2\delta) \cdot C_{10} + \beta \cdot C_{11} + \gamma \cdot C_7$$

$$\frac{dC_{11}}{dt} = -(\alpha + \beta + \gamma + 2\delta) \cdot C_{11} + 2\alpha \cdot C_{10} + 2\beta \cdot C_{12} + 2\gamma \cdot C_8 + 3\delta \cdot C_{13}$$

$$\frac{dC_{12}}{dt} = -(2\beta + 2\gamma + 2\delta) \cdot C_{12} + \alpha \cdot C_{11} + 3\gamma \cdot C_9 + 3\delta \cdot C_{14}$$

$$\frac{dC_{13}}{dt} = -(\alpha + 3\delta) \cdot C_{13} + \beta \cdot C_{14} + \gamma \cdot C_{11}$$

$$\frac{dC_{14}}{dt} = -(\beta + \gamma + 3\delta) \cdot C_{14} + \alpha \cdot C_{13} + 2\gamma \cdot C_{12} + 4\delta \cdot C_{15}$$

$$\frac{dC_{15}}{dt} = -(4\delta + \theta) \cdot C_{15} + \gamma \cdot C_{14} + \eta \cdot O_1$$

$$\frac{dO_1}{dt} = -(\eta + \psi) \cdot O_1 + \theta \cdot C_{15} + \omega \cdot O_2$$

$$\frac{dO_2}{dt} = -(\omega) \cdot O_2 + \psi \cdot O_1$$

**Current:**

$$\bar{G}_{Ks} = 0.0826 \cdot \left( 1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_i} \right)^{1.4}} \right)$$

$$O_{Ks} = O_1 + O_2$$

$$E_{Ks} = \frac{R \cdot T}{F} \cdot \log \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_o}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i}$$

$$I_{Ks} = \bar{G}_{Ks} \cdot O_{Ks} \cdot (V_m - E_{Ks})$$

**4. I<sub>NaK</sub>**

See Chapter 2 for description of model and experimental fits.

$$V_{1/2} = -92 \text{ mV}$$

$$\sigma = 1$$

$$\phi = \sigma \cdot (V_m - V_{1/2}) \cdot \frac{F}{RT}$$

$$f_v = \frac{1}{1 + \exp(-\phi)}$$

$$K_{Ko} = 1.5 \text{ mM}$$

$$P_K = \frac{[K^+]_o}{[K^+]_o + K_{Ko}}$$

$$K_{Nai} = 2.6 \text{ mM}$$

$$P_{Na} = \left( \frac{[Na^+]_i}{[Na^+]_i + K_{Nai}} \right)^3$$

$$\bar{I}_{NaK} = 1.4 \text{ mS}/\mu\text{F}$$



$$I_{NaK} = \bar{I}_{NaK} \cdot f_v \cdot P_{Na} \cdot P_K$$

## 5. $I_{to1}$

See Chapter 2 for description of model and experimental fits.

$$E_{to1} = \frac{RT}{F} \cdot \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)$$

$$R_{to1} = \exp\left(\frac{V_m}{550}\right)$$

$$\beta_a = \frac{3.5}{\left(1 + \exp\left(\frac{(V_m + 100)}{29.3814}\right)\right)}$$

$$\alpha_a = \frac{1}{1.2089 \cdot \left(1 + \exp\left(\frac{(V_m - 18.4099)}{-29.3814}\right)\right)}$$

$$\tau_a = \frac{1}{\alpha_a + \beta_a}$$

$$a_\infty = \frac{1}{\left(1 + \exp\left(\frac{(V_m + 9.437)}{-7.133}\right)\right)}$$

$$\alpha_{if} = \frac{0.025}{\left(1 + \exp\left(\frac{(V_m + 58)}{5}\right)\right)}$$

$$\beta_{if} = \frac{1}{9.7953 \cdot \left(1 + \exp\left(\frac{(V_m + 19)}{-9}\right)\right)}$$

$$\alpha_{is} = \frac{1}{250 \cdot \left(1 + \exp\left(\frac{(V_m + 60)}{5}\right)\right)}$$

$$\beta_{is} = \beta_{if}$$

$$\bar{G}_{to1} = 0.4975 \text{ mS}/\mu\text{F}$$

$$I_{to1} = \bar{G}_{to1} \cdot a^3 \cdot i_{1f} \cdot i_{1s} \cdot R_{to1} \cdot (V_m - E_{to1})$$

## 6. $I_{to2}$

See Chapter 2 for description of model and experimental fits.

$$k_{Ca,ito2} = 0.4$$

$$K_{Ca,ito2} = 1 - \frac{1}{1 + \left( \frac{I_{rel}}{k_{Ca,ito2}} \right)^2}$$

$$\tau_{i2} = 8$$

$$\alpha_{i2} = \frac{0.025}{\left( 1 + \exp\left( \frac{(V_m + 58)}{5} \right) \right)}$$

$$\beta_{i2} = \frac{1}{5 \cdot \left( 1 + \exp\left( \frac{(V_m + 19)}{-9} \right) \right)}$$

$$i_{2,\infty} = \frac{\alpha_{i2}}{\alpha_{i2} + \beta_{i2}}$$

$$P_{Cl} = 9e-7 \text{ cm/s}$$

$$\bar{I}_{to2} = P_{Cl} \cdot z_{Cl}^2 \cdot \frac{V_m F^2}{RT} \cdot \frac{[Cl]_i - [Cl]_o \cdot \exp(-z_{Cl} \cdot V_m \cdot F / (RT))}{1 - \exp(-z_{Cl} \cdot V_m \cdot F / (RT))}$$

$$I_{to2} = \bar{I}_{to2} \cdot i_{2f} \cdot K_{Ca,ito2}$$

## 7. $I_{NaCa}$

The  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger formulation was adopted from Weber and Bers. 20% of exchangers were located in the restricted space, consistent with experimental results and previous work from our lab (Faber et al., 2007).

$$I_{\text{NaCa},x} = \frac{f_x \cdot v_{\text{max}} \cdot \left( [\text{Na}^+]_x^3 \cdot [\text{Ca}^{2+}]_o \cdot \exp\left(\frac{\eta \cdot VF}{RT}\right) - [\text{Na}^+]_o^3 \cdot [\text{Ca}^{2+}]_x \cdot \exp\left(\frac{(\eta-1) \cdot VF}{RT}\right) \right)}{\left( 1 + \left( \frac{K_{m\text{Ca},\text{act}}}{[\text{Ca}^{2+}]_x} \right)^2 \right) \cdot \left( 1 + k_{\text{sat}} \exp\left(\frac{(\eta-1) \cdot VF}{RT}\right) \right) \cdot \left( \begin{array}{l} K_{m,\text{Cao}}[\text{Na}^+]_x^3 + K_{m,\text{Nao}}^3[\text{Ca}^{2+}]_x + \\ K_{m,\text{Nai}}^3[\text{Ca}^{2+}]_o \cdot \left( 1 + \frac{[\text{Ca}^{2+}]_x}{K_{m,\text{Cai}}} \right) + \\ K_{m,\text{Cai}}[\text{Na}^+]_o^3 \cdot \left( 1 + \frac{[\text{Na}^+]_x^3}{K_{m,\text{Nai}}^3} \right) + \\ [\text{Na}^+]_x^3 \cdot [\text{Ca}^{2+}]_o + [\text{Na}^+]_o^3 \cdot [\text{Ca}^{2+}]_x \end{array} \right)}$$

where

$$I_{\text{NaCa}} = I_{\text{NaCa},\text{SS},\text{SR}} + I_{\text{NaCa},i}$$

and if

$$x = \text{SS}, \text{SR} \text{ then } f_x = f_{\text{SS},\text{SR}} = 0.2, [\text{Na}^+]_x = [\text{Na}^+]_{\text{SS},\text{SR}} \text{ and } [\text{Ca}^{2+}]_x = [\text{Ca}^{2+}]_{\text{SS},\text{SR}}$$

$$x = i \text{ then } f_x = f_i = 0.8, [\text{Na}^+]_x = [\text{Na}^+]_i \text{ and } [\text{Ca}^{2+}]_x = [\text{Ca}^{2+}]_i$$

## 8. $I_{\text{Na}}$

Fast  $\text{Na}^+$  current density ( $I_{\text{Na}}$ ) was adjusted to achieve conduction velocity (Spach et al., 2000), maximum upstroke velocity (Di Diego et al., 1996) and maximum upstroke voltage (Di Diego et al., 1996) consistent with data in canine epicardial tissue.  $\text{Na}^+$  current recovery from inactivation was adjusted to fit data on recovery of upstroke velocity in canine epicardial myocytes (Lue and Boyden, 1992). Note that this formulation was modified in the NZ model (see Appendix B).

$$\bar{G}_{\text{Na}} = 9.075 \text{ mS}/\mu\text{F}$$

$$E_{Na} = \frac{RT}{F} \cdot \ln \left( \frac{[Na^+]_o}{[Na^+]_i} \right)$$

$$\alpha_m = \frac{0.32 \cdot (V_m + 47.13)}{1 - \exp(-0.1 \cdot (V_m + 47.13))}$$

$$\beta_m = 0.08 \cdot \exp\left(\frac{-V_m}{11}\right)$$

If  $V_m \geq -40.0$  mV

$$\alpha_h = 0.0$$

$$\beta_h = \frac{1}{0.13 \cdot \left( 1 + \exp\left(\frac{V_m + 10.66}{-11.1}\right) \right)}$$

$$\alpha_j = 0.0$$

$$\beta_j = \frac{0.3 \cdot (-2.535 \times 10^{-7}) \cdot V_m}{\exp(-0.1 \cdot (V_m + 32)) + 1}$$

else

$$\alpha_h = 0.135 \cdot \exp\left(\frac{(80.0 + V_m)}{-6.8}\right)$$

$$\beta_h = 3.56 \cdot \exp(0.079 \cdot V_m) + 3.1 \times 10^5 \cdot \exp(0.35 \cdot V_m)$$

$$\alpha_j = \frac{(-1.2714 \times 10^5 \cdot \exp(0.2444 \cdot V_m) - 6.948 \times 10^{-5} \cdot \exp(-0.04391 \cdot V_m)) \cdot (V_m + 37.78)}{1 + \exp(0.311 \cdot (V_m + 79.23))}$$

$$\beta_j = \frac{0.1212 \cdot \exp(-0.01052 \cdot V_m)}{1 + \exp(-0.1378 \cdot (V_m + 40.14))}$$

$$I_{Na} = \bar{G}_{Na} \cdot m^3 \cdot h \cdot j \cdot (V_m - E_{Na})$$

## 9. $I_{Nab}$ , $CT_{NaCl}$ , and $CT_{KCl}$

Background  $Na^+$  current ( $I_{Nab}$ ) and co-transporter fluxes were required to maintain a resting  $[Na^+]_i$  concentration consistent with measurements in canine epicardial myocytes (Gao et

al., 2005).  $\text{Na}^+$  permeability for the background  $\text{Na}^+$  current was given by the constant field equation, following the formulation used by Gao et al. (Gao et al., 2005):

$$\phi = \frac{F \cdot V_m}{R \cdot T}$$

$$P_{\text{Nab}} = 0.32 \times 10^{-8} \text{ cm/s}$$

$$I_{\text{Nab}} = F \cdot P_{\text{nab}} \cdot \phi \cdot \frac{([\text{Na}^+]_i \cdot \exp(\phi) - [\text{Na}^+]_o)}{\exp(\phi) - 1}$$

$\text{K}^+$ - $\text{Cl}^-$  ( $\text{CT}_{\text{KCl}}$ ) and  $\text{Na}^+$ - $\text{Cl}^-$  ( $\text{CT}_{\text{NaCl}}$ ) cotransporter formulations were based on those in the original HRd model, with modified density.

$$E_{\text{Na}} = \frac{RT}{F} \cdot \ln \left( \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right)$$

$$E_{\text{K}} = \frac{RT}{F} \cdot \ln \left( \frac{[\text{K}^+]_o}{[\text{K}^+]_i} \right)$$

$$E_{\text{Cl}} = -\frac{RT}{F} \cdot \ln \left( \frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i} \right)$$

$$\overline{\text{CT}}_{\text{NaCl}} = 2.46108\text{e-}05 \text{ mmol/L per ms}$$

$$\text{CT}_{\text{NaCl}} = \overline{\text{CT}}_{\text{NaCl}} \cdot \frac{(E_{\text{Na}} - E_{\text{Cl}})^4}{(E_{\text{Na}} - E_{\text{Cl}})^4 + 87.8251^4}$$

$$\overline{\text{CT}}_{\text{KCl}} = 1.77\text{e-}05 \text{ mmol/L per ms}$$

$$\text{CT}_{\text{KCl}} = \overline{\text{CT}}_{\text{KCl}} \cdot \frac{(E_{\text{K}} - E_{\text{Cl}})}{(E_{\text{K}} - E_{\text{Cl}}) + 87.8251}$$

## 10. $\mathbf{I_{Kr}}$

The formulation for the rapid component of the delayed rectifier potassium current is equivalent to that in the original HRd model.

$$\bar{G}_{Kr} = 0.0138542 \cdot \sqrt{\frac{[K^+]_o}{5.4}}$$

$$E_{Kr} = \frac{RT}{F} \cdot \ln\left(\frac{[K^+]_o}{[K^+]_i}\right)$$

$$\tau_{Xr} = \frac{1}{\frac{0.6 \times 10^{-3} \cdot (V_m - 1.7384)}{1 - \exp(-0.136 \cdot (V_m - 1.7384))} + \frac{3 \times 10^{-4} \cdot (V_m + 38.3608)}{\exp(0.1522 \cdot (V_m + 38.3608)) - 1}}$$

$$X_{r\infty} = \frac{1}{1 + \exp(-(V_m + 10.0805) / 4.25)}$$

$$R_{Kr} = \frac{1}{1 + \exp((V_m + 10) / 15.4)}$$

$$I_{Kr} = \bar{G}_{Kr} \cdot X_r \cdot R_{Kr} \cdot (V_m - E_{Kr})$$

## 11. $I_{K1}$

The formulation for the inward rectifier potassium current is equivalent to that in the original HRd model.

$$E_{K1} = \frac{RT}{F} \cdot \ln\left(\frac{[K^+]_o}{[K^+]_i}\right)$$

$$\alpha_{K1} = \frac{1.02}{1 + \exp(0.2385 \cdot (V_m - E_{K1} - 59.215))}$$

$$\beta_{K1} = \frac{0.49124 \cdot \exp(0.08032 \cdot (V_m - E_{K1} + 5.476)) + \exp(0.06175 \cdot (V_m - E_{K1} - 594.31))}{1 + \exp(-0.5143 \cdot (V_m - E_{K1} + 4.753))}$$

$$K_1 = \frac{\alpha_{K1}}{\alpha_{K1} + \beta_{K1}}$$

$$\bar{G}_{K1} = 0.5 \cdot \sqrt{\frac{[K^+]_o}{5.4}}$$

$$I_{K1} = \bar{G}_{K1} \cdot K_1 \cdot (V_m - E_{K1})$$

## 12. $I_{Kp}$

The formulation for the plateau potassium current is equivalent to that in the original HRd model.

$$E_{Kp} = \frac{RT}{F} \cdot \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)$$
$$K_p = \frac{1}{1 + \exp \left( \frac{7.488 - V_m}{5.98} \right)}$$
$$\bar{G}_{Kp} = 2.76 \times 10^{-3} \text{ mS}/\mu\text{F}$$
$$I_{Kp} = \bar{G}_{Kp} \cdot K_p \cdot (V_m - E_{Kp})$$

## 13. $I_{pca}$

The formulation for the sarcolemmal calcium pump is equivalent to that in the original HRd model.

$$\bar{G}_{p,Ca} = 0.0575 \text{ mS}/\mu\text{F}; K_{m,pCa} = 0.0005 \text{ mM}$$
$$I_{p,Ca} = \bar{G}_{p,Ca} \cdot \frac{[Ca^{2+}]_i}{K_{m,pCa} + [Ca^{2+}]_i}$$

## 14. $I_{ca,b}$

The formulation for the background calcium current is equivalent to that in the original HRd model.

$$P_{Ca,b} = 1.995 \times 10^{-7} \text{ cm/s}; \gamma_{Cai} = 1; \gamma_{Cao} = 0.341$$
$$I_{Ca,b} = P_{Ca,b} \cdot z_{Ca}^2 \cdot \frac{V_m \cdot F^2}{RT} \cdot \frac{\gamma_{Cai} \cdot [Ca^{2+}]_i \cdot \exp(z_{Ca} \cdot V_m \cdot F / (RT)) - \gamma_{Cao} \cdot [Ca^{2+}]_o}{\exp(z_{Ca} \cdot V_m \cdot F / (RT)) - 1}$$

## 15. $I_{Cl,b}$

The formulation for the background chloride current is equivalent to that in the original HRd model.

$$\bar{G}_{Cl,b} = 2.25 \times 10^{-4} \mu A/\mu F$$

$$E_{Cl} = -\left(\frac{RT}{F}\right) \cdot \ln\left(\frac{[Cl^-]_o}{[Cl^-]_i}\right)$$

$$I_{Cl,b} = \bar{G}_{Cl,b} \cdot (V_m - E_{Cl})$$

## 16. $I_{Na,L}$

The formulation for the late  $Na^+$  current is equivalent to that in the original HRd model. Note that a new  $I_{Na,L}$  formulation was developed for the NZ model (see Appendix B).

$$E_{Na,L} = \frac{RT}{F} \cdot \ln\left(\frac{[Na^+]_o}{[Na^+]_i}\right)$$

$$\alpha_{m,L} = \frac{0.32 \cdot (V_m + 47.13)}{1 - \exp(-0.1 \cdot (V_m + 47.13))}$$

$$\beta_{m,L} = 0.08 \cdot \exp\left(\frac{-V_m}{11.0}\right)$$

$$h_{L,\infty} = \frac{1}{1 + \exp((V_m + 91)/6.1)}$$

$$\tau_{hL} = 600 \text{ ms}$$

$$\bar{G}_{Na,L} = 0.0065 \text{ ms}/\mu F$$

$$I_{Na,L} = \bar{G}_{Na,L} \cdot m_L^3 \cdot h_L \cdot (V_m - E_{Na,L})$$

## 17. SR $Ca^{2+}$ fluxes

Formulations for SR  $Ca^{2+}$  fluxes were adopted from Hund et al. (Hund and Rudy, 2004) and Livshitz et al. (Livshitz and Rudy, 2007)



## SR Release

Model SR release formulation is equivalent to that in Livshitz et al. (Livshitz and Rudy, 2007).

$$\Delta\beta_{\tau, CaMK} = \Delta\beta_0 / [1 + (K_{\beta} / CaMK_{active})^{h_{\beta}}]$$

$\alpha_{Rel} = \kappa\beta_{\tau} mmol \cdot L^{-1} \cdot ms^{-1}$  Type equation here.

$$\beta_{\tau} = \beta_0 (1 + \Delta\beta_{\tau, CaMK})$$

$$I_{rel, \tau} = \frac{\beta_{\tau}}{1 + (K_{Rel, \tau} / [Ca^{2+}]_{JSR})}$$

$$I_{rel, \infty} = \frac{\alpha_{Rel} I_{Ca, L}}{1 + (K_{Rel, \infty} / [Ca^{2+}]_{JSR})^{h_{Rel}}}$$

$$\frac{dI_{rel}}{dt} = - \frac{I_{rel, \infty} + I_{rel}}{I_{rel, \tau}}$$

## SR Uptake

Model SR release formulation is equivalent to that in Livshitz et al. (Livshitz and Rudy, 2007).

$$\Delta K_{m, PLB} = \bar{\Delta} K_{m, PLB} \cdot \frac{CaMK_{active}}{K_{m, CaMK} + CaMK_{active}}$$

$$\Delta I_{up, CaMK} = \bar{\Delta} I_{up, CaMK} \cdot \frac{CaMK_{active}}{K_{m, CaMK} + CaMK_{active}}$$

$$I_{up} = (\Delta I_{up, CaMK} + 1) \cdot \bar{I}_{up} \cdot \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + K_{m, up} - \Delta K_{m, PLB}}$$

## SR Leak

Model SR leak is equivalent to that in Livshitz et al. (Livshitz and Rudy, 2007).

$$I_{leak} = \frac{0.004375}{NSR} \cdot [Ca^{2+}]_{NSR}; \overline{NSR} = 15$$

### JSR/NSR transfer

Time constant of NSR/JSR Ca<sup>2+</sup> transfer was adjusted to 25 ms.

$$I_{tr} = \frac{([Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR})}{\tau_{tr}}$$

### 18. Intracellular subspaces and myoplasmic concentrations

L-type Ca<sup>2+</sup> channels are located in a local subspace (SS,CaL). Ryanodine receptors, I<sub>to2</sub> channels and 20% of Na<sup>+</sup>-Ca<sup>2+</sup> exchangers are located in a Ca<sup>2+</sup> release subspace (SS,SR). Ca<sup>2+</sup> diffusion occurs between SS,CaL and SS,SR (I<sub>diff,SS</sub>) and diffusion of Ca<sup>2+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> occur between the SS,SR and the bulk myoplasm (I<sub>diff,Ca</sub>, I<sub>diff,Na</sub> and I<sub>diff,Cl</sub>).

### Calcium

$$\tau_{Diff} = 0.2 \text{ ms}$$

$$\tau_{Diff,SS} = 2 \text{ ms}$$

$$I_{Diff,SS} = \frac{[Ca^{2+}]_{SS,SR} - [Ca^{2+}]_{SS,CaL}}{\tau_{Diff,SS}}$$

$$I_{Diff} = \frac{[Ca^{2+}]_{SS,SR} - [Ca^{2+}]_i}{\tau_{Diff}}$$

$$\frac{d[Ca^{2+}]_i}{dt} = - \left( \frac{(I_{cab} + I_{pca} - 2 \cdot I_{NaCa,i}) \cdot A_{cap} \cdot C_m}{z_{Ca} \cdot F \cdot V_{myo}} + (I_{up} - I_{leak}) \frac{V_{nsr}}{V_{myo}} - I_{Diff} \frac{V_{SS,SR}}{V_{myo}} \right)$$

$$[Ca^{2+}]_{i,t} = [trpn] + [cmdn] + [Ca^{2+}]_i + \Delta[Ca^{2+}]_i$$

$$b = \overline{cmdn} + \overline{trpn} - [Ca^{2+}]_{i,t} + K_{m,trpn} + K_{m,cmdn}$$

$$c = K_{m,cmdn} \cdot K_{m,trpn} - [Ca^{2+}]_{i,t} \cdot (K_{m,trpn} + K_{m,cmdn}) + \overline{trpn} \cdot K_{m,cmdn} + \overline{cmdn} \cdot K_{m,trpn}$$

$$d = -K_{m,tripn} \cdot K_{m,cmdn} \cdot [Ca^{2+}]_{i,t}$$

$$[Ca^{2+}]_i = \frac{2}{3} \sqrt{b^2 - 3c} \cdot \cos \left( \frac{1}{3} \cdot \cos^{-1} \left( \frac{9bc - 2b^3 - 27d}{2(b^2 - 3c)^{1.5}} \right) \right) - \frac{b}{3}$$

$$\beta_{SS} = \frac{1}{1 + \frac{\overline{BSR} \cdot K_{m,BSR}}{(K_{m,BSR} + [Ca^{2+}]_X)^2} + \frac{\overline{BSL} \cdot K_{m,BSL}}{(K_{m,BSL} + [Ca^{2+}]_X)^2}}$$

$$\overline{BSR} = 0.047 \text{mM}; K_{m,BSR} = 0.00087 \text{mM}$$

$$\overline{BSL} = 1.124 \text{mM}; K_{m,BSL} = 0.0087 \text{mM}$$

$$\frac{d[Ca^{2+}]_{SS}}{dt} = \beta_{SS} \left( \frac{2 \cdot I_{NaCa,ss} \cdot A_{cap} \cdot C_m}{z_{Ca} \cdot F \cdot V_{SS}} + I_{rel} \cdot \frac{V_{JSR}}{V_{SS}} - I_{Diff} - I_{Diff,SS} \right)$$

$$\frac{d[Ca^{2+}]_{SS(CaL)}}{dt} = \beta_{SS} \left( -\frac{I_{Ca(L)} \cdot A_{cap} \cdot C_m}{z_{Ca} \cdot F \cdot V_{SS(CaL)}} + \frac{V_{SS}}{V_{SS(CaL)}} \cdot I_{Diff,SS} \right)$$

$$\frac{d[Ca^{2+}]_{JSR}}{dt} = I_{tr} - I_{rel}$$

$$b = [\overline{csqn}] - [csqn] - [Ca^{2+}]_{JSR} - \Delta[Ca^{2+}]_{JSR} + K_{m,csqn}$$

$$c = K_{m,csqn} \cdot ([csqn] + [Ca^{2+}]_{JSR} + \Delta[Ca^{2+}]_{JSR})$$

$$[Ca^{2+}]_{JSR} = \frac{\sqrt{b^2 + 4c} - b}{2}$$

$$\frac{d[Ca^{2+}]_{NSR}}{dt} = I_{up} - I_{leak} - I_{tr} \cdot \frac{V_{JSR}}{V_{NSR}}$$

## Sodium

$$I_{Diff,Na} = \frac{[Na^+]_{SS} - [Na^+]_i}{\tau_{Diff,SS}}$$

$$\frac{d[Na^+]_i}{dt} = - \left( \frac{(3 \cdot I_{NaCa,i} + 3 \cdot I_{NaK} + I_{Na} + I_{NaL} + I_{Nab}) \cdot A_{Cap} \cdot C_m}{z_{Na} \cdot F \cdot V_{myo}} - CT_{NaCl} - I_{Diff,Na} \cdot \frac{V_{SS}}{V_{myo}} \right)$$

$$\frac{d[Na^+]_{SS}}{dt} = - \left( \frac{3 \cdot I_{NaCa,SS} \cdot A_{Cap} \cdot C_m}{z_{Na} \cdot F \cdot V_{SS}} + I_{Diff,Na} \right)$$

## Chloride

$$I_{Diff,Cl} = \frac{[Cl^-]_{SS} - [Cl^-]_i}{\tau_{Diff,SS}}$$

$$\frac{d[Cl^-]_i}{dt} = - \left( \frac{I_{Clb} \cdot A_{Cap} \cdot C_m}{z_{Cl} \cdot F \cdot V_{myo}} - CT_{NaCl} - CT_{KCl} - I_{Diff,Cl} \cdot \frac{V_{SS}}{V_{myo}} \right)$$

$$\frac{d[Cl^-]_{SS}}{dt} = - \left( \frac{I_{to2} \cdot A_{Cap} \cdot C_m}{z_{Cl} \cdot F \cdot V_{SS}} + I_{Diff,Cl} \right)$$

## Potassium

$$\frac{d[K^+]_i}{dt} = - \left( \frac{I_{K,tot} \cdot A_{Cap} \cdot C_m}{z_{Cl} \cdot F \cdot V_{myo}} - CT_{KCl} \right)$$

## CaMKII

Model CaMKII formulation is equivalent to that in the original HRd model.

$$\alpha_{CaMK} = 0.05 \text{ ms}^{-1}; \alpha_{CaMK} = 0.00068 \text{ ms}^{-1}$$

$$CaMK_0 = 0.05; K_{m,CaM} = 0.0015 \text{ mmol/L}; K_{m,CaMK} = 0.15$$

$$CaMK_{bound} = CaMK_o \cdot (1 - CaMK_{trap}) \cdot \frac{1}{1 + \frac{K_{m,CaM}}{[Ca^{2+}]_{SS}}}$$

$$\frac{dCaMK_{trap}}{dt} = \alpha_{CaMK} \cdot CaMK_{bound} \cdot (CaMK_{bound} + CaMK_{trap}) - \beta_{CaMK} \cdot CaMK_{trap}$$

$$CaMK_{active} = CaMK_{bound} + CaMK_{trap}$$

## Appendix B. NZ and BZ Model formulations

### Note on updates to the NZ model

The control normal zone (NZ) model is based on a previously published model (Decker et al., 2009) of the canine epicardial myocyte. The late  $\text{Na}^+$  current ( $I_{\text{NaL}}$ ) in the NZ model was reformulated to reflect more detailed characterization of channel kinetics in canine (Maltsev et al., 2008b) and human ventricular (Maltsev and Undrovinas, 2006) cardiomyocytes as described in Section S3. NZ  $I_{\text{Na}}$  steady state inactivation was shifted to better reproduce the time constant of recovery of  $dVdt_{\text{max}}$  recorded in NZ cells (Lue and Boyden, 1992). The NZ  $I_{\text{CaL}}$  formulation was adjusted to reproduce the effects of CaMKII overexpression (Kohlhaas et al., 2006). All other NZ current formulations are equivalent to those in the previously published model (Decker et al., 2009). The EBZ model was derived from the NZ model by incorporating available remodeling data as described below.

#### 1. $I_{\text{Na}}$

Fast  $\text{Na}^+$  current formulation is equivalent to that in a previous canine epicardial cell model (Decker et al., 2009). An additional parameter ( $V_{\text{SSI},\text{shift}}$ ) was introduced to facilitate fits to the relative shift in voltage dependent inactivation observed in NZ (Lue and Boyden, 1992), EBZ (Lue and Boyden, 1992) and SkM1  $I_{\text{Na}}$ . NZ  $V_{\text{SSI},\text{shift}} = 2$  mV and EBZ  $V_{\text{SSI},\text{shift}} = -7$  mV resulted in a relative shift in steady state inactivation (9 mV) matching that observed experimentally (Lue and Boyden, 1992). This shift in steady state activation also resulted in a time constant of recovery of maximum upstroke velocity in NZ and EBZ cells that matched experiments (conditioning potential of -80 mV). In addition,  $\bar{G}_{\text{Na}}$  in the EBZ was reduced in order to fit the decrease in peak upstroke velocity (conditioning potential of -110 mV) observed experimentally

(Lue and Boyden, 1992).  $\bar{G}_{Na}$  of SkM1 in EBZ tissue was adjusted to reproduce the 80% increase in EBZ  $dV_{dt_{max}}$  observed experimentally (Lau et al., 2009).

$V_{SSI,shift}$  Shift in steady state  $I_{Na}$  steady state inactivation, (mV)

$\bar{G}_{Na}$  Maximal conductance of  $I_{Na}$ , ms/ $\mu$ F

	$\bar{G}_{Na}$	$V_{SSI,shift}$
NZ	9.075 mS/ $\mu$ F	2 mV
EBZ	7.71375 mS/ $\mu$ F	-7 mV
SkM1	1.65 mS/ $\mu$ F	9 mV

$$E_{Na} = \frac{RT}{F} \cdot \ln \left( \frac{[Na^+]_o}{[Na^+]_i} \right)$$

$$\alpha_m = \frac{0.32 \cdot (V_m + 47.13)}{1 - \exp(-0.1 \cdot (V_m + 47.13))}$$

$$\beta_m = 0.08 \cdot \exp \left( \frac{-V_m}{11} \right)$$

If  $V_m \geq -40.0$  mV

$$\alpha_h = 0.0$$

$$\beta_h = \frac{1}{0.13 \cdot \left( 1 + \exp \left( \frac{(V_m - V_{SSI,shift}) + 10.66}{-11.1} \right) \right)}$$

$$\alpha_j = 0.0$$

$$\beta_j = \frac{0.3 \cdot (-2.535 \times 10^{-7}) \cdot (V_m - V_{SSI,shift})}{\exp(-0.1 \cdot ((V_m - V_{SSI,shift}) + 32)) + 1}$$

else

$$\alpha_h = 0.135 \cdot \exp \left( \frac{(80.0 + (V_m - V_{SSI,shift}))}{-6.8} \right)$$

$$\beta_h = 3.56 \cdot \exp(0.079 \cdot (V_m - V_{SSI,shift})) + 3.1 \times 10^5 \cdot \exp(0.35 \cdot (V_m - V_{SSI,shift}))$$

$$\alpha_j = \frac{(-1.2714 \times 10^5 \cdot \exp(0.2444 \cdot (V_m - V_{SSI,shift})))}{1 + \exp(0.311 \cdot ((V_m - V_{SSI,shift}) + 79.23))} - \frac{6.948 \times 10^{-5} \cdot \exp(-0.04391 \cdot (V_m - V_{SSI,shift})) \cdot ((V_m - V_{SSI,shift}) + 37.78)}{1 + \exp(0.311 \cdot ((V_m - V_{SSI,shift}) + 79.23))}$$

$$\beta_j = \frac{0.1212 \cdot \exp(-0.01052 \cdot (V_m - V_{SSI,shift}))}{1 + \exp(-0.1378 \cdot ((V_m - V_{SSI,shift}) + 40.14))}$$

$$I_{Na} = \bar{G}_{Na} \cdot m^3 \cdot h \cdot j \cdot (V_m - E_{Na})$$

## 2. $I_{CaL}$

NZ  $I_{CaL}$  current formulation is derived from that in a previous published canine epicardial cell model (Decker et al., 2009). The NZ model was updated so that maximal CaMKII activity increases peak  $I_{CaL}$  by 30% and slows  $Ca^{2+}$  dependent inactivation (Kohlhaas et al., 2006). EBZ current parameters were altered to reproduce a) decreased density ( $P_{Ca}$ ), b) rightward shift in the I-V relationship ( $ACT_{\infty,shift}$ ) and c) faster voltage dependent inactivation ( $\tau_{VDL,SS}$ ) (Aggarwal and Boyden, 1995).

$P_{Ca}$	Maximum $Ca^{2+}$ permeability of $I_{CaL}$ , cm/s
$ACT_{\infty,shift}$	Shift in $I_{CaL}$ activation parameter, mV
$\tau_{VDL,SS}$	Minimal $I_{CaL}$ inactivation time constant parameter, ms
$\Delta\tau_{Ca}^{CaMK}$	Maximal change in $I_{CaL}$ inactivation time constant due to CaMKII, ms
$\Delta P_{Ca}^{CaMK}$	Maximal change in $I_{CaL}$ $Ca^{2+}$ permeability due to CaMKII, cm/s

	$P_{Ca}$	$ACT_{\infty,shift}$	$\tau_{VDL,SS}$
NZ	1.5555x10 <sup>-4</sup> cm/s	13.56 mV	30.553 ms
EBZ	1.187x10 <sup>-4</sup> cm/s	17.5 mV	15.0 ms

$$ACT_{\infty} = \frac{1}{1.0 + \exp(-(V_m - ACT_{\infty,shift})/9.45)}$$

$$I_{V,\tau} = \frac{1}{\frac{1}{24.828 \cdot (1 + \exp((V_m + 49.10)/10.349))} + \frac{1}{\tau_{VDL,SS} \cdot (1 + \exp(-(V_m + 0.213)/10.807))}}$$

$$ACT_\tau = 0.59 + 0.8 \cdot \frac{\exp(0.052 \cdot (V_m + 13))}{1 + \exp(0.132 \cdot (V_m + 13))}$$

$$\alpha = \frac{ACT_\infty}{ACT_\tau}$$

$$\beta = \frac{1 - ACT_\infty}{ACT_\tau}$$

$$I_{V,\infty} = \frac{1/(1 + \exp((V_m + 17.5)/3)) + 0.25}{1.25}$$

$$x = \frac{I_{V,\infty}}{I_{V,\tau}}$$

$$y = \frac{1 - I_{V,\infty}}{I_{V,\tau}}$$

$$\Delta\tau_{Ca}^{CaMK} = \frac{7.5}{1 + \left(\frac{0.7}{[CaMK]_{active}}\right)^4}$$

$$I_{Ca}^* = 25 - \frac{17.5 - \Delta\tau_{Ca}^{CaMK}}{1 + \left(\frac{0.003}{[Ca^{2+}]_{SS,Cal}}\right)^4}$$

$$I_{V,\tau}^* = \frac{1}{\frac{1}{24.828 \cdot (1 + \exp((V_m + 49.10)/10.349))} + \frac{1}{I_{Ca}^* \cdot (1 + \exp(-(V_m + 0.213)/10.807))}}$$

$$I_{V,\infty}^* = \frac{1/(1 + \exp((V_m + 17.5)/3)) + 0.0001}{1.0001}$$

$$x^* = \frac{I_{V,\infty}^*}{I_{V,\tau}^*}$$

$$y^* = \frac{1 - I_{V,\infty}^*}{I_{V,\tau}^*}$$



$$\delta = \frac{3}{1 + \left( \frac{0.003}{[Ca^{2+}]_{SS, CaL}} \right)^4}$$

$$\theta = 1$$

$$\theta_I = 0.000001$$

$$\delta_I = \theta_I \cdot \left( \frac{x \cdot y^* \cdot \delta}{y \cdot x^* \cdot \theta} \right)$$

**State transitions:**

$$\frac{dC}{dt} = -(\alpha + \delta + y) \cdot C + \beta \cdot O + \theta \cdot C^* + x \cdot CI$$

$$\frac{dO}{dt} = -(\beta + \delta + y) \cdot O + \alpha \cdot C + \theta \cdot O^* + x \cdot OI$$

$$\frac{dC^*}{dt} = -(\alpha + \theta + y^*) \cdot C^* + \delta \cdot C + \beta \cdot O^* + x^* \cdot CI^*$$

$$\frac{dO^*}{dt} = -(\beta + \theta + y^*) \cdot O^* + \delta \cdot O + \alpha \cdot C^* + x^* \cdot OI^*$$

$$\frac{dCI}{dt} = -(\alpha + \delta_I + x) \cdot CI + y \cdot C + \theta_I \cdot CI^* + \beta \cdot OI$$

$$\frac{dOI}{dt} = -(\beta + \delta_I + x) \cdot OI + y \cdot O + \theta_I \cdot OI^* + \alpha \cdot CI$$

$$\frac{dCI^*}{dt} = -(\alpha + \theta_I + x^*) \cdot CI^* + \delta_I \cdot CI + y^* \cdot C^* + \beta \cdot OI^*$$

$$\frac{dOI^*}{dt} = -(\beta + \theta_I + x^*) \cdot OI^* + \delta_I \cdot OI + y^* \cdot O^* + \alpha \cdot CI^*$$

$$\Delta P_{Ca}^{CaMK} = \frac{0.4}{1 + \left( \frac{0.7}{CaMK_{active}} \right)^4}$$

$$\gamma_{Cai} = 1; \gamma_{Ca0} = 0.341$$

$$\bar{I}_{CaL} = (1 + \Delta P_{Ca}^{CaMK}) \cdot P_{Ca} \cdot z_{Ca}^2 \cdot \frac{V_m \cdot F^2}{RT} \left( \frac{\gamma_{Ca i} \cdot [Ca^{2+}]_{SS, CaL} \cdot \exp\left(z_{Ca} \cdot \frac{V_m \cdot F}{RT}\right) - \gamma_{Ca o} \cdot [Ca^{2+}]_o}{\exp\left(z_{Ca} \cdot \frac{V_m \cdot F}{RT}\right) - 1} \right)$$

$$I_{CaL} = \bar{I}_{CaL} \cdot (O + O^*)$$

### 3. $I_{K1}$

The approximately 70% reduction in density of  $I_{K1}$  recorded in the EBZ during ramp (Pinto and Boyden, 1999) and clamp (Lue and Boyden, 1992) protocols was reproduced by reducing the parameter  $\bar{G}_{K1,5.4}$

$\bar{G}_{K1,5.4}$  Maximal  $I_{K1}$  conductance at  $[K^+]_o = 5.4$  mM, ms/ $\mu$ F

	$\bar{G}_{K1,5.4}$
NZ	0.5 ms/ $\mu$ F
EBZ	0.15 ms/ $\mu$ F

$$E_{K1} = \frac{RT}{F} \cdot \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)$$

$$\alpha_{K1} = \frac{1.02}{1 + \exp(0.2385 \cdot (V_m - E_{K1} - 59.215))}$$

$$\beta_{K1} = \frac{0.49124 \cdot \exp(0.08032 \cdot (V_m - E_{K1} + 5.476)) + \exp(0.06175 \cdot (V_m - E_{K1} - 594.31))}{1 + \exp(-0.5143 \cdot (V_m - E_{K1} + 4.753))}$$

$$K_1 = \frac{\alpha_{K1}}{\alpha_{K1} + \beta_{K1}}$$

$$\bar{G}_{K1} = \bar{G}_{K1,5.4} \cdot \sqrt{\frac{[K^+]_o}{5.4}}$$

$$I_{K1} = \bar{G}_{K1} \cdot K_1 \cdot (V_m - E_{K1})$$

### 4. $I_{Kr}$

$I_{Kr}$  in the EBZ model was altered to fit experimentally observed reduction in density and more rapid activation (Jiang et al., 2000).

$\bar{G}_{Kr,5.4}$  Maximal  $I_{Kr}$  conductance at  $[K^+]_o = 5.4$  mM, ms/ $\mu$ F  
 $Xr_{\tau,MAG}$   $I_{Kr}$  activation time constant parameter,  $ms^{-1}mV^{-1}$

	$\bar{G}_{Kr,5.4}$	$Xr_{\tau,MAG}$
NZ	0.0138542 ms/ $\mu$ F	0.0006 $ms^{-1}mV^{-1}$
EBZ	0.009698 ms/ $\mu$ F	0.001 $ms^{-1}mV^{-1}$

$$\bar{G}_{Kr} = 0.0138542 \cdot \sqrt{\frac{[K^+]_o}{5.4}}$$

$$E_{Kr} = \frac{RT}{F} \cdot \ln\left(\frac{[K^+]_o}{[K^+]_i}\right)$$

$$\tau_{Kr} = \frac{1}{\frac{Xr_{\tau,MAG} \cdot (V_m - 1.7384)}{1 - \exp(-0.136 \cdot (V_m - 1.7384))} + \frac{3 \times 10^{-4} \cdot (V_m + 38.3608)}{\exp(0.1522 \cdot (V_m + 38.3608)) - 1}}$$

$$X_{r\infty} = \frac{1}{1 + \exp(-(V_m + 10.0805) / 4.25)}$$

$$R_{Kr} = \frac{1}{1 + \exp((V_m + 10) / 15.4)}$$

$$I_{Kr} = \bar{G}_{Kr} \cdot X_r \cdot R_{Kr} \cdot (V_m - E_{Kr})$$

## 5. $I_{Ks}$

$I_{Ks}$  density in the EBZ was reduced to match experiments (Jiang et al., 2000).

$\bar{G}_{Ks,\infty}$  Minimum  $I_{Ks}$  conductance, ms/ $\mu$ F

	$\bar{G}_{Ks,\infty}$
NZ	0.0826 ms/ $\mu$ F
EBZ	0.01652 ms/ $\mu$ F

$$\begin{aligned}
\alpha &= 1.4864 \cdot 10^{-2} \cdot \exp\left(2.9877 \cdot 10^{-2} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\beta &= 8.3986 \cdot 10^{-2} \cdot \exp\left(-5.5461 \cdot 10^{-2} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\gamma &= 1.4601 \cdot 10^{-2} \cdot \exp\left(2.4465 \cdot 10^{-1} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\delta &= 3.1173 \cdot 10^{-3} \cdot \exp\left(-4.2625 \cdot 10^{-1} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\eta &= 7.7320 \cdot 10^{-2} \cdot \exp\left(-6.4726 \cdot 10^{-2} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\theta &= 8.9538 \cdot 10^{-2} \\
\omega &= 7.9405 \cdot 10^{-1} \cdot \exp\left(-8.0174 \cdot 10^{-2} \cdot V_m \cdot (F/(R \cdot T))\right) \\
\psi &= 5.8638 \cdot 10^{-1} \cdot \exp\left(2.8206 \cdot 10^{-1} \cdot V_m \cdot (F/(R \cdot T))\right)
\end{aligned}$$

**State transitions:**

$$\begin{aligned}
\frac{dC_1}{dt} &= -(4\alpha) \cdot C_1 + \beta \cdot C_2 \\
\frac{dC_2}{dt} &= -(3\alpha + \beta + \gamma) \cdot C_2 + 4\alpha \cdot C_1 + 2\beta \cdot C_3 + \delta \cdot C_6 \\
\frac{dC_3}{dt} &= -(2\alpha + 2\beta + 2\gamma) \cdot C_3 + 3\alpha \cdot C_2 + 3\beta \cdot C_4 + \delta \cdot C_7 \\
\frac{dC_4}{dt} &= -(\alpha + 3\beta + 3\gamma) \cdot C_4 + 2\alpha \cdot C_3 + 4\beta \cdot C_5 + \delta \cdot C_8 \\
\frac{dC_5}{dt} &= -(4\beta + 4\gamma) \cdot C_5 + \alpha \cdot C_4 + \delta \cdot C_9 \\
\frac{dC_6}{dt} &= -(3\alpha + \delta) \cdot C_6 + \beta \cdot C_7 + \gamma \cdot C_2 \\
\frac{dC_7}{dt} &= -(2\alpha + \beta + \gamma + \delta) \cdot C_7 + 3\alpha \cdot C_6 + 2\beta \cdot C_8 + 2\gamma \cdot C_3 + 2\delta \cdot C_{10} \\
\frac{dC_8}{dt} &= -(\alpha + 2\beta + 2\gamma + \delta) \cdot C_8 + 2\alpha \cdot C_7 + 3\beta \cdot C_9 + 3\gamma \cdot C_4 + 2\delta \cdot C_{11} \\
\frac{dC_9}{dt} &= -(3\beta + 3\gamma + \delta) \cdot C_9 + \alpha \cdot C_8 + 4\gamma \cdot C_5 + 2\delta \cdot C_{12} \\
\frac{dC_{10}}{dt} &= -(2\alpha + 2\delta) \cdot C_{10} + \beta \cdot C_{11} + \gamma \cdot C_7 \\
\frac{dC_{11}}{dt} &= -(\alpha + \beta + \gamma + 2\delta) \cdot C_{11} + 2\alpha \cdot C_{10} + 2\beta \cdot C_{12} + 2\gamma \cdot C_8 + 3\delta \cdot C_{13}
\end{aligned}$$

$$\frac{dC_{12}}{dt} = -(2\beta + 2\gamma + 2\delta) \cdot C_{12} + \alpha \cdot C_{11} + 3\gamma \cdot C_9 + 3\delta \cdot C_{14}$$

$$\frac{dC_{13}}{dt} = -(\alpha + 3\delta) \cdot C_{13} + \beta \cdot C_{14} + \gamma \cdot C_{11}$$

$$\frac{dC_{14}}{dt} = -(\beta + \gamma + 3\delta) \cdot C_{14} + \alpha \cdot C_{13} + 2\gamma \cdot C_{12} + 4\delta \cdot C_{15}$$

$$\frac{dC_{15}}{dt} = -(4\delta + \theta) \cdot C_{15} + \gamma \cdot C_{14} + \eta \cdot O_1$$

$$\frac{dO_1}{dt} = -(\eta + \psi) \cdot O_1 + \theta \cdot C_{15} + \omega \cdot O_2$$

$$\frac{dO_2}{dt} = -(\omega) \cdot O_2 + \psi \cdot O_1$$

### Current:

$$\bar{G}_{Ks} = \bar{G}_{Ks,\infty} \cdot \left( 1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_i} \right)^{1.4}} \right)$$

$$O_{Ks} = O_1 + O_2$$

$$E_{Ks} = \frac{R \cdot T}{F} \cdot \log \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_o}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i}$$

$$I_{Ks} = \bar{G}_{Ks} \cdot O_{Ks} \cdot (V_m - E_{Ks})$$

### 6. $I_{to1}$

$I_{to1}$  density in the EBZ was reduced by 90% (Lue and Boyden, 1992).

$\bar{G}_{to1}$  Maximal  $I_{to1}$  conductance, ms/ $\mu$ F

	$\bar{G}_{to1}$
NZ	0.4975 ms/ $\mu$ F
EBZ	0.04975 ms/ $\mu$ F

### G. $I_{to2}$

$I_{to2}$  density in the EBZ was reduced to match experiments (Aggarwal et al., 1997).

$P_{Cl}(I_{to2})$  Maximum  $Cl^-$  permeability of  $I_{to2}$ , cm/s

	$P_{Cl}(I_{to2})$
NZ	9e-7 cm/s
EBZ	7.767e-7 cm/s

## 7. $I_{NaL}$

$I_{NaL}$  in the NZ and EBZ models was reformulated to better fit recent data from human and canine ventricular myocytes and reflect present understanding of the current (Zaza et al., 2008). At 24°C, human (Maltsev and Undrovinas, 2006) and canine (Maltsev et al., 2008a) ventricular  $I_{NaL}$  inactivates with fast ( $\tau \sim 50$  ms) and slow ( $\tau \sim 500$  ms) time constants during a pulse to -30 mV. These fast and slow time constants are thought to represent burst and late activating modes of the cardiac  $Na^+$  channel isoform ( $Na_v1.5$ ), respectively. Total  $I_{NaL}$  in the model is calculated as the sum of channels in the late bursting (*LB*) and late scattered (*LS*) modes. Time and voltage dependent inactivation of the burst and late activating modes was implemented using Hodgkin-Huxley type gates  $h_{LB}$  and  $h_{LS}$ . Considering the similarities in reported behavior in human (Maltsev and Undrovinas, 2006) and canine ventricle (Maltsev et al., 2008a),  $I_{NaL}$  onset of inactivation is based on characterization of the time course of inactivation in human ventricular myocytes (Maltsev and Undrovinas, 2006), as adjusted to 37°C based on Q10 data (Maltsev and Undrovinas, 2006). The relative contribution of the late scattered mode ( $f_{LS} = 0.27$ ) and steady state voltage dependence of inactivation is based on canine ventricular myocyte data (Maltsev et al., 2008a). The time constant of recovery from inactivation was adapted from data in human ventricular myocytes (Maltsev et al., 1998), also adjusted to 37°C based on Q10 of 2.2 (Maltsev and Undrovinas, 2006). Current density was adjusted to reproduce the experimentally measured

APD prolongation of approximately 40 ms after application of low concentrations of TTX in canine epicardial myocytes (Zygmunt et al., 2001). No experimental data are available on  $I_{NaL}$  in the EBZ.

$LS$	Late scattered mode, $I_{NaL}$
$LB$	Late bursting mode, $I_{NaL}$
$m_L$	activation gate, $I_{NaL}$
$h_{LB}$	late bursting inactivation gate, $I_{NaL}$
$h_{LS}$	late scattered inactivation gate, $I_{NaL}$
$\alpha_{m,L}, \beta_{m,L}$	Opening and closing rate constants of $I_{NaL}$ activation gate
$\tau_{h,LB}$	Time constant of late bursting $I_{NaL}$ inactivation gate
$\tau_{h,LS}$	Time constant of late scattered $I_{NaL}$ inactivation gate
$h_{LB,\infty}$	Steady state value of late bursting $I_{NaL}$ inactivation gate
$h_{LS,\infty}$	Steady state value of late scattered $I_{NaL}$ inactivation gate
$h_{LB}$	late bursting $I_{NaL}$ inactivation gate
$f_{LS}$	Relative contribution of $I_{NaL}$ late scattered mode

$$\alpha_{m,L} = \frac{0.32 \cdot (V_m + 47.13)}{1 - \exp(-0.1 \cdot (V_m + 47.13))}$$

$$\beta_{m,L} = 0.08 \cdot \exp\left(\frac{-V_m}{11.0}\right)$$

$$\tau_{h,LS} = \frac{1}{\frac{1}{215.4339 \cdot \left(1 + \exp\left(\frac{V_m + 85.9963}{8.9337}\right)\right)} + \frac{1}{168.6230 \cdot \left(1 + \exp\left(\frac{-(V_m + 83.1026)}{10.8195}\right)\right)}}$$

$$\tau_{h,LB} = \frac{1}{\frac{1}{215.4339 \cdot \left(1 + \exp\left(\frac{V_m + 85.9963}{8.9337}\right)\right)} + \frac{1}{11.1135 \cdot \left(1 + \exp\left(\frac{-(V_m + 33.3651)}{14.9459}\right)\right)}}$$

$$h_{LB,\infty} = h_{LS,\infty} = \frac{1}{1 + \exp((V_m + 91)/6.1)}$$

$$f_{LS} = 0.27$$

$$\bar{G}_{Na,L} = 0.0065 \text{ ms}/\mu\text{F}$$

$$E_{Na,L} = \frac{RT}{F} \cdot \ln\left(\frac{[Na^+]_o}{[Na^+]_i}\right)$$

$$I_{Na,L} = \bar{G}_{Na,L} \cdot m_L^3 \cdot (V_m - E_{Na,L}) \cdot ((1 - f_{LS}) \cdot h_{LB} + f_{LS} \cdot h_{LS})$$



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