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Mathematical modeling of the sinoatrial node cell in the rabbit heart

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by

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Mathematical Modeling of the Sinoatrial Node Cell in the Rabbit Heart

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Abstract

A mathematical model has been developed for the rabbit sinoatrial node (SAN) cardiac pacemaker cell, that is based on experimental results from whole-cell voltage clamp and action potential studies on single enzymatically-isolated pacemaker cells. The model consists of two parts, a model of sarcolemma, and a fluid compartment model of the intra and extracellular media. The sarcolemmal membrane model is of Hodgkin-Huxley form, and includes mathematical descriptions of the major ion channels present in mammalian cardiac pacemaker cells, as well as, descriptions of the $Na^+/K^+$ and $Ca^{2+}$ pump and the $Na^+/Ca^{2+}$ exchanger currents. The fluid compartment model is based on a material balance for the ion concentrations in the intracellular medium ($Na^+$, $K^+$ and $Ca^{2+}$); concentrations of these ions are considered constant in the extracellular medium. Provision is made in this model for $Ca^{2+}$ buffering in the intracellular medium, as well as the uptake and release of $Ca^{2+}$ by the sarcoplasmic reticulum (SR). Our model is able to provide good fits to both voltage clamp and action potential data from several published sources, and has provided insights into the biophysical interactions and the functional roles of several currents underlying the pacemaker potential of the rabbit SAN cell.
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Chapter 1

Introduction

The sinoatrial node (SAN), which is the phylogenetic remnant of the sinus venosus of lower vertebrate hearts, was first described for the mammalian hearts in 1906 by Keith and Flack [35]. The SAN in the human is 15mm long, 2mm thick and 5mm wide; and the SAN is near the junction between the superior vena cava and the right atrium (Fig 1.1A). The sinus node artery runs lengthwise through the center of the node.

SAN was discovered in the mole’s heart by Flack in 1906. Keith and Flack soon found out that the sinoatrial node was present in the many mammalian hearts [35]. The first physiological studies after the anatomical discovery of the SAN were done between 1910 and 1920. Lewis et al performed a study correlating the morphology and the electrophysiology of the SAN [38]. In 1921, Eyster et al [19] published the first review on the origin and conduction of the heart beat in the first volume of the Physiological Reviews. The first SAN action potentials were recorded by West in 1955 [60]. He showed the typical SAN action potential and different action potentials within the same node. Moreover he showed the phenomenon of pacemaker shift after the addition of acetylcholine (ACh). The first study correlated the sinoatrial node’s ultrastructural and electrophysiological characteristics was performed in the rabbit by Trautwein and Uchizono [57] in 1963.
Figure 1.1: Diagrams of the human sinoatrial node and the rabbit sinoatrial node
Panel A indicates the location and the shape of the normal human sinoatrial node. The heavy lines show exiting Purkinje tracts, anteriorly to Bachmann’s bundle (B.B.) and posteriorly to the sinus intercavarum (S.I.) and interarterial septum (I.A.S.) and to crista terminalis (C.T.) and Eustachian ridge (E.R.). Reproduced from [34]. Panel B is the activation map of the rabbit sinoatrial node taken from [2].
1.1 Ultrastructure of the Sinoatrial Node

The sinoatrial node was identified in the intercaval region as a large group of cells which differed from the atrial cells by their staining properties [3]. Different architectural zones were identified in the nodal cell group. In the central portion of the node a dense arrangement of interweaving cells embedded in a network of collagen fibers was present. Cells in a more or less parallel fashion surround the central nodal cells. The zone of the peripheral nodal cells was broad towards the crista terminalis. Fig 1.1B shows the map of the rabbit sinoatrial node along with the action potentials recorded in the route of preferential conduction towards the crista terminalis (CT). The impulse originates at 0 and the numbers in the map give the activation times after the primary pacemaker in milliseconds [2]. Action potentials show an increase in maximum upstroke velocity and amplitude and a decrease in diastolic depolarization rate towards the periphery.

Three main types of myocardial cells are distinguished in the node: (1) Primary nodal cells which are located in the center of the SAN. These cells have empty appearance on micrographs. Myofilaments are scarcely present and run in every direction and are not properly organized into myofibrils; (2) Transitional cells which surround the typical nodal cells. Their ultrastructure has aspects intermediate between that of typical nodal cells and that of the atrial cells. The myofilaments and their organization into myofibrils become more dense as one goes from the center of the node to the periphery; (3) Atrial cells which contain granules in the perinuclear region, and these granules are not present in the nodal and transitional cells [3].

1.1.1 The Rhythmic Cardiac Impulse Conduction

The rhythmic cardiac electrical impulse originates in the pacemaker cells in the SAN. Figure 1.2A shows the distribution of the specialized conduction tissues in the human
heart. The electrical impulse passes from the SAN through three conducting tracts in the atria to atrioventricular (AV) node. These tracts also supply branches to activate the right and left atria. The specialized conduction tracts between the SAN and the atrioventricular (AV) node are called internodal tracts: anterior, middle and posterior. The impulse is delayed at the AV node before it goes through the bundle of His, the right bundle branch, the common left bundle branch, the anterior and posterior parts of the left bundle branch and the Purkinje fiber network (see Fig 1.2A).

1.1.2 Morphology of Different Cell Types from the SAN

Even though several different SAN cell types have been observed in the rabbit heart, only a number of these cells exhibited regular spontaneous beating. Figure 1.2B shows the phase-contrast micrograph of the different cell types observed in cell suspensions following enzymatic dispersion of the rabbit SAN [59]. Type I cells are round (~5-8μm in diameter) and do not exhibit spontaneous activity and have weak structures. Type II cells which, are large and round (~20μm in diameter), do not exhibit spontaneous activity and do not contract, either. Since the SAN is densely innervated, type II cells can be nerve or endothelial cells [59]. Type III and IV cells, which are slender and elongated, exhibit regular contractions and spontaneous electrophysiological activity. We have used these cells, type III and IV, to model the SAN cell in the rabbit heart.

1.2 Previous Models of the Rabbit SAN Cell

Enzymatic dispersion techniques coupled with whole-cell voltage clamp methods have provided experimentalists with essential tools for investigating the types of ionic currents present in different types of single isolated cardiac myocytes (see e.g. [31, 32, 36, 37, 52, 58]). Although quantitative data regarding types of ionic currents,
Figure 1.2: Diagrams of the human heart and different sinoatrial node cells in the rabbit
Panel A illustrates the distribution of the specialized conduction tissues in the human heart (from [39]). Panel B is the phase-contrast micrograph of the different cell types observed in cell suspensions following enzymatic dispersion of the rabbit sinoatrial node (from [59]).
as well as their size and kinetics remains incomplete in many cases, data of this type is being utilized in Hodgkin-Huxley type models [30] to provide biophysical explanations for the action potential waveshapes exhibited by different types of cardiac cells (see e.g. [1, 14, 42, 53, 54]). In the past 12 – 15 years, a number of Hodgkin-Huxley (HH) type models have been developed to describe the electrophysiological behavior of the rabbit sinoatrial node (SAN) cell [4, 5, 11, 18, 43, 44, 48, 61]. In 1980, Yanigahara, Noma and Irisawa (YNI) [61] formulated a seventh-order HH-type model of the rabbit SAN that included a sodium current ($I_{Na}$), a slow-inward (calcium) current ($I_{S}$), a delayed-rectifier $K^+$ current ($I_K$), a hyperpolarization-activated current ($I_h$ or $I_f$) and a background leakage current ($I_L$). In addition, a mathematical model of the independent ACh-sensitive $K^+$ channel, that had been previously developed by Osterrieder, Noma and Trautwein (ONT) [51], was also included in the original YNI model to simulate the effects of different bath concentrations of ACh on the electrical activity of the SAN cell [61]. In 1984, Michaels et al [43] also employed the combined YNI-ONT model to simulate the effects of single and repetitive square pulses of ACh on the electrical activity of the sinus node cell. Dexter et al [11, 12] have since utilized the YNI-ONT model of the individual rabbit SAN cell, to represent the effects of ACh on the sinus period of the heart in the intact animal (dog), under conditions of $\beta$-adrenergic blockade. Their study included the development of a new model for generating of an appropriate [ACh(t)] waveform to drive the single [ACh]-dependent rate constant ($\alpha_{ACh}$) used in the ONT muscarinic channel model. In 1990, Guevara and Jongsma [24] have utilized a modified version of the YNI model [33] in studies of phase-resetting of the SAN cell.

A significant advance in simulations of the electrophysiology of cardiac cells was made in 1985, with the development of a model of the mammalian Purkinje fiber by DiFrancesco and Noble (DN) [14]. This model consists of a number of component
parts: a membrane (sarcolemmal) model together with a model of the intracellular, interstitial (cleft) and extracellular fluid compartments. It includes descriptions for mechanisms of the uptake and release of \([Ca^{2+}]_i\), by the sarcoplasmic reticulum, as well as mathematical descriptions of the \(Na^+/K^+\) pump and \(Na^+/Ca^{2+}\) exchanger. The addition of these new elements provided a much better characterization of the resting state of the membrane, and made possible simulations of a much greater range of biophysical phenomena, was possible using than previous models (e.g. the McAllister, Noble, Tsien (MNT) model [42] or the Beeler-Reuter (BR) model [1]). Noble and Noble (NN) [48] subsequently modified the DN Purkinje fiber model to simulate pacemaker activity in the rabbit SAN cell. Egan and Noble [18] have utilized this model together with a model for the ACh-sensitive \(K^+\) channel current \((I_{K,ACh})\) to study the effect of ACh on the activity of the SAN cell. They propose two possible actions of ACh on this cell, one being an increase of \(I_{K,ACh}\) with an increase in [ACh] in the bathing medium, and the other an ACh-dependent block of the calcium current. Murphey and Clark [44] have also used a model that combines modified versions of both the ONT muscarinic \(K^+\) channel [4, 5] and the NN model [48], to study the dynamic effects of vagal stimulation on SAN cell activity, in the presence and absence of background sympathetic stimulation.

Recently, a number of quite extensive experimental studies on single myocytes from the rabbit SAN have been published. For example, Hagiwara, Irisawa and Kameyama [27] have provided both data and mathematical descriptions of two types of calcium channels in the SAN cell membrane, the transient (T-type) and long-lasting (L-type) calcium channels. Shibasaki [56] has published data on the delayed rectifier channel in isolated rabbit SAN cells. More recently, several laboratories have investigated the selectivity and kinetics of the hyperpolarization-activated current \(I_f\), particularly DiFrancesco et al [15, 16], Hagiwara and Irisawa [25], Denyer and Brown
[9, 10], and van Ginneken and Giles [59]. Microanatomical studies of this cell-type are available [3, 41, 40, 46, 50], and inclusion of this data should have an impact on the development of accurate models of the electrophysiological behavior of the SAN cell.

1.3 The New SAN Model

The main objective of our study was to develop a new mathematical model of the primary pacemaker cell from the rabbit SAN that is based on quantitative whole-cell voltage clamp data from enzymatically-isolated SAN cells. Our model is more similar in structure to the DN-type models than the earlier YNI-type models [61, 33] of rabbit SAN cells. It includes fluid compartment formulations for the myoplasm and extracellular medium, as well as the $Na^+ / K^+$ and $Ca^{2+}$ pumps, and the $Na^+ / Ca^{2+}$ exchanger. Provisions are made for intracellular $Ca^{2+}$ buffering in this model, and it contains a description of the mechanisms for $Ca^{2+}$ uptake and release by the sarcoplasmic reticulum (SR), that is similar to the one presented by Hilgemann and Noble (HN) [28]. Details of our modification of the HN calcium uptake and release mechanism model are given in a related study by Murphey et al [45], that deals with a model of the rabbit atrial myocyte.

The incorporation of recently published whole-cell voltage-clamp data in this model, allows more accurate simulations of the spontaneous activity of the SAN cell, than was possible using previous models. For example, the YNI model did not deal with issues such as calcium buffering in the intracellular medium, two different types of calcium channels in the sarcolemma, or membrane pumps and exchangers. Additions of these currents can have significant effects on the pacemaker waveform, as will be discussed later.
Our model is similar to the DN-type model [48, 14] for the rabbit SAN cell. However, there are important differences between these models. For example, (1) Two calcium channels (T type and L-type) are used in our model; (2) intracellular $Ca^{2+}$ buffering (via calmodulin and to a lesser extent troponin) is not included in the DN-type model, but is present in our model; and (3) the DN-type model is based mainly on experimental data from multicellular preparations, while in our model, the descriptions of the size and kinetics of the ionic membrane currents are based, whenever possible, on quantitative voltage clamp data from myocytes of the rabbit SAN [10, 27, 56, 59].

1.4 Summary

Our main goals in this work were to build the rabbit SAN cell that incorporates recently published experimental data and that can mimic the voltage clamp data upon which it is based and the waveshape of the action potential study [26]. The model has two major components (the sarcolemmal model and the fluid compartment model of the intra- and extracellular media) and these components will be explained in Chapter 2. Mathematical descriptions of the ionic channels ($I_{Ca,L}$, $I_{Ca,T}$, $I_{K}$, $I_{f}$) and background, pump, exchanger, chloride currents ($I_{B}$, $I_{Na}$, $I_{Ca,P}$, $I_{NaCa}$, $I_{Cl}$) which form the sarcolemmal model are given in Tables A.1- A.4, while the equations in Tables A.5- A.6 represent the subcompartments of the fluid compartment model; the mass balance descriptions for ionic species ($Na^{+}$, $K^{+}$, $Ca^{2+}$), $Ca^{2+}$ buffering proteins (calmodulin and troponin) and the uptake and release mechanism of $Ca^{2+}$ by the sarcoplasmic reticulum. The numerical methods to solve the SAN cell model will also be discussed in Chapter 2. The results of our simulation study will be given in Chapter 3. We will discuss the model fit to the action potential data, the effects of the membrane currents present in the model, the fluxes in the fluid compartment
model, the usefulness of the instantaneous current-voltage plots, the effects of the membrane pump and exchanger currents, and the blockade of $I_f$ and $I_{Ca,T}$. Chapter 4 compares our model with the previous SAN models, summarizes its advantages over the previous models, and discusses the limitations of the modeling work, while Chapter 5 states the conclusions of the SAN model presented in this thesis, and summarizes the extents of this work.
Chapter 2

The Rabbit SAN Cell Model

2.1 Model Development

The electrical equivalent circuit for the sarcolemma of the isolated rabbit SAN cell is shown in Figure 2.1A. Under space clamp conditions, the differential equation describing the membrane potential (V) is:

\[
\frac{dV}{dt} = \frac{-[I_{Ca,T} + I_{Ca,L} + I_K + I_f + I_{Cl} + I_B + I_{NaK} + I_{NaCa} + I_{CaP}]}{C_m}
\]  

(2.1)

Here \(I_{Ca,T}\) and \(I_{Ca,L}\) are the time and voltage dependent "transient" and "long-lasting" calcium currents, respectively; \(I_K\) is the time- and voltage-dependent delayed-rectifier potassium current; \(I_f\), the hyperpolarization-activated current; \(I_{Cl}\) is the outwardly-rectifying chloride current; \(I_B\), the linear background current; \(I_{NaK}\), the electrogenic \(Na^+/K^+\) pump current; \(I_{NaCa}\), the electrogenic \(Na^+/Ca^+\) exchanger current; \(I_{CaP}\), the ATP-dependent calcium pump current and \(C_m\), the whole-cell membrane capacitance (pF). The background current \(I_B\) is comprised of two linear components: \(I_{Na,B}\), a sodium current and \(I_{Ca,B}\) a calcium current. \(I_{Ca,B}\) is assumed to be quite small relative to the magnitude of \(I_{Na,B}\).

The specific equations and parameter values necessary for the simulation of these currents are given in Tables A.1- A.7 in the Appendix. Figure 2.1B shows the lumped fluid compartment models of the intracellular and bulk extracellular spaces. Material balance expressions are given for the ionic species (\(Na^+\), \(K^+\) and \(Ca^{2+}\)) in the intracellular medium (Table A.5), along with equations that describe the binding
Figure 2.1: Diagram of the rabbit SAN cell model
(A) electrical equivalent circuit of the cell membrane, and (B) fluid compartmental model
of the cell including intra- and extracellular spaces. See text for definitions of symbols.
of intracellular calcium to specific myoplasmic proteins (calmodulin and troponin) [53]. The concentrations of the three ionic species are considered constant in the extracellular compartment. Equations describing the mechanisms for uptake and release of $Ca^{2+}$ by sarcoplasmic reticulum (SR) are given in Table A.6. These equations have been adopted from Murphey et al [45] for the rabbit atrial cell, and represent modifications of the SR model of Hilgemann and Noble [28]. The nominal parameter values used to obtain the model-generated cell action potential for the rabbit SAN cell are given in Tables A.1- A.7.

The Hodgkin-Huxley type activation and inactivation gating variables [30] are solutions of the first order differential equations of the form:

$$\frac{dz(V, t)}{dt} = \frac{[\bar{z}(V) - z(V, t)]}{\tau_z(V)} \tag{2.2}$$

where $\bar{z}(V)$ is the steady-state value of the generic gating variable $z$ at transmembrane voltage $V$, and $\tau_z(V)$ is the associated time constant. Here $z = d_T, f_T, d_L, f_L, p_a, p_i$ and $y$ and the formulas for the functions $\bar{z}(V)$ and $\tau_z(V)$ are given in Tables A.1, A.2 and A.4. The activation gating variables for the $I_{Ca,T}$ and $I_{Ca,L}$ currents are $d_T$ and $d_L$, respectively, while $f_T$ and $f_L$ are the inactivation gating variables for the same currents. The delayed rectifier current $I_K$ has both an activation ($p_a$) and an inactivation ($p_i$) gating variable, while $I_f$ has only an activation variable ($y$). The parameters associated with the gating variables in these tables are those chosen to yield a good fit (by eye) to voltage clamp data from Hagiwara et al [26], Nilius [47], Fermini and Nathan [21], Shibasaki [56]; and van Ginneken and Giles [59]; and the action potential data from Hagiwara et al [26], which were obtained from isolated single cell rabbit SAN preparations.
2.2 Microanatomical Considerations

Primary SAN pacemaker cells in the rabbit heart are characterized by their empty appearance in micrographs. This is due to the poor development of the contractile apparatus in these cells [3, 40]. In primary pacemaker cells, the myofilaments occupy \( \sim 20\% \) of the cell volume, while in atrial cells the contractile elements occupy \( \sim 54\% \) of the cell volume [41, 40]. Only a small fraction of SAN cells are primary pacemaking cells. They are located in the central part of the node (this central region containing primary pacemaking cells of the SAN is estimated to be \( 0.1\text{mm}^2 \) and contain fewer than 5000 cells; [2]). Transitional cells surround this primary pacemaking region, and these cells exhibit an increase in the density of myofilaments they contain (as well as, their organization into myofibrils), with increasing distance from the central pacemaking region to the periphery of the node. Cells within the the SAN may also be classified according to their electrophysiology; primary pacemaker cells exhibit a lower upstroke velocity (2-4V/sec) than transitional cells (7-13V/sec). Thus, on both anatomical and electrophysiological grounds, the SAN exhibits a heterogeneous structure. Due to its small size and variable location, the primary pacemaking region is difficult to locate in a given SAN without extensive prior testing. Hence, specific isolation of primary pacemaking cells via enzymatic dispersion techniques is difficult, and the majority of cells normally isolated via these procedures yield transitional cells, since they are much more abundant within the node.

In the study at hand, we attempt to mimic the action potential data of Hagiwara et al [26, Fig 1], which exhibits the electrophysiological characteristics (MDP, upstroke velocity) of a transitional cell, rather than a primary pacemaker cell. In our studies, we also refer to the experimental studies of Denyer and Brown [10] and van Ginneken and Giles [59]; the cells investigated in these works also exhibit MDPs in the range (-58 to -67mV) and upstroke velocities (7.9 to 12.8V/sec), which are characteristic
of transitional cells. These cells may have been taken from regions of SAN known to contain the primary pacemaking center, however, due to the small size and variable location of this center, the probability of harvesting a primary pacemaking cell via enzymatic dispersion techniques is low.

In our modeling study, we assume that our SAN cell is a representative transitional cell, that exhibits anatomical and electrophysiological properties that are close to those of the primary pacemaker cell. Specifically, we assume that 20% of the cell volume is occupied by the contractile elements. Therefore, the effective intracellular volume in which free calcium ions are available to enter into reactions, is approximately 80% in the rabbit SAN cell (compared with 46% in the atrial myocyte). This reasoning with regard to contractile element volume has been applied in our previous modeling work for the atrial myocyte [45], and volume-dependent values associated with the contractile protein buffers and the calcium uptake and release mechanisms of the SR, have been derived in that study. These values are appropriately scaled for use in the intracellular medium equations for calcium buffering and the mechanisms for $Ca^{2+}$ uptake and release by the SR, in the present model of the SAN cell.

2.3 Computational Aspects

The rabbit SAN model is assumed to have an idealized cylindrical geometry ($12\mu m$ diameter, $32\mu m$ length; [59]). The electrical equivalent circuit model (Fig 2.1) is described by a set of 20 first-order differential equations, which are given in Tables A.1- A.6. Eight of these equations describe the ion fluxes across the sarcolemma; the remaining twelve equations are associated with: (1) the material balance equations for three ionic species in the intracellular medium ($Na^+$, $K^+$ and $Ca^{2+}$); (2) calcium buffering equations; and (3) the equations describing the mechanisms for the uptake and release of $Ca^{2+}$ by the SR. The nominal model parameter values used in this study
(Tables A.1- A.7), were obtained as the results of fits to various types of experimental data: (1) isolated single SAN cell voltage clamp data, (2) action potential data, and (3) anatomical measurements. These have either been reported in the literature or obtained in the laboratory of one of us (W.R.G).

When applying a conventional numerical integration method to the differential equations of the model, one must be aware that the time constants involved vary by approximately 3 orders of magnitude from 150 $\mu$s (ICa,T kinetics) to 100 ms (calcium diffusion between the uptake and release compartments in the SR). Hence double precision arithmetic must be used for all calculations, so that round-off errors due to finite precision arithmetic can be minimized. A Runge–Kutta–Merson numerical integration algorithm, which is equipped with an automatic step size adjustment that is based on an error estimate, was employed in these simulations [23]. The accuracy of the computation of voltage and concentration changes in the model was maintained via the adjustment of temporal step-size, which resulted in stepsizes ranging from 342 $\mu$s to 1.2 ms. The relative local error in the estimate of every state variable including membrane voltage, gating variables and concentrations, was maintained below $10^{-6}$. The model equations were coded in the C language and a Sequent Systems Symmetry S 81 parallel computer was used for all computations.

2.4 Descriptions of the Membrane Currents

The complete model of the rabbit SAN cell consists of two major components, a membrane model (Figure 2.1A) and a lumped fluid compartment model (Figure 2.1B); each of these, in turn, consists of several subcomponents. This section describes each of these components and the contribution of each to the model-generated waveforms of transmembrane voltage and/or current during the pacemaker depolarization and the subsequent action potential. Emphasis here will be placed on the more novel
components of this model, which were not discussed in previously published simulation studies by either our group \cite{45, 53, 54}, or DiFrancesco, Noble and Hilgemann \cite{14, 28}.

2.4.1 The Inward Calcium Currents, $I_{Ca,T}$ and $I_{Ca,L}$

In cardiac pacemaker tissue, the last part of the diastolic depolarization, as well as the rapid upstroke of the action potential, is strongly influenced by calcium currents. The smaller low-threshold calcium current $I_{Ca,T}$ is activated in early diastole at the level of the MDP, while the larger, higher-threshold current $I_{Ca,L}$ is activated during the upstroke of the action potential. The gating characteristics of these calcium currents are different in that both activate rapidly, but inactivate differently. For example, $I_{Ca,T}$ inactivates very rapidly according to a conventional voltage-dependent process that is well characterized mathematically by a Boltzmann relationship. However, $I_{Ca,L}$ inactivates much more slowly and according to a different process. In particular, the steady-state voltage-dependent inactivation gating variable $\bar{F}_L(V)$ has been shown to have skewed "U-shape" rather than the conventional sigmoidal shape that is easily characterized by a Boltzmann relationship (for a review see \cite{29, 7}). The "U-shaped" function $\bar{F}_L(V)$ does not go to zero at relatively depolarized levels of potential; rather it increases. This is known as the phenomenon of incomplete inactivation \cite{7, 29}. This phenomenon produces a secondary shoulder in the waveform of $I_{Ca,L}$, which provides a longer lasting inward current that supports the plateau phase of the SAN cell action potential. This secondary shoulder in the $I_{Ca,L}$ waveform, mimics recent data from action potential clamp experiments on rabbit SAN myocytes \cite{17}.

In our model, $I_{Ca,T}$ is small relative to the peak magnitude of $I_{Ca,L}$ (0.4%), and is blocked in a selective fashion by 40$\mu$M Ni$^{2+}$ \cite{17, 27}. Total block of $I_{Ca,T}$ at 40$\mu$M Ni$^{2+}$ was confirmed in whole-cell voltage clamp experiments by Hagiwara et
al [27]. The effect on spontaneous pacemaker activity produced by 40μM Ni\textsuperscript{2+},
was to prolong the cycle length by 24%. \textit{I}_{Ca,L} is selectively blocked by appropriate
concentrations of either Nifedipine or D600. Both \textit{I}_{Ca,T} and \textit{I}_{Ca,L} are blocked by
appropriate concentrations of Cd\textsuperscript{2+}.

Equations for the voltage-dependent activation and inactivation gating variables
for \textit{I}_{Ca,T} and \textit{I}_{Ca,L} are given in Table A.1. These equations are based on the
experimental data of Hagiwara et al [27], Nilius [47], and Fermini and Nathan [21].
However, in fitting the action potential data of Hagiwara et al [26, Fig 1], we have
produced much better fits to this action potential by shifting the steady-state ac-
tivation curves \textit{d}_T and \textit{f}_T (given by Fermini and Nathan [21, Fig 2]) by -10.6mV
and +1.7 mV, respectively. Similarly, \textit{d}_L and \textit{f}_L (given by Fermini and Nathan [21,
Fig 2]) were shifted by -5.4mV and -4.2mV, respectively. In addition, the slopes of
the Boltzmann relations representing \textit{d}_L and \textit{f}_T were made steeper for a better fit to
the action potential data. The rate constant data for \textit{d}_T and \textit{f}_T (i.e. \textit{τ}_d^{-1}\text{ and } \textit{τ}_f^{-1}\text{)}
were given by Hagiwara et al [27], however the same data was not provided for \textit{τ}_d^{-1}\text{ and }
\textit{τ}_f^{-1}\text{. Therefore, we chose voltage-dependent rate constant relationships for \textit{τ}_d\text{ and }
\textit{τ}_f\text{, which were based on the whole-cell data of Nilius [47] on guinea-pig SAN
myocytes at 25°C. A } Q_{10} \text{ factor of 2.3 was applied to this data which is appropriate
for a temperature of 37°C. The rate constant relationships used for \textit{τ}_d^{-1}\text{ and } \textit{τ}_f^{-1}\text{ were}
those given by Hagiwara et al for rabbit SAN cells at 37°C. These relationships were
shifted in a hyperpolarizing direction in order to align them with the adjusted \textit{d}_T and
\textit{f}_T curves adopted from Fermini and Nathan [21]. Fig 2.2A shows the voltage de-
pendent steady-state activation (\textit{d}_L) and inactivation (\textit{f}_L) curves for the \textit{I}_{Ca,L} current
used in our model, while Fig 2.2B shows the same curves for the \textit{I}_{Ca,T} current. The
time constants associated with the gating variables for \textit{I}_{Ca,L} are shown in Fig 2.2C,
whereas those associated with \textit{I}_{Ca,T} are shown in Panel D of this figure.
Figure 2.2: The steady-state curves and the time constants for $I_{Ca,L}$ and $I_{Ca,T}$

Panel A shows the voltage dependent steady-state activation ($\tilde{f}_L$) and inactivation ($\tilde{d}_L$) curves for the $I_{Ca,L}$ and Panel B shows those ($\tilde{d}_T$, $\tilde{f}_T$) for the $I_{Ca,T}$ current, in our model. The time constants associated with the gating variables for $I_{Ca,L}$ are shown in Panel C of this figure whereas those associated with $I_{Ca,T}$ are in Panel D.
2.4.2 The Outward Delayed Rectifier Current, $I_K$

The delayed-rectifier current $I_K$ plays an important role in the initiation of action potential repolarization. Its rate of decay during phase 4 is also an important factor in determining the rate of diastolic depolarization, particularly in the first half of the pacemaker potential. The equations for the activation and inactivation gating variables associated with $I_K$ are based on the experimental data of Shibasaki [56] on rabbit SAN myocytes at 37°C, and are given in Table A.2. The equations employed in our model for describing the activation gating variable $p_a$, are similar to those of Shibasaki [56], however the steady-state activation curve $\bar{p}_a(V)$ has been shifted by 20mV in the depolarizing direction. In addition, the rate function $\tau_{pa}^{-1}$ has been multiplied by 10 in the voltage range $-100mV \leq V \leq -20mV$. However, for $V > -20mV$, our $\tau_{pa}^{-1}(V)$ relationship reproduces the voltage-dependent curve specified by Shibasaki [56]. These changes were made in order to better fit the action potential data of Hagiwara et al [26, Fig 1]. On the other hand, the steady-state inactivation variable $p_i$ and the rate constant $\tau_{pi}^{-1}$ are those specified by Shibasaki [56]. Fig 2.3A shows the voltage-dependence of the steady-state activation ($\bar{p}_a$) and inactivation ($\bar{p}_i$) variables for the $I_K$ current employed in the model, while Panel B shows the voltage-dependent rate constants ($\tau_{pa}^{-1}$ and $\tau_{pi}^{-1}$) associated with these gating variables.

2.4.3 Pump and Exchanger Currents

The equations we have used for the sarcolemmal pumps ($I_{NaK}$, $I_{CaP}$), and the exchanger current ($I_{NaCa}$), are taken from the related modeling work of Murphey et al [45] for the rabbit atrial myocyte. These pump and exchanger formulations are based in part, on the earlier work of DiFrancesco and Noble [14] on the mammalian Purkinje fiber, and in part, on the work of Rasmusson et al [53, 54] which simulates the pacemaker activity in isolated myocytes from the amphibian heart.
Figure 2.3: The voltage-dependent steady-state curves and rate constants for the $I_K$ current

Panel A shows the steady-state activation ($\bar{p}_a$) and inactivation ($\bar{p}_i$) curves for $I_K$ used in the model. In Panel B the voltage-dependent rate constants ($\tau_{p_a}^{-1}$ and $\tau_{p_i}^{-1}$) associated with these gating variables are shown.
The $Na^+/K^+$ pump current ($I_{NaK}$) is an outward current which is responsible for the maintenance of $Na^+$ and $K^+$ electrochemical gradients in the cell. This current is of sufficient magnitude to be a contributing factor during diastole and it can also influence the rate of the pacemaker cell (e.g. see Rasmussen et al [53]). The sodium-calcium exchanger current ($I_{NaCa}$) is due to the electrogenic co-transport mechanism involved in the homeostatsis of $[Ca^{2+}]_i$. In our model it is also a contributing factor in determining the magnitude of the maximum diastolic potential (MDP) and the rate of diastolic depolarization. The calcium extrusion pump ($I_{CaP}$) has a high affinity for calcium, but a relatively low turn-over rate. This pump current is significant in setting and maintaining the relatively low levels of $[Ca^{2+}]_i$ occurring during diastolic depolarization. The specific equations used for $I_{NaK}$, $I_{NaCa}$ and $I_{CaP}$ are given in Table A.3.

2.4.4 The Hyperpolarization-Activated Current, $I_f$

The inward current $I_f$ is activated when the cell membrane is hyperpolarized. However, the extent to which $I_f$ contributes to pacemaking in the SAN cell is a question that remains somewhat controversial. For example, Brown et al [6] and Noma et al [49] have found that $Cs^+$ (a blocker of $I_f$) does not produce a strong influence on the spontaneous rate of the rabbit SAN cell. On the other hand, other investigators [10, 59] have found, that when $I_f$ is blocked by application of $Cs^+$, spontaneous pacemaking is slowed significantly, but not abolished (see e.g. [9, 59]). Specifically, Denyer and Brown [10] found that the spontaneous rate changed by 29% (for 2mM-CsCl application) while van Ginneken and Giles reported a change of 57% (for 1mM-CsCl application). These findings support the hypothesis that $I_f$ normally makes an important contribution to pacemaker depolarization in SAN cells. However, since spontaneous pacemaking is not abolished under $Cs^+$ block, it is unlikely that $I_f$ is the definitive
"pacemaker current". Rather, it most likely serves as an important inward current that normally supports diastolic depolarization, and which may be modulated by receptor-mediated agonists, such as ACh [13].

The mathematical expressions for $I_f$ appearing in Table A.4 are modified from those given by van Ginneken and Giles [59]. These modified equations provide a good fit (by eye) to the whole-cell voltage clamp data obtained by van Ginneken and Giles [59], as will be shown later.

2.4.5 The Chloride Current, $I_{Cl}$

A renewed interest in $Cl^-$ currents in the heart has followed recent suggestions that there may be a chloride-dependent component to the transient-outward current [62], and that an outwardly rectifying chloride current ($I_{Cl}$) may be responsible for action potential repolarization in rabbit ventricular myocytes [20]. Earlier, Seyama [55] reported a chloride channel in the rabbit SA node cell. Therefore, we have included $I_{Cl}$ in our model and the equation describing this current is given in Table A.4.

2.4.6 Background Currents

Since we have mentioned earlier that neither $Cs^+$-block of $I_f$ nor $Ni^{2+}$-block of $I_{Ca,T}$ produced cessation of spontaneous pacemaking, it is unlikely that either of these currents are fundamentally involved in pacemaking. An interesting alternative is that the linear background current $I_B$ could provide the inward current necessary to sustain diastolic depolarization, and hence would serve as a necessary "pacemaker" current. This possibility is investigated later in our modeling study. Although $I_B$ is a small current that is included in most models of SAN cells, there is little quantitative experimental data upon which to base its mathematical characterization. We assume
the background current $I_B$ to be comprised of two linear components, a sodium current $I_{Na,B}$, and a calcium current $I_{Ca,B}$. The equations that we have used for $I_B$ are given in Table A.4.

2.5 Ionic Concentrations and Buffering

In this SAN cell model, the cell surface area, volume and membrane capacitance values were taken from the measurements of van Ginneken and Giles [59]. Assuming that caveolae increase the surface area of the sarcolemma by 115 % [41], the whole cell capacitance of 55pF reported by van Ginneken and Giles [59], corresponds to a specific membrane capacitance of 2.1 $\mu F/cm^2$. As discussed earlier, our typical nodal cell is assumed to have a smaller amount of contractile protein (20 % by volume) than a typical atrial myocardial cell (54 % by volume). We have therefore reduced the amount of contractile protein and SR, compared with that contained in our atrial cell model. Thus, the amount of troponin available for calcium binding has been taken to be 37 % of the values used in the rabbit atrial myocyte model [45]. Correspondingly, the fractional volumes of the sarcoplasmic reticulum in the calcium uptake and release mechanism were also taken to be 37 % of the values given in the atrial cell model. We assumed however, that the amount of calmodulin available for $Ca^{2+}$-binding in the SAN cell, was essentially the same as in the atrial myocyte model [45].

Table A.5 includes the mass balance equations in the intracellular medium for $Na^+$, $K^+$ and $Ca^{2+}$, as well as, the equations describing the binding of intracellular $Ca^{2+}$ to specific myoplasmic proteins (calmodulin and troponin). The mechanisms describing the uptake and release of $[Ca^{2+}]$, by the sarcoplasmic reticulum (SR) were also taken from Murphey et al [45]; as previously mentioned, this mechanism is a modified version of the SR model by Hilgemann and Noble [28], and these equations are given in Table A.6. The rate constants associated with these equations were
scaled from the temperature of the rabbit atrial model (23°C) to the temperature of our SAN model (37°C) using the a $Q_{10}$ factor of 2.3 [8].
Chapter 3

Results

3.1 Model-generated Spontaneous Activity and the Data

Fig 3.1A shows the comparison of a model-generated pacemaker depolarization and action potential waveform, with experimental data from an isolated rabbit SAN cell [26, Fig 1]. The single action potential shown consists of digitized data points which lie between the points labeled "a" and "b" in Fig. 3.1A. The time derivatives (dV/dt) of the model-generated and experimental action potentials are shown in Panel B. The model-generated action potential has a peak upstroke velocity of 9.8 V/sec for this pacemaker waveform, which agrees well with the experimental data of Hagiwara et al [26], as well as, that of van Ginneken and Giles [59]. A numerical comparison of selected indices (maximum upstroke velocity, overshoot, etc.) calculated from the experimental and model-generated action potential waveforms is given in Table A.8, which shows very close similarities in these values.

3.2 Membrane Currents

The SAN cell action potential and corresponding membrane currents are shown in Fig 3.2. The action potential shown in Fig 3.2A is identical to that shown in Fig 3.1A. Panel B shows that $I_{Ca,L}$ consists of a spike and a secondary shoulder of inward current. The spike of this current is responsible for the upstroke of the action potential, while the secondary shoulder supports the plateau of the action potential. The model-generated $I_{Ca,L}$ waveform seen in Fig 3.2B mimics the calcium 'compensation' current.
Figure 3.1: Comparison of a simulated and an experimentally recorded pacemaker action potential from SAN in rabbit

(A) Comparison of the model-generated waveform to the data [26, Fig 1] (one cycle between points "a" and "b"). A numerical comparison of these waveforms is given in Table A.8; and (B) comparison of the time derivative of the model-generated and experimental $dV/dt$ waveforms. The maximum upstroke velocity of the model-generated action potential waveform is $9.8V/sec$. 
waveforms recorded recently in single rabbit SAN cells using a special action potential clamp technique [17]. The depolarization brought about by $I_{Ca,L}$ is opposed by the outward current $I_{Ci}$ which peaks with the peak of the action potential and decreases gradually during the plateau phase (Panel B). Ultimately, repolarization is initiated by the delayed increase in $I_K$ (Panel B), which then declines during the remainder of phase 3, and the first half of phase 4 (Panel B). Note that during phase 3 repolarization, the $I_{Ca,L}$ current returns to zero.

Fig 3.2C shows the waveforms for the $I_{Ca,T}$ and $I_f$ currents which play an important role during the pacemaker depolarization. During phase 4, membrane potential changes slowly. The small, low-threshold calcium current $I_{Ca,T}$ is activated in this potential range, increasing slowly at first and then more rapidly, as it rises to a peak that occurs 60msec prior to the $I_{Ca,L}$ peak. Thereafter, it inactivates very rapidly, and is reduced to a level of 0.76pA (28% of peak $I_{Ca,T}$) by the time that $I_{Ca,L}$ peaks (see Fig 3.2B and C). Thus, the membrane depolarization produced by the activation of $I_{Ca,T}$ contributes to diastolic depolarization and eventually the activation of $I_{Ca,L}$, which when activated, further augments the net calcium current to produce a more rapid increase in the upstroke velocity (Fig 3.2). The small inward hump seen in the $I_{Ca,T}$ waveform during phase 3 repolarization (Fig 3.2C) is due to deactivation of this current as membrane potential passes through the $I_{Ca,T}$ activation window.

The $I_f$ current shown in Fig 3.2C is as a small (-1.75pA peak) inward current with an activation threshold of -36mV. It activates slowly, late in phase 3 and is present as a small inward current supporting diastolic depolarization during phase 4; it peaks in the middle of phase 4 depolarization. With the rapid upstroke, $I_f$ develops as a small outward current as membrane potential exceeds the reversal potential for this current (-30mV).
Figure 3.2: Model-generated pacemaker depolarization, action potential and transmembrane currents
(A) Pacemaker and action potential waveforms, (B) Transmembrane currents $I_{Ca,L}$, $I_K$ and $I_{Cl}$; (C) hyperpolarization-activated current ($I_f$) and the low-threshold transient calcium channel current ($I_{Ca,T}$) and (D) the background $I_B$, pump ($I_{Na,K}$, $I_{Ca,P}$) and exchanger ($I_{Na, Ca}$) currents.
Fig 3.2D shows that other currents besides $I_{Ca,T}$ and $I_f$ contribute to the total current during phase 4. For example, the $Na^+$/Ca$^{2+}$ exchanger current $I_{NaCa}$, increases during the upstroke of the action potential to produce a small net outward current at the peak of the action potential (Fig 3.2D). Thereafter, $I_{NaCa}$ becomes a net inward current during the action potential plateau and phase 3 repolarization. This current produces a relatively large (-79pA) inward peak during the first third of the pacemaker potential, and then declines slowly during the remainder of phase 4.

In Fig 3.2D, the total background current ($I_B = I_{Na,B} + I_{Ca,B}$) produces a very small net inward current at the peak of the action potential and during the plateau phase (reversal potential for the background current is 89mV). During phase 4 it is a slowly declining inward current having a magnitude of approximately -2.4pA. Thus, both $I_{NaCa}$ and $I_B$ provide inward currents that support membrane depolarization during the pacemaker potential. These inward currents are opposed by the outward pump currents ($I_{NaK}$ and $I_{CaP}$) which are also shown in Panel D.

Thus, during the early portion of the pacemaker potential (at the level of the MDP), a number of currents contribute to diastolic depolarization: (1) declining $I_K$, (2) increasing $I_{NaCa}$, (3) declining $I_{CaP}$, (4) increasing $I_f$, (5) increasing $I_{Ca,T}$, and (6) increasing $I_B$ (Figs 3.2 B,C,D). $I_K$ contributes a decaying outward current over the first half of phase 4, while the outward $I_{CaP}$ current continues to decline throughout this phase. The $I_f$ current peaks in the middle of phase 4, while $I_{Ca,T}$ peaks in the final third of this interval. Both $I_f$ and $I_{Ca,T}$ are active throughout diastolic depolarization. With the occurrence of the upstroke of the action potential, $I_{Ca,T}$ inactivates abruptly, while $I_f$ contributes a small transient phase of outward current. During phase 4, $I_B$ contributes an inward current that is maximum at the MDP and declines slowly during diastolic depolarization. It too is a small but significant current throughout phase 4.
3.3 Fluxes in the Fluid Compartment Model

Figures 3.3 A and B show the change in \( [Ca^{2+}] \) relative to events in the action potential waveform, while Panel C shows the associated change in the occupancy of the protein buffering sites for calcium. Note here that the majority of intracellular \( Ca^{2+} \) is buffered by the competitive \( Mg^{2+}-Ca^{2+} \) site on the troponin molecule (TMgC), rather than the calcium specific site (TC), or calmodulin (C). Figure 3.3D shows the model-generated release \( (I_{rel}) \), uptake \( (I_{up}) \) and transfer \( (I_{tr}) \) currents associated with the compartmental model of the SR. The spike seen in the release site waveform \( I_{rel} \) is due to calcium-induced calcium release (CICR) and it coincides with both the upstroke of the action potential and the spike portion of \( I_{Ca,L} \) waveform seen in Fig 3.2B. These waveforms represent model predictions of quantities that are essentially unobservable via present-day measurement techniques.

3.4 The \( I_f \) Current

Quantitative formulation of the hyperpolarization-activated inward current \( I_f \) is essential for any model of pacemaking activity in the SAN. Figure 3.4A and B show the voltage-dependent steady-state activation \( (\bar{y}) \) and reciprocal time constant \( (\tau_y^{-1}) \) curves for the gating variable \( y \) associated with the \( I_f \) current. Since \( \bar{y}^2 \) is also of interest in the equation for \( I_f \) (Table A.4), it is plotted along with \( \bar{y} \) in Panel A. The expression used for \( I_f \) in this study is based on a modified version of the analysis of whole-cell voltage clamp data on \( I_f \) by van Ginneken and Giles [59]. To validate this model of \( I_f \), we calculated the response of the membrane to a two pulse protocol of voltage clamp steps, and compared the model-generated results with the experimental data reported in van Ginneken and Giles [59, Fig 6]. The first pulse \( P_1 \) of 1 second duration is applied from a holding potential of \(-40mV\) to a level of \(-90mV\) in order to activate \( I_f \). At the end of this conditioning step, the membrane
Figure 3.3: Model-generated action potential, intracellular calcium concentration, buffer occupancies and SR compartment currents

(A) Action potential waveform; (B) intracellular calcium concentration $[Ca^{2+}]_i$ as a function of time; (C) changes in the fractional occupancy of the protein binding sites (calmodulin, $\Phi_C$; troponin-specific, $\Phi_{TC}$; and troponin-nonspecific competitive, $\Phi_{TMgC}$). The range of $[Ca]_i$ is $0.22\mu M$ - $2.95\mu M$ while the ranges of the fractional occupancy of the protein binding sites are: $\%8-54$ for $\Phi_C$; $\%4-36$ for $\Phi_{TC}$; and $\%63-87$ for $\Phi_{TMgC}$; and (D) currents ($I_{rel}$, $I_{up}$ and $I_{tr}$) involved in the the uptake and release mechanisms of the sarcoplasmic reticulum.
Figure 3.4: Voltage-dependence of the activation ($\bar{y}$) and time constant ($\tau_y$) functions associated with $I_f$

(A) $\bar{y}$ and $\bar{y}^2$ as a function of membrane voltage $V$; and (B) the reciprocal time constant $\tau_y^{-1}$ as a function of $V$. The experimental data from van Ginneken and Giles [59, Fig 8] and its comparison with experimental data [59, Fig 6]. The modified equation for $I_f$ is given in Table A.4.
Figure 3.5: Voltage-clamp study of $I_f$ in the rabbit SAN cell model. Panel A illustrates the response of the membrane model to a two pulse protocol of voltage-clamp steps. The first pulse $P_1$ of 1 second duration is applied from a holding potential of $-40\,\text{mV}$ to a level of $-90\,\text{mV}$ in order to activate $I_f$. At the end of this conditioning step $P_1$ the membrane potential is clamped via a depolarizing step $P_2$ to a number of voltages (-80, -70, -60, -50, -20 mV) for two seconds. In Panel B, the initial amplitude of the current tail was plotted versus the $P_2$ voltages. The resulting I-V relation is linear with a slope conductance of 14.9 nS.
potential is subsequently clamped via a second depolarizing step \( P_2 \), to a number of voltages (-80, -70, -60, -50, -20 mV) for two seconds. The total membrane current in response to this two pulse protocol is shown in Fig 3.5 A. We also plot the initial amplitude of the current tail versus the \( P_2 \) voltages (Fig 3.5B), which results in a linear relationship with a slope conductance \( (g_f) \) of 14.9 nS. This value is close to the value (12.0±0.88nS) that was reported by van Ginneken and Giles [59]. The reversal potential for this simulation is -30 mV. Thus, the modified equations developed for \( I_f \) are given in Table A.4. They were included in the model based on the close fit that they produce to the experimental voltage-clamp data of Figs 3.4 and 3.5.

### 3.5 Instantaneous Current-Voltage Plots

In fitting the model-generated data to spontaneous pacemaking waveforms from rabbit SAN cells, we have found it very beneficial to plot the data as an instantaneous current-voltage (I-V) plot. In the construction of this plot we first take the first derivative of the membrane voltage \( \dot{V} \) using a central difference algorithm. We then multiply \( \dot{V} \) by the whole-cell membrane capacitance \( C_m \) (pF) to form the capacitative displacement current \( (I_d = C_m \dot{V}) \). Under space clamped conditions:

\[
-I_d = I_{Ca,T} + I_{Ca,L} + I_K + I_{NaK} + I_{NaCa} + I_{CaP} + I_f + I_{Cl} + I_B. \tag{3.1}
\]

In other words, \(-I_d\) is the negative sum of the net transmembrane (sarcolemma) currents. Plotting \(-I_d\) vs. \( V \) yields a closed-loop plot that is quite similar to a phase-plane plot [22] (see Fig 3.6A). Note that time is implicit on the diagrams (see arrows), and that the maximum excursion of the plot along the negative \( V \)-axis represents the maximum diastolic potential (MDP). Similarly the largest excursion along the positive \( V \)-axis represents the peak overshoot (PO) of the action potential. Fig 3.6A is a representative I-V plot showing both the experimental data [26, Fig 1] and the model-generated I-V plots. Similar instantaneous I-V plots for each of the
Figure 3.6: Instantaneous current-voltage (I-V) plots
Panel A shows the plot of the experimental data and the model generated (I_d-V) plot. I-V plots for the major component currents indicated on the diagram are in Panel B. Time (t) increases in the direction of the arrows. The time of occurrence of the MDP and the time of occurrence of the peak overshoot potential (PO) are represented by t_{MDP} and t_{PO}, respectively. The time of the onset of the rapid upstroke of the action potential is t_{UP} and the onset of the exponential 'foot' of the action potential is t_{foot}. 
major component voltage-dependent currents of the model are shown in Fig 3.6B. By superimposing I-V plots on the same coordinate axis as the data (V, -Cm \( V \)), one can observe how a given current component contributes to the overall I-V plot.

To aid in the interpretation of these plots we define the following times in the pacemaker cycle: (1) the time associated with the maximum diastolic potential (MDP) of the action potential, \( t_{MDP} \); (2) the onset of the exponential 'foot' of the action potential, \( t_{foot} \); (3) the beginning of the upstroke of the action potential, \( t_{UP} \); and (4) the peak overshoot (PO) of the action potential, \( t_{PO} \). The associated potentials at these times are \( V_{MDP}, V_{foot}, V_{UP} \) and \( V_{PO} \), respectively. These times are indicated on the I-V plot of Fig 3.6, and time increases in the direction indicated by the arrows.

Note from Fig 3.6A that the values of these potentials as measured from the data waveform are \( V_{MDP} = -63.1 \); \( V_{foot} = -40.2 \); \( V_{UP} = -31.3 \); and \( V_{PO} = 35.5 \), mV, respectively. Note from Fig 3.6 A that the exponential foot of the action potential in the interval \( t_{foot} \leq t \leq t_{UP} \) appears as a straight line in the phase-plane plot. This I-V plot clearly delineates the occurrence of the upstroke as the point of departure from this short linear segment (see interval between \( t_{foot} \) and \( t_{UP} \)). To aid in the further interpretation of the I-V plot, we divide the plot into two segments: (1) from the time of occurrence of the MDP (\( t_{MDP} \)) to the time of occurrence of the peak overshoot potential (\( t_{PO} \)) (Fig 3.7 A), and (2) from \( t_{PO} \) to \( t_{MDP} \) on the succeeding cycle (Fig 3.7 B). Figure 3.7 A illustrates the time period \( t_{MDP} < t < t_{PO} \), which may be further subdivided into three intervals based on the detection of \( t_{foot} \) and \( t_{UP} \). Note from Figs 3.7A and B, that at the MDP, the major currents at play are \( I_K \) and \( I_{NaCa} \). On the other hand, at \( V_{PO} \), the major currents involved are the inward current \( I_{Ca,L} \) and the outward currents \( I_{Cl} \) and \( I_K \). The upstroke is formed largely by \( I_{Ca,L} \) which is opposed by an increasing \( I_{Cl} \) current and to a lesser extent \( I_K \).
Figure 3.7: Instantaneous current-voltage (I-V) plots for two divisions of the pacemaker cycle
(A) I-V plots from the time of occurrence of the MDP \((t_{MDP})\) to the time of occurrence of the peak overshoot potential \((t_{PO})\); (B) I-V plots belonging to the period from \(t_{PO}\) to \(t_{MDP}\) on the succeeding cycle. Time \((t)\) increases in the direction indicated by the arrows.
Events occurring between the peak overshoot (PO) and the maximum diastolic potential (MDP), ie. the repolarization phase \( t_{PO} < t < t_{MDP} \) are depicted in Panel B of Fig 3.7. Note from the direction of the arrows that both the outward current \( I_K \) and the inward current \( I_{NaCa} \) increase as time increases. Note also that the secondary hump of \( I_{Ca,L} \) is evident in the voltage range \(-51 \leq V \leq 35.5 \text{ mV}\). This current is relatively constant in the plateau range of potentials \( 0 \leq V \leq 35.5 \text{ mV} \) and is effectively zero negative to -51 mV; in contrast, \( I_K \) increases significantly in the plateau range of potentials and is therefore the controlling influence responsible for the initiation of repolarization. The \( I_{Ca,T} \) current contributes only a very small current (maximum \(-2.71pA\)) in the range \(-60.5 \leq V \leq 35.5 \text{ mV}\). Thus, Fig 3.7B clearly shows that \( I_K \) is the dominant current in the repolarization limb of the instantaneous I-V plot.

Fig 3.8 shows that the instantaneous I-V plot on a more sensitive scale during the interval \( t_{MDP} \leq t \leq t_{PO} \). Here the I-V relationships of the smaller currents such as \( I_f \), \( I_{Ca,T} \), \( I_B \), \( I_{NaK} \) and \( I_{CaP} \) may be observed, particularly during the diastolic depolarization phase and the upstroke of the action potential. Increasing time is indicated in this plot by the arrows. Here we observe within the interval \( t_{MDP} \leq t \leq t_{UP} \), \( I_{NaK} \) increases slowly with increasing \( V \); \( I_{CaP} \) drops sharply since \([Ca^{2+}]_i\) is low in this interval; \( I_f \) increases, peaks at \( V=-54\text{ mV} \) and decays thereafter; \( I_{Ca,T} \) increases, peaks at \( V=-47\text{ mV} \), and decreases with further increases in \( V \); and \( I_B \) decreases linearly with \( V \).

In the interval \( t_{UP} \leq t \leq t_{PO} \) \( I_{Ca,L} \) increases dramatically, and as a result \([Ca^{2+}]_i\) increases as well. This causes \( I_{CaP} \) to strengthen as an outward current (Fig 3.8). \( I_{NaK} \) increases gradually with increasing \( V \). Note that \( I_f \) is outward in this voltage range, \( I_{Ca,T} \) decreases and is quite small, and \( I_B \) decreases linearly with increasing transmembrane potential.
Figure 3.8: The instantaneous I-V plot of small currents
This figure shows the I-V relationships of the smaller currents such as $I_f$, $I_{Ca,T}$, $I_B$, $I_{NaK}$ and $I_{CaP}$ on a more sensitive scale during the interval $t_{MDP} \leq t \leq t_{PO}$. 
3.6 Effects of Background Pumps and the Exchanger

The effects of the background pump and exchanger currents on the action potential waveform can be seen in Figure 3.9, where the action potential generated by a reduced or simplified model that contains no pump or exchanger currents (i.e. $I_{NaK} = I_{CaP} = I_{NaCa} = 0$), is compared with that of the complete model. Note that spontaneous pacemaking activity continues with removal of the pump and exchanger currents, but at a faster rate. Thus, Fig 3.9 suggests that $I_{NaK}$, $I_{CaP}$ and $I_{NaCa}$ may have a significant influence on both the magnitude of the maximum diastolic depolarization (MDP) and the rate of the diastolic depolarization. Also, the removal of $I_{NaK}$, $I_{CaP}$ and $I_{NaCa}$ increases the action potential duration very slightly. Table A.9 provides a numerical comparison of selected action potential indices, obtained from the output of the simplified and complete models.

3.7 Simulated Blockade of $I_f$

We have simulated the effect of selective $Cs^+$ blockade of the $I_f$ current by setting $I_f = 0$. Note from Fig 3.10 that complete blockade ($I_f = 0$) produced a 17% change in the cycle length of the spontaneous activity, but did not bring about a cessation of spontaneous pacemaking. Experimental results reported by Denyer and Brown [9] and van Ginneken and Giles [59] on the rabbit SAN myocytes, produced a 23% and a 57% increase in pacemaker cycle length, respectively. Note from Fig 3.10 that the main change in the waveform is in the rate of diastolic depolarization; it is lowered when $I_f$ is blocked.

3.8 Simulated Blockade of $I_{Ca,T}$

$Ni^{2+}$ and tetramethrin (TMR) in appropriate concentrations have been shown to be selective blockers of $I_{Ca,T}$ [27]. We simulated complete blockade in the model
Figure 3.9: Comparison of the model-generated waveforms for the "complete" and "simplified" models

Panel A shows two spontaneous action potential waveforms; the more rapid pacemaker activity is generated by the "simplified" model in the absence of pump and exchanger currents. Panel B shows the pump and exchanger currents of the "complete" model that are responsible for the differences observed in the action potential waveforms in Panel A. A numerical comparison of the "complete" and "simplified" models is given in Table A.9.
Figure 3.10: Effect of $Cs^+$ on the spontaneous activity in the SAN model. This figure shows the model-generated pacemaking waveforms during control (C) and $Cs^+$ blockade of $I_f$ ($I_f=0$).
by setting $I_{Ca,T}=0$. The resulting spontaneous pacemaking waveform is shown in Fig 3.11 A, along with an experimental waveform from Hagiwara et al [26, Fig 1], which was obtained under conditions of $I_{Ca,T}$ blockade using 40μM $Ni^{2+}$. One cycle of this data waveform is shown between the points a and b indicated in this figure. In Panel B, the model-generated pacemaking waveforms during control (C) and complete block of $I_{Ca,T}$ (labeled $Ni^{2+}$-block) are compared. Overall there is a 23% increase in cycle length, which compares very favorably with the 24% increase, measured from the experiments of Hagiwara et al [26]. Note from Fig 3.11B that the main change in the pacemaker waveform under conditions of $Ni^{2+}$-block, is in the lowered rate of phase 4 depolarization. The action potential is affected only slightly as a result of $I_{Ca,T}$ blockade, which implies that it is $I_{Ca,L}$ alone, that is mainly responsible for the shape of the action potential. Thus, our simulations, which are based on the action potential data of Hagiwara et al [26] indicate that there is a separation in function between the sarcolemmal calcium currents: $I_{Ca,T}$ is concerned with the pacemaker potential in phase 4, while $I_{Ca,L}$ is concerned with the action potential.

3.9 Simulated Blockade of $I_B$

If both $I_{Ca,T}$ and $I_f$ are blocked in our model, we observe an 83% increase in the cycle length of the spontaneous oscillations. Earlier we showed that if $I_f$ and $I_{Ca,T}$ are intact and the pump and exchanger currents ($I_{NaK}$, $I_{CaP}$ and $I_{NaCa}$) are blocked, pacemaking activity continues, but at a higher rate (Fig 3.9). Thus removal of either set of these currents did not produce cessation of spontaneous activity. Fig 3.12 shows however, that when the total background current $I_B$ was reduced by 10% (Labeled 0.9C; C=control) pacemaking ceased and membrane potential was steady at -77.1mV. As $I_B$ was reduced further, membrane potential was increased in the hyperpolarizing direction, until for $I_B=0$, it held at a steady level of -97.2mV.
Figure 3.11: Effect of Ni\textsuperscript{2+} on the spontaneous activity in the SAN model. Panel A shows the model-generated and data waveforms during 40\mu M Ni\textsuperscript{2+} application. The data [26, Fig 1] waveform is shown between points "a" and "b". Panel B compares the model-generated pacemaking waveforms during control (C) and Ni\textsuperscript{2+} block of \( I_{Ca,T} \) (\( I_{Ca,T}=0 \)).
Figure 3.12: Effect of blocking the background currents
Control (C) case shows that $I_f$ and $I_{Ca,T}$ are blocked ($I_f = I_{Ca,T} = 0$) at the same time and $I_{B,Na}$ and $I_{B,Ca}$ are not blocked; the period increased by 83%. 0.9C and 0C show the cases where the background currents ($I_{B,Na}$ and $I_{B,Ca}$) are block by 10% and totally, respectively. As it can be seen even small blockades of the background currents along with total blockades of the $I_f$ and $I_{Ca,T}$ abolished the spontaneous activity.
Thus, our model predicts that in the absence of currents such as $I_I$ and $I_{Ca,T}$ during diastolic depolarization, $I_B$ is still an important inward current that can sustain spontaneous pacemaking activity. The net effect produced by the pump and exchanger currents is a hyperpolarizing influence that opposes $I_B$. When these currents are removed as in the case of the 'simplified' model of Fig 3.9, the influence of $I_B$ is stronger and pacemaker rate increases. However, pacemaking ceases and membrane potential is hyperpolarized under the influence of $I_{NaK}$ and $I_{CaP}$, as the magnitude of $I_B$ is significantly decreased.
Chapter 4

Discussions

We have developed a Hodgkin-Huxley-(HH) type model that is based as closely as possible on published voltage clamp data from isolated single rabbit SAN cells. This model contains mathematical descriptions of the ionic transmembrane currents in the SAN cell, as well as characterizations of the membrane pump currents ($I_{NaK}$ and $I_{CaP}$) and a sodium-calcium exchanger current ($I_{NaCa}$). Provision is made for intracellular buffering of $Ca^{2+}$, as well as, the uptake and release of calcium by the sarcoplasmic reticulum (SR). Material balance equations describe the homeostasis of the cytosolic concentrations of $Na^+$, $K^+$ and $Ca^{2+}$. The model can: (1) accurately mimic the voltage clamp data upon which it is based, and with suitable scaling of the transmembrane currents, it can also mimic the waveshape of the SAN cell pacemaker depolarization and action potential (see Fig 3.1); (2) accurately mimic whole-cell voltage-clamp data for the hyperpolarization-activated $I_f$ current (Figs 3.4 and 3.5); (3) simulate the experimental effect of $I_f$ blockade using $Cs^+$ (Fig 3.10), and $I_{Ca,T}$ blockade using $Ni^{2+}$ (Fig 3.11); and (4) simulate the total cessation of spontaneous pacemaking in the SAN cell via reduction in $I_B$, following blockade of both $I_f$ and $I_{Ca,T}$ (Fig 3.12). In fitting SAN action potential data, we have found it most useful to employ the instantaneous current-voltage plot (Figs 3.6, 3.7 and 3.8), which provides a graphical means of supervising the scaling of membrane currents so as to achieve very good nominal fits to both $V$ and $\dot{V}$. This procedure was used in this study to obtain the fits shown for the pacemaking waveform under normal conditions (Fig 3.1), as well as, conditions of $I_{Ca,T}$ blockade using $Ni^{2+}$ (Fig 3.11 A). The data
for both of these spontaneous pacemaking waveforms was taken from Hagiwara et al [26, Fig 1].

4.1 Comparison of SAN Cell Models

Early Hodgkin Huxley (HH) type models of the rabbit SAN cell were based on qualitative descriptions of the $I_{Ca}$, $I_K$ and $I_f$ currents from multicellular preparations. For example, the YNI model and its modified form (Irisawa and Noma (IN) [33]), as well as, the DiFrancesco-Noble model of the mammalian Purkinje fiber [14] and its modification for the rabbit SAN cell by Noble and Noble [48] all were developed before significant single cell voltage clamp results were available. The quantitive data from individual isolated SAN myocytes, which has become available in the last 5 years, has motivated us to develop a more quantitative model of the pacemaker depolarization and action potential of this cell. Our model is similar in structure to that of the DN and NN models, and yet differs from these previous works in the following respects: (1) the DN and NN models are based on data from multicellular preparations rather than individual cells; (2) two calcium currents (T and L-type) rather than one are employed in our rabbit SAN cell model; (3) intracellular buffering of $Ca^{2+}$ by calmodulin and troponin are incorporated in our model; (4) models of $Ca^{2+}$ sequestration by the SR are provided in both models, however our model employs a modified version of the new formulation by Hilgemann and Noble (HN) [28], rather than the SR model contained in the original DN paper [14]; in addition it includes a provision for buffering of calcium by calsequestrin in the SR release compartment; and (5) in our formulation we have not assumed that the intracellular buffers are in steady-state as was assumed in the HN model. In contrast with steady-state formulations, the response of the buffers to transient changes in $[Ca^{2+}]$ can
have a marked effect on both the intracellular calcium waveform and the intracellular calcium-dependent currents, such as $I_{NaCa}$, during the initial rise in $[Ca^{2+}]_i$.

The models discussed above are more complex than the YNI-type model that has been used extensively in studies of the parasympathetic stimulation of pacemaker cells [11, 12, 24, 43, 61]. It is our opinion, that the additional complexity of our model is warranted, if indeed, a better understanding of the pacemaker mechanism, as well as its modulation by autonomic transmitters, is to be achieved. As new techniques provide different forms of data (e.g. $[Ca^{2+}]_i$ as a function of time), more detailed questions will be asked in experimental investigations, regarding the electrochemical behavior of cardiac cells. In fact it seems inevitable, that in the future, even more complex models will be required which are capable of providing more detailed explanations of biophysical phenomena, e.g. intracellular $Ca^{2+}$ transients, and the role of intracellular second messengers.

4.2 Limitations of the Present Model

Some of the more important limitations of our rabbit SAN cell model are as follows:

1. Quantitative measurements of $[Ca^{2+}]_i$ during the pacemaker depolarization and action potential of the rabbit SAN cell, as well as data on calcium-induced release of calcium by the SR to our knowledge have not been published. The Hilgemann and Noble (HIN) [14] model of the mechanisms of calcium uptake and release by the SR to our knowledge mimics both calcium-induced calcium release (CICR) and slow-inward calcium current magnitude. Nonetheless, such formulations of these uptake and release mechanisms is considered only a first approximation, since they cannot be tested and improved upon, on the basis of quantitative experimental data in this cell type;
2. At present, no data is available on $[Ca^{2+}]_i$ in the myoplasm of the rabbit SAN cell. Thus, our assumption of a single lumped intracellular fluid compartment may have to be modified. For example, if spatio-temporal calcium gradients are found within the myoplasm, we will have to adopt a multiple-compartment (shell-type) model to characterize diffusion of $Ca^{2+}$ in the intracellular medium;

3. There is also limited experimental data available on this cell type regarding the magnitude and time course of the sodium-calcium exchanger current ($I_{NaCa}$) and no information regarding the size or voltage dependence of the sarcolemmal calcium pump ($I_{CaP}$) currents;

4. When the cell isolation techniques are applied to nodal tissue, the heterogeneity of cell types within the node, make it difficult to discern exactly which cell type is being recorded from e.g. primary pacemaker cell, a transitional cell or an atrial 'working' cell. More work needs to be done in classifying the various cell types, as well as their electrophysiological behavior. As mentioned previously, our model is based on action potential data that is more characteristic of a transitional pacemaker cell, rather than a primary nodal cell; and

5. Our model would benefit from data obtained using the newly developed 'action potential' clamp technique Doerr et al [17], particularly in the identification of the sizes and waveshapes of some of the important currents in this cell.

Nevertheless, our simulations provide well-integrated explanations of both the electrical activity of the rabbit SAN cell and associated cytosolic ion concentration changes, and point the direction to future modeling work on the cardiac pacemaker cells.
Chapter 5

Conclusions

In short, we have developed a mathematical SAN cell model that consists of two major components (the sarcolemmal model and the fluid compartment model). The sarcolemmal model is composed of the membrane capacitance, the ionic channels \( I_{Ca,L}, I_{Ca,T}, I_K, I_f \) and background, pump, exchanger and chloride currents \( I_B, I_{NaK}, I_{Ca,P}, I_{NaCa}, I_{Cl} \). On the other hand, the mass balance descriptions for ionic species \( Na^+, K^+, Ca^{2+} \), \( Ca^{2+} \) buffering proteins (calmodulin and troponin) and the uptake and release mechanism of \( Ca^{2+} \) by the sarcoplasmic reticulum (SR) define the fluid compartment model for the cellular medium. Each element of our model incorporates recently published data and is mathematically well-defined. The conclusions of our SAN model are:

1. Our model can mimic (a) the voltage-clamp data on which it is based and (b) the action potential data of Hagiwara et al [26, Fig 1]. Also, in these fits, we found it very beneficial to use the instantaneous current-voltage plots (Figs 3.6, 3.7 and 3.8); in these plots the contribution of each current to the action potential can be seen quite well along with the current-time plots (Fig 3.2);

2. Our model can mimic the newly acquired voltage-clamp data for the hyperpolarization activated \( I_f \) current [59] (see Figs 3.4 and 3.5);

3. Our model can show the effect of \( I_f \) blockade using \( Cs^+ \). The main effect of \( I_f \) blockade is the change in the rate of diastolic depolarization. Total \( I_f \) blockade
caused 17% increase in the cycle length of the spontaneous activity in our model (Fig 3.10);

4. Our model can simulate the effect of $I_{Ca,T}$ blockade using $Ni^{2+}$ and it mimics the $Ni^{2+}$ block data of Hagiwara et al [26, Fig 1] quite well (see Fig 3.11). The effect of this blockade, derived from our work is that $I_{Ca,T}$ changes the rate of phase 4 depolarization; in our model $Ni^{2+}$ blockade caused 23% increase in the cycle length of the spontaneous activity. Most important conclusion of this $Ni^{2+}$ blockade simulation is that effectively, $I_{Ca,T}$ is concerned with the pacemaker potential whereas $I_{Ca,L}$ is concerned the action potential;

5. Our model can also simulate the effects of the membrane pump and exchanger currents ($I_{NaK}$, $I_{CaP}$, $I_{NaCa}$) on the spontaneous pacemaker activity. When $I_{NaK}$, $I_{CaP}$ and $I_{NaCa}$ are removed from the model, the SAN cell oscillates at a higher rate (Fig 3.9); and

6. Our model can simulate the total cessation of the spontaneous pacemaking by the partial blockade of the total background current $I_B$, in the presence of total blockades of the $I_{Ca,T}$ and $I_f$ currents (Fig 3.12).

Since our model is well defined in many aspects and incorporates recently published experimental results from whole-cell voltage-clamp and action potential studies, it can have the following important extensions:

1. The models of the other rabbit pacemaker cells (the atrioventricular node (AV) and the crista terminalis (CT)) can be developed on the basis of our SAN cell model;

2. The effects of the the $\beta$-adrenergic agonists (NE,ISO) and of the cholinergic agonists (ACh) on the SAN cell activity can be investigated.
Appendix A

SAN Cell Model Equations

The equations describing the rabbit SAN cell model are contained within this Appendix. Expressions for the inward and outward transmembrane currents are contained in Tables A.1 and A.2, respectively. The equations in Table A.3 represent the pump and exchanger currents. The background current, $I_B$, and the hyperpolarization-activated current $I_f$ are given in Table A.4 and the compartmental ion flux equations in Table A.5. Equations in Table A.6 describe the calcium uptake and release mechanism in the sarcoplasmic reticulum. A listing of model parameter values and initial conditions necessary to run the model is provided in Table A.7. Table A.8 gives the comparison between the experimental and model-generated action potentials and Table A.9 has the comparison of the action potentials in the "complete" and "simplified" SAN cell models.
Table A.1: Calcium Currents: $I_{Ca,L}$ and $I_{Ca,T}$

\[
I_{Ca,L} = g_{Ca,L} (d_L f_L + 0.065d')(V - 46.4)
\]

\[
\alpha_{dL} = \frac{-10.45(V+35)}{\exp((V+35)/-2.5)-1} + \frac{-31.25V}{\exp(-0.208V-1)}
\]

\[
\tau_{dL} = \frac{1}{\alpha_{dL} + \beta_{dL}}
\]

\[
\alpha_{fL} = \frac{3.125(V+28)}{\exp((V+28)/4)-1}
\]

\[
\tau_{fL} = \frac{1}{\alpha_{fL} + \beta_{fL}} - 0.002
\]

\[
\alpha_{dT} = 100 \exp((V + 26.3)/12)
\]

\[
\tau_{dT} = \frac{1}{\alpha_{dT} + \beta_{dT}}
\]

\[
\alpha_{fT} = 15 \exp((V + 61.7)/ -11.2)
\]

\[
\tau_{fT} = \frac{1}{\alpha_{fT} + \beta_{fT}} + 0.002
\]

\[
I_{Ca} = I_{Ca,L} + I_{Ca,T}
\]

\[
I_{Ca,T} = g_{Ca,T} d_T f_T (V - 45.0)
\]

\[
\beta_{dL} = \frac{4.21(V-5)}{\exp(0.4(V-5))-1}
\]

\[
\beta_{fL} = \frac{25}{1 + \exp((V+28)/-4)}
\]

\[
\beta_{dT} = \frac{100 \exp((V + 26.3)/-12)}{1 + \exp((V+30)/5)}
\]

\[
\beta_{fT} = 15 \exp((V + 61.7)/11.2)
\]

\[
\beta_{fT} = \frac{\alpha_{fT}}{\alpha_{fT} + \beta_{fT}}
\]
### Table A.2: \( I_K \): Delayed Rectifier \( K^+ \) Current

\[
I_K = g_K p_a p_i (V - E_K) \quad \alpha_{p_i} = 100 \exp(-0.0183V)
\]

\[
\bar{p}_a = \frac{1}{1 + \exp\left(\frac{V - E_K}{\tau_{p_a}}\right)} \quad \beta_{p_i} = 656 \exp(0.00942V)
\]

\[
\tau_{p_a^{-1}} = 17 \exp(0.0398V) + 2.11 \exp(-0.0510V) \quad \dot{p}_a = \frac{(\bar{p}_a - p_a)}{\tau_{p_a}}
\]

\[
E_K = 26.71 \log \frac{[K_i]}{[K_o]} \quad \dot{p}_i = \alpha_{p_i}(1 - p_i) - \beta_{p_i} p_i
\]

### Table A.3: \( Na^+/K^+ \) and \( Ca^{2+} \) Pump and \( Na^+/Ca^{2+} \) Exchanger Currents

#### \( I_{NaK} \): Sodium Potassium Pump

\[
I_{NaK} = \bar{I}_{NaK} \left( \frac{[Na]_i}{[Na]_i + k_m Na} \right)^3 \left( \frac{[K]_o}{[K]_o + k_m K} \right)^2 \left( \frac{V + 150}{V + 200} \right)
\]

#### \( I_{CaP} \): Calcium Pump

\[
I_{CaP} = \bar{I}_{CaP} \left( \frac{[Ca]_i}{[Ca]_i + 0.001} \right)
\]

#### \( I_{NaCa} \): Sodium Calcium Exchanger

\[
I_{NaCa} = \frac{k_{NaCa} ([Na]_i^3 [Ca]_o \exp(0.03743V \gamma) - [Na]_o^3 [Ca]_i \exp(0.03743V(\gamma - 1)))}{1 + d_{NaCa} ([Ca]_i [Na]_o^3 + [Ca]_o [Na]_i^3)}
\]
Table A.4: Background ($I_B$), Outwardly-rectifying Chloride ($I_{CI}$) and Hyperpolarization-Activated ($I_f$) Currents

<table>
<thead>
<tr>
<th>Sodium ($I_{B_{Na}}$) and Calcium ($I_{B_{Ca}}$) Background Currents</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{B_{Na}} = g_{B_{Na}}(V - E_{Na})$</td>
</tr>
<tr>
<td>$I_{B_{Ca}} = g_{B_{Ca}}(V - E_{Ca})$</td>
</tr>
<tr>
<td>$I_B = I_{B_{Na}} + I_{B_{Ca}}$</td>
</tr>
</tbody>
</table>

$I_{CI}$: Outwardly-rectifying Chloride Current

$I_{CI} = g_{CI}(V + 33.6789) \frac{1}{1 + \exp((V + 59.9)/-73.1)}$

$I_f$: Hyperpolarization-Activated Current

$I_f = g_f \cdot y^2(V - 30)$

$\bar{y} = \frac{1}{1 + \exp((V + 72.2)/9)}$

$\tau_y^{-1} = \exp((V + 54.06)/ - 24.33) + \frac{8.5}{0.7 + \exp((V + 60)/-5.5)}$
### Table A.5: Compartmental Equations

<table>
<thead>
<tr>
<th>Intracellular Concentrations of $Na^+$ and $K^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[Na]<em>i = \frac{-3I</em>{NaK} - 3I_{NaCa} - I_{BNa}}{FV_i}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intracellular Calcium Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{\Phi}_C = 10^5 [Ca]_i (1 - \Phi_C) - 238 \Phi_C$</td>
</tr>
<tr>
<td>$\dot{\Phi}<em>{TC} = 39000 [Ca]<em>i (1 - \Phi</em>{TC}) - 196 \Phi</em>{TC}$</td>
</tr>
<tr>
<td>$\dot{\Phi}<em>{TMgC} = 10^5 [Ca]<em>i (1 - \Phi</em>{TMgC} - \Phi</em>{TMgM}) - 3.3 \Phi_{TMgC}$</td>
</tr>
<tr>
<td>$\dot{\Phi}<em>{TMgM} = 1000 [Mg]<em>i (1 - \Phi</em>{TMgC} - \Phi</em>{TMgM}) - 333 \Phi_{TMgM}$</td>
</tr>
<tr>
<td>$\Phi_B = 0.045 \dot{\Phi}<em>C + 0.031 \dot{\Phi}</em>{TC} + 0.062 \dot{\Phi}_{TMgC}$</td>
</tr>
<tr>
<td>$[\dot{Ca}]<em>i = \frac{2I</em>{NaCa} - I_{Ca} - I_{CaP} - I_{B,Zn} - I_{rel}}{2V_{Ca F}} - \Phi_B$</td>
</tr>
</tbody>
</table>
### Table A.6: SR Calcium Uptake and Release

\[
\begin{align*}
[Ca]_{up} &= \frac{I_{up} - I_{tr}}{2V_{up} F} \\
[Ca]_{rel} &= \frac{I_{tr} - I_{rel}}{2V_{rel} F} \\
I_{rel} &= \alpha_{rel} \left( \frac{F_3}{F_2 + 0.25} \right)^2 [Ca]_{rel} \\
I_{tr} &= ( [Ca]_{up} - [Ca]_{rel} ) \frac{2F_{V_{eal}}}{0.08} \\
K_1 &= \frac{k_{cyca} k_{xes}}{k_{sca}} \\
K_2 &= [Ca]_i + [Ca]_{up} K_1 + k_{cyca} k_{xes} + k_{cyca} \\
r_{act} &= 25(\exp(V - 40)/12.5) + 75\left( [Ca]_{rel} \right)^2 \\
r_{inact} &= 8.3 + 125\left( \frac{[Ca]_i}{[Ca]_i + k_r} \right)^2 \\
\dot{F}_1 &= 0.625 \cdot F_3 - r_{act} F_1 \\
\dot{F}_2 &= r_{act} F_1 - r_{inact} F_2 \\
\dot{F}_3 &= r_{inact} F_2 - 0.625 \cdot F_3 \\
I_{up} &= \frac{\alpha_{up}[Ca_i] - \beta_{up}[Ca]_{up} K_1}{K_2}
\end{align*}
\]
Table A.7: Model Constants and Initial Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>$V_i$</td>
<td>3.49 picoliters</td>
</tr>
<tr>
<td>$V_{Ca}$</td>
<td>0.8$V_i$ = 1.29 picoliters</td>
</tr>
<tr>
<td>$F$</td>
<td>96487 coul/mole</td>
</tr>
<tr>
<td>$g_{Ca,i}$</td>
<td>21.27 nS</td>
</tr>
<tr>
<td>$g_{Na,B}$</td>
<td>12.49 pS</td>
</tr>
<tr>
<td>$g_K$</td>
<td>0.0103 $[K^+]_o^{0.59}$</td>
</tr>
<tr>
<td>$k_{NaCa}$</td>
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</tr>
<tr>
<td>$k_{mNa,a}$</td>
<td>40 mM</td>
</tr>
<tr>
<td>$T_{Na,K}$</td>
<td>7.446 nA</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>140 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>2 mM</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>7.5 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_i$</td>
<td>0.1 μM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{rel}$</td>
<td>1 mM</td>
</tr>
<tr>
<td>$F_2$</td>
<td>0</td>
</tr>
<tr>
<td>$\alpha_{up}$</td>
<td>0.05 nA</td>
</tr>
<tr>
<td>$k_{cyc}a$</td>
<td>0.002 mM</td>
</tr>
<tr>
<td>$k_{xcs}$</td>
<td>0.4</td>
</tr>
<tr>
<td>$k_r$</td>
<td>0.008 mM</td>
</tr>
<tr>
<td>$g_f$</td>
<td>7.85 nS</td>
</tr>
<tr>
<td>$V_i$</td>
<td>0.46$V_i$ = 1.62 picoliters</td>
</tr>
<tr>
<td>Temperature</td>
<td>$37^\circ C$</td>
</tr>
<tr>
<td>$R$</td>
<td>8.314 J/mole · K</td>
</tr>
<tr>
<td>$g_{Ca,t}$</td>
<td>8.18 nS</td>
</tr>
<tr>
<td>$g_{Ca,B}$</td>
<td>5.08 pS</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>0.5</td>
</tr>
<tr>
<td>$d_{NaCa}$</td>
<td>0.001</td>
</tr>
<tr>
<td>$k_{mK}$</td>
<td>1 mM</td>
</tr>
<tr>
<td>$T_{Ca,P}$</td>
<td>0.036 nA</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>2.4 mM</td>
</tr>
<tr>
<td>$C_m$</td>
<td>55 pF</td>
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<tr>
<td>$[K^+]_i$</td>
<td>140 mM</td>
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<tr>
<td>$[Ca^{2+}]_{up}$</td>
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</tr>
<tr>
<td>$F_1$</td>
<td>0.1</td>
</tr>
<tr>
<td>$F_1$</td>
<td>0.9</td>
</tr>
<tr>
<td>$\beta_{up}$</td>
<td>0.4$\alpha_{up}$</td>
</tr>
<tr>
<td>$k_{srcs}$</td>
<td>2.0 mM</td>
</tr>
<tr>
<td>$\alpha_{rel}$</td>
<td>9 nA/mM</td>
</tr>
<tr>
<td>$k_r$</td>
<td>0.008 mM</td>
</tr>
<tr>
<td>$[Mg^{2+}]_i$</td>
<td>2.5 mM</td>
</tr>
<tr>
<td>$g_{Cl}$</td>
<td>0.254 nS</td>
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</table>
Table A.8: Comparison of Experimental and Model-generated Action Potentials

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Max. Diastolic Potential</td>
<td>$-63.1mV$</td>
<td>$-60.5mV$</td>
<td>$-57.9\pm1.8$</td>
</tr>
<tr>
<td>Overshoot</td>
<td>$35.5mV$</td>
<td>$34.5mV$</td>
<td>$31.9\pm1.95mV$</td>
</tr>
<tr>
<td>Duration</td>
<td>$272\text{msec}$</td>
<td>$279\text{msec}$</td>
<td>$335.4\pm15\text{msec}$</td>
</tr>
<tr>
<td>Max. Upstroke Velocity</td>
<td>$9.6\text{ V/sec}$</td>
<td>$9.8\text{V/sec}$</td>
<td>$12.8\pm1.65\text{ V/sec}$</td>
</tr>
</tbody>
</table>

Table A.9: Comparison of Action Potentials in Complete and Simplified SAN Models

<table>
<thead>
<tr>
<th></th>
<th>Complete Model</th>
<th>Simplified Model</th>
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</thead>
<tbody>
<tr>
<td>Max. Diastolic Potential</td>
<td>$-60.5mV$</td>
<td>$-71.4mV$</td>
</tr>
<tr>
<td>Overshoot</td>
<td>$34.5mV$</td>
<td>$37.9mV$</td>
</tr>
<tr>
<td>Duration</td>
<td>$279\text{msec}$</td>
<td>$263\text{msec}$</td>
</tr>
</tbody>
</table>
Bibliography


