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**A model of the rate dependence of the atrial action potential in
rabbit**

Lindblad, Douglas S., M.S.

Rice University, 1994

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300 N. Zeeb Rd.
Ann Arbor, MI 48106

RICE UNIVERSITY

**A Model of the Rate Dependence of the Atrial
Action Potential in Rabbit**

by

Douglas S. Lindblad

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
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Master of Science

APPROVED, THESIS COMMITTEE:

John W. Clark, Chairman
Professor of Electrical & Computer
Engineering

Ian D. Walker
Assistant Professor of Electrical &
Computer Engineering

Raymon M. Glantz
Professor of Biochemistry & Cell Biology

David Caprette
Lab Coordinator, Ecology & Evolutionary
Biology

Houston, Texas

April, 1994

A Model of the Rate Dependence of the Atrial Action Potential in Rabbit

Douglas S. Lindblad

Abstract

We have developed a mathematical model of the rabbit atrial myocyte in order to investigate the ionic bases of rate dependent changes in action potential (AP) wave-shape. Postulated mediators of such changes include rate dependencies of ionic currents (incomplete reactivation) and ionic concentration gradients. The model utilizes biophysical data to quantify the specific morphology and electrophysiology of the rabbit atrial cell. Ionic current descriptions incorporate whole-cell voltage-clamp data from enzymatically isolated rabbit cardiomyocytes, and account for the reactivation timecourse of the largest ionic currents. Our model can simulate both the whole-cell voltage-clamp data upon which it is based and the steady-state AP wave-shapes observed over a range of stimulus rates. It also predicts the intracellular $[Ca^{2+}]_i$ -transient that accompanies the AP, the “premature stimulus” response, and the effect of rapid stimulation on the AP. These responses provide insight into the electrophysiological mediators of atrial refractoriness and arrhythmia.

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Chapter 1

Introduction

Atrial myocardium constitutes one of two types of cardiac tissue responsible for the blood pumping function of the heart. Contractions of the atrial and ventricular chambers, which exist as two pairs in the mammalian heart, are coordinated during each pump cycle so as to produce a continuous flow of blood through the systemic circulation. Coordination is achieved through a coupling of the mechanical (contractile) response with an excitatory electrical response known as the action potential (AP). The latter stimulates contraction as it propagates from its source in the sino-atrial (SA) node through the atrial and ventricular tissues. Modulation of the rate of generation of SA nodal stimuli provides a primary mechanism for regulating the mean volume flow rate or cardiac output (CO) of the heart. This and other mechanisms adjust the cardiac output, nominally 5 liters/min in the healthy human adult, to meet the physiological oxygen, nutrient, and waste disposal demands of the systemic tissues.

Atrial tissue presents an interesting target for studying the role of rate in the excitatory and contractile functions of the heart. Although the muscular cells or myocytes of the atrium differ somewhat from ventricular myocytes in size and in the distribution of cellular constituents, both cell types share the same general structural components. Functionally, atrial myocytes follow a similar physiological process to ventricular myocytes during the cardiac cycle. Both cell types are excited by stimuli originating in the SA node. The stimulus induces an action potential which couples to an intracellular process to produce cell shortening and thus contraction. Moreover,

as we shall discuss, pacing rate exerts a significant effect on the shape of atrial action potentials. The mechanism(s) by which these changes occur and their implications for contraction present significant questions for investigation. Further, in the pathologic state both atrium and ventricle are known to exhibit abnormal patterns of beating rate and rhythm otherwise known as arrhythmias. It is therefore important to understand the normal mechanisms of rate-dependent regulation in order to provide appropriate treatment for the clinically abnormal condition.

In this work we investigate through modeling the influence of stimulus rate on the AP of single myocytes isolated from the rabbit atrium. Compared to other commonly studied species, the rabbit atrium offers more similar electrophysiological characteristics to those of human tissue. Nevertheless, the electrophysiology of rabbit atrial myocytes is far from simple. Both the cell membrane and interior contain numerous functional components, the dynamic behavior of which may depend in a non-linear fashion on several cell parameters. In the laboratory, a given experimental technique can only isolate a few of these components at one time. Consequently, a modeling study is required to examine the integrated effects of all components on cell responses.

In our model we examine the physiology behind such responses by providing mathematical descriptions of the cellular constituents known to participate in rabbit atrial excitation and contraction. To ensure the validity of our model representation, we have utilized experimental data to quantify various model components. The explanations offered by our model are therefore presented in physiologically meaningful terms.

The chapters which follow present the development, results and interpretations of our novel rabbit atrial model. Since these discussions assume some familiarity with the structural and functional components of mammalian heart cells, we present a brief review of these details below. This is followed by a short outline of cardiac

experimentation and modeling to provide some historical perspective for this work. We close with a summary of the objectives and focus of the present study.

1.1 Structure and Function in the Mammalian Atrium

As in the ventricle, atrial myocardium is structurally well-organized to serve its mechanical and electrical functions (Fig. 1.1(A)). Atrial muscle consists of a syncytium of muscular cells or myocytes with discrete mechanical and electrical interconnections. The latter, known as gap junctions, provide low-resistance pathways between cell interiors which allow for AP propagation from cell to cell. Each individual myocyte, however, contains the structural elements required for both electrical and mechanical cell functions. Electrical excitability derives from properties of the cell membrane, while mechanical contractility arises from interactions between intracellular ions and proteins. We consider each of these functions separately.

1.1.1 Electrical Activity

The electrical response known as the action potential (AP) is generated and controlled by several different types of proteins located within the lipid bilayer membrane which surrounds the cell. At rest the cell membrane or sarcolemma is relatively impermeable to ions. This impermeability, in combination with the activity of sarcolemmal ion-transporting pumps and exchangers, enables cells to maintain ionic concentrations gradients across the sarcolemma. Ion transport activity is oriented such that the intracellular concentrations of sodium ($[Na^+]_i$) and calcium ($[Ca^{2+}]_i$) are maintained at low levels and potassium ($[K^+]_i$) at high levels relative to the extracellular environment. Since these ions are charged particles, a polarizing electric field develops across the cell membrane such that the cell interior attains a more negative potential than the exterior. The transmembrane potential difference, known as the resting potential

(RP), is typically -60 to -90 mV in resting mammalian cardiac cells. An “active” AP response results when sufficient positive charge is introduced to the cell interior to raise the transmembrane potential (V) to a suitably less negative value. Such charge is introduced to the cell either by currents propagating from adjacent cells in vivo or by stimulation through an electrode accessing the intracellular medium in vitro. Under a suitable stimulus current, depolarization of V past a threshold potential results in the transient opening of sarcolemmal protein pores known as ion channels. These channels allow passive flux of specific ions across the cell membrane according to the direction of the particular ion’s concentration gradient. Opening and closing of channels is arranged such that Na^+ and Ca^{2+} ion influx precedes K^+ ion efflux, and consequently the cell depolarizes to a relatively positive V and then “repolarizes” back toward the RP. Transmembrane ion balances are restored between action potentials due to the activity of the pump and exchanger mechanisms. This transient pattern of changes in V is termed the action potential.

Figure 1.1: Structural Organization of Cardiac Muscle. Top: Diagram illustrating the cellular arrangement of heart muscle. From Junqueira et. al. [62, Fig 10-15]. Bottom: Illustration of the ultrastructure of cardiac muscle, showing the arrangement of myofibers into sarcomeres and the interspersed SR network. From Berne and Levy [4, Fig 28-2].

1.1.2 Contractile Activity and Excitation-Contraction Coupling

The muscular cells or myocytes of the atrium contain numerous filamentous bundles known as myofibrils. Each myofibril consists of a collection of myosin, actin, and troponin filaments organized into contractile segments known as the sarcomere. In the latter, myosin filaments occupy a dark A band within the myofibril, while actin filaments occupy a similar I band (Fig. 1.1(B)). Smaller troponin filaments arranged along the actin filaments act as regulators of contraction. Contraction results from the physical interaction of myosin and actin molecules which occurs when free intracellular (Ca^{2+}) ions bind to troponin and activate the actin-troponin complex [4, 63]. In mammalian heart, increases in intracellular Ca^{2+} ion concentration ($[Ca^{2+}]_i$) which induce contraction result primarily from a large efflux of Ca^{2+} ions from an intracellular Ca^{2+} sequestering organelle known as the sarcoplasmic reticulum (SR). This organelle forms a network of tubular structures which surround the myofibrils (Fig. 1.1(B),1.2). SR Ca^{2+} release is triggered mainly by small increases in $[Ca^{2+}]_i$ levels, a phenomenon known as Ca^{2+} -induced Ca^{2+} -release (CICR) [27, 28, 29].

Under resting conditions, contraction is prevented by maintaining $[Ca^{2+}]_i$ at extremely low levels (< 100 nM). Membrane excitation is coupled to contraction through a flux of Ca^{2+} ions into the cell during the action potential. Entry of Ca^{2+} ions via Ca^{2+} channels and perhaps exchanger mechanisms as well provides the transient $[Ca^{2+}]_i$ signal responsible for CICR [29]. The size and shape of this signal depends upon the activity of Ca^{2+} channels and transporters and thus indirectly upon the action potential itself. With cell repolarization contraction terminates due to re-sequestration of Ca^{2+} by the SR and Ca^{2+} extrusion by sarcolemmal transporters.

1.1.3 Control of Cardiac Output

Atrial action potentials and the consequent contractions are repetitive events initiated by rhythmic electrical impulses originating from a specialized region of the right atrium known as the sinoatrial (SA) node. The rate at which impulses are generated is determined by a neuroendocrine control system which responds to the physiological demands of systemic tissues. Net cardiac output (CO) results from the product of heart rate (HR) and the stroke volume (SV) of blood pumped during each contraction. Although the heart has some capability to alter CO by modulating SV (the Frank-Starling effect), changes in HR act as the primary mechanism for modulating cardiac output. Consequently, the effect of rate on the atrial action potential is functionally related to the normal heart's ability to respond to changing pump requirements. In the diseased heart, abnormalities of rate and rhythm generate the impaired electrophysiological responses and pump function observed in cardiac arrhythmia.

Figure 1.2: Electron Micrograph of Feline Atrium. The banded myofibers are visible, as are mitochondria (Mit), gap junctions (GJ), and mechanical interconnections known as desmosomes (D). From Weiss [105, Fig 11-8].

1.2 History of Cardiac Experimentation

Cardiac function has a long history of scientific study. The role of the heart as a pump was proposed by William Harvey as early as 1628 [64]. Galvani and Volta demonstrated a link between cardiac electrical and mechanical activity over two hundred years ago [4]. In this century, a progression of new experimental techniques has produced new studies and knowledge at an increasingly microscopic level. Electron microscopy has provided information on the ultrastructural arrangements of myocytes (Fig. 1.2) not visible under the light microscope (e.g. [61]). The advent of voltage- and ion-sensitive dyes in combination with "skinned" muscle fiber and myocyte techniques has uncovered the role of CICR in excitation-contraction coupling [27, 28]. Early voltage-clamp studies on membrane electrophysiology utilized a "sucrose gap" technique employing a macroscopic electrode [55]. Subsequent development of "conventional" microelectrodes and the whole-cell patch clamp technique [47] has greatly supplemented our knowledge of ion channel function.

The whole-cell patch-clamp method [47], in particular, has provided considerable experimental information concerning the ionic currents of the cardiac sarcolemma. In the technique, isolated single cells are obtained from tissue sections by enzymatic dispersion [58] and placed in an ionic bathing solution. A glass microelectrode having a tip diameter of a few microns or less is lowered onto the cell until the tip contacts the cell membrane. Application of gentle suction to the microelectrode causes the membrane to "seal" to the microelectrode tip, producing a gigohm resistance between the bathing and intracellular media. At this point, several techniques may be used to gain a relatively low-resistance access to the cell interior. In the "ruptured patch" technique, additional suction creates a hole in the sarcolemma within the interior of the microelectrode tip. In the "nystatin patch" technique, an enzyme within the solution filling the microelectrode digests microscopic pieces of the sarcolemma exposed

within the pipette tip. These techniques may be combined to increase (ruptured patch) or decrease (nystatin patch) the degree to which the cell interior is dialyzed by the microelectrode filling solution. Both techniques allow membrane currents to be recorded during controlled manipulation of membrane potential (voltage-clamp), or alternatively potential to be recorded during controlled manipulation of net membrane current (current-clamp).

The existence of rate-related effects on the cardiac action potential has been known for several decades; however, investigation using patch clamp techniques of the changes in specific membrane currents with rate has begun only recently. A study of rabbit atrial muscle preparations by Tanaka et. al. [99] in 1967 reported effects on the plateau of the rabbit atrial action potential which followed various periods of rest. Upon stimulation after rest the initial action potential had a somewhat triangular appearance, while the later action potentials that followed demonstrated a gradual increase in the prominence of a plateau. More recent studies in cardiac fiber preparations have correlated action potential waveshape (APW) changes to similar changes in the transient outward current (I_t). Boyett et. al. [9, 8, 7] have proposed that changes in the early repolarization phase of the action potential with rate reflect rate-dependent alterations in the completeness of recovery from inactivation of I_t . In sheep Purkinje fibers, Boyett et. al. [8, 7] have shown that increases in rate decrease the size of I_t and that changes in early APW with rate correspond to the rate- and potential-dependence of I_t . Moreover, 4-aminopyridine (4-AP), a blocker of I_t , has been shown to inhibit rate-related changes in early repolarization [7] and action potential amplitude [62] (canine Purkinje fibers). However, the interpretation of rate-dependent APW changes in fiber preparations has been complicated somewhat by the possible role of accumulation/depletion effects in the extracellular space [9]. Rate-dependent changes in other currents, e.g. calcium currents (I_{Ca}) or Ca^{2+} -

dependent currents [9, 6], have also been reported to contribute to the modulation of APW. More recent studies of isolated rabbit atrial myocytes by Giles and Imaizumi [39, 17] have supported the dominant role of I_t in modulating action potential wave-shape. Pronounced changes in the size of I_t with rate were reported in single atrial myocytes, both at room [39, 23 °C] and at a near-physiological [32, 36 °C] temperature (Fig. 1.3). Corresponding changes were reflected in action potential configuration.

A limited number of studies have also reported the effects of premature stimulation on action potential configuration. Boyett and Jewell [9] have the effects of premature stimuli on the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential. As premature stimuli are delayed, $\dot{V}_{max}$ generally increases reflecting more complete reactivation of I_{Na} . In isolated human atrial myocytes, Fermini et. al. [32] have reported the premature stimulus response following sustained low-rate stimulation (Fig. 1.4(A)). These premature action potentials demonstrate a gradual recovery of action potential height with time while showing little change in the character of mid or late repolarization (Fig. 1.4(A)).

1.3 History of Electrophysiological Modeling

Advances in the modeling of cardiac electrophysiology have to some extent paralleled advances in experimental measurement techniques. Hodgkin and Huxley [55] published a classical series of papers in 1952 describing a model of the squid axon action potential which could explain responses observed in voltage-clamp experiments. In 1977, Beeler and Reuter [3] applied Hodgkin Huxley (HH)-type models to a reconstruction of the ventricular action potential. DiFrancesco and Noble [21] advanced this work considerably in 1985 by integrating membrane and compartmental mass-balance models and providing more complete descriptions of membrane ion fluxes. This work was extended in 1987 by Hilgemann and Noble [52] with the publication

Figure 1.3: Frequency Dependence of the Transient Outward Current (I_t) in Rabbit Atrium. Left: Frequency dependence of the action potential (A) and I_t (B) at 23 °C, where the latter is measured by voltage-clamp steps to +20 mV applied at various rates. From Giles and Imaizumi [38, Fig 4]. Right: Frequency dependence of I_t at 36 °C, measured by voltage clamp steps to 0 mV at various rates. From Fermini et. al. [31, Fig 2].

of an SR compartmental model which mimicked the observed responses of extracellular Ca^{2+} transients. Unfortunately, each of these models were based largely on data from multicellular preparations and utilized somewhat qualitative descriptions of membrane currents. Notable cardiac models directed toward a single-cell data-based approach were provided by Rasmusson et. al. [86, 87] on bullfrog pacemaker and atrial cells.

1.4 Objective of Thesis

The primary objective of our present study is to develop a mathematical model of the rabbit atrial myocyte which accurately represents the rate-dependent behavior of action potentials. The opportunity to present a significantly improved description of the atrial action potential has been provided by the proliferation of patch-clamp studies in the recent literature. We adopt a data-based approach similar to that of Rasmusson et. al. [86, 87] in the model formulation process in order to explain the rate-dependent effect in biophysical terms as quantitatively as possible.

Toward this end, Chapter 2 will discuss the development of our model and its basis in experimental data. Chapter 3 presents the simulation results of our model including its capacity to represent rate-dependent changes in the rabbit atrial action potential. Chapter 4 discusses the interpretations, capabilities, and limitations of our model results. Chapter 5 summarizes the conclusions of this thesis. Details related to the process of model-based simulation and the the model equations themselves are included in Appendices A and B.

Figure 1.4: (A) Effect of Premature Stimuli on the Human Atrial Action Potential. The control action potential recorded under a 1.0 Hz stimulus frequency is followed by responses elicited by variously delayed "premature" stimuli. Reproduced from Fermini et. al. [32, Fig. 5].

Chapter 2

Model Development

2.1 Introduction

We have developed a mathematical model of the electrophysiological responses of an enzymatically-isolated rabbit atrial myocyte at 35 °C. It describes the nonlinear voltage- and time-dependent behavior of membrane potential, intracellular ion concentrations, and the known transmembrane ionic fluxes using mathematical expressions that are based on physiological data. The model has been designed to mimic the measured (in vitro) responses of single atrial cells reported in the literature or recorded by a collaborative laboratory (W. R. Giles, University of Calgary Medical School, Calgary, Alberta, Canada).

Our primary goal throughout the model development process has been to mimic action potentials recorded from rabbit atrial cells using physiologically-based model components. Wherever possible, we have used quantitative information (e.g. voltage-clamp data from isolated rabbit cardiac myocytes) to describe specific model elements (e.g. a particular ionic current). In some cases, however, suitable quantitative data was either unavailable or incomplete, and subtle modifications to the ionic current descriptions were required to explain observed action potential behavior. The complete atrial model, explained in the following sections, therefore gives a semi-quantitative representation of rabbit atrial cell electrophysiology. Notwithstanding these limitations, the model will be shown to have useful capabilities including the ability

to simulate both whole-cell voltage-clamp responses and action potential waveshape changes that occur with changes in stimulus frequency.

2.2 Model Components

The structure of our model consists of two components: an equivalent circuit model of the sarcolemma, and a fluid compartment model of the intra- and extracellular media of the cell. The former includes descriptions of transmembrane ionic channels, pumps, and exchangers that have been identified in the rabbit atrial cell membrane. The latter provides for material balances of Na^+ , K^+ and Ca^{2+} ions within the intracellular medium. In particular, the intracellular Ca^{2+} balance accounts for the buffering of Ca^{2+} by troponin and calmodulin as well as the uptake and release of Ca^{2+} by the associated sarcoplasmic reticulum (SR). The model also describes the binding of Ca^{2+} to calsequestrin within the release compartment of the SR. Fig. 2.1 illustrates each of these model components.

A. Membrane Equivalent Circuit

B. Fluid Compartment Model

Figure 2.1: Components of Atrial Model. (A) Membrane equivalent circuit. The “boxes” around resistors indicate voltage-dependent conductances; shading indicates time-varying conductances. Symbol definitions are provided in a glossary (APPENDIX B). (B) Compartmental model. $[Ca^{2+}]_i$ is buffered by calmodulin and troponin in the cytosol and by calsequestrin in the release compartment of the sarcoplasmic reticulum (SR).

The circuit representation of Fig. 2.1(A) considers the cell membrane as a whole-cell capacitance (C_m ; pF) shunted by several ion-specific conductances. The membrane also contains ion pumps driven by adenosine triphosphate (ATP) and a $Na^+ - Ca^{2+}$ ion exchanger driven by appropriate concentration gradients. Under “space-clamp” conditions, wherein transmembrane potential is spatially uniform (but time-varying), the net ionic current (I_{net}) is linearly related to the time derivative of membrane potential, i.e.

$$I_{net} = \sum I_{ionic} = -C_m \frac{dV}{dt}. \quad (2.1)$$

The minus sign above arises from the convention that considers outward current positive and membrane potential defined as:

$$V \equiv V_{in} - V_{out} \quad . \quad (2.2)$$

Current flow through ion channels is generally highly selective for an ion specific to that channel type; the associated conductance may vary with time, membrane potential, or both (see Fig. 2.1 legend). Our model uses Hodgkin-Huxley (HH)-based expressions [55] to describe each ionic current; these have the general form:

$$I_i = \bar{g} y_{i,1}^{k_1} \cdots y_{i,n}^{k_n} (V - E_i) \quad (2.3)$$

where E_i is the reversal (Nernst) potential for the i^{th} , species, $\bar{g}$ is the maximum achievable whole cell conductance for that cell and channel type, and the $y_{i,j}$ terms are HH-type “gating variables” for the i^{th} ionic species which contribute to the variation of channel conductance with time and membrane potential. In the HH formalism these follow first-order kinetics described by the equation

$$\frac{dy}{dt}(V, t) = \frac{y_{\infty}(V) - y(V, t)}{\tau_y(V)} \quad (2.4)$$

where y_∞ represents the steady-state value of y as a function of V and τ_y is the voltage-dependent time constant of y characterizing the “step response” of y to a square step in membrane potential.

Our model provides expressions for six identified ion channel currents in rabbit atrial myocytes. These include a fast sodium current (I_{Na}), a transient outward current (I_t), transient and long-lasting calcium currents ($I_{Ca,T}$ and $I_{Ca,L}$), an inwardly rectifying K^+ current (I_{K1}) and a delayed-rectifier K^+ current (I_K).

The remaining sarcolemmal currents consist of background currents, ionic pumps and an ion exchanger current. The background current I_B contains two linear components, a Na^+ ($I_{B,Na}$) and a Ca^{2+} ($I_{B,Ca}$) current, and a nonlinear rectifying Cl^- current ($I_{B,Cl}$). Two sarcolemmal ATPases are described in the model: a $Na^+ - K^+$ pump (I_{NaK}) and a Ca^{2+} pump (I_{CaP}). Finally, a $Na^+ - Ca^{2+}$ exchanger current (I_{NaCa}) is also provided.

Our description of the intracellular medium provides a single compartment for distribution of Na^+ , K^+ and Ca^{2+} ions. An additional pair of compartments account for the uptake, sequestering, and release of Ca^{2+} by the SR. The model also accounts for Ca^{2+} -binding sites located on troponin and calmodulin molecules within the intracellular compartment. Two sites are described on the troponin molecule: a calcium-specific site (T_C) and a competitive $Ca^{2+} - Mg^{2+}$ site (T_{MgC}).

The handling of Ca^{2+} by the SR is described using a two-compartment model. Appropriate levels of either $[Ca^{2+}]_i$ or V open Ca^{2+} -release channels, allowing a unidirectional flux of Ca^{2+} from the release compartment into the intracellular medium. An active extrusion pump recovers Ca^{2+} to the SR uptake compartment where Ca^{2+} normally returns to the release compartment via passive diffusion. If Ca^{2+} concentration in the release compartment ($[Ca^{2+}]_{rel}$) is very high, Ca^{2+} may return to the cytosol via passive diffusion and reversal of the uptake mechanism. Calsequestrin

molecules located in the release compartment enhance the Ca^{2+} storage capacity of the SR and modify the Ca^{2+} release waveform.

The complete set of equations describing the membrane and fluid compartment models are given in Tables B.1-B.12. Associated parameter values and initial conditions are specified in Tables B.13 and B.14, respectively. Except where otherwise indicated in the text, these tables together with Equation 2.1 provide a sufficient mathematical context to simulate the model-generated results of this paper. A more detailed explanation of the computational aspects involved in model simulations is provided in an appendix (see COMPUTATIONAL DETAILS).

2.2.1 Sarcolemmal Currents

The cardiac sarcolemma contains a variety of ion channels and transporters which are responsible for controlling cell excitation and intracellular ionic homeostasis. Some differences exist in the population and distribution of channels among different species or even among cell subtype within a species. Certain ionic currents contribute to the action potential only in the presence of pharmacologic modulators. The currents included in our model are those known or considered to participate in the rabbit atrial myocyte based on whole-cell voltage-clamp data. These include six ionic currents (I_{Na} , I_t , $I_{Ca,L}$, $I_{Ca,T}$, $I_{K,fast}$, $I_{K,slow}$) and seven background, pump, and exchanger currents (I_{K1} , I_{NaK} , $I_{B,Na}$, $I_{B,Ca}$, $I_{B,Cl}$, I_{CaP} , I_{NaCa}). Our mathematical descriptions of each ionic current and the majority of background currents are based on whole-cell voltage-clamp data from enzymatically isolated rabbit cardiac myocytes.

Sodium Current

Sodium channels generate the large inward current responsible for the upstroke of the action potential. The size and speed of I_{Na} activation makes it difficult to measure experimentally, and voltage clamp recordings which show signs of adequate voltage control have generally been performed under reduced temperatures and/or sodium gradients [16, 33, 49, 48, 94, 74, 73, 91, 106, 107, 89, 90]. Even under more favorable experimental conditions, many of these studies report the steady-state gating or peak current-voltage (I-V) behavior of I_{Na} without supplying corresponding information on channel kinetics. Consequently, modeling projections are required to approximate I_{Na} properties under physiological conditions.

A previous formulation for I_{Na} based on Colatsky's two-microelectrode voltage clamp data in 19 °C in multicellular rabbit Purkinje fiber preparations [19] was presented by DiFrancesco and Noble [21]. As in Colatsky's paper, the model that was

developed used a Goldman-Hodgkin-Katz (GHK) [56] expression to describe the dependence of I_{Na} on V and the Na^+ concentration gradient. More significantly, the DN model considered inactivation to follow a single exponential time course under voltage clamp conditions, and thus adopted a traditional HH m^3h form with associated alpha-beta expressions to describe I_{Na} gating. The small contribution ($< 10\%$) of a possible second slow component of inactivation was left unmodeled [21, 19]. More recent single-cell studies of cardiac I_{Na} have supported the notion of a second component for both inactivation and reactivation [16, 33, 73, 91, 107], where the fast component generally accounts for 80-90% of total I_{Na} and τ_{fast} is 4 to 8 times smaller than τ_{slow} . Given the large size of I_{Na} ($\geq 5 \text{ nA}$), a slowly inactivating component responsible for 10% of total current could theoretically supply several hundred picoamperes of inward current during the early plateau. Such a current would prove comparable in size to other plateau currents and therefore might play a significant role in determining action potential waveshape.

We have developed a new model of I_{Na} which includes both fast and slow components of inactivation. In forming this model we have utilized the perforated patch-clamp data of Wendt et. al. [107] on enzymatically-isolated rabbit atrial myocytes (17 °C) to guide descriptions of both the steady-state gating and the peak I-V behavior of I_{Na} . However, we have allowed the steady-state activation ($\overline{m^3}$) and inactivation ($\overline{h}$) curves used by the model to deviate somewhat from the data reported by Wendt et. al. [107], so as to (1) approximate physiological operating conditions at 35 °C and (2) achieve an improved fit to action potential data from isolated rabbit atrial myocytes.

Fig. 2.2 summarizes the complete steady-state and peak I-V properties of our I_{Na} model. Panel A compares the model-generated steady-state activation ($\overline{m^3}$) and inactivation ($\overline{h}$) curves to data from Wendt et. al. [107] and Colatsky [19]. In

projecting our I_{Na} description to 35 °C, the steady-state gating variable curves have been shifted to a depolarized position relative to the data of Wendt et al. The adjustment in steady-state inactivation corresponds to a +4 mV/10 °C shift relative to the -73.5 mV average value of $V_{1/2}$ stated in [107]. This shift rate compares favorably with the +3 mV/10 °C value reported by Colatsky [19]. Action potential fits were improved by decreasing the slope factor for inactivation (obtained from Boltzmann fits to the data in Fig. 2.2(A)) from 6.4 mV in [107] to 4.0 mV in the model. A comparable shift rate of +4.5 mV/10 °C relative to the experimental data [107] was applied to the steady-state activation curve at 35 °C, yielding a $V_{1/2}$ of -21.8 mV. In addition, the slope factor was decreased from 8.5 mV to 7.1 mV. The resulting overlap between the steady-state $\overline{m^3}$ and $\overline{h}$ curves is minimal, resulting in a negligible (< 2 pA) “window current” [16, 19, 33, 107, 73].

The complete set of equations for I_{Na} are shown in Table B.1. Many of the mathematical forms used to describe the steady-state gating behavior of I_{Na} are similar to those used in the DN model [21], reflecting the similarity of steady-state curves between the Wendt et. al. [107] and Colatsky [19] data. As in the DN model, we have used a GHK [56] form to model the Na^+ and voltage dependence of I_{Na} . However, I_{Na} is assumed to be perfectly selective to Na^+ ions and therefore current reversal occurs at E_{Na} . Expressions used to describe activation gating were also modified from those presented in the DN model; following adjustment of steady-state activation as discussed above, the kinetics were adjusted using a Q_{10} of 1.7 relative to the 19 °C formulation in the DN model (Fig. 2.2(C)).

Due to the lack of suitable dual time constant data to quantify inactivation kinetics at 35 °C in a rabbit preparation, we have been obliged to model the fast and slow time constants for I_{Na} in qualitative terms. In this regard, the expressions for τ_{h1} and τ_{h2} presented in Table B.1 and displayed graphically in Fig. 2.2(D,E) only

qualitatively reflect the relative rates and voltage dependencies observed under typical voltage clamp conditions. At plateau potentials the inactivation time constants attain values of $350 \mu\text{s}$ (τ_{h1}) and 2.8 ms (τ_{h2}). By comparison, Colatsky projected a τ_h of $485 - 728 \mu\text{s}$ at 37°C based on a monoexponential decay (at -20 mV). This is consistent with our model, since a monoexponential representation of biexponential data generates a τ intermediate to corresponding dual time constant values. At more hyperpolarized potentials (i.e. near $V_{1/2}$) the model values for inactivation time constants estimate the relatively slow kinetics of recovery from inactivation typically observed in paired-pulse experiments (e.g. [41]). Consequently τ_{h1} and τ_{h2} become faster with depolarization and slower with hyperpolarization, reaching maximum values of 30.3 ms and 122.8 ms , respectively. The fast reactivation time constant is comparable to the monoexponential projection by Colatsky of $34 - 48 \text{ ms}$ at potentials between -73 and -86 mV . In our model, we have assumed a similar voltage dependence for τ_{h2} as τ_{h1} (Fig. 2.2(D,E)), so that τ_{h2} attains values up to 7.5 times slower at plateau potentials.

An assessment of the voltage-dependence and current density of model I_{Na} is provided in Fig. 2.2(B). The figure compares the 17°C peak I-V data of Wendt et al. [107, Fig. 4B], normalized to cell capacitance, with the model-generated peak I-V curve at 35°C obtained by simulating the same voltage-clamp protocol under the same reduced sodium gradient. At the higher temperature the density of model I_{Na} is slightly greater than the data of Wendt et al [107]. Overall, the model generates a similar I-V response to the data with minimal ($[Na^+]_o$) parameter adjustment.

The decomposition of I_{Na} inactivation into two components, one large and fast and the other smaller and slower, suggests that these may play distinct functional roles in influencing action potential waveshape. Our descriptions of the inactivation gating variables h_1 and h_2 reflects this idea. As we will show, the fast current (related

to h_1) contributes primarily to the action potential upstroke while the slow current (h_2 -related) interacts late in the upstroke with the early currents of repolarization.

Figure 2.2: Steady-state model of I_{Na} . (A) Activation ($\bar{m}^3$) and inactivation ($\bar{h}$) curves. Data from Wendt et. al. (circles) [107, Fig. 4A(normalized),B(inset)] and Colatsky (squares) [19, Fig. 5] (B) Model-generated (solid, 35 °C, 50 pF) and recorded (circles, 17 °C, 14 pF, perforated patch) rabbit atrial peak I-V relations, normalized for cell capacitance, obtained using identical “test pulse” parameters (HP=-90 mV; $[Na^+]_o=75$ mM). Data from Wendt et. al. [107, Fig. 4B]. (C,D,E) Time constants for activation (τ_m), fast (τ_{h1}) & slow (τ_{h2}) inactivation, respectively.

Transient Outward and Steady Currents

The transient-outward K^+ current (I_t) develops to support repolarization immediately after the action potential upstroke. Its development early in the action potential, a consequence of its rapid activation kinetics, provides the opportunity to modulate action potential waveshape. Differences in action potential waveshape between different cardiac cell types and with variations in stimulus rate (in the same cell type) have been correlated with the strength of I_t [8, 7, 9, 39, 69]. These correlations have identified a need to elucidate the contribution of I_t to the production of such waveshape differences.

Voltage clamp experiments have distinguished I_t from other early outward currents as a large and rapidly activating voltage-dependent K^+ current with complex inactivation and reactivation (i.e. recovery from inactivation) properties [39, 30, 97, 32]. In rabbit atrial and ventricular myocytes, I_t can supply hundreds of picoamperes of outward repolarizing current, and peak I_t (when fully recovered from inactivation) is substantially larger than other (I_K , $I_{K,Ca}$, $I_{K,Na}$) outward K^+ currents [39, 30]. Other notable features of this current include: (1) it is blocked selectively by 4-aminopyridine (4-AP); (2) it is highly temperature-sensitive; (3) steady-state activation and inactivation curves measured by standard protocols vary sigmoidally with membrane voltage; (4) two time constants are required to describe inactivation and reactivation kinetics; (5) reactivation is very slow; and (6) the size of peak current is very sensitive to depolarization frequency. The latter arises from the slowness of recovery from inactivation; dual-pulse data has shown that as long as 30 seconds may be required to achieve full recovery [97, 32].

We have formulated a model of I_t based fundamentally on voltage-clamp studies from two sources: (1) from our collaborating laboratory using cardiac myocytes from the rabbit atrium, ventricle, and crista terminalis [17, 39, 40, 30, 31] and (2) from

studies of Fermini et. al. [32] on rabbit atrium. Table B.3 gives the equations describing I_t , which include one activation (r) and three inactivation (s_1 , s_2 , s_3) gating variables to describe the complex voltage- and time-dependent behavior of this current. Fig. 2.3 shows the voltage-dependent steady-state and time constant curves for r , s_1 , s_2 , and s_3 along with discrete points obtained from voltage clamp protocols. The data points presented for τ_r , τ_{s_1} , τ_{s_2} , and τ_{s_3} at potentials between -20 and +20 mV derive from a least-squares fit [72] of our mathematical description of I_t to the voltage clamp records of Fedida and Giles [30, Fig. 6A]. This has enabled our model to mimic the entire transient obtained under voltage clamp, a capability found lacking in the standard practice of fitting only the decay phase of the voltage clamp transient to a sum of exponentials (e.g. [11]). To fit the voltage clamp records of Fedida and Giles [30, Fig. 6A] and match 35 °C atrial action potential repolarization trajectories [10], the steady-state activation curve ($\bar{r}$) was formulated using a -3.0 mV shift in the half-activation value and a 70.4 % broadening in the slope at half-activation relative to values stated for rabbit atrium at 23 °C by Clark, Giles and Imaizumi [17]. Half-values and slope-factors for the steady-state inactivation of variables s_1 and s_2 were taken directly from the “standard inactivation protocol” measurement of Fedida, Giles, and Shimoni [31, Fig. 9]; corresponding parameters for s_3 were adjusted to achieve a least-squares fit to the “cumulative steady-state inactivation” data of Fedida, Giles, and Shimoni [31, Fig. 11]. Subtle modifications to the expressions governing I_t availability have been made based on the rate-sensitivity of early repolarization velocity observed in rabbit atrial action potentials [10].

Figure 2.3: Steady-state model of transient outward current. (A) Activation ($\bar{r}$) and inactivation ($\bar{s}_1^3$, $\bar{s}_2^3$, $\bar{s}_3^4$) gating variable curves. Data (circles) from Fedida et. al. [31, Figs. 9,11(rescaled)] has been shifted by -10 mV to correct for tip potential. (B,C,D,E) Time constants for activation (τ_r), fast inactivation (τ_{s1}), slow inactivation (τ_{s2}) and “slow reactivation” (τ_{s3}), respectively. Data points (squares) represent discrete measurements from voltage clamp protocols via least-squares parameter estimation of time constants (B-E, see text) using the model equations to fit the data of Fedida and Giles (Fig. 2.5) [30].

Activation of I_t has proved difficult to characterize at physiological temperature due to the speed and size of the activating current [30]. In myocytes from rabbit crista terminalis at 23 °C [40], τ_r has been found to have a “bell-shaped” dependence on membrane potential with the peak occurring close to the half-activation voltage. Accordingly, we have adopted a Gaussian form to describe τ_r in our model and adjusted the parameters to achieve a least-squares fit [72] to the ventricular myocyte data of Fedida and Giles at 35 °C [30]. Compared to the data of Van Ginneken, the resultant “bell-shaped” curve has a narrower voltage-dependence, places peak τ_r at a more depolarized potential, and specifies an approximately 3 times faster value for peak τ_r .

Time constants for the inactivation gating variables model the complexity of both the inactivation and reactivation processes. At depolarized potentials τ_{s1} and τ_{s2} reflect the two components of inactivation observed for I_t [17]. Analytical forms (Table B.3) have been adopted to represent the voltage-dependent behavior of these constant functions. Parameters associated with these forms have been adjusted to achieve least-squares fits [72] to inactivation time constants estimated from the whole-cell voltage clamp data of Fedida and Giles [30]. Values for time constants at potentials near or below the resting potential (RP) represent the slow multiple time constant reactivation of I_t . Reactivation of I_t has been measured in rabbit atrium at a holding potential of -80 mV (at 23 °C) [39, 17, 97] and at $V=-60$ mV (30 °C) [32]. It has been found to consist of a slow and fast component. Giles and Imaizumi found no voltage-dependence to the recovery [39]. Therefore, neglecting any voltage dependence, rate constants can be projected from these values (23 °C, 30 °C) to 35 °C assuming an exponential dependence of rate upon temperature, i.e.

$$\tau = B \exp\left(\frac{E_a}{RT}\right) \quad (2.5)$$

where E_a is the activation energy and B is a constant [53, p. 272]. Under this assumption, Equation 2.5 and the hypothermic kinetic data provide uniquely determined values for B and E_a which then yield calculated reactivation time constants at 35 °C. These values, provided in Table 2.1, have been used to guide the model-generated diastolic reactivation response (a combined effect of τ_{s1} , τ_{s2} , and τ_{s3}) in the “warm” condition. Further adjustments have been made in selecting values for τ_{s1} , τ_{s2} , and τ_{s3} to better reflect the rate-sensitive behavior of I_t as it affects action potential waveshape. The somewhat unusual voltage-dependencies of τ_{s1} , τ_{s2} , and τ_{s3} in Panels C-E of Fig. 2.3 reflect strong differences in systolic inactivation and diastolic reactivation kinetics of I_t as measured by standard voltage clamp protocols.

Table 2.1: Time constants for I_t recovery from inactivation.

Temperature	23 °C	30 °C	35 °C	E_a (kcal/mol)	B	Q_{10} **
τ_{fast} (sec)	3.93	0.650	0.189*	191.8	5.66e-34	12.75
τ_{slow} (sec)	14.6	8.4	5.75*	58.9	5.86e-10	2.18

*Calculated from equation 2.5.

**Calculated from 23 to 33 °C.

Voltage-clamp measurements of I_t are found to contain a steady as well as a transient component, even at depolarized potentials where I_t should be fully inactivated [30, 22]. The steady current increases in size with depolarization [30, 22], is both 4-AP and rate-insensitive [30, 32, 22], is not eliminated by 10 mM TEA, is attenuated by Cl^- transport blockers, and is sensitive to $[Cl^-]$ in the recording pipette. We have formulated a model of the steady current as an instantaneous background Cl^- current ($I_{B,Cl}$) based on the current-voltage relation of Duan et. al. [22, Fig. 3A]. Model-generated and experimental I-V data are shown together in Fig. 2.4. An empirical

relation was used to describe the reversal potential of this current which deviates somewhat from predicted E_{Cl} values (see $E_{B,Cl}$ in Table B.6).

Figure 2.4: I-V Relation of Chloride Current (I_{Cl}) Model fits (solid lines) to I-V relation of the chloride background current identified by Duan et. al. [22, Fig. 3a] parameterized by pipette chloride concentration. $[Cl^-]_o$ is 137.4 mM; $[Cl^-]_{pipette}$ is 22 (triangles), 67 (circles), or 132 (squares) mM. The model expression (Table B.6) assumes complete intracellular dialysis, i.e. $[Cl^-]_i \approx [Cl^-]_{pipette}$.

Our models of I_t and $I_{B,Cl}$ were summed to fit the data of Fedida and Giles [30]. Fig. 2.5 shows the model-generated and recorded currents in response to 400 msec voltage clamp steps from a holding potential of -80 mV to clamp potentials of -20 , -10 , 0 , $+10$, and $+20$ mV applied at 10 second intervals. Addition of a delayed rectifier current (I_K) does not improve the quality of the fits (not shown); however, modest improvements to the steady-state and overall fit can be achieved by inclusion of a Na^+ background current ($I_{B,Na}$, not shown). The agreement between the data and model suggests that I_t and $I_{B,Cl}$ provide the majority of the current responsible for the measured response of this cell.

The size of I_t evoked under a voltage-clamp or action potential setting varies with rate as well as membrane potential. In sheep Purkinje fibers, Boyett et. al. [9, 8, 7] have demonstrated that I_t decreases as the fiber is stimulated more rapidly, and that rate-dependent changes in I_t size correlate with alterations in the prominence and velocity of early repolarization in the action potential. Similar findings have been reported in isolated rabbit atrial myocytes by Giles and Imaizumi [39, 17].

Boyett et. al. [9, 8, 7] have postulated that the "fast process" governing rate-dependent changes in I_t result from an incomplete reactivation phenomenon. In this scheme, changes in stimulus rate modify the balance between inactivation and reactivation and thus alter the availability (s_{total}) of I_t immediately prior to the stimulus. One may consider the availability balance to reflect the relative durations of systole versus diastole. Consequently, a longer diastolic interval between stimuli leads to a larger, more fully reactivated I_t and a stronger phase of early repolarization. This phenomenon is represented in our model through the inactivation gating variables s_1 and s_2 .

A "slow process" governing I_t availability is also described by Boyett et. al. [9, 8, 7]. Although the mechanism(s) for this process are somewhat unclear, the

relative sizes of the kinetics involved are most closely related to the gating variable s_3 in our model.

Figure 2.5: Voltage clamp responses of transient outward current. Shown are model-generated fits (solid) to the time-dependent transient outward current recorded by Fedida and Giles in 35 °C ventricular epicardium [30, Fig. 6a]. Protocol consists of 400 msec depolarizing steps to -20, -10, 0, 10, and 20 mV applied at 10 second intervals. The current is modeled as the sum of I_t and $I_{B,Cl}$ (see text).

Low-Threshold Calcium Current

Bean [2] has identified two types of Ca^{2+} currents in mammalian cardiac myocytes, a high-threshold long-lasting “L-type” ($I_{Ca,L}$) and a low-threshold transient “T-type” ($I_{Ca,T}$) current. Both $I_{Ca,T}$ and $I_{Ca,L}$ have been found and studied in isolated rabbit sinoatrial node cells by Hagiwara [46] and Nathan [77] using whole-cell and cell-attached patch clamp techniques at 37 °C. Hagiwara et. al. [46] found $I_{Ca,T}$ differs from $I_{Ca,L}$ in its activation and inactivation behavior, conductance properties and sensitivity to blocking agents. For example, $NiCl_2$ (40 μ M) or tetramethrin (0.1 μ M) provide a selective block of $I_{Ca,T}$; conversely, nifedipine and D600 block $I_{Ca,L}$ without affecting $I_{Ca,T}$. Cadmium (20 μ M) and cobalt (2 mM) can inhibit both T- and L-type calcium currents. Hagiwara et. al. [46] have reported, however, that the current density of $I_{Ca,T}$ in rabbit SA node cells appears to be about ten times larger than that found in either atrial or ventricular cells. Moreover, in our simulation studies $I_{Ca,T}$ has a negligible effect on either intracellular calcium handling or the action potential waveshape and provides a tiny contribution (relative to I_{Na}) to the action potential upstroke. For completeness we have included equations adapted from Hagiwara et. al. [46] for $I_{Ca,T}$ in our model (Table B.2), where a Q_{10} factor of 1.8 was used to adjust rate expressions for the gating variables d_t and f_t . However, both our modeling assessment and Hagiwara’s results support the view that $I_{Ca,T}$ has functional importance in pacemaker rather than working tissue [84].

High-Threshold Calcium Current

Following depolarization, the high-threshold or long-lasting calcium current ($I_{Ca,L}$) provides a sustained influx of calcium ions which affects both intracellular calcium handling and action potential waveshape. By opposing the repolarizing influences of I_t and I_K , $I_{Ca,L}$ contributes to the development of a plateau in the action potential

and delays the onset of final repolarization. By increasing $[Ca^{2+}]_i$ early in the action potential $I_{Ca,L}$ may provide a significant trigger signal for calcium-induced calcium-release (CICR) by the SR [28, 29]. Consequently, an understanding of the behavior of $I_{Ca,L}$ in quantitative terms is important to an understanding the electrical and contractile behavior of the atrial myocyte.

Under suitable voltage clamp conditions, $I_{Ca,L}$ is recorded as a transient inward current whose size is maximal near a clamp potential of 0 mV. The inactivation time course of $I_{Ca,L}$ is complex and has been reported to follow various patterns of decay (monoexponential, biexponential, or neither) depending on the temperature, divalent carrier ion, and species used in the preparation [84]. Paired-pulse experiments reveal a similarly complicated timecourse of recovery from inactivation. While both Ca^{2+} and V have been implicated as important factors influencing $I_{Ca,L}$ inactivation [44, 45], the precise mechanism(s) governing the process of inactivation remain unclear as do the quantitative roles of each factor in the process.

In the absence of detailed quantitative information describing how $I_{Ca,L}$ inactivation develops, we have adopted a relatively simple model of $I_{Ca,L}$ in which inactivation is treated as a purely voltage-dependent process. Our model uses a single activation (d_L) and inactivation (f_L) gating variable to describe channel gating. The complete expressions, given in Table B.2, are based jointly on data from Hagiwara et. al. [46], Nilius et. [79], Banno et. al. [1], and Kawano et. al. [65]. A Boltzmann function has been used to describe steady-state activation based on data from Hagiwara in 37 °C SAN cells [46]; however, the $V_{1/2}$ for activation has been shifted by +5.55 mV from Hagiwara's results placing the activation curve closer to that measured by Banno [1] in 35 °C rabbit ventricular myocytes. Conventional α and β rate constant forms have been used to describe steady-state inactivation as based on fits to Hagiwara's data.

Note that the data provided in Hagiwara et. al. [46] did not include the voltage-dependence of the time constants and therefore an approximation had to be made. Our equations for τ_{dl} have been adapted from Nilius et. al. [79], which derived originally from forms of Yanigahara [108] used to describe two-microelectrode data from a 35-36 °C rabbit SA nodal preparation [82]. We have shifted the Nilius time constants by +1.5 mV (in the depolarizing direction) in order to align the time constant curves with the current window of the steady state gating variable curves and scaled the peak τ_{dl} to agree with the original data from Noma et. al. [82].

The time constant for inactivation (τ_{fi}) has been formulated based on data from Banno et. al. [1], Hagiwara et. al. [46], and Kawano et. al. [65]. Values of τ_{fi} at depolarized potentials represent the average time constants resulting from single exponential fits to $I_{Ca,L}$ transients recorded from 35 °C rabbit ventricle. At more hyperpolarized potentials, time constants reflecting the time course of recovery from inactivation are represented. In particular, data from and Kawano et. al. [65] represent time constants from single-exponential fits of recordings from 33-35 °C rabbit ventricle.

To match the behavior of action potentials at high rates of stimulation, we have found it necessary to include a non-inactivating term (d') in our description of $I_{Ca,L}$. The $V_{1/2}$ and slope factors used to describe this component have been taken from the steady-state inactivation curve recorded by Banno et. al. [1] which was found to demonstrate incomplete inactivation and a rising “N-type” voltage-dependence above +10 mV. The resultant current, which may be likened to an “N-type” Ca^{2+} channel, has a threshold near -25 mV and a $V_{1/2}$ of +33 mV.

Fig. 2.6 gives the complete steady-state curves for the 35 °C $I_{Ca,L}$ model as well as the model-generated peak I-V responses obtained from a holding potential of -

75 mV. As shown in Fig. 2.6(D), the model generated current-voltage curve provides an acceptable representation of the experimentally recorded I-V relationship for $I_{Ca,L}$.

Figure 2.6: Steady-state model of the long-lasting calcium current. (A) Activation ($\overline{d_L}$) and inactivation ($\overline{f_L}$) gating variable curves. (B) Model (solid curve) and experimental peak I-V data from HP=-75 mV. Data from Hagiwara (triangles, scaled by 15%) [46, Fig. 1B] and Banno (circles, current rescaled for 27.5 pF ventricular cell) [1]. (C,D) Time constants for activation (τ_{dL}) and inactivation (τ_{fL}), respectively. Data from Kawano (squares) [65, text], Hagiwara (triangles) [46, Fig. 8B], Banno (circles) [1].

Inward Rectifier

The inward rectifier (I_{K1}) supplies a functionally important K^+ current in working rabbit cardiac cells. Functionally, I_{K1} helps to establish both the resting potential (RP) and input resistance (R_{IN}) of the quiescent cell. In addition, I_{K1} contributes to the action potential by supplying outward current during late repolarization. Each of these roles can be understood from the strong inward rectification of the current-voltage relation for I_{K1} (Fig. 2.7). Inspection of the I_{K1} -V characteristic at rest (nominally $V = -71.0$ mV) reveals an outward current and a relatively large slope conductance. This outward current helps to balance resting inward leakage currents, and the large slope strongly influences cell conductance in the quiescent state.

Experimental studies by Giles et. al. [39, 98] have identified I_{K1} in both rabbit atrial and ventricular cells and have demonstrated the modulatory effect of $[K^+]_o$. However, the size of I_{K1} in atrium, measured as the Ba^{2+} -sensitive ($100 \mu\text{M}$) current, was found to be an order of magnitude smaller than in ventricle [39]. Moreover, the atrial measurements generated an I_{K1} -V curve containing a small outward component at depolarized potentials [39], as if to suggest a less strongly rectified current in atrial than ventricular cells. The degree of I_{K1} rectification in atrial and ventricular cells is summarized in Fig. 2.7, which shows the normalized I_{K1} -V data recorded by Giles et. al. [39, 98] from various preparations.

As the figure illustrates, the negative slope region above -40 mV is less prominent in the atrial than ventricular I_{K1} -V data. Due to the small size of I_{K1} recorded from atrial myocytes, these I-V differences may not be distinguishable from measurement error resulting from a low signal-to-noise ratio. Alternatively the lessened negative slope in the atrial data may represent a genuine variation in channel behavior with cell subtype. In our modeling study, we have adopted an I-V relation which represents a compromise between the atrial and ventricular recordings (Fig. 2.7), and scaled the

model current to reflect its smaller size in atrial cells. This compromise has improved the model's representation of action potentials over the more strongly rectified form based solely on ventricular data.

Shimoni et. al. [98] have reported the gating associated with I_{K1} to be relatively rapid. Consequently, we have treated the inward rectifier as an instantaneous current in our model.

Figure 2.7: Normalized I-V relations for the inward rectifier K^+ current. Currents have been normalized for the maximum outward current occurring near $V = -60$ mV (typically ~ 30 pA in atrial and 200 pA in ventricular cells [39, 98]). I-V curves are shown for the model (solid), ventricular myocytes (dotted, Shimoni et. al. [98, Fig. 8B]; triangles, Giles & Imaizumi [39, Fig. 11D]), and atrial myocytes (circles, Giles & Imaizumi [39, Fig. 11B]).

Delayed Rectifier

The delayed rectifier K^+ current (I_K) plays an important role in repolarization in a variety of cardiac cell types and species [80]. Classically, I_K is identified under whole-cell voltage clamp conditions as a slowly developing outward current observed during a depolarizing clamp step (e.g. [95]). Due to its gradual activation, I_K has been thought to contribute to the initiation of repolarization during phase 2 of the action potential. The support to repolarization provided by I_K serves to limit action potential duration (APD) and thus may have indirect modulatory effects on cell refractoriness.

Recent studies have demonstrated that at least two and possibly three subtypes of delayed rectifier currents exist in mammalian cardiac myocytes. In guinea pig atrial and ventricular myocytes, Sanguinetti and Jurkiewicz [92, 93] have distinguished fast (I_{Kr}) and slow (I_{Ks}) delayed rectifier currents based on differential sensitivity to the class III antiarrhythmic agent E-4031. Habuchi and Giles [43, 42] have identified similar currents, $I_{K,fast}$ and $I_{K,slow}$, in rabbit SA and AV nodal cells. The fast type of I_K current has also been found in rabbit ventricle by both Carmeliet [14] and Veldkamp et. al. [101] and in rabbit nodal cells by Shibasaki [95]. A possible third type of delayed rectifier with even faster kinetics and a more depolarized activation threshold has been identified in guinea pig ventricle [109] and human atrium [104].

The two types of delayed rectifiers identified in rabbit preparations have been shown to possess distinct pharmacologic, ion-transfer and kinetic properties (e.g. [18] for review). $I_{K,fast}$ is blocked selectively by class III antiarrhythmic agents (e.g. sotalol, dofetilide, E-4031), has an inwardly rectified ion-transfer characteristic, and has relatively fast activation/deactivation kinetics. In contrast, $I_{K,slow}$ is insensitive to E-4031, has a linear ion-transfer characteristic and has slower activation/deactivation kinetics. [92, 93, 43, 42]

In 23 °C rabbit atrial cells, Giles and Imaizumi [39] have reported I_K to be small in comparison with other outward currents (I_t , I_{K1}). However, the amplitude [103] and kinetics [102] of I_K have been reported to depend strongly on temperature, such that I_K may contribute a significant outward current in 35 °C rabbit atrial cells [23]. Duan et. al. [23] have suggested that $I_{K,fast}$ provides the major delayed rectifier current in rabbit atrial myocytes. In our modeling study, however, we have found that both fast and slow currents are required to model the rabbit atrial action potential. In particular, as we will demonstrate, a contribution from $I_{K,slow}$ is required to supply the necessary outward current to initiate early repolarization, a task for which the fast current proves inadequate due to its strong inward rectification.

We have developed models of $I_{K,slow}$ and $I_{K,fast}$ based on the data of Habuchi et. al. [43, 42] from rabbit SA and AV nodal cells. Our model of $I_{K,fast}$, provided in Table B.4, utilizes the general mathematical forms of Shibasaki [95] to describe its voltage- and time-dependence. In contrast to previous models of DiFrancesco [21] and Rasmusson [86, 87], these expressions include a fast inactivation variable (p_i) in the description of I_K channel gating. The inclusion of p_i in the model produces inward rectification in the fully-activated ion transfer (I-V) curve (Fig. 2.8(B), discussed below), thus attenuating the role of $I_{K,fast}$ at more depolarized potentials. Moreover, the rapid reactivation kinetics of p_i generates a transient increase in outward current during a tail current voltage-clamp protocol (not shown) [101].

The complete steady-state model of $I_{K,fast}$ is illustrated in Fig. 2.8. As illustrated in panel A, the steady-state activation curve measured using whole-cell methods by Habuchi et. al. ($V_{1/2}$ -14.6 mV, slope factor k 6.9 mV) [43, 42] is in good agreement with the single-channel derived measurement of Veldkamp et. al. ($V_{1/2}$ -7.3 mV, k 7.7 mV) [101] but differs by roughly +20 mV from the relatively hyperpolarized Shibasaki measurement ($V_{1/2}$ -25.1 mV, k 7.4 mV) [95]. The kinetic and steady-state

parameters associated with a possible rapid inactivation process rectifying $I_{K,fast}$ were not measured by Habuchi et. al. [43, 42]. However, assuming rapid inactivation kinetics, the steady-state inactivation curve ($\bar{p}_i$) can be estimated from the fully-activated ion-transfer relation for $I_{K,fast}$. We therefore adopted the Shibasaki kinetics for p_i and adjusted the associated $V_{1/2}$ and the slope factor values by least-squares parameter estimation [72] to fit to the peak I-V measurement of [42] (Fig. 2.8(B)). Activation kinetics were initially set using time constants measured by Habuchi et. al. [42]. However, the slowness of deactivation associated with $I_{K,fast}$ tails proved unsatisfactory for representing rabbit atrial action potentials, and it was necessary to increase the β_{pa} associated with τ_{pa} by a factor of 12. The resultant voltage-dependence of τ_{pa} is comparable in peak magnitude to guinea pig atrial measurements by Sanguinetti et. al. [93], but generates somewhat more rapid deactivation at diastolic membrane potentials.

Figure 2.9 provides the steady-state model for $I_{K,slow}$ associated with the equations of Table B.4. Curves for steady-state activation are given in panel A, while panel B illustrates the associated time constant. Since $I_{K,slow}$ has been proposed to be modulated by $[Ca^{2+}]_i$ in a fashion not yet fully characterized, our model undoubtedly represents a simplification of the in-vivo situation. Nevertheless the simplified current, based on activation data and reasonably slow kinetics, provides a qualitative model with which to explore the relative contributions of slow and fast I_K components to the rabbit atrial action potential.

We have omitted from consideration the contribution of a third delayed rectifier component due to (1) a lack of evidence for its existence in rabbit preparations, (2) a lack of sufficient data in other preparations to construct a quantitative model, and (3) a lack of a clear functional role for this component in our existing model. Total delayed rectifier current (I_K) is then represented as the sum of $I_{K,slow}$ and $I_{K,fast}$.

Figure 2.8: Fast Delayed Rectifier K^+ Current. (A) Activation ($\bar{p}_a$) and inactivation ($\bar{p}_i$) gating variable curves. Data from Habuchi and Giles (circles) [42, Fig. 4B] and Veldkamp et al. (triangles) [101, Fig. 8]. (B) Model-generated fully-activated current-voltage relation, normalized to the maximum outward current. Data from Habuchi and Giles [42, Fig. 1B]. (C) Activation time constant (τ_{pa}). The model kinetics (solid, 35 °C) are compared to kinetic measurements in rabbit by Habuchi and Giles (circles and squares, 32 °C S-A and A-V node) [42, Fig. 7A,9B] and in guinea pig by Sanguinetti (triangles, 34-35 °C) [93]. (D) Fast inactivation time constant (τ_{pi}).

Figure 2.9: Slow Delayed Rectifier K^+ Current. (A) Activation ($\bar{n}$) gating variable curve. Data from Habuchi Giles [42, Fig. 5C, normalized to peak current] shows nystatin-permeabilized (triangles) and EGTA-dialyzed (circles) results. (B) Time constant for activation (τ_n). Model kinetics (solid) shown at 35 °C, data (squares) at 32 °C. Data from Habuchi and Giles [42, Fig. 7B,9B].

Calcium-Activated Potassium Current

The calcium-activated K^+ current ($I_{K,Ca}$) produces a 4-AP insensitive transient outward current in response to the intracellular calcium transient that accompanies the cardiac action potential. $I_{K,Ca}$ has been described in rabbit ventricular and atrial myocytes by Hiraoka and Kawano [54] and Giles and Imaizumi [39], respectively. Since Giles and Imaizumi [39] found $I_{K,Ca}$ to be much smaller than other outward currents, we have neglected it in our atrial model.

Sodium-Calcium Exchanger

The sodium-calcium exchanger couples together the electrical and Ca^{2+} transport functions of the sarcolemmal membrane. While the current carried by the sodium-calcium exchanger is usually small compared to major ionic currents, I_{NaCa} may influence action potential waveshape under conditions where $[Ca^{2+}]_i$ is high, repolarization velocity (an indicator of net membrane current) is low, or membrane potential is strongly negative. Sodium-calcium exchange also provides a significant pathway for extrusion of Ca^{2+} ions from the intracellular medium. At rest, sodium-calcium exchange transports relatively little Ca^{2+} due to low $[Ca^{2+}]_i$ levels and the relatively low Ca^{2+} -binding affinity of the sodium-calcium exchanger compared to the sarcolemmal calcium pump (I_{CaP}). However, when I_{NaCa} is enhanced by high $[Ca^{2+}]_i$ or negative potentials, Ca^{2+} removal from the intracellular medium via I_{NaCa} may greatly exceed that via I_{CaP} [13].

A simplified form of the Mullins [75] formulation for I_{NaCa} , which has been used in both the DN model [21] as well as in our previous studies on bullfrog [86, 87] and rabbit ventricular [38] myocytes is used in this rabbit atrial cell model. When a 3:1 stoichiometry is assumed, the exchanger reversal potential, as measured by Kimura [66], is in close agreement with the estimated value for the reversal potential given

by $E_{NaCa} = (n_{NaCa}E_{Na} - 2E_{Ca})/(n_{NaCa} - 2)$, where n_{NaCa} is the stoichiometry. In addition, Kimura has reported Hill coefficient values for the dependence of I_{NaCa} on extracellular Na^+ and Ca^{2+} of 2.9 and 0.9, respectively, suggesting that a stoichiometry of $3Na^+:1Ca^{2+}$ is appropriate. Noble [81] has introduced a partition parameter, γ , from Eyring rate theory, which determines the symmetry between the voltage dependence of outward and inward fluxes of the exchanger. A value of 0.5 specifies symmetric voltage dependence of the inward and outward fluxes about the reversal potential. Earm [25] and Kimura [66] have suggested that this value is less than 0.5, since the inward and outward components of the I_{NaCa} -V curve are not symmetrical. We have used a nonlinear least-squares parameter estimation method [72] to fit the general form of the I_{NaCa} equation (given in Table B.9) to experimental data of Earm et. al. [25] from single rabbit atrial cells. In applying the method, we have fixed the stoichiometry of I_{NaCa} at the value used by both Earm and Kimura ($n_{NaCa} = 3$). Least-squares estimates for two parameter values, k_{NaCa} and γ , were then obtained for various the values of d_{NaCa} and $[Ca^{2+}]_i$ until an acceptable representation was achieved. In particular, since Earm et. al. measured I_{NaCa} as a Ca^{2+} -sensitive difference current, an attempt was made to utilize a physiologically realistic value of $[Ca^{2+}]_i$ in the fit. The resulting “fully-activated” I_{NaCa} -V curve, shown in Fig. 2.10, was obtained using $[Ca^{2+}]_i = 2 \mu M$, $d_{NaCa} = 0.0003$, $k_{NaCa} = 0.141$, $\gamma = 0.470$. However, in our action potential simulations we have modified k_{NaCa} and γ to 0.016 and 0.45, respectively.

Figure 2.10: I-V Relation for Sodium Calcium Exchanger Under High ($2 \mu\text{M}$) $[\text{Ca}^{2+}]_i$. Shown is the model fit (solid) to the data (circles) of Earm et. al. [24, Fig. 10]. Model parameter values used: $n_{\text{NaCa}} = 3$, $[\text{Ca}^{2+}]_i = 2 \mu\text{M}$, $d_{\text{NaCa}} = 0.0003$, $k_{\text{NaCa}} = 0.141$, $\gamma = 0.470$.

Sodium-Potassium Pump

The sodium-potassium pump current (I_{NaK}) utilizes energy derived from ATP to maintain Na^+ and K^+ electrochemical gradients across the cell membrane. This current has been described in detail in guinea-pig ventricle [15, 36, 76] and has also been studied in bullfrog atrium [96]. The detailed models of Chapman et. al. [15] and Lemieux et. al. [67] account for the dependence of pump activity on $[Na^+]_i$, $[K^+]_o$, ATPase activity, membrane potential, and time. We have adopted a less sophisticated model which considers I_{NaK} as an instantaneous function of $[Na^+]_i$, $[K^+]_o$ and V (Table B.8). In this expression, the voltage-dependent term mimics the data of Gadsby and Nakao [36] from 36 °C guinea pig ventricular myocytes. The voltage-dependent term is a hyperbolic function scaled to match the relative changes in I_{NaK} as a function of membrane potential (c.f. Gadsby et al [35]). Concentration-dependent terms are raised to powers (2 for activation by $[K^+]_o$ and 3 for activation by $[Na^+]_i$) to reflect the well-known 3:2 stoichiometry of the $Na^+ - K^+$ pump [26]. The expression used for I_{NaK} in the present study is similar to that used by Rasmusson et. al. [86, 87]. In contrast, the expression for I_{NaK} (or i_p) in DiFrancesco and Noble [21] assumed a 1:1 stoichiometry for $Na^+ - K^+$ coupling and did not include any voltage dependence.

In modeling the rabbit atrial action potential we have adjusted the maximum transport capacity ($\overline{I_{NaK}}$) of the sodium-potassium pump to reflect current densities measured in isolated cardiac myocytes. In our model, I_{NaK} supplies 12-22 pA or 0.24-0.45 pA/pF of outward current over the range of atrial membrane potentials. Comparable ouabain-sensitive current densities of I_{NaK} have been measured in rabbit ventricular (W.R. Giles, personal communication, 15-20 pA; 0.32 pA/pF, Hool et. al. [57]) and Purkinje (W.R. Giles, personal communication, 30-40 pA) cells.

The $[Na^+]_i$ and V dependence of I_{NaK} (Table B.8) suggest that different $[Na^+]_i$ levels are appropriate for the quiescent and actively stimulated cell. In our model, membrane current balances have been adjusted such that a true resting condition occurs when $[Na^+]_i$ is 6.8 mM and V is -71.7 mV. In the actively stimulated cell, the magnitude of $\overline{I_{NaK}}$ is sufficient to approximately balance inward Na^+ fluxes through membrane channels when $[Na^+]_i$ equals the Désilets and Baumgarten average (8.4 mM) [20] from ion-sensitive electrode recordings. For example, cycle-to-cycle variation in $[Na^+]_i$ is minimal ($< 2 \mu\text{M}/\text{sec}$) during model simulations at 2.0 Hz. However, cessation of stimulation leads to a slow decrease in $[Na^+]_i$ (roughly $18 \mu\text{M}/\text{sec}$) and slow membrane hyperpolarization toward the resting state as the absence of action potentials allows I_{NaK} to effect a net extrusion of Na^+ ions.

The Calcium Pump

The calcium pump (I_{CaP}) is a low capacity, high affinity electrogenic transport mechanism that helps to maintain the low resting level of $[Ca^{2+}]_i$. We employ the formulation used in our previous studies [86] which is also listed in Table B.7. This pump current is not considered in the DN model [21], while a nonelectrogenic formulation was employed in the HN model [52].

2.2.2 Myocyte Ultrastructure

We have utilized experimental data to provide reasonably accurate values of model parameters related to the ultrastructural geometry of the rabbit atrial myocyte. Structurally, the myocyte was assumed to have an idealized cylindrical geometry, and model assignments of cell diameter (11 μm), length (130 μm), volume, surface area, and capacitance were obtained from average values reported by Giles and Imaizumi [39]. Component volumes for the cytoplasm, organelles, SR and fractional volumes

for the SR subcompartments were taken from the ultrastructural analysis of Page [83] in rat left ventricular myocardium.

2.2.3 Ionic Composition

Intracellular concentrations of Na^+ and K^+ ions were based on the average activities measured by Désilets and Baumgarten [20] using ion-sensitive microelectrodes. Model equations describing the mass balance of these ions are given in Table B.10.

2.2.4 Calcium Buffering

The rate constants for calcium binding to calmodulin and troponin were taken from Robertson, Johnson, and Potter [88], modified for troponin according to Potter and Zott [85], and scaled from 23 to 35 °C using a Q_{10} factor of 1.8. Specifically, Potter and Zott have reported an ten-fold reduction in Ca^{2+} -binding affinity for the Ca^{2+} -specific site on troponin associated with the presence of actin; consequently, we have raised the off-rate in our model by ten times. On and off rates for Ca^{2+} binding to calsequestrin within the SR release compartment were based on the study of Cannell and Allen [12] (K_d 800 μ M, k_{on} 240 $mM^{-1}s^{-1}$) and adjusted to 35 °C via a Q_{10} factor of 1.6. Tables B.11 and B.10 give the model equations governing the buffering and mass balance of intracellular Ca^{2+} ions.

2.2.5 Sarcoplasmic Reticulum

The mathematical description of the uptake and release of calcium by the SR is similar to that used by Hilgemann and Noble [52] and Earm and Noble [25]. However, there are several important differences between the HN formulation [52] and our mathematical description of the SR. These differences are listed below:

- (1) We have used measurements of the Ca^{2+} -uptake and release compartment volumes of the SR by Page et. al. [83]. Page reports that the Ca^{2+} release compartment is ten times smaller than the Ca^{2+} uptake compartment, while the reverse holds in the HN model.
- (2) The HN model assumes that cytosolic Ca^{2+} buffers are in steady-state, while our model includes simulations of the dynamics of these buffers [88]. The time constant for changes in the occupancy by Ca^{2+} on the calcium-specific site of troponin varies between 5.1 msec when $[Ca^{2+}]_i = 50$ nM and 4.3 msec when $[Ca^{2+}]_i = 1.0$ μ M during the action potential. The assumption that this buffer is in steady-state during the action potential is not valid.
- (3) We have included calsequestrin in the Ca^{2+} -release compartment of the SR as an additional Ca^{2+} buffering agent.
- (4) To prevent Ca^{2+} -induced Ca^{2+} -release at the resting potential (-71.0 mV) and resting Ca^{2+} concentration (50.0 nM), we have increased the Hill-coefficient for Ca^{2+} binding to the sarcoplasmic Ca^{2+} release channel from 2 to 4. This increase reduces the sensitivity to calcium at concentrations below 0.1 μ M while maintaining it at concentrations above 0.5 μ M.
- (5) We have modified several parameters associated with size and kinetics of SR uptake and release currents to give an appropriately triggered release of Ca^{2+} in response to influx through high-threshold Ca^{2+} channels ($I_{Ca,L}$). The parameters in question include the Ca^{2+} binding affinity of the release gate (lowered from 500 to 300 μ M), the maximum uptake and release currents, and the scaling of rate constants associated with the three state release gate mechanism.

Table B.12 provides the equations used to describe SR Ca^{2+} handling and SR release compartment buffering by calsequestrin.

Chapter 3

Results

3.1 Introduction

In the previous chapter, we developed the biophysical components of our rabbit atrial model and showed that component properties match in-vitro data from rabbit cardiomyocytes. Assembly of these ionic current components into the complete whole-cell model yields a tool for investigating integrated responses (e.g. action potentials) of the rabbit atrial myocyte. To the extent that model-generated action potentials agree with in-vitro recordings, we can assess roles of important contributors to action potential waveshape (APW). Similarity of model-generated and experimental responses over a range of stimulus frequencies therefore provides a basis to explain rate-dependent changes in APW.

To achieve satisfactory mimicry of such integrated responses, some parameter adjustment and additional model development was required. For instance, voltage-clamp experiments which provided steady-state and/or kinetic data for a particular ionic current may have limited use in determining an appropriate model current density or, equivalently, a maximal whole-cell conductance. In some cases the data originated from a different cardiac cell subtype; moreover, action potential and voltage-clamp data for a particular cell type were always obtained from different cells. We have therefore treated ionic conductances as free parameters in model fits [72] to atrial action potentials. However, in modeling action potentials we have attempted to minimize changes to individual model components, particularly if this caused deviation

from the component data described previously. Nevertheless, in some cases small modifications (e.g. to τ_{pa}) proved necessary for adequate action potential mimicry. It should be noted that the model-generated responses presented below result from simulations using a single set of parameter values given in Tables B.1-B.13.

In the following sections we demonstrate the ability of our model to mimic the electrophysiological properties of the rabbit atrial myocyte. Our model is shown to mimic the passive electrical properties of the resting cell, consistent with an adequate representation of rabbit atrial background currents. Moreover, it mimics the steady-state APW observed at a typical, physiological rate of stimulation in the active cell. Further, our model is shown to mimic the variation in steady-state APW observed over a range of stimulus frequencies that exceeds the normal physiological range. The correlation between changes in APW and the size of I_t is confirmed in the model, supporting the Boyett hypothesis [9]. However, rate-dependent contributions by other ionic currents (e.g. I_{Na} , I_K) are also suggested by our modeling studies. Model simulations suggest that the latter currents interact with I_t in governing atrial refractoriness, as revealed by the model-generated atrial response to a second “premature” action potential stimulus.

3.2 Quiescent Properties of the Atrial Myocyte

Unlike pacing cells, healthy enzymatically-isolated atrial myocytes are both electrically and mechanically silent and must be stimulated to evoke action potentials. In the absence of external stimuli, the cell maintains a stable resting membrane potential (RP) and the membrane currents are balanced. Although quiescent, the resting cell is nevertheless important to characterize since: (1) passive electrical properties influence the behavior of the active cell, and (2) these properties differ with each cell type

in the heart [39]. It is therefore of interest to guarantee that the model accurately mimics the background state of the quiescent atrial myocyte.

Table B.15 compares the whole-cell capacitance (C_m), resting potential (RP), and input resistance (R_{IN}) of the model cell with reported experimental values. The model C_m and RP represent nominal values for a typical atrial myocyte (50 pF and -71 mV) and the model value for R_{IN} falls within the range given by Giles and Imaizumi [39]. Model R_{IN} has a strong dependence on the sizes of the background currents and in particular I_{K1} , a consequence of the slope of the inward rectifier ion transfer (I-V) characteristic near E_K .

The sizes of background currents at rest affect not only the diastolic properties of the sarcolemmal membrane (RP, R_{IN}) but also the stability of ion concentrations within the intracellular medium. Several membrane currents (I_{NaK} , I_{NaCa} , I_{CaP}) are influenced directly by intracellular ion concentrations, and all ionic currents depend on Nernst potentials for their specific carrier ions. Consequently, the absence of spontaneous electrical or mechanical activity in the resting myocyte depends on ionic stability within the intracellular compartment. However, a similar question of ionic stability arises in the actively paced or stimulated cell. Under suitable conditions, the influx and efflux of a particular ion balance over the cardiac cycle and a “pseudo steady state” mean ion concentration is achieved.

In our model, parameters associated with the sizes of background currents (conductances and maximum transport capacities; see Table B.13) have been adjusted to achieve stability at an assumed resting state ($[Na^+]_i = 6.8$ mM, $[Ca^{2+}]_i = 50.0$ nM, $V_m = -71.0$ mV). The low value of $[Na^+]_i$ relative to that (8.4 mM) reported by reported by Désilets and Baumgarten [20] reflects a difference in $[Na^+]_i$ stability in the resting and working state. Stability of Na^+ ions in the working state is achieved at a higher mean $[Na^+]_i$ level than at rest. The phenomenon results from the added Na^+

load due to I_{Na} and I_{NaCa} during systole. At a physiological pacing rate (2.0 Hz), the total Na^+ load is approximately balanced by I_{NaK} ; however, this results in a slow extrusion of Na^+ ions once cell stimulation is terminated. Consequently we have assumed a modestly lower $[Na^+]_i$ level in the quiescent cell and tolerated a small downward drift in $[Na^+]_i$ and V_m at the nominal resting condition ($[Na^+]_i = 8.4$ mM, $[Ca^{2+}]_i = 50.0$ nM, $V_m = -71$ mV).

3.3 Action Potential Simulations

The rabbit atrial action potential undergoes pronounced changes in waveshape as the frequency of cell stimulation is varied. The extent of this variation is presented in Fig. 3.1, which shows the response of a typical 35 °C rabbit atrial myocyte to a range of stimulus frequencies. At normal physiologic heart rates, the waveshape has a triangular appearance characteristic of an atrial cell (e.g. Boyett et. al. [9]). As the stimulus frequency is lowered, the early portion of the action potential narrows progressively giving rise to a relatively hyperpolarized plateau and a “spike and dome” appearance to the waveshape at very low frequencies [68].

3.3.1 Action Potential Waveshape at 2.0 Hz

Fig. 3.2(A) displays the recorded and model-generated action potentials (35 °C) at a stimulus rate of 2.0 Hz or 120 beats/min (bpm). For comparison, spontaneous pacemaker activity in isolated rabbit SA nodal cells (37 °C) has been reported over a frequency range of 120-260 bpm [60]. Intact rabbit heart typically beats at a rate of 166 bpm [78, Fig. 12.6(A)]. Thus the imposed stimulus frequency represents a physiologically relevant and slightly bradycardic rate of pacing. The recorded waveshape illustrates three of the four conventional systolic phases (labeled in Fig. 3.7) in the cardiac action potential [4]: a rapid upstroke (phase 0), a modest plateau during

Figure 3.1: Steady-state action potentials from a typical 35 °C rabbit atrial myocyte at stimulus frequencies (in Hz): 0.2 (a), 0.5 (b), 1.0 (c), 2.0 (d), 3.0 (e). Numbers indicate classical phases of the action potential. Raw data from Braun and Giles [10] adjusted as described in COMPUTATIONAL DETAILS.

middle repolarization (phase 2), and a noticeable increase in repolarization velocity during late repolarization (phase 3). The phase of early rapid repolarization (phase 1) is not prominent, but is present in action potential waveforms at lower stimulus rates (Fig. 3.7). The prominence of phase 1 repolarization has been correlated with the strength of I_t [7, 39], suggesting a minimal role for I_t at this stimulus rate (2 Hz).

Panels B-D of Fig. 3.2 illustrate the model-generated responses of membrane currents which underlie the model-generated action potential. In Panel A the action potential upstroke, produced by I_{Na} , has a maximum velocity ($\dot{V}_{max}$) of 131 V/s and corresponds to a peak I_{Na} of roughly 6.0 nA. Since peak I_{Na} is significantly larger than all other ionic currents, we have omitted displaying the I_{Na} transient in Fig. 3.2. Panel B displays transients for the next two largest ionic currents, I_t and $I_{Ca,L}$. Consistent with the relative weakness of early repolarization, the model-generated transient in I_t is not large but has a comparable magnitude and timecourse to $I_{Ca,L}$. Nevertheless a small phase 1 repolarization “notch” is evident in both model-generated and recorded action potentials. As repolarization progresses through the plateau phase, the largest ionic currents (I_t and $I_{Ca,L}$) inactivate and delayed K^+ currents ($I_{K,slow}$ and $I_{K,fast}$) slowly activate to support repolarization (Panel C). Although the activation kinetics of $I_{K,slow}$ are less rapid than $I_{K,fast}$, the slow delayed rectifier is less fully deactivated prior to the action potential upstroke. Consequently, both $I_{K,slow}$ and $I_{K,fast}$ provide support to the second and third repolarization phases. Late repolarization is supported by an increase in I_{K1} and opposed by declines in I_K (deactivation) and I_{NaK} (voltage-dependence) as well as slow increases in I_{NaCa} and I_B (Panels C and D). The size of I_{NaK} is consistent with ouabain-sensitive measurements (see MODEL DEVELOPMENT) and is comparable in magnitude to the outward K^+ currents. Thus I_{NaK} provides significant support to repolarization throughout our simulated rabbit atrial action potential. Interestingly, our model is

Figure 3.2: Model-simulated action potential at 2.0 Hz stimulus rate. (A) Model-generated and recorded action potentials (B-C) Ionic currents (D) Background pump and exchanger currents

Figure 3.3: Model-simulated action potential at 2.0 Hz stimulus rate. (A) Model-generated and recorded action potentials (B) $[Ca^{2+}]_i$ transient (C) Ca^{2+} buffer occupancies for calmodulin (O_C), the Ca^{2+} -specific (O_{TC}) and competitive (O_{TMgC}) sites on troponin, and calsequestrin (O_{Calse}) (D) SR release current (E) SR uptake current (F) Transients in Ca^{2+} concentration in the SR release ($[Ca^{2+}]_{rel}$) and uptake ($[Ca^{2+}]_{up}$) compartments.

able to mimic the long “tail” present in the latest phases of atrial repolarization. The simulated tail is developed solely by currents contributing to the resting state of the cell (I_{K1} , I_{NaK} , I_{CaP} , I_B , I_{NaCa}).

Fig. 3.3 presents model-generated transients associated with Ca^{2+} -handling components of the rabbit atrial model for the same 2.0 Hz simulation as above. The predicted $[Ca^{2+}]_i$ transient is given in panel B. Following the action potential upstroke, free $[Ca^{2+}]_i$ levels rise rapidly from a low diastolic level to a peak. The maximum rate of rise of $[Ca^{2+}]_i$ corresponds temporally to the peak Ca^{2+} release signal from the SR (panel D). As previously discussed, the transient rise in $[Ca^{2+}]_i$ results primarily from SR Ca^{2+} release. However, the increase in free intracellular Ca^{2+} falls significantly short of the 65 μ M load released by the SR due primarily to fast Ca^{2+} binding by calmodulin and troponin (panel C). Flux of Ca^{2+} ions into the uptake compartment (panel E) also limits peak $[Ca^{2+}]_i$. At this stimulus rate the SR release compartment retains a portion of its diastolic Ca^{2+} load (panel F) and calsequestrin buffer sites remain partially occupied throughout the action potential (panel C). The diastolic interval is sufficiently short in comparison to the SR uptake-to-release transit time to prevent equilibration of Ca^{2+} ions. Hence a Ca^{2+} concentration gradient between SR uptake and release compartments exists at all times during the action potential (Panel F).

3.3.2 Waveshape changes at 0.2 Hz

The importance of several currents to repolarization is substantially altered at low frequencies of stimulation. Fig. 3.4(A) compares the recorded and model-generated steady-state action potentials at a stimulus rate of 0.5 Hz. The low-rate action potential demonstrates the “spike and dome” features described by Litovsky et. al. [68, 69, 70]. Early repolarization is extremely rapid, and following phase 1 a “dome-

Figure 3.4: Model-simulated action potential at 0.2 Hz stimulus rate. (A) Model-generated and recorded action potentials (B-C) Ionic currents (D) Background pump and exchanger currents

Figure 3.5: Model-simulated action potential at 0.2 Hz stimulus rate. (A) Model-generated and recorded action potentials (B) $[Ca^{2+}]_i$ transient (C) Ca^{2+} buffer occupancies for calmodulin (O_C), the Ca^{2+} -specific (O_{TC}) and competitive (O_{TMgC}) sites on troponin, and calsequestrin (O_{Calse}) (D) SR release current (E) SR uptake current (F) Transients in Ca^{2+} concentration in the SR release ($[Ca^{2+}]_{rel}$) and uptake ($[Ca^{2+}]_{up}$) compartments.

like” or nearly flat plateau develops which delays final repolarization. The plateau occurs at a substantially more hyperpolarized potential than for the high-rate action potential, and both peak overshoot and the area under the action potential are reduced (Table 3.1).

The changes in APW that occur with a lowered stimulus rate can be appreciated by comparing panels B-D of Figs. 3.2 and 3.4. In this case the model-generated upstroke is somewhat faster than at high rates of stimulation ($\dot{V}_{max}$ 152 V/s, peak I_{Na} 7.3 nA), an effect we will consider shortly. More significantly, a lowered stimulus rate allows a more complete recovery from inactivation of I_t , resulting in a larger I_t and consequently a stronger phase 1 repolarization. A significant difference in the magnitudes of I_t and $I_{Ca,L}$ is evident in Fig. 3.4(B), wherein the larger I_t contributes to a dominant early repolarization phase (compare with Fig. 3.2(B)). The quick return of membrane potential to a relatively hyperpolarized plateau value deactivates both I_t and $I_{Ca,L}$ and allows only minimal activation of delayed rectifier currents ($I_{K,slow}$ and $I_{K,fast}$). In the latter case, the relatively longer diastolic interval under a 0.2 than 2.0 Hz stimulus facilitates a more complete deactivation of $I_{K,slow}$ prior to the upstroke (compare diastolic currents in panel C of Figs. 3.2 and 3.4). Hence, I_K makes only a small contribution to repolarization at 0.2 Hz, and this contribution results primarily from $I_{K,fast}$. The primary mediators of the plateau and late repolarization are currents contributing to the resting state of the cell (I_{K1} , I_{NaK} , I_{CaP} , I_B , I_{NaCa}). Moreover, the termination of the early repolarization phase in Fig. 3.4(A) corresponds temporally to a point of high $[Ca^{2+}]_i$ (Fig. 3.5(B)). This results in a more significant recruitment of I_{NaCa} as compared to the earlier high rate simulation (compare panel D in Figs. 3.2 and 3.4). Mimicry of the plateau and late repolarization phases in this case involves a decline in I_{NaCa} , coupled with relatively small changes (~ 10 pA) in other background currents. As in the 2.0 Hz case, a model-generated balance in

Table 3.1: Rate-dependence of Atrial Action Potential: Classical Features

Stimulus Frequency (Hz)	Peak Overshoot* (mV)		APD50** (msec)		A.P. Integral*** (mV-sec)	
	data	model	data	model	data	model
0.2	~90.4	101.4	3.2	6.66	3.59	6.54
0.5	~95.0	104.7	5.7	10.18	3.96	4.01
1.0	102.0	107.4	21.4	21.84	4.82	4.67
2.0	108.0	111.1	45.9	47.81	6.84	6.80
3.0	111.1	111.4	55.4	55.53	7.46	7.38

*Relative to potential before stimulus applied

**Measured at $V = -15$ mV

***Calculated as $\int_{S.P.} (V_m(t) - RP)dt$ where S.P. = one stimulus period.

“resting currents” provides excellent mimicry of the long “tail” in the 0.2 Hz action potential.

The changes in repolarization observed in the 0.2 Hz action potential are associated with corresponding changes in model intracellular Ca^{2+} handling. As illustrated in Fig. 3.5(B), the model-generated $[Ca^{2+}]_i$ transient rises from a low diastolic level to a peak in a manner similar to the 2.0 Hz case. However, diastolic $[Ca^{2+}]_i$ levels are somewhat lower (50 instead of 70 nM) and peak $[Ca^{2+}]_i$ levels are somewhat higher (0.93 instead of 0.79 μ M) under a 0.2 Hz stimulus. The decreased diastolic $[Ca^{2+}]_i$ levels result from an increased time for Ca^{2+} extrusion via $Na^+ - Ca^{2+}$ exchange. The increased peak $[Ca^{2+}]_i$ during the action potential follows from a more complete release of Ca^{2+} from the SR, as evident by the increased release current (Fig. 3.5(D)) and decreased $[Ca^{2+}]_{rel}$ (Panel F), O_{Calse} (Panel C) and uptake current (Panel E). Release current, in turn, is increased due to the lower diastolic $[Ca^{2+}]_i$ levels which produce less diastolic inhibition of the modified HN Ca^{2+} release gate. Although the Ca^{2+} release load is somewhat larger at 0.2 than 2.0 Hz (109 rather than 65 μ M),

the mean occupancy of the competitive Ca^{2+} -binding site on troponin is somewhat lower (16.6 versus 28.5%) reflecting once again the larger diastolic interval in this case. Interpreting the mean value of O_{TMgC} as an indicator of contraction, the model demonstrates a positive inotropic effect with stimulus rate even though peak $[Ca^{2+}]_i$ levels decline slightly as rate increases. The longer diastolic period also permits equilibration of Ca^{2+} between SR subcompartments during diastole (Panel F) and results in a slightly higher Ca^{2+} “filling” of the SR release compartment.

Collectively Figs. 3.2 and 3.4 demonstrate that the model can mimic APW changes that occur with varying stimulus rates. The complete set of action potential fits is shown in Fig. 3.6. A single set of model parameters (given in Table B.13) was used to provide the fit to the range of APW changes shown in Fig. 3.6. Individual fits can be further improved (at the expense of overall rate-dependent mimicry) by adjustment of individual model conductances using a Marquardt parameter estimation scheme [72]. However, without such adjustments the model succeeds in mimicking the major features of rate-dependent APW changes. As summarized in Table 3.1, the model also succeeds in mimicking classical features of the observed APW changes such as action potential duration (APD), area, and peak overshoot (POS).

3.3.3 I-V Trajectories

Since the variation in APW with stimulus frequency (see Fig. 3.1) involves significant changes in repolarization velocity, a model of such changes should mimic the frequency-dependence of repolarization rates. From Equation 2.1 (p. 18), the repolarization velocity provides a direct measure of the net current passing through the membrane (I_{net}). In turn the behavior of I_{net} , calculated from the time derivative of membrane potential, may suggest the responses of underlying membrane currents.

Since many of these currents undergo voltage-dependent gating [55], it is convenient and even

Figure 3.6: Simulated (solid) and recorded (dotted) action potentials at stimulus frequencies (labeled) of 0.2, 0.5, 1.0, 2.0, 3.0 Hz. Data from Braun and Giles [10].

Figure 3.7: (A) Steady-state action potentials from a typical 35 °C rabbit atrial myocyte at stimulus frequencies (in Hz): 0.2 (a), 0.5 (b), 1.0 (c), 2.0 (d), 3.0 (e). Numbers indicate classical phases of the action potential. Raw data from Braun and Giles [10] adjusted as described in COMPUTATIONAL DETAILS. (B,inset) Calculated I-V trajectories.

insightful to examine this behavior as an instantaneous current-voltage (I_{net} -V) trajectory.

Fig. 3.7(B) provides the calculated I-V trajectories for the corresponding action potentials shown in Fig. 3.1 and repeated in Fig. 3.7(A). In this diagram, time is implicit as the behavior of the net sum of membrane currents (I_{net}) is displayed as a function of membrane potential. For convenience, only the repolarization phases of the trajectories are shown and the trajectories proceed in time from right to left. The depolarization phase of each I-V trajectory is omitted due to the presence of a “stimulus artifact” in the raw data and the relatively low rate of sampling used in the recording (see COMPUTATIONAL DETAILS). The decrease in the prominence of phase 1 repolarization with increasing stimulus rate apparent in the time-domain plots is reflected in the decline of the outward phase of I_{net} at depolarized potentials. Most of the decrease occurs over 0.2-1.0 Hz frequency range, in which peak outward I_{net} varies in graded fashion from roughly 800 pA to 150 pA. An increase in the peak overshoot voltage accompanies the increase in stimulus rate. Due to the relatively low sampling rate used in the recording (2 kHz), these increases are most reliable over a frequency range of 1.0-3.0 Hz. Fig. 3.7(B) also demonstrates an apparent overlap of repolarization velocities negative to -55 mV, which corresponds temporally to late repolarization. Possible explanations for this effect are considered in the Discussion.

Fig. 3.8 demonstrates the capacity of the model to mimic the I-V trajectories of Fig. 3.7(B) at corresponding rates of stimulation. The various panels show that the much of the agreement in action potentials presented in Fig. 3.6 is supported by mimicry in the I-V “phase plane” plots. Of particular interest is the variation in model-generated early repolarization velocity over the 0.2-1.0 Hz stimulus frequency range. As illustrated in Panels C-E, the maximum early repolarization current in the model grades in a similar fashion to the data. Following repolarization to approx-

Figure 3.8: Instantaneous I-V trajectories for the model (solid) and data (dotted) at stimulus rates (Hz) of: (A) 3.0 (B) 2.0 (C) 1.0 (D) 0.5 (E) 0.2. Plots show repolarization phase only. Data trajectories calculated from action potential records of Braun and Giles [10].

imately -35 mV, net repolarizing currents are relatively weak but are well matched by the model-generated trajectories. At higher rates of stimulation (Panels A-B), the net repolarizing membrane current (I_{net}) is relatively small early in repolarization but becomes more strongly outward later in the action potential. The slope change apparent in the action potential records (traces 1 and 2 of Fig. 3.7(A)) is reflected as a “notch” or transient decrease in the outward I_{net} in both model-generated and calculated phase plane plots (Fig. 3.8(A-B)). A companion figure, Fig. 3.9, displays the decomposition of model-generated I_{net} -V trajectories into component membrane currents at two different stimulus rates (corresponding to those of Figs. 3.2-3.5). This diagram clarifies the mechanisms behind the model-generated rate-dependent formation of early repolarization velocity, peak overshoot, the action potential “notch,” the “spike and dome” waveshape, and phase of late repolarization.

In our study the phase of early repolarization develops from a balance of I_{Na} , I_t , and to a lesser extent $I_{Ca,L}$. With a low stimulus frequency, illustrated in Fig. 3.9(A), rapid early repolarization to approximately -35 mV is supported by a larger net outward current (I_{net}). This net current is the sum of a much larger outward I_t , a relatively small $I_{Ca,L}$, and a sizable tail in I_{Na} . The tail results from the slowly inactivating (h_2) component of I_{Na} , so that I_{Na} inactivation is completed at relatively hyperpolarized potentials when the action potential has a “spike and dome” morphology. In contrast at high stimulus rates incomplete reactivation reduces the magnitude of I_t and early repolarization is less prominent (Fig. 3.9(B)).

Panels A and B of Fig. 3.9 also illustrate the model construction of the the peak overshoot voltage and the action potential notch. The model mimics an increase in peak overshoot voltage with increasing stimulus rate observed in the action potential data (Fig. 3.7) via a balance of I_t and the slow (h_2) component of I_{Na} . At a low stimulus rate I_t is sufficiently recovered to oppose I_{Na} during the action potential

Figure 3.9: Comparison of I-V trajectories during repolarization at low (0.2 Hz; panels A & C) and (2.0 Hz; B & D) high stimulus rates. (A,B) Major currents (I_{Na} , I_t , $I_{Ca,L}$); (C,D) Minor and background currents (I_K , I_{K1} , I_{NaCa} , I_{NaK} , I_B) Trajectories proceed in time from right to left.

upstroke. As stimulus rate is increased, incomplete reactivation reduces the peak I_t observed in the action potential (Fig. 3.9(B)) and allows the slow component of I_{Na} to generate a higher peak overshoot. This overshoot occurs later in time than at a lowered stimulus rate (Figs. 3.7(A) and 3.6). Consequently, at peak overshoot I_{Na} is more fully inactivated and supplies a smaller current than at low stimulus rates (Fig. 3.9(B)). The relative timings of the decays of I_{Na} (slow inactivation), I_t (fast inactivation) and $I_{Ca,L}$ provides model mimicry of the slope change or “notch” present near $V=+30$ mV.

Our model mimics the “spike and dome” character of the low stimulus rate action potential in both the time (Fig. 3.6) and phase-plane (Fig. 3.9(A,C)) plots. Model termination of the “spike” phase of rapid early repolarization results primarily from the decay of major ionic currents, with minor or background currents playing a more significant role in “dome” repolarization. During early repolarization model-generated major currents (I_{Na} , I_t , $I_{Ca,L}$) achieve magnitudes eight to fifty times larger than all lesser cell currents. Following this phase, repolarization over potentials negative to -35 mV involves a net current balance of less than 20 pA in magnitude. The model transition from the “spike” to “dome” results from the coordinated deactivation of I_t and $I_{Ca,L}$ and to a lesser extent inactivation of remnant I_{Na} . Due to the large size of I_t in early repolarization, I_{NaCa} has relatively little influence on the termination of early repolarization. However, I_{NaCa} does contribute to the plateau and late repolarization phases associated with the “dome” since its size is comparable to other minor currents.

The phase of late repolarization, viewed from the phase plane plots of Fig. 3.7(B), has similar characteristics at both low and high rates of stimulation with the most notable differences occurring in the early portions of this phase. At potentials negative to -55 mV, repolarization currents overlap and final repolarization appears rate independent. In the model, changes in late repolarization arise solely from differences in

the recruitment of I_{NaCa} and the delayed rectifiers ($I_{K,slow}$ and $I_{K,fast}$). As illustrated in panels B and D of Fig. 3.9, the relatively broad action potential at high stimulus rates allows more significant recruitment of I_K currents than at low stimulus rates. A more substantial $I_{K,slow}$ is generated as a result of incomplete deactivation during rapid cell stimulation, while $I_{K,fast}$ recruits from a nearly fully deactivated diastolic level to generate outward current later in repolarization. The combination of $I_{K,slow}$ and $I_{K,fast}$ increases the net repolarization current over the low rate case at potentials between -55 and -35 mV. This effect is enhanced by the larger recruitment of I_{NaCa} with the “spike and dome” action potential. However, at potentials negative to -55 mV I_K has deactivated and recruitment of I_{NaCa} is virtually identical, thus mimicking the apparent rate-insensitive portion of late repolarization.

We are reminded that a stimulus rate of 2.0 Hz lies within the normal physiological range of rabbit atrial beating. A model which purports to explain the atrial action potential should therefore necessarily mimic the changes in net repolarization current throughout the various phases of repolarization. Since our model closely mimics the 2.0 Hz action potential in both the time and phase-plane domains, we can suggest the extent to which various currents may participate in repolarization during the normal rabbit atrial cardiac cycle.

The phase plane trajectories of Fig. 3.9(D) prove useful in addressing this issue. From the figure, our model would suggest that I_{NaK} , as well as $I_{K,fast}$ and $K^+I_{K,slow}$, provide comparable repolarizing currents at normal stimulus rates. Contributions from each current are required, however, to adequately represent the action potential. At depolarized potentials, the strongly rectifying characteristic of the recently identified $I_{K,fast}$ precludes a significant role for this current in initiating repolarization at potentials above -20 mV. Consequently, the outward peak of roughly 80 pA net repolarization current in the data must be supplied in our model by other out-

ward currents, in this case I_{NaK} and $I_{K,slow}$. Thus model I_{NaK} not only affects the intracellular ionic equilibrium but also provides the most substantial support to the initiation of repolarization of the “minor” outward currents. The contribution of $I_{K,slow}$ at depolarized potentials is also required in our model to bring about an appropriate initiation of repolarization. As an additional effect, $I_{K,slow}$ exerts a subtle influence on the model-generated “notch” produced by the interaction of major ionic currents (I_{Na} , I_t , and $I_{Ca,L}$).

If the fast delayed rectifier does not initiate repolarization, the apparent rate-independence of final repolarization would suggest its lack of participation in this phase as well. At potentials below -55 mV, net repolarization current appears independent of rate as are all model-generated I-V trajectories except I_{NaCa} , $I_{K,slow}$, and $I_{K,fast}$ (compare Panels C-D of Fig. 3.9). Our model predicts that the uptake of intracellular Ca^{2+} by the SR suffices to reduce $[Ca^{2+}]_i$ to low levels by the final repolarization phase, producing a rate-independence of I_{NaCa} current during final repolarization. Consequently, I_K must also be independent of rate and hence contribute zero current in final repolarization. This scheme has been satisfied in our model by accelerating the deactivation kinetics of $I_{K,fast}$ (i.e. τ_{pa}) as presented in the MODEL DEVELOPMENT.

3.3.4 SR Ca^{2+} Handling

Under our modified SR model, the sarcoplasmic reticulum acts as the most significant modulator of $[Ca^{2+}]_i$ during the action potential. For example, at a stimulus rate of 2.0 Hz approximately 93.6% of the Ca^{2+} load added to the intracellular medium during the action potential (systolic phase only) results from SR Ca^{2+} release. The remaining fractions (4.3%, 0.7% and 1.5%, respectively) arise from transmembrane Ca^{2+} influx (via I_{Ca} , $I_{B,Ca}$, and I_{NaCa} , respectively). Membrane currents therefore

provide only a small contribution to the $[Ca^{2+}]_i$ transient, giving general agreement with the reported dominant role of SR Ca^{2+} release in rat ventricular cells [100].

Release of SR Ca^{2+} at the same stimulus rate is associated with a transient reduction in $[Ca^{2+}]_{rel}$ to 32% of its initial value. Following the systolic Ca^{2+} release, $[Ca^{2+}]_i$ is restored to low levels through a combination of uptake by the SR and extrusion via the calcium pump and sodium-calcium exchanger. The amount of Ca^{2+} removed by the SR uptake mechanism balances the previous SR release load. Transsarcolemmal Ca^{2+} extrusion via I_{NaCa} and I_{CaP} balances the preceding transmembrane Ca^{2+} influx, such that roughly 73% of the extrusion flux results from sodium-calcium exchange.

The maximum release capacity of our SR model agrees closely with measurements reported in rat ventricle [100]. Varro et. al. [100] have estimated that caffeine induces a 120 μM contribution to intracellular Ca^{2+} from the SR. For comparison, our model predicts a Ca^{2+} release load of 109 μM under a 0.2 Hz stimulus rate. Our model further predicts that the amount of Ca^{2+} released by the SR is maximal at low stimulus rates (0.2 Hz), while at higher stimulus rates net SR Ca^{2+} release is reduced due to the inhibitory effect of elevated diastolic $[Ca^{2+}]_i$ levels on SR release current (I_{rel}). Consequently, at 2.0 Hz the net SR Ca^{2+} release drops to 65 μM .

3.4 Transient Outward Current Voltage-Clamp Simulations

Our model's capacity to simulate rate-dependent APW changes, shown in Figs. 3.2-3.9, includes the ability to mimic the graded variation in the prominence and velocity of early repolarization. Since the latter have been correlated experimentally with rate-dependence effects on I_t [9, 8, 7, 39], the question arises as to the extent to which I_t may participate in such changes. In order to address such a question with our model, some verification of our mathematical description of I_t is desirable. If the

rate-dependent variation in model I_t could be shown to correspond to experimental data, our confidence in a model interpretation of the role of I_t would be enhanced. We have therefore conducted simulation studies to assess our mathematical description of I_t .

An assessment of the timecourse of recovery from inactivation of I_t is provided in Fig. 3.10. As discussed in the MODEL DEVELOPMENT, we have utilized data from both Giles and Imaizumi [39] and Fermini et. al. [32] to assess the dual time constant recovery of I_t . However, the inclusion of a third gating variable (s_3) in our model description complicates the reactivation behavior of our mathematical model such that it no longer follows a purely biexponential timecourse. Consequently, we have simulated the recovery of I_t under voltage-clamp and compared these results directly with the data of Fermini et. al. [32]. In these simulations, the time constants for activation and inactivation gating variables have been adjusted for 30 °C using Q_{10} factors. Activation (τ_r) kinetic adjustments utilized a Q_{10} factor of 2.3. Inactivation (τ_{s_1} , τ_{s_2} , τ_{s_3}) kinetic adjustments utilized separate Q_{10} factors for the Boltzmann (2.3, 2.3, and 0.1) and offset (2.18, 12.75, 5.0) terms of the time constant expressions. Since the temperature adjustment of s_3 slows an already slow gating variable, the deviation of the model response from a double exponential is small and the model can closely mimic the observed data ([32]). However, the recovery behavior of I_t changes somewhat for the 35 °C model as shown by the semi-solid line of Fig. 3.10.

Fig. 3.11 compares the rate-dependent availability of I_t generated by the model (adjusted to 30 °C as above) with the data from Fermini et. al. [22]. In this figure, the peak current (in steady-state) elicited by a voltage clamp step to 0 mV from -60 mV is displayed as a function of the period at which the voltage-clamp protocol is applied. Currents have been normalized by the response at a period of 10 seconds (0.1 Hz).

Figure 3.10: Timecourse of I_t Reactivation. A double-pulse protocol (inset) probes the reactivation timecourse of the measured (circles) and model-generated (solid: 30 °C, semi-solid: 35 °C) I_t responses. The current evoked during pulse P2, normalized to that during pulse P1, is shown as a function of the interpulse interval. The P2/P1 ratio measures changes in I_t availability (s_{total}) with time. In all traces, longer interpulse intervals lead to a more fully reactivated I_t (higher s_{total}).

In contrast, panel B compares the rate-dependent changes in model I_t elicited during an action potential with changes in the maximum phase 1 repolarization velocity (taken from repolarization currents of Fig. 3.7(B)). Two observations are evident from the figure. First, the apparent sensitivity of early repolarization velocity to frequency is somewhat higher than that of I_t under voltage clamp (at 30 °C) over low frequency ranges (0.1 to 0.9 Hz). Second, the model mimics the higher level of frequency sensitivity over this range, giving some disagreement with the observed voltage-clamp results ([22]). We have found this modeling result to be a consequence of the behavior of the third inactivation variable (s_3), whose slow changes “enhance” the frequency sensitivity of I_t availability at low stimulus rates.

Our simulation studies of I_t demonstrate qualitative agreement of our mathematical model with experimental measurements of its reactivation timecourse and rate dependence. In the model these phenomena share a common dependence on a collection of inactivation gating variables (s_1, s_2, s_3). While our description of I_t inactivation is somewhat complicated, our modeling efforts suggest that we have presented roughly the minimal complexity necessary to mimic both voltage-clamp and action potential results. In particular, the inclusion of a third inactivation variable (s_3) was required to mimic the graded changes in early repolarization velocity observed over low stimulus frequencies (0.2-1.0 Hz).

The apparent differences in frequency sensitivities of I_t availability and peak early repolarization velocity are somewhat puzzling. Certainly the comparison must be between action potential and voltage clamp results must be made with caution; however, it is notable that the duration of the depolarizing clamp step used by Duan et. al. [22] was close to the rabbit atrial APD. Unarguably, some incompleteness of representation may be ascribed to our I_t model and some uncertainty to the extrapolation of temperature (from 30 to 35 °C). Nevertheless, considering only the low frequency

range (0.2-1.0 Hz), we find it interesting that a greater rate-sensitivity may apply to early repolarization velocity than to the size of I_t . If a slowly inactivating component of I_{Na} participates in the atrial action potential, as suggested by our model, then at low stimulus rates such a component might oppose I_t throughout all or a portion of rapid early repolarization. Consequently, the rate sensitivity of currents generating the peak in early repolarization velocity might be greater than suggested by our action potential records. Moreover, the differentiation process may be considered to slightly underestimate the peak $\dot{V}_{max}$ during early repolarization, thus suggesting a somewhat underestimated rate-sensitivity of currents in our action potential results.

If during early repolarization changes in the peak $\dot{V}_{max}$ are in fact greater than can be attributed to changes in I_t , then some other process(es) must be prescribed for the difference. Note in this case that incomplete reactivation of an inward current cannot account for such an effect. Such a phenomenon would produce a more positive (less inward) current as rate increases and would therefore decrease, not increase, the "rate sensitivity of $\dot{V}_{max}$."

Figure 3.11: Frequency Dependence of I_t and Early Repolarization Current (A) Frequency-dependence of peak I_t evoked in steady-state from a train of voltage clamp steps (illustrated in inset), normalized to peak current under a 0.1 Hz step frequency. Model-generated (solid, adjusted to 30 °C) and recorded (squares, 30 °C) [22, Fig. X] rabbit atrial responses are shown. (B) Frequency-dependence of peak outward I_{net} observed during the early repolarization phase of the action potential. Results are shown as a function of stimulus frequency. The model-generated I_{net} (solid) is compared with the peak repolarization current (triangles) estimated from action potential records [10].

3.5 Application: Response to a “Premature” Stimulus

The interaction between inactivation and reactivation which generates rate-dependent variations in the size I_t should not be considered a phenomenon unique to I_t . Instead, these changes reflect more general characteristics of HH-type voltage-dependent gating variables. “Rate-dependent effects” on a particular gating variable y are observed whenever y_{ss} changes substantially more than y during the action potential cycle. This occurs whenever diastolic (or systolic) duration, defined relative to the $V_{1/2}$ value of y_{ss} , is short compared to the diastolic (or systolic) kinetics of y , so as to produce an incomplete transition in y during diastole (or systole). In such a case, alterations in diastolic (or systolic) duration modulate the steady-state end-diastolic (systolic) value of y . The direction of the rate-dependent modulation differs between activation and inactivation variables. For instance, with relatively slow diastolic kinetics, shortening of diastole produces increases in activation and decreases in inactivation gating variables at end-diastole.

One would therefore expect similar incomplete transition phenomena to occur in other slowly inactivating ionic currents such as I_{Na} and $I_{Ca,L}$ and slowly deactivating currents such as $I_{K,slow}$. However, the time constants involved for these currents are significantly shorter than those for I_t . For instance, from the maximum value of τ_{h2} in our model (roughly 120 msec), one would not expect to observe I_{Na} -related incomplete recovery effects on APW until the stimulus frequency enters the 4-8 Hz frequency range. Since the rabbit atrial APD_{90} under a 3 Hz stimulus is somewhat more than 100 msec, our model would suggest a stimulus period shorter than the action potential duration would not be required to generate incomplete recovery effects on I_{Na} . Preliminary model studies using high stimulus frequencies have shown this to be the case (not shown). Moreover, at very high stimulus frequencies (e.g. 8 Hz), the periodicity of APW observed over the 0.2-3.0 Hz frequency range breaks down and

peak overshoot varies from action potential to action potential (not shown). The lack of periodicity results from an unstable feedback interaction between peak overshoot, end-diastolic I_{Na} availability, and the size of the subsequent I_{Na} transient.

While the more rapid reactivation kinetics associated with I_{Na} and $I_{Ca,L}$ may not produce significant rate-dependent APW changes over the normal heart rates, the closeness of the magnitude of τ_{h2} and τ_{f1} to the normal APD suggest that these may affect action potential refractoriness in a rate-dependent manner. A simple way to consider this possibility is to examine the response of our model to the application of a "premature" stimulus, i.e. one which occurs earlier than expected based on the previously applied stimulus period.

Numerous studies have shown that normal cardiac tissue undergoes a "refractory period" following the action potential in which the "excitability" or ability to generate a second action potential response is transiently reduced [4]. This period is divided classically into "absolute" and "relative" refractory periods. During the former, the tissue is completely unexcitable while more intense stimuli than normal are required to evoke a response during the latter.

Simulation of the response to a "premature" stimulus therefore tests cell refractoriness and the role of ionic gating phenomena in its modulation. Fig. 3.12 illustrates the model-generated premature stimulus response following steady low-rate stimulation (at 0.2 Hz). Panel A shows the membrane potential responses to various premature stimuli (5 msec duration, 285 pA amplitude) applied at different times following the initial (periodic) stimulus applied at time $t=0$ msec. Panels B-C illustrate associated transient changes in sodium current availability (h_{total}) and the corresponding I_{Na} response. Premature stimuli applied earlier than $t=100$ msec fail to invoke an "active" response in the cell membrane and consequently repolarization continues after stimulus termination. Over the time period between $t=100$ and $t=500$ msec, a

Figure 3.12: Premature Stimulus Response Following Low-Rate Stimulation (0.2 Hz). Shown are the responses to premature stimuli applied at various times following the initial (periodic) stimulus (at $t=0$ msec). (A) Membrane Potential (B) I_{Na} Availability (h_{total}) (C) I_{Na} . Predicted absolute and relative refractory periods (ARP and RRP) are labeled below the time axis. APD_{-50mV} is 90 msec.

gradually stronger action potential response is elicited as the premature stimulus is delayed. Following $t=500$ msec, action potential waveshape approximately matches the initial “control” response. Consequently, the time interval between $t=0$ and $t=100$ msec corresponds to the “absolute refractory period (ARP)” and the interval that follows corresponds to the “relative refractory period (RRP)” in Fig. 3.12. The recovery of APW to the “control” condition parallels changes in I_{Na} size (Panel C) and availability (Panel B). Consequently, refractoriness in our model is tied directly to I_{Na} reactivation and is distinguished through h_{total} into absolute and relative refractory periods. Moreover, the model-generated response is qualitatively similar to that recorded from human atrium (Fig. 1.4) under conditions which generate a similar steady-state waveshape.

Since APW and the underlying currents of repolarization vary with stimulus rate, one might expect to find rate-dependent changes in atrial refractoriness as well. Model simulations of the premature stimulus response following high-rate (3.0 Hz) stimulation (Fig. 3.13), however, suggest this is not the case. In spite of differences in the “control” waveshape, the high-rate response follows a similar timecourse of APW recovery to the low-rate case (compare Panel A of Figs. 3.12-3.13). Moreover, both I_{Na} availability and size (Panels B-C) recover in a similar manner to the low-rate simulation. Consequently, the low and high-rate cases have the same absolute refractory period (100 msec duration). Subtle differences exist between relative refractory periods due to the lower initial value of h_{total} (incomplete reactivation of h_2) under a 3.0 Hz stimulus. Nevertheless, I_{Na} recovers with approximately the same kinetics in each case.

Colatsky et. al. [18] have suggested that certain newly identified class III antiarrhythmic agents may prove useful in modulating cell refractoriness. Specifically, they propose that the lengthening of APD achieved through inhibition of delayed K^+

Figure 3.13: Premature Stimulus Response Following High-Rate Stimulation (3.0 Hz). Shown are the responses to premature stimuli applied at various times following the initial (periodic) stimulus (at $t=0$ msec). (A) Membrane Potential (B) I_{Na} Availability (h_{total}) (C) I_{Na} . Predicted absolute and relative refractory periods (ARP and RRP) are labeled below the time axis. APD_{-50mV} is 92 msec, resulting in nearly identical refractoriness (ARP and RRP) to the low-rate case (Fig. 3.12).

currents may increase cell refractoriness. We have tested this hypothesis by simulating the premature stimulus response under 100% block of both types of delayed rectifier currents (Fig. 3.14). Blockade of $I_{K,slow}$ and $I_{K,fast}$ lengthens action potential duration (Panel A) and delays reactivation of sodium current availability (Panel B). Consequently, the ARP increases by 100 msec. Restoration of APW after the ARP follows a similar timecourse to the unblocked case (compare Figs. 3.13-3.14). As Panel B illustrates, reactivation of h_{total} is simply delayed in time (compare dotted and solid curves) and responds in the same manner as the control after the delay. Consequently, the relative refractory period is also delayed but has a similar duration to the control case.

Mechanistically, we can explain the effects on refractoriness discussed above in terms of the interaction between h_{total} and APD_{-50mV} , the duration of the action potential over which membrane potential is positive to -55 mV. Note that this potential represents the approximate threshold for complete inactivation on the steady-state inactivation curve for I_{Na} (Fig. 2.2(A)). Consequently, h_{total} tends to zero (inactivates) during the APD_{-50mV} interval and tends to positive values (reactivates) outside this interval. Variation of APD_{-50mV} therefore directly modulates refractoriness by changing the time of initiation of I_{Na} reactivation during the action potential. The apparent rate-independence of absolute refractory periods discussed above reflects the approximate rate-independence of APD_{-50mV} over stimulus rates of 0.2-3.0 Hz (Fig. 3.7). Complete block of $I_{K,slow}$ and $I_{K,fast}$ increases APD_{-50mV} by 92 msec, i.e. approximately the same increase as for the ARP.

Figure 3.14: Premature Stimulus Response Following High-Rate Stimulation (3.0 Hz) under 100% I_K Block. Shown are model-generated responses to premature stimuli applied at various times following the initial (periodic) stimulus (at $t=0$ msec). (A) Membrane Potential (B) I_{Na} Availability (h_{total}) (C) I_{Na} . Predicted absolute and relative refractory periods (ARP and RRP) are labeled below the time axis. The dotted curves indicate the control responses elicited in the absence of I_K block and premature stimuli. Complete block of $I_{K,slow}$ and $I_{K,fast}$ increases APD_{-50mV} from 92 to 184 msec and lengthens the ARP by roughly 100 msec. Reactivation of h_{total} is delayed but follows a similar timecourse to the control; consequently RRP duration is unaffected by I_K block.

Chapter 4

Discussion

We have developed a novel mathematical model of the rabbit atrial myocyte which has an extensive basis on published data from isolated rabbit cardiac myocytes. Our model mimics many of the in-vitro electrophysiological responses of the enzymatically isolated cell, including (1) the behavior of individual ionic currents in voltage clamp experiments, (2) the rabbit atrial action potential waveshape at a nominal, physiological rate of stimulation, and (3) the variation in action potential waveshape that occurs as stimulus rate is varied. Adoption of a data-based approach to model development has provided a biologically relevant correspondence between model and cellular elements. Consequently, the quality of mimicry demonstrated in our model has allowed these responses to be interpreted in biophysical terms. Further, the semi-quantitative nature of our model has provided the opportunity to examine atrial myocyte excitability vis-a-vis the response to “premature” stimuli. In the context of the clinical cardiology, the ionic contributors to such responses are frequently offered as targets for antiarrhythmic drugs. Our model therefore provides a useful tool by which to investigate the factors governing atrial repolarization and which may be applied in a variety of clinically relevant contexts.

4.1 Data-Based Approach

In the process of model development, we have utilized physiological data collected by our collaborative laboratory and/or recently published in the literature. In most cases the data derives from recordings from isolated cardiac myocytes in rabbit heart.

Where possible, we have used data obtained specifically from single rabbit atrial myocytes. The mathematical components within our model framework have been required to correspond to biophysical components known to exist in rabbit atrial myocytes. Considerable attention has been paid to representing the dynamic behavior of specific components (e.g. ion channel currents) as quantitatively as possible. The relative contributions of these components in the complete model have been constrained to agree with direct (e.g. $\overline{I_{NaK}}$) or indirect (e.g. $\dot{V}_{max}$) estimates of their functional size. We have also required that model properties resulting from an aggregate of model components (e.g. input resistance) fall within the known physiological range. Our approach has therefore provided a mathematical description of the rabbit atrial myocyte in both structurally and functionally relevant terms. We consider the incorporation of biophysical data within a biologically-based mathematical framework to be essential in providing a realistic explanation of the behavior and properties of the rabbit atrial cell.

4.2 Rate Dependent APW Changes: Model Interpretations

Rabbit atrial action potentials undergo pronounced changes in waveshape as the rate of cell stimulation is varied. Our model has simulated such changes observed over the 0.2-3.0 Hz stimulus frequency range at 35 °C. Model-generated results agree closely with the simulated responses in both the time- and phase-plane domains, thus supporting the notion that our model accurately represents the rate-dependent changes in membrane currents that produce APW variations. The extensive basis of our model in published data and the semi-quantitative nature of our model representations allows the roles of various membrane currents in repolarization to be interpreted through our model. These interpretations are facilitated by our use of instantaneous I-V plots which in turn highlight the effects of voltage-dependent gating on the behav-

ior of various membrane currents. While our model interpretations ultimately require experimental verification to prove how repolarization occurs, we can provide insight into the interaction between various currents and the likely ways in which these may combine to regulate the action potential.

4.2.1 I_t and Early APW Changes

Our simulation studies corroborate the notion proposed by Boyett [9] and supported by single-cell studies [39, 17, 32] that early APW changes reflect predominantly the rate-sensitivity of I_t . At the lowest stimulus rate of this study (0.2 Hz), the model-generated peak I_t is significantly larger than all other currents except I_{Na} . Following the upstroke, I_t contributes significantly to repolarization over exactly the same potential range as where rapid early repolarization occurs. This is particularly evident in the phase plane plots (Fig. 3.9) [8]. With rapid early repolarization, I_t decays primarily through deactivation, i.e. the activation gating variable r decays to zero. In so doing, the potential at which early repolarization terminates provides a rough indication of the activation threshold of I_t . With an increase in stimulus frequency to 1.0 Hz, graded changes in the maximum velocity of early repolarization are reflected in graded changes in the size of I_t (Fig. 3.8-3.9,3.11). Further increases in rate lead to further reduction in I_t ; however, even at the highest rate studied (3.0 Hz), phase plane analysis would suggest a contribution of I_t to the a small early repolarization phase (Fig. 3.8). Increases in peak overshoot accompany increases in stimulus rate, an effect consistent with the incomplete reactivation of I_t and/or other outward membrane currents.

However, our studies suggest that the rate dependence of I_t may be distinguished somewhat from the rate-dependence of early repolarization. Over the 0.2-1.0 Hz stimulus frequency range, I_t appears to change less rapidly as observed in a voltage

clamp protocol than the maximum velocity of early repolarization calculated from action potential data. The cause of this difference is uncertain; however, it may reflect yet unidentified contributions of membrane currents other than I_t to the rate-dependence of APW. It should be noted that the incomplete reactivation of an inward current such as I_{Na} or I_{Ca} cannot account for this difference, since this would tend to reduce the gradation of early repolarization velocity produced by I_t .

4.2.2 Slowly Inactivating Component of I_{Na}

Our studies suggest that a slowly inactivating component of I_{Na} may have functional roles in the modulation of peak overshoot voltage and secondarily in early repolarization at low stimulus rates. The rabbit atrial action potentials recorded by our collaborators [10] demonstrate a graded increase in peak overshoot voltage with stimulus rate. This increase occurs in spite of a lack of any significant decline in upstroke velocity until membrane potential reaches within 5-10 mV of the peak. One might assume that these such changes are generated by the incomplete recovery of outward currents such as I_t . In such a case the contribution of counterbalancing inward currents to the final phase of the upstroke must be small enough to be opposed by I_t or other outward currents. Further, the changes in outward currents with rate must have sufficient magnitude to enhance the action potential height by the appropriate amount as rate increases. Given the 5-10 nA size of peak I_{Na} , we have found a single component of inactivation description to be inadequate in simulating peak overshoot changes. Conversely, use of a biexponential decay for I_{Na} , as supported by experimental evidence, provides a modest rate-dependent increase in peak overshoot with rate. This slow decay also provides a contribution for I_{Na} to early repolarization at low stimulus rates (e.g. 0.2 Hz), since I_{Na} activates but fails to inactivate completely during rapid early repolarization (Fig. 3.9).

4.2.3 Rate Independence of Final Repolarization

Final repolarization velocity calculated from our rabbit atrial action potential data appears to vary little with rate at potentials negative to -55 mV. The contributors to repolarization below this potential include a number of instantaneous background currents, I_{NaK} , I_{CaP} , I_{NaCa} , and the residual contributions of membrane ionic currents. The first two of these have no direct dependence on membrane potential. Assuming that the $[Ca^{2+}]_i$ level has been reduced significantly below its peak value by final repolarization, one may expect to find little change in current contributed by I_{CaP} or I_{NaCa} to this stage. The assumption requires only a modestly active SR Ca^{2+} uptake mechanism, as is the case in our model. Consequently, our model shows that the rate-independence of final repolarization can be represented by the complete decay of major ionic currents prior to this phase.

4.2.4 Ca^{2+} and K^+ Currents and Mid Repolarization

The plateau and initial repolarization phases of the action potential is usually considered to result from a combination of a decay in $I_{Ca,L}$ and increases in K^+ currents. In our study $I_{Ca,L}$ supports the plateau and helps to create a “notch” in the action potential at high stimulus rates. The contribution of $I_{Ca,L}$ is most significant at potentials above -10 mV and at high stimulus rates. Under low stimulus rates the “turn-off” of $I_{Ca,L}$ is accomplished through deactivation (i.e. decay of d_L). With high stimulus rates and a significant plateau an inactivation process attenuates the $I_{Ca,L}$ transient. The peak value of our simulated $I_{Ca,L}$ transient changes relatively little with stimulus rate; rather, $I_{Ca,L}$ exerts a greater influence on APW when outward currents are reduced due to I_t inactivation.

Recent studies of delayed rectifier currents have suggested a role for I_K and particularly its fast component in the repolarization of rabbit atrium. In our model, $I_{K,fast}$,

$I_{K,slow}$, and I_{NaK} generate comparable peak currents during repolarization. However, the potential range over which these contributions occur differ. While I_{NaK} supports repolarization over the entire range of repolarization potentials, $I_{K,fast}$ contributes primarily to late repolarization $I_{K,slow}$ contributes to an earlier mid repolarization. This somewhat paradoxical behavior of $I_{K,slow}$ and $I_{K,fast}$ results from the strongly inwardly rectifying I-V characteristic of $I_{K,fast}$ which limits its contribution at depolarized potentials. Our simulations suggest that both $I_{K,slow}$ and $I_{K,fast}$ affect atrial refractoriness, based on the response of our atrial model to premature stimuli.

4.2.5 Refractoriness, Reactivation of I_{Na} , and Rate

Our simulations of the premature stimulus response suggest a relatively straightforward link between the classically defined refractory periods and the recovery from inactivation process for I_{Na} . In this view, the period of absolute refractoriness represents that time interval over which I_{Na} is fully inactivated and the sarcolemma is too depolarized to allow recovery from inactivation. Relative refractoriness applies to the period that follows over which I_{Na} recovers from inactivation. Our model predicts virtually identical ARP's over the 0.2-3.0 Hz stimulus frequency range due to the apparent rate-independence of APD_{-50mV} . Consequently, I_{Na} recovers over exactly the same timecourse following repolarization regardless of rate, but varies only slightly in peak size due to a small incomplete recovery phenomenon associated with the h_2 -component of I_{Na} . However, complete blockade of $I_{K,slow}$ and $I_{K,fast}$ doubles the ARP by doubling the APD_{-50mV} , in accordance with recent predictions by Colatsky et. al. [18].

4.3 Comparison to Previous Cardiac Models

The novel model of the rabbit atrial myocyte presented here shares many qualitative similarities with earlier cardiac models such as the Beeler-Reuter (BR) [3], DiFrancesco and Noble (DN) [21], Hilgemann and Noble (HN) [52], Luo and Rudy (LR) [71], and Rasmusson, Clark, and Giles (RCG) [86, 87]. Each of these models provided descriptions of sodium, potassium, and calcium membrane currents and utilized the membrane model structure to account for electrophysiological responses of interest. The DN, HN, and RCG models also provided compartmental descriptions in order to consider mass-balance issues. Hilgemann and Noble [52] developed a relatively stable compartmental model of the SR to account for Ca^{2+} uptake and release phenomena. In general terms, the structural components of our model share the same components as the HN model. Our model differs significantly, however, from these earlier models in the emphasis on a single-cell characterization and the extent to which this characterization depends on physiological data.

Our model was developed specifically to simulate action potentials recorded from single rabbit atrial myocytes. Its development was motivated by the proliferation of voltage-clamp data recorded from isolated cells using the patch clamp technique [47]. We were therefore able to provide descriptions of membrane currents based on single-cell recordings from isolated rabbit cardiomyocytes. We have also incorporated anatomical data describing the ultrastructural geometry of the rabbit myocyte. In contrast, the BR, DN and HN models described multicellular or fiber preparations [3, 21, 52]. Phenomena such as ion accumulation-depletion within cleft spaces or effects due to intercellular coupling or to the spatial arrangement of cells do not occur in our model. We are therefore able to study the responses of the cell in isolation and to separate single-cell effects from those dependent on cell-to-cell interactions.

Our objective of simulating action potentials in a quantitative manner also differs from the approaches of most earlier cardiac models. For instance, in spite of the utilization of cleft $[Ca^{2+}]$ data in the HN atrial model, neither the DN nor the HN model attempts to mimic action potentials quantitatively. The LR model presents some data basis with respect to membrane currents, but makes no direct comparison of simulated and recorded action potentials. In this regard, the bullfrog models by Rasmusson et. al. [86, 87] are more closely related to the present rabbit model in that both membrane components and overall action potential responses are compared to physiological data.

While the RCG bullfrog models follow a similar data-based approach to the modeling process, they are nevertheless significantly different from our present model due to the role of the SR in mammalian tissue. Modified forms of the HN expressions [52] for SR Ca^{2+} uptake and release are an essential component of this model. Since histological investigations have shown that the bullfrog atrial myocyte does not have a transverse tubular system and possesses very little SR [58, 59], previous models of the bullfrog atrial and sinus venosus cells [86, 87] did not include these intracellular organelles.

Membrane current descriptions also differ in both quantitative and qualitative aspects from earlier models. Qualitatively, we have attempted to ensure that the mathematical components essential to describing rate-dependent phenomena are described by our model. Consequently, we have accounted for slow recovery from inactivation within each of the three largest membrane currents (I_{Na} , I_t , and $I_{Ca,L}$) in the rabbit atrial myocyte. Quantitatively, we have supplied a basis in single-cell data for each ionic current presently described in the rabbit atrial myocyte. Many of the details of these current descriptions differ between this and previous models. Some important points of distinction are as follows.

- (1) I_{Na} . We have included a slowly inactivating component (h_2) in our gating description and suggested a possible functional role for $I_{Na,slow}$ in the rate-dependent modulation of peak overshoot. This component is mentioned but not included in the DN or the later HN model.
- (2) I_t . We have provided extensive mimicry of the steady-state, time- and rate-dependent behavior of I_t based on physiological data. In particular, we have provided simulated fits to the entire set of transients evoked from a standard voltage clamp protocol. Previous models, in contrast, only represent information derived from exponential fits to a portion of the voltage clamp response and are therefore unable to reproduce this response quantitatively by simulation.
- (3) $I_{Ca,L}$. We have mimicked measurements of the steady-state and time-dependent properties of $I_{Ca,L}$ using a single Ca^{2+} -independent inactivation gating variable. Our formulation therefore differs from the HN description which included a Ca^{2+} -dependent modulation of inactivation. However, we believe that this simplification may not have a significant functional impact on rate-dependent APW changes. At low stimulus rates, deactivation of $I_{Ca,L}$ is primarily responsible for the subsequent decline in $I_{Ca,L}$ as a result of the very rapid early repolarization phase. Inactivation of $I_{Ca,L}$ plays a significant role in attenuating $I_{Ca,L}$ only at rapid stimulus rates when the plateau is large. We have found a monoexponential description of $I_{Ca,L}$ inactivation to be adequate in describing the rabbit atrial plateau. It is possible, however, that a multiexponential response may prove more suitable for cells with a longer APD, e.g. ventricular cells.
- (4) I_K . We have included data-based descriptions of recently identified [92, 93, 42, 43] slow and fast ($I_{K,slow}$ and $I_{K,fast}$) delayed K^+ currents. Earlier car-

diac models (DN,HN,RCG) included only a single type of I_K in the membrane model. Our description of $I_{K,slow}$ is qualitatively similar to the classical delayed rectifier within the LR model which has been alleged to generate Wenckebach phenomena [71].

- (5) I_{K1} . We have provided a basis in experimental data for the ion-transfer behavior of I_{K1} , and illustrated several functional roles for I_{K1} in atrial tissue. Agreement between model-generated and measured passive cell properties, such as resting potential (RP) and input resistance (R_{IN}), was achieved using an appropriately sized I_{K1} current. The resulting conductance (G_{K1}) allowed a rather small peak current comparable in size to I_{NaK} . During periods of stimulation, the size and instantaneous gating of model I_{K1} facilitated mimicry of the observed rate-independence of final repolarization. Our model therefore differs from the HN I_{K1} current which did not reverse at E_K and from previous models (DN,HN,LR) which did not utilize input resistance measurements to help define the background state [21, 52, 71, 86, 87].
- (6) $I_{B,Cl}$. We have included a recently identified background current, alleged to be carried by Cl^- ions, in our model to explain the steady-current response in voltage-clamp experiments designed to measure I_t [22]. Although sympathetically modulated Cl^- currents have been described in mammalian cardiac myocytes [51, 50], a background Cl^- current present in the absence of sympathetic stimulation has not been a component of earlier models.
- (7) Background currents. Consideration has been given in our model to adequately representing the background or resting state of the rabbit atrial myocyte. We have therefore provided expressions for I_{NaCa} , I_{NaK} , I_{CaP} , and I_B currents. These currents were not included in the LR model. We have also constrained

the size of certain currents, such as I_{NaK} and I_{K1} , to reflect measured properties of the background state. A 3:2 ratio of Hill coefficients was assumed for the Na^+ and K^+ binding sites of I_{NaK} rather than the 1:1 ratio used in the HN model.

Our description of the intracellular compartment also differs somewhat from the DN and HN models. The HN model assumed the binding of Ca^{2+} to various buffers was in the steady-state, while we have modeled the dynamics of buffer binding. These kinetics are slow enough to significantly effect the waveshape of the $[Ca^{2+}]_i$ transient during the action potential.

We have also placed some attention on the issue of ionic homeostasis within our model. As a result, $[Na^+]_i$ differs by approximately 2.5 mM between the quiescent and actively stimulated cell. This value is somewhat smaller than the 3-4 mM difference predicted by the HN model.

4.4 Limitations of Our Model

While our model succeeds in many areas of voltage clamp and action potential mimicry as discussed above, its results must nevertheless be considered in the context of known model limitations. Some particularly important limitations to the present model are as follows.

- (1) Limited experimental data on the transient in $[Ca^{2+}]_i$ during the action potential is currently available. The modified Hilgemann and Noble [52] description of the SR included in our model qualitatively mimics the phenomenon of calcium-induced calcium-release (CICR) and provides for a relatively stable interaction between transmembrane, SR uptake, and SR release Ca^{2+} fluxes. Moreover, $[Ca^{2+}]_i$ transients predicted by our model have a similar shape to some published fluorescent dye recordings [5]. However, the data presently available do

not provide the basis to elucidate the quantitative behavior of various intracellular Ca^{2+} handling mechanisms.

- (2) Limited experimental data is available concerning rate-dependent changes in $[Ca^{2+}]_i$. Although some evidence suggests that diastolic $[Ca^{2+}]_i$ levels rise as rate increases, as mimicked by our model, the rate-dependence of SR Ca^{2+} release is unknown and may increase, decrease, or remain constant with rate [34]. Our present model provides a highly qualitative suggestion that the known positive inotropic effect associated with increased rate may not require a positive SR Ca^{2+} release staircase [34]. However, our uncertainty concerning SR mechanisms precludes a conclusive statement in this regard.
- (3) Experimental data is also limited concerning the mechanisms and roles of the sarcolemmal calcium pump and sodium-calcium exchanger. Uncertainties in the behavior $[Ca^{2+}]_i$ and I_{NaCa} during the action potential have generated some uncertainty in the roles of delayed K^+ currents during middle and final repolarization.
- (4) Although our descriptions of the major rabbit atrial ionic currents have been based extensively on single-cell data, certain limitations apply to each formulation. In future models, alternatives to the Hodgkin-Huxley formalism may be required to overcome some of these difficulties.
 - (a) As discussed extensively in the MODEL DEVELOPMENT, quantitative information on the physiological kinetics of I_{Na} is limited. Suitable data on physiological activation kinetics is lacking, and the available publications do not provide quantitative information on the voltage-dependence or interaction of dual time constant inactivation kinetics. Model reactivation kinetics are similarly qualitative.

- (b) The mechanisms governing the rate-dependence of I_t are not fully understood. Our model quantitatively mimics steady-state gating variable curves and standard voltage clamp transients, and we can qualitatively mimic frequency-dependent effects and multiexponential recovery from inactivation. Nevertheless, our understanding of the interaction between inactivation and reactivation is somewhat incomplete. Despite considerable modeling effort, we have been unable to mimic the two types of inactivation curves observed in 23 °C rabbit atrium [98] without significantly degradatory model changes. The mechanisms producing biexponential inactivation and recovery of I_t have also not been elucidated. Significant projections in temperature have been made in some cases from data recorded at room temperature (23 °C); considering the high overall temperature sensitivity of I_t , our understanding of the physiological behavior of I_t must be considered incomplete. Finally, the modulatory effects of slower processes, such as those mediated by second messengers [31], on I_t have not been examined quantitatively. Such processes may exert rate-dependent effects due to the slowness of their kinetics and the possibility of interaction with intracellular Ca^{2+} .
- (c) Limited information is available concerning the mechanisms governing inactivation of $I_{Ca,L}$. Experimental evidence exists for both voltage- and calcium-dependent regulation [44, 45]. In their multicellular atrial fiber model, Hilgemann and Noble [52] included a Ca^{2+} -dependence to $I_{Ca,L}$ inactivation. However, in spite of considerable investigation, the nature of the interaction between Ca^{2+} and V in $I_{Ca,L}$ inactivation remains unknown [84]. Since our goal was to mimic rate-dependent APW changes, we have utilized a simplified $I_{Ca,L}$ model in which inactivation does not depend on

$[Ca^{2+}]_i$. The absence of dual inactivation mechanisms in our model does not seem to play any significant role in rate-dependent APW changes, but may prove significant if the objective is to study Ca^{2+} overload induced by high $[Ca^{2+}]_o$ [52].

- (d) The role of rate-dependent variations in $I_{Ca,L}$ on APW is not fully explored in this model. While we do estimate the reactivation kinetics of $I_{Ca,L}$ within our τ_{FI} representation, we have not chosen to fit the voltage clamp recordings from Banno et. al. [1] directly. Moreover, we have introduced a non-inactivating "N-type" component in our $I_{Ca,L}$ description to participate in plateau and "notch" formation during the action potential. Such a component may or may not be reflective of the activity of rabbit atrial Ca^{2+} channels at plateau potentials.
 - (e) Our models of the outward currents of repolarization may be considered semi-quantitative. We have incorporated into our model quantitative data on the voltage-dependence of I_{K1} as well as recently acquired data on the two types of delayed rectifiers ($I_{K,fast}$ and $I_{K,slow}$). However, Habuchi et. al. [43, 42] have suggested a Ca^{2+} -dependence to $I_{K,slow}$ which is not represented in our model. In addition, modifications to the activation kinetics of $I_{K,fast}$ were required in our model to adequately simulate rate-dependent APW changes. The latter is related to our uncertainties concerning I_{NaCa} , discussed above.
- (5) Some uncertainty exists concerning the completeness of our description of intracellular ionic balances. Although the size of model I_{NaK} adequately balances inward Na^+ fluxes during simulated periods of cell stimulation, the efflux of Na^+ ions produced by cessation of stimulation suffices to reduce $[Na^+]_i$ by 2-3 mM and to produce a slow hyperpolarizing drift in membrane potential.

- (6) Extrapolations with regard to temperature have been made in the present model in order to characterize the atrial waveshape at 35 °C. We have found this process to be far from simple. While scaling adjustments via Q_{10} factors are obviously required to increase reaction kinetics (including gating and binding reactions) at an elevated temperature, such changes have not always proved sufficient. A number of investigators report temperature-dependent shifts in activation curves, inactivation curves, or both. Separately, we have found that conductance increases (relative to an earlier, 23 °C atrial model) have also sometimes been required to yield our present model. Although the latter can be justified on a biophysical basis (increased diffusion rates), consideration of this effect complicates the model analysis and development process.

Chapter 5

Conclusions

We have developed a novel mathematical model of the rabbit atrial myocyte which accounts for the waveshape of the rabbit atrial action potential over a considerable range of stimulus frequencies (0.2-3.0 Hz). Our model contains two component models which provide an equivalent circuit description of the sarcolemma and a fluid compartment representation of the intracellular medium. The membrane model describes a whole-cell capacitance (C_m), six ionic currents (I_{Na} , I_t , $I_{Ca,L}$, $I_{Ca,T}$, $I_{K,fast}$, $I_{K,slow}$) and seven background, pump, and exchanger currents (I_{K1} , I_{NaK} , $I_{B,Na}$, $I_{B,Ca}$, $I_{B,Cl}$, I_{CaP} , I_{NaCa}). The fluid compartment model describes mass balances of free intracellular Na^+ , Ca^{2+} , and K^+ ion concentrations, accounts for Ca^{2+} buffering by calmodulin and troponin within the intracellular medium, and represents the uptake, transport, and release of Ca^{2+} by the SR as well as Ca^{2+} buffering by calsequestrin within the SR release compartment. Parameter values describing each model element are based wherever possible on quantitative data obtained from single rabbit cardiac myocytes.

This work shares many similarities with previous Hodgkin-Huxley [55] based descriptions [3, 21, 52, 71, 86, 87] of the cardiac action potential. It differs significantly from these in its single-cell orientation and in its extensive basis in experimental data. Adoption of a data-based approach has enabled our model to quantitatively mimic experimentally recorded (action potential and voltage clamp) waveforms. Since our description uses biologically based model components, model predictions and interpretations are offered in biophysically credible terms.

Our approach to model development has provided remarkable agreement between the recorded and model-generated responses of the rabbit atrial myocyte. We find that:

- (1) Our model can mimic both the voltage clamp data upon which it is based and the rabbit atrial action potential waveshapes recorded over the 0.2-3.0 Hz range of stimulus frequencies. The reasonable agreement between model-generated and recorded action potentials is preserved in the phase-plane plots, suggesting that the rate-dependent changes in underlying membrane currents have been plausibly represented.
- (2) Several different kinds of voltage-clamp responses of I_t are mimicked by our model. Under a “standard” voltage-clamp protocol, model simulations mimic the entire timecourse of the evoked transient current. The model also mimics the transient observed in a “recovery from inactivation” experiment and frequency-dependent changes in peak current observed as clamp steps are applied at different rates.
- (3) Our model can mimic the changes in early repolarization velocity with stimulus rate observed in the experimental data through phase plane plots. These changes largely reflect rate-dependent variations in the availability (s_{total}) of I_t , although secondary contributions are attributed to I_{Na} and $I_{Ca,L}$.
- (4) Model simulations support the view that incomplete reactivation effects can account for the dominant changes in APW over the frequencies studied. However, other currents such as a slowly decaying component of I_{Na} may also participate in this effect. Other factors may also contribute to differences in the “frequency dependence” of I_t and early repolarization velocity over the low frequency range, where the latter changes more rapidly than the former with rate.

- (5) A slowly inactivating component of I_{Na} is predicted to have a functional role in the rate-dependent modulation of maximal upstroke velocity ($\dot{V}_{max}$) and peak overshoot (POS).
- (6) Our model mimics the apparent rate-independence of late repolarization velocity negative to -55 mV observed from the AP data. This effect is generated in our model through the complete decay of ionic currents and the significant attenuation of the $[Ca^{2+}]_i$ -transient by the time V repolarizes to -55 mV. Consequently I_{NaCa} appears rate-independent in final repolarization as do all other background currents.
- (7) Comparable maximal contributions of I_{NaK} , $I_{K,fast}$, $I_{K,slow}$, and I_{K1} to the repolarization of the rabbit atrial myocyte are predicted by this study.
- (8) Our model can simulate the effect of a “premature stimulus” in evoking a “premature action potential” (PAP). We relate the PAP response to slow reactivation of I_{Na} , and from this account for both the absolute and relative refractory periods (ARP and RRP) of the rabbit atrial myocyte. Our model predicts a rate-independence of refractoriness over the 0.2-3.0 Hz range but a near 100% increase in ARP under complete block of $I_{K,fast}$ and $I_{K,slow}$. This prediction concords with the view expressed by Colatsky et. al. [18] that class III antiarrhythmic agents may prove useful in lengthening refractoriness.

As a successful representation of the rabbit atrial action potential, the present model offers many opportunities for future investigation and extension. Our model may be combined with more sophisticated descriptions of the intracellular medium to investigate the cellular basis of excitation-contraction coupling, contraction, or effects related to changing pH, osmolarity, or energetic state. It may also be included in a multicellular model to investigate myocardial conduction, for example, as it relates to

local circuits for reentrant arrhythmias. Effects related to pharmacologic modulation may be investigated at the subcellular, cellular, or multicellular level. The scope of these extensions will undoubtedly expand as more powerful experimental techniques and computing resources become available.

Appendix A

Computational Aspects and Data Analysis

The simulation results presented in this model were obtained by forward integration of the equations given in Tables B.1-B.12 using a Runge Kutta Merson algorithm [37] with a variable stepsize. Double precision floating point arithmetic was used in all calculations to minimize roundoff errors due to finite precision. This was necessary to accurately integrate the relatively stiff system of differential equations, whose time constants vary from $14.6 \mu\text{s}$ (I_{Na} activation kinetics) to 8 sec (slowest reactivation component of I_t). The accuracy of membrane voltage, ion concentration, and gating variable values was further maintained by requiring the local error of each state variable satisfy a relative error constraint. Model code was developed in the C programming language under the Unix operating system, and simulations performed on Sun Microsystems Sparc workstation (Sparc 2, IPX, SLC) platforms. The execution time for action potential simulations varied somewhat depending on the stimulus rate and the workstation platform. On an IPX platform two seconds of simulated response at a 1.0 Hz stimulus rate required approximately 3 1/2 minutes of execution time.

The atrial action potential data presented in this paper was recorded by a collaborative laboratory (W.R. Giles [10]) and corrected to facilitate comparison with model-generated results. Specifically, the integer output from A/D conversion of the membrane voltage waveform was scaled by the A/D gain factor to achieve an appropriate range of excursion (peak overshoot) of the output values. The data was then offset by -10.5 mV to correct for tip potential and achieve an minimum diastolic potential (MDP) level at low stimulus rates close to the nominal resting potential

(-71 mV). Data points associated with the stimulus artifact in the raw data were removed and a Boltzman expression with an offset was used to fit the remaining rest level and points on the action potential upstroke and to “fill in” an appropriate upstroke phase for the data. However, due to the presence of the stimulus artifact and the low sampling rate (2 kHz) used in the action potential recordings, no attempt was made to analyze the action potential upstroke in the phase plane.

To obtain a suitable approximation of I_{net} for use in I-V trajectories, the slow phases of repolarization of the action potential data were processed through a Butterworth filter. The reconstructed action potential was median filtered before and after differentiation to limit noise associated with differentiation, which was performed using difference algorithms. Correctness of the derived repolarization currents was verified by comparing the unfiltered action potential repolarization phase to the waveshape predicted by integrating repolarization currents from the point of peak overshoot using Simpson’s rule.

The raw data was recorded by Braun and Giles [10] from enzymatically isolated rabbit atrial cells in a bath maintained at 34-35 °C. The action potentials presented at each rate represent a typical steady-state waveshape achieved after stimulation for several tens of seconds at that rate.

Appendix B

Glossary and Model Equations

The following tables present complete set of equations, parameter values, and initial conditions used in our atrial model. Unless otherwise specified, model quantities are given in standard units: time in seconds, potential in millivolts (mV), concentrations in millimolar (mM), currents in picoamperes (pA), capacitance in nanofarads (nF), volumes in nanoliters (nl), temperature in Kelvin (°K). The rate-dependent action potential behavior shown in this paper may be simulated by forward-integration of the system of coupled differential equations. The initial conditions given in Table B.14 specify the state vector 100 milliseconds prior to application of the stimulus current, which should consist of a rectangular pulse $650 - 800 \mu s$ in duration and 2 nA in amplitude. This stimulus provides depolarization of the membrane to just above the threshold for I_{Na} , thus evoking an action potential.

Glossary

APD	Action potential duration
APD ₅₀	Action potential duration, measured halfway between RP and POS
APD _{-50 mV}	Action potential duration, measured at $V = -50$ mV
C_m	Whole cell membrane capacitance
$[Ca]_{up}$	$[Ca^{2+}]$ in the uptake compartment
$[Ca]_{rel}$	$[Ca^{2+}]$ in the release compartment
d_L	Activation gating variable for $I_{Ca,L}$
d_T	Activation gating variable for $I_{Ca,T}$
d_{NaCa}	Denominator constant for I_{NaCa}
$E_{Na}, E_{Ca,L}, \dots$	Reversal potential for Na^+ channel, long-lasting Ca^{2+} calcium channel,
F	Faraday's constant
f_L	Inactivation gating variable for $I_{Ca,L}$
f_T	Inactivation gating variable for $I_{Ca,T}$
F_1	The relative amount of inactive precursor in the SR release compartment
F_2	The relative amount of activator in the SR release compartment
F_3	The relative amount of inactive product in the SR release compartment
$g_{Na}, g_{Ca,L}, \dots$	Na^+ channel conductance, long-lasting Ca^{2+} channel conductance, ...
h_1	Fast inactivation gating variable for I_{Na}

h_2	Slow inactivation gating variable for I_{Na}
$h_{total}, s_{total}, \dots$	Net fractional availability of I_{Na} channels, of I_t channels, ...
I_B	Background current ($I_{B,Na} + I_{B,Ca} + I_{B,Cl}$)
$I_{B,Ca}$	Ca^{2+} background current
$I_{B,Cl}$	Cl^- background current
$I_{B,Na}$	Na^+ background current
I_{Ca}	Ca^{2+} current ($I_{Ca,L} + I_{Ca,T}$)
$I_{Ca,L}$	Long lasting Ca^{2+} current
$I_{Ca,T}$	Transient Ca^{2+} current
I_{CaP}	Ca^{2+} pump current
$\bar{I}_{CaP}$	Maximum Ca^{2+} pump current
I_K	Delayed rectifier K^+ current ($I_{K,Fast} + I_{K,Slow}$)
$I_{K,fast}$	Fast subtype of delayed rectifier K^+ current
$I_{K,slow}$	Slow subtype of delayed rectifier K^+ current
I_{K1}	Inward rectifier K^+ current
I_{Na}	Na^+ current
I_{NaCa}	$Na^+ - Ca^{2+}$ ion exchanger current
I_{NaK}	$Na^+ - K^+$ pump current
$\bar{I}_{NaK}$	Maximum $Na^+ - K^+$ pump current
I_{rel}	SR release compartment current
I_t	Transient outward K^+ current
I_{tr}	SR transfer current
I_{up}	SR uptake compartment current
$[K^+]_i, [Na^+]_i, [Ca^{2+}]_i, [Mg^{2+}]_i$	$K^+, Na^+, Ca^{2+}, Mg^{2+}$ concentrations

	in the intracellular medium
$[K^+]_o, [Na^+]_o, [Ca^{2+}]_o$	K^+, Na^+, Ca^{2+} concentrations in the extracellular bathing medium
$k_{m,Na}$	Equilibrium binding constant for Na^+ activation of the I_{NaK} pump
$k_{m,K}$	Equilibrium binding constant for K^+ activation of the I_{NaK} pump
$k_{m,K1}$	Equilibrium binding constant for $[K^+]_o$ dependence of the I_{K1} current
$K_{M,rel}$	Equilibrium binding constant of the SR Ca^{2+} release gate for $[Ca^{2+}]_i$
k_{NaCa}	Scaling factor for I_{NaCa}
k_{cyca}	Equilibrium binding concentration for $[Ca^{2+}]$ on the cytosol side
k_{srca}	Equilibrium binding concentration for $[Ca^{2+}]$ on the uptake side
k_{xcs}	Translocation constant
m	Activation gating variable for I_{Na}
$\bar{m}, \bar{f}_L, \dots$	Steady-state of activation gating variable m , of inactivation gating variable f_L , ...
$\frac{dm}{dt}, \frac{df_L}{dt}, \dots$	First time derivative of activation gating variable m , of inactivation gating variable f_L , ...
n	Activation gating variable for $I_{K,slow}$
n_{NaCa}	Coupling ratio for $Na^+ - Ca^{2+}$ exchanger
$[\dot{Na}]_i, [\dot{K}]_i, \dots$	First time derivative of intracellular

	concentration of Na^+ ions, K^+ ions, ...
O_{Calse}	Fractional occupancy of calsequestrin by Ca^{2+} ions
O_{TC}	Fractional occupancy of the troponin-Ca complex by Ca^{2+} ions
O_{TMgC}	Fractional occupancy of the troponin-Mg complex by Ca^{2+} ions
O_{TMgMg}	Fractional occupancy of troponin-Mg complex by Mg^{2+} ions
$\dot{O}_C, \dot{O}_{TC}, \dot{O}_{TMgC}, \dots$	First time derivative of $O_C, O_{TC}, O_{TMgC}, \dots$
p_a	Activation gating variable for $I_{K,fast}$
p_i	Inactivation gating variable for $I_{K,fast}$
P_{Na}	Na^+ permeability
POS	Peak overshoot potential
R	Universal gas constant
r	Activation gating variable for I_t
r_{act}	Activation rate constant
r_{inact}	Inactivation rate constant
RP	Resting potential
s_1	Fast inactivation gating variable for I_t
s_2	Slow inactivation gating variable for I_t
s_3	Third inactivation gating variable for I_t
SR	Sarcoplasmic reticulum
T	Temperature (Kelvin)
V	Transmembrane potential (voltage)
$\dot{V}_{max}$	Maximum upstroke velocity

Vol_{Ca}	Intracellular volume for the Ca^{2+} ions
Vol_i	Intracellular volume
Vol_{rel}	Volume of the release compartment
Vol_{up}	Volume of the uptake compartment
$\alpha_m, \alpha_{f_L}, \dots$	Voltage-dependent opening rate constant of gating variable m, f_L , ...
α_{up}	Forward reaction rate constant between the cytosol and the uptake compartment
$\beta_m, \beta_{f_L}, \dots$	Voltage-dependent closing rate constant of gating variable m, f_L , ...
β_{up}	Backward reaction rate constant between the cytosol and the uptake compartment
γ	Position of Eyring rate theory energy barrier controlling the voltage-dependence of I_{NaCa}
$\Phi_{[Ca]_i}$	Rate of flux of Ca^{2+} ions onto intracellular buffers
ϕ_F	Forward term in voltage-dependence of I_{NaCa}
ϕ_R	Reverse term in voltage-dependence of I_{NaCa}
$\tau_m, \tau_{f_L}, \dots$	Voltage-dependent time constant of gating variable m, f_L , ...

Table B.1: Sodium Current I_{Na}

$$\begin{aligned}
I_{Na} &= P_{Na} m^3 (0.611 h_1 + 0.389 h_2) [Na]_o V \frac{F^2}{RT} \frac{e^{(V-E_{Na})F/RT} - 1}{e^{VF/RT} - 1} \\
\frac{dm}{dt} &= \alpha_m (1 - m) - \beta_m m & \bar{h} &= \frac{\alpha_h}{\alpha_h + \beta_h} \\
\frac{dh_1}{dt} &= (\bar{h} - h_1)/\tau_{h_1} & \frac{dh_2}{dt} &= (\bar{h} - h_2)/\tau_{h_2} \\
\alpha_m &= \frac{-460.000(V+44.4)}{e^{(V+44.4)/-12.673} - 1} & \beta_m &= 18400.000 e^{(V+44.4)/-12.673} \\
\alpha_h &= 44.900 e^{(V+66.9)/-5.570} & \beta_h &= \frac{1491.000}{1+323.300 e^{(V+94.6)/-12.900}} \\
\tau_{h_1} &= \frac{0.03000}{1+e^{(V+40.00)/6.000}} + 0.00035 & \tau_{h_2} &= \frac{0.12000}{1+e^{(V+60.00)/2.000}} + 0.00295 \\
h_{total} &= 0.611 h_1 + 0.389 h_2
\end{aligned}$$

Table B.2: Calcium Currents $I_{Ca,L}$ and $I_{Ca,T}$

$I_{Ca} = I_{Ca,L} + I_{Ca,T}$	
$I_{Ca,L} = g_{Ca,L} (d_L f_L + d')(V - E_{Ca,L})$	
$\frac{dd_L}{dt} = (\bar{d}_L - d_L)/\tau_{dL}$	$\frac{df_L}{dt} = (\bar{f}_L - f_L)/\tau_{fL}$
$\alpha_{dL} = \frac{-16.720(V+35.0)}{e^{(V+35.0)/-2.500}-1} + \frac{-50.000V}{e^{V/-4.808}-1}$	$\beta_{dL} = \frac{4.480(V-5.0)}{e^{(V-5.0)/2.500}-1}$
$\tau_{dL} = \frac{1}{\alpha_{dL} + \beta_{dL}}$	$\bar{d}_L = \frac{1.000}{1 + e^{(V+0.95)/-6.600}}$
$\tau_{fL} = 0.211 \exp(-(\frac{V+37.427}{20.213})^2) + 0.015$	$\bar{f}_L = \frac{\alpha_{fL}}{\alpha_{fL} + \beta_{fL}}$
$\alpha_{fL} = \frac{8.490(V+28.0)}{e^{(V+28.0)/4.000}-1}$	$\beta_{fL} = \frac{67.922}{1 + e^{(V+28.0)/-4.000}}$
$d' = \frac{1.000}{1 + e^{(V-33.0)/-12.000}}$	
$I_{Ca,T} = g_{Ca,T} d_T f_T (V - E_{Ca,T})$	
$\frac{dd_T}{dt} = (\bar{d}_T - d_T)/\tau_{dT}$	$\frac{df_T}{dt} = (\bar{f}_T - f_T)/\tau_{fT}$
$\alpha_{dT} = 674.173 e^{(V+23.0)/30.000}$	$\beta_{dT} = 674.173 e^{(V+23.0)/-30.000}$
$\tau_{dT} = \frac{1}{\alpha_{dT} + \beta_{dT}}$	$\bar{d}_T = \frac{1.000}{1 + e^{(V+23.0)/-6.100}}$
$\alpha_{fT} = 9.637 e^{(V+75.0)/-83.333}$	$\beta_{fT} = 9.637 e^{(V+75.0)/15.385}$
$\tau_{fT} = \frac{1}{\alpha_{fT} + \beta_{fT}}$	$\bar{f}_T = \frac{\alpha_{fT}}{\alpha_{fT} + \beta_{fT}}$

Table B.3: Transient Outward Current I_t

$$I_t = g_t r (0.590 s_1^3 + 0.410 s_2^3)(0.600 s_3^6 + 0.400)(V - E_K)$$

$$\frac{dr}{dt} = (\bar{r} - r)/\tau_r \quad \alpha_r = 386.6 \exp(V/12.00)$$

$$\frac{ds_1}{dt} = (\bar{s}_1 - s_1)/\tau_{s_1} \quad \beta_r = 8.011 \exp(V/ - 7.20)$$

$$\frac{ds_2}{dt} = (\bar{s}_2 - s_2)/\tau_{s_2} \quad \tau_{s_1} = \frac{0.189}{1+e^{(V+32.80)/0.10}} + 0.0204$$

$$\frac{ds_3}{dt} = (\bar{s}_3 - s_3)/\tau_{s_3} \quad \tau_{s_2} = \frac{5.750}{1+e^{(V+32.80)/0.10}} + 0.45 \exp\left(-\left(\frac{V-13.54}{-13.97}\right)^2\right) + 0.020$$

$$\bar{r} = \frac{1.0}{1+e^{(V+15.00)/-5.633}} \quad \tau_{s_3} = \frac{7.500}{1+e^{(V+23.00)/0.5000}} + 0.5000$$

$$\tau_r = \frac{1}{\alpha_r + \beta_r} + 0.00040$$

$$\bar{s}_1 = \frac{1.0}{1+e^{(V+28.29)/7.06}} \quad \bar{s}_3 = \left(\frac{1.0}{1+e^{(V+50.67)/27.38}} + 0.666\right)/1.666$$

$$\bar{s}_2 = \frac{1.0}{1+e^{(V+28.29)/7.06}} \quad s_{total} = (0.59 s_1^3 + 0.41 s_2^3)(0.60 s_3^6 + 0.40)$$

Table B.4: Delayed Rectifier I_K

$$\begin{aligned}
I_K &= I_{K, Fast} + I_{K, Slow} \\
I_{K, Fast} &= g_{K, Fast} p_a p_i (V - E_K) \\
\frac{dp_a}{dt} &= (\overline{p_a} - p_a) / \tau_{p_a} & \frac{dp_i}{dt} &= (\overline{p_i} - p_i) / \tau_{p_i} \\
\alpha_{p_a} &= 9.00 e^{V/25.371} & \beta_{p_a} &= 1.3 e^{V/-13.026} \\
\tau_{p_a} &= \frac{1}{\alpha_{p_a} + \beta_{p_a}} & \overline{p_a} &= \frac{1.000}{1 + e^{(V+5.1)/-7.400}} \\
\alpha_{p_i} &= 100.0 e^{V/-54.645} & \beta_{p_i} &= 656.0 e^{V/106.157} \\
\tau_{p_i} &= \frac{1}{\alpha_{p_i} + \beta_{p_i}} & \overline{p_i} &= \frac{\alpha_{p_i}}{\alpha_{p_i} + \beta_{p_i}} \\
I_{K, Slow} &= g_{K, Slow} n (V - E_K) \\
\frac{dn}{dt} &= (\overline{n} - n) / \tau_n & \overline{n} &= \frac{1.000}{1 + e^{(V-0.9)/-13.800}} \\
\alpha_n &= 1.66 e^{V/69.452} & \beta_n &= 0.3 e^{V/-21.826} \\
\tau_n &= \frac{1}{\alpha_n + \beta_n} + 0.060
\end{aligned}$$

Table B.5: Inward Rectifier I_{K1}

$$I_{K1} = g_{K1} \left[\frac{[K]_o}{[K]_o + k_{m, K1}} \right]^3 \frac{V - E_K}{1 + e^{1.393(V - E_K + 3.6)F/RT}}$$

Table B.6: Background Currents $I_{B,Na}$, $I_{B,Ca}$, $I_{B,Cl}$

$I_{B,Na} = g_{B,Na} (V - E_{Na})$	$E_{Na} = \frac{RT}{F} \ln\left(\frac{[Na]_o}{[Na]_i}\right)$
$I_{B,Ca} = g_{B,Ca} (V - E_{Ca})$	$E_{Ca} = \frac{RT}{2F} \ln\left(\frac{[Ca]_o}{[Ca]_i}\right)$
$I_{B,Cl} = g_{B,Cl} (V - E_{B,Cl}) (1 + \exp(\frac{V - (E_{Cl} + 36.95)}{74.514}))$	$E_{Cl} = \frac{RT}{F} \ln\left(\frac{[Cl]_{pipette}}{[Cl]_o}\right)$
$E_{B,Cl} = E_{Cl} - 0.49 (E_{Cl} - 30.59)$	$[Cl]_{pipette} = [Cl]_i$

Table B.7: Calcium Pump I_{CaP}

$$I_{CaP} = \overline{I_{CaP}} \frac{[Ca]_i}{[Ca]_i + 0.200 \mu M}$$

Table B.8: Sodium Potassium Pump I_{NaK}

$$I_{NaK} = \overline{I_{NaK}} \left(\frac{[K]_o}{[K]_o + k_{m,K}} \right)^2 \left(\frac{[Na]_i}{[Na]_i + k_{m,Na}} \right)^3 \frac{V + 150.0}{V + 200.0}$$

Table B.9: Sodium Calcium Exchanger I_{NaCa}

$$I_{NaCa} = k_{NaCa} \frac{([Na]_i^{n_{NaCa}} [Ca]_o \phi_F - [Na]_o^{n_{NaCa}} [Ca]_i \phi_R)}{(1 + d_{NaCa} ([Na]_o^{n_{NaCa}} [Ca]_i + [Na]_i^{n_{NaCa}} [Ca]_o))}$$

$$\phi_F = e^{\gamma V (n_{NaCa} - 2) z_{NaCa} F / RT} \quad \phi_R = e^{(\gamma - 1) V (n_{NaCa} - 2) z_{NaCa} F / RT}$$

Table B.10: Ion Concentrations $[Na]_i$, $[K]_i$, $[Ca]_i$

$$\begin{aligned}
[\dot{Na}]_i &= -\frac{I_{Na}+I_{B,Na}+3I_{NaK}+3I_{NaCa}}{Vol_i F} \\
[\dot{K}]_i &= -\frac{I_t+I_{K1}+I_{K2}-2I_{NaK}}{Vol_i F} \\
[\dot{Ca}]_i &= -\frac{I_{Ca}+I_{B,Ca}+I_{CaP}-2I_{NaCa}+I_{up}-I_{rel}}{2Vol_{ca}F} - \Phi_{[Ca]_i} \\
\Phi_{[Ca]_i} &= 0.080 \dot{O}_{TnCa} + 0.160 \dot{O}_{TnMgCa} + 0.045 \dot{O}_{Cal}
\end{aligned}$$

Table B.11: Ca^{2+} Buffering

$$\begin{aligned}
\dot{O}_{Cal} &= 200000.0 [Ca]_i (1 - O_{Cal}) - 476.0 O_{Cal} \\
\dot{O}_{TnCa} &= 78400.0 [Ca]_i (1 - O_{TnCa}) - 392.0 O_{TnCa} \\
\dot{O}_{TnMgCa} &= 200000.0 [Ca]_i (1 - O_{TnMgCa} - O_{TnMgMg}) - 6.6 O_{TnMgCa} \\
\dot{O}_{TnMgMg} &= 2000.0 [Mg]_i (1 - O_{TnMgCa} - O_{TnMgMg}) - 666.0 O_{TnMgMg}
\end{aligned}$$

Table B.12: SR Ca^{2+} Handling

$$\begin{aligned}
I_{up} &= \overline{I_{up}} \frac{[Ca]_i/k_{cyca} - k_{xcs}^2 [Ca]_{up}/k_{srca}}{([Ca]_i + k_{cyca})/k_{cyca} + k_{xcs}([Ca]_{up} + k_{srca})/k_{srca}} \\
I_{tr} &= ([Ca]_{up} - [Ca]_{rel}) \frac{2F Vol_{rel}}{\tau_{tr}} \quad I_{rel} = \alpha_{rel} \left(\frac{F_2}{F_2 + 0.250} \right)^2 [Ca]_{rel} \\
[\dot{Ca}]_{up} &= \frac{I_{up} - I_{tr}}{2 Vol_{up} F} \quad [\dot{Ca}]_{rel} = \frac{I_{tr} - I_{rel}}{2 Vol_{rel} F} - 31.0 \dot{O}_{Calseq} \\
\dot{O}_{Calseq} &= 480.0 [Ca]_{rel} (1 - O_{Calseq}) - 400.0 O_{Calseq} \\
\dot{F}_1 &= r_{recov} F_3 - r_{act} F_1 \quad r_{act} = 203.8 \left[\frac{[Ca]_i}{[Ca]_i + K_{M,rel}} \right]^4 + 203.8 e^{(V-40.0)/12.5} \\
\dot{F}_2 &= r_{act} F_1 - r_{inact} F_2 \quad r_{inact} = 33.96 + 339.6 \left[\frac{[Ca]_i}{[Ca]_i + K_{M,rel}} \right]^4 \\
\dot{F}_3 &= r_{inact} F_2 - r_{recov} F_3
\end{aligned}$$

Table B.13: Model Parameter Values

$R = 8314.0 \text{ mJ}/(\text{mol}^\circ\text{C})$	$T = 35.0^\circ\text{C}$	$F = 96487.0 \text{ C/mol}$
$P_{Na} = 0.0014 \mu\text{l}/\text{sec}$	$C_m = 0.05 \text{ nF}$	$n_{NaCa} = 3.0$
$g_{Ca,L} = 4.000 \text{ nS}$	$Vol_i = 0.0126 \text{ nl}$	$\gamma = 0.450$
$g_{Ca,T} = 120.000 \text{ nS}$	$Vol_{ca} = 5.884 \text{ pl}$	$z_{NaCa} = 1.0$
$g_t = 50.002 \text{ nS}$	$Vol_{up} = 0.39690 \text{ pl}$	$d_{NaCa} = 0.00030$
$g_{K,Fast} = 3.500 \text{ nS}$	$Vol_{rel} = 0.04410 \text{ pl}$	$\overline{I_{up}} = 2800.0 \text{ pA}$
$g_{K,Slow} = 2.500 \text{ nS}$	$[Na]_o = 140.0 \text{ mM}$	$\tau_{tr} = 0.0100 \text{ sec}$
$g_{K1} = 5.455 \text{ nS}$	$[K]_o = 5.0 \text{ mM}$	$\alpha_{rel} = 200000.0 \text{ pA/mM}$
$g_{B,Na} = 0.070 \text{ nS}$	$[Ca]_o = 2.5 \text{ mM}$	$k_{cyca} = 0.00030 \text{ mM}$
$g_{B,Ca} = 0.031 \text{ nS}$	$[Mg]_i = 2.5 \text{ mM}$	$k_{xcs} = 0.400$
$g_{B,Cl} = 0.120 \text{ nS}$	$[Cl]_o = 132.0 \text{ mM}$	$k_{srca} = 0.500 \text{ mM}$
$\overline{I_{NaK}} = 124.34 \text{ pA}$	$[Cl]_i = 30.0 \text{ mM}$	$r_{recov} = 0.815 \text{ sec}^{-1}$
$\overline{I_{CaP}} = 9.509 \text{ nS}$	$k_{m,K} = 0.621 \text{ mM}$	$k_{NaCa} = 0.020 \text{ nS}$
$E_{CaT} = 38.0 \text{ mV}$	$k_{m,Na} = 5.460 \text{ mM}$	$K_{M,rel} = 0.300 \mu\text{M}$
$E_{CaL} = 60.0 \text{ mV}$	$k_{m,K1} = 0.590 \text{ mM}$	

Table B.14: Initial State Values

$V = -68.72\text{ mV}$	$s_2 = 0.57936$	$O_{Cal} = 0.03528$
$m = 0.01095$	$s_3 = 0.57655$	$O_{TnCa} = 0.01712$
$h_1 = 0.63530$	$p_a = 0.00741$	$O_{TnMgCa} = 0.27446$
$h_2 = 0.54127$	$p_i = 0.50580$	$O_{TnMgMg} = 0.64020$
$d_L = 0.00003$	$n = 0.02286$	$O_{Calseq} = 0.33399$
$f_L = 0.99950$	$[Na]_i = 8.40\text{ mM}$	$F_1 = 0.19790$
$d_T = 0.00056$	$[K]_i = 100.0\text{ mM}$	$F_2 = 0.00324$
$f_T = 0.26774$	$[Ca]_i = 0.08681\text{ }\mu\text{M}$	$F_3 = 0.70185$
$r = 0.00443$	$[Ca]_{up} = 0.49271\text{ mM}$	
$s_1 = 0.40557$	$[Ca]_{rel} = 0.41845\text{ mM}$	

Table B.15: Model Properties

Model Property	Literature Value	Model Value
Resting Potential	-71.0 mV	-71.70 mV
Input Resistance	$617.0 \pm 401.0\text{ M}\Omega$	$707.4\text{ M}\Omega$
Cell Capacitance	$54.3 \pm 5.9\text{ pF}$	50.00 pF

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