A Novel Computational Model of Pacemaker Activity in the Mouse Atrioventricular Node Cell

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Abstract

Nowadays, mathematical modeling has been one of the improvements in technologically advanced science in supporting decision-making in different healthcare scenarios. In the field of numerical modelling of heart electrophysiology, several models of action potential (AP) have been developed for cardiac chambers of different species. The atrioventricular node (AVN) acts as a subsidiary pacemaker and controls impulse conduction between the atria and ventricles. Despite its physiological importance, limited data are available for computing AVN cellular electrophysiology. Further, the ionic mechanisms underlying the automaticity of AVN myocytes are incompletely understood.

Only two computational models of AVN have been developed in the last decades (one for rabbit, the other for mouse but without calcium handling). We aimed to develop a new mouse AVN model.

We thus build on the preliminary AP mouse AVN model published by Marger et al., which has been updated and improved, by implementing more realistic cellular compartments and calculation of dynamics and handling of intracellular Ca^{2+}. The new model reproduces almost all the AVN AP hallmarks and has been used to simulate the effects of blockade of ionic currents involved in AVN pacemaking.

1. Introduction

Computational cardiology is one of the different fields where mathematical modeling has a translational interest. Numerical modelling allows integration of experimental data of cardiac electrophysiology in models raging from nano- to macro-scale scale levels. Therefore, computational cardiology is a powerful diagnostic and research tool for the treatment of heart disease.

The atrioventricular node (AVN) is a key component of the cardiac pacemaker-conduction system in mammalian hearts. It is normally the sole pathway for impulse propagation from atria to ventricles and its slow conduction properties can also serve a protective function during some supraventricular tachyarrhythmias [1]. However, the cellular electrophysiological basis of AVN pacemaking is still poorly understood.

Nowadays, the development of computer electrophysiology can reproduce action potential (AP) and conduction properties in a variety of species and cell types. Although many AP models have been developed for most heart regions [2], the AVN node has not been widely studied from the computational cardiology point of view.

In 2009, the first biophysically detailed model of the AVN was published by Inada et al., based on experimental data from the rabbit AVN [3]. To identify and distinguish general and species-specific properties, however, it is desirable to have AVN cellular electrophysiology data from additional model species accessible to gene targeting techniques. In 2011, Marger et al. [4] proposed a mouse AVN cell model to provide insights into the roles of L-type $Ca_v1.3$, T-type $Ca_v3.1$, and "funny" f-(HCN) channels. This model simulated the AP of isolated mouse AVN myocytes, but neglected cell compartmentalization and did not provide calculation of intracellular Ca^{2+} concentration and dynamics.

The purpose of this work is to obtain a more detailed and complete computational model of the AP of mouse single AVN cells, by building from the Marger et al. model [4]. The introduction of calculations of calcium handling is paramount to generate a full set of AVN single-cell APs, due to the role that calcium has related to cellular pacemaker activity and contractility. Implementation of cellular compartments and calculation of intracellular Ca^{2+} dynamics allowed us to generate a 2^{nd} -generation model of mouse AVN pacemaking. Importantly, the model reproduced experimental AVN AP hallmarks. We used the model to simulate experimentally observed genetic deletion or blockade of ionic currents involved in the pacemaking of AVN cells.

2. Methods

The present work used as a starting point the mouse AVN model published by Marger et al. [4], which was elaborated by adapting the mouse sinoatrial node cell model (SAN) [5], by implementing the densities and activation of ionic currents recorded in native mouse AVN cells. This model has been used to simulate the AP behavior of an AVN cell but lacked two important missing components: a realistic cell's compartmentalization (which includes the sarcoplasmic reticulum (SR) and the subsarcolemmal space) and dynamic calculation of intracellular ion concentrations.

To improve the description, we inserted in the preliminary AVN model, the equations to calculate calcium handling. The equations describing calcium handling were taken from the AP mouse SAN single-cell model published by Kharche et al. [6].

Figure 1 shows a schematic representation of the different elements included in the present model. Four compartments are considered: the SR, divided into junctional and network spaces (JSR and NSR, 0.12% and 1.16% of the whole cell volume, respectively), the subsarcolemmal calcium subspace, and the cytosol. The calcium subspace is considered as an independent intracellular compartment (1% of the total cell volume) because of the transient local calcium accumulation taking place upon release from the SR.

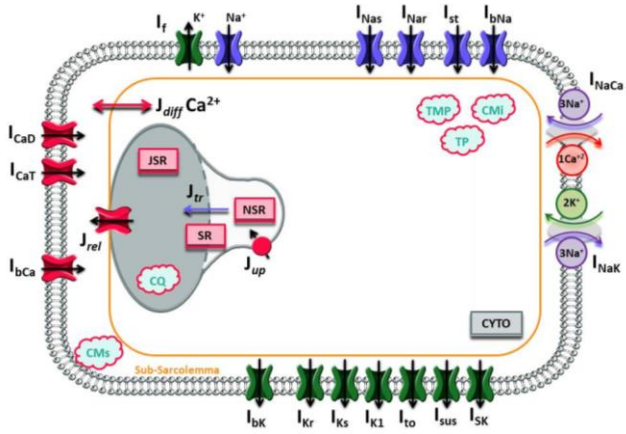

Figure 1. Schematic diagram of the mouse AVN single-cell model.

The cell geometry was kept as a cylindrical structure where the length and radius of the cell were derived from the total membrane capacitance. This was accomplished by fixing both the membrane capacitance (to 22pF, as reported in experiments [7,8]) and the cell radius (to 5µm), and by calculating the cell length using the cylindrical description and the specific membrane capacitance of 1 µF/cm^2.

All the intracellular concentrations (Na_i^+, Ca_i^{2+}, K_i^+) have been described with dynamic equations and calcium buffering (troponin Ca^{2+} site, [TC] and troponin Mg^{2+} site [TMC] in the cytosol, calmodulin located in the subspace [CM_s] and cytosol [CM_i] and calsequestrin (CQ) in JSR) was included. Because of subspace and SR existence, four fluxes controlling calcium handling and balance have been added: the Sarco-Endoplasmic Reticulum Calcium ATPase pump (SERCA, J_{up}), the flux between NSR and JSR (J_{tr}), the release from JSR to the subsarcolemmal subspace (J_{rel}), and the Ca^{2+} diffusion from subspace to the whole cytosol (J_{diffCa}).

The formulations for the ionic currents were taken from the preliminary mouse AVN single-cell model [5], as Hodking-Hukley type of formulation. Experimental data [8], as current-to-voltage (I-V) curves from mouse AVN single cells, were used to reformulate ionic currents in the new model to obtain a more realistic description. The ionic currents considered for reformulation are as follows:

- I_{Nar}, inward TTX-resistant component of Na^+ current
- I_{Nas}, TTX-sensitive component of Na^+ current
- I_{CaD}, inward Ca^{2+} current through L-type $Cav1.3$ channels
- I_{CaT}, inward Ca^{2+} current through T-type $Cav3.1$ channels
- I_{Kr}, outward rapid delayed rectifier K^+ current
- I_{to}, transient outward K^+ current
- I_f, $Na^+$and K^+ currents activated by hyperpolarization ("funny" current).

Model differential equations were implemented in Matlab (Mathworks Inc., Natick, MA, USA) and solved with a variable order solver (*ode23s*), based on numerical differentiation formulas [9], with a time step of 1ms. The pacemaking was calculated for 300s to ensure reaching of steady-state. Figures show the last second of the simulation. The AP hallmarks are: beating rate (beats per minutes, bpm), maximum diastolic potential (MDP), AP amplitude (APA), and AP duration (APD_X measured once membrane voltage reached X% of the resting). AP hallmarks were calculated as averaged values from the last second of the steady-state simulation at 300s.

3. Results

The present model simulates the AP of a mouse AVN cell, as shown in Figure 2. Simulated AP hallmarks are compared to experimental data in Table 1. Despite the lack of experimental data on intracellular Ca^{2+} transients in mouse AVN cells, the model did compute Ca^{2+} dynamics, as reported in Figure 2B-E.

With this updated model we have tested the effects of blockade of some ionic currents involved in pacemaking.

In particular, the impact of a 50% and 90% block of the I_f current is analyzed in Figure 3. Although this current is not the only one responsible for automaticity, it is considered one of the most important for generating pacemaker activity.

Therefore, it is expected that reducing its conductance makes beating frequency slow down. As expected, a reduction in I_f considerably slows down the rate of pacemaker activity (CL= 318 vs 457ms), and a 90% block stops the cell from beating (Figure 3, red line).

We tested the block of I_{CaD} current in Figure 4: with a 60% reduction is confirmed the cessation of the pacemaker activity and the cell membrane potential depolarized around -30mV, as experimentally observed [8].

The block of the I_{Kr} current slows AP beating as shown in Figure 5 (CL=318 vs 343ms), as experimentally reported in [10]. As expected, APD_{90} is prolonged with respect to the control. Moreover, a total block of I_{Kr} stops beating.

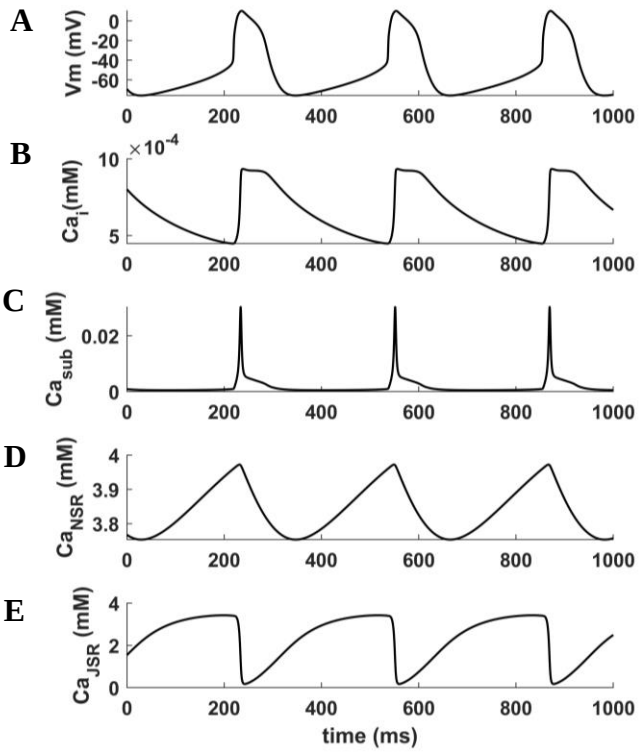

Figure 2. (A) Simulated action potential of AVN single-cell model. **(B)** Ca^{2+} transient in the cytosol. **(C)** Ca^{2+} transient in the subspace. **(D-E)** Ca^{2+} transient in the NSR and JSR.

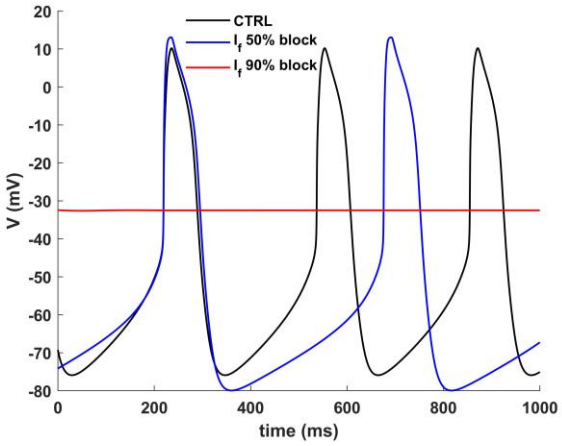

Figure 3. AP with I_f in normal condition (black line), with 50% I_f block (blue line) and 90% I_f block (red line).

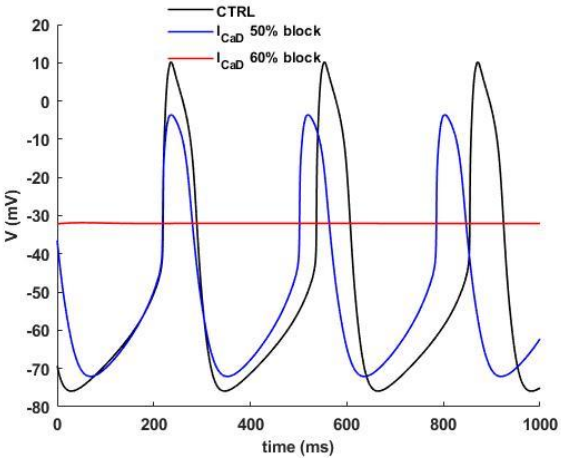

Figure 4. AP with I_{CaD} in normal condition (black line), and with 50% I_{CaD} block (blue line) and 60% (red line).

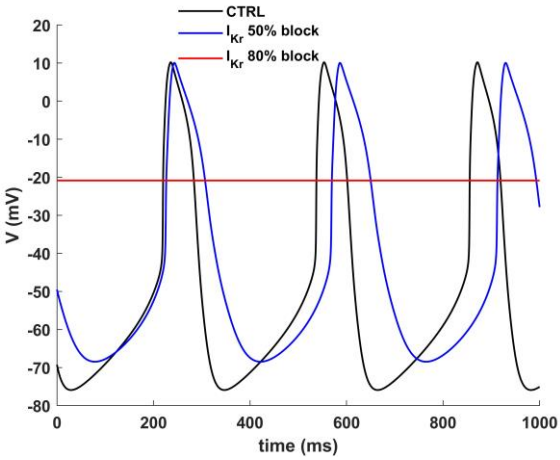

Figure 5. AP with I_{Kr} in normal condition (black line), with 50% I_{Kr} block (blue line), and with 80% I_{Kr} block (red line).

	Experimental Data		Present Model
	[8]	[7]	
Rate (bpm)	173±27	--	189
MDP (mV)	-57±1	-62±1	-76
APA (mV)	91±7	76±3	86
Eth (mV)	-41±2	--	-46
dV/dt (mV/ms)	13±3	--	18
APD_{30} (ms)	--	25±4	68
APD_{50} (ms)	--	37±6	77
APD_{70} (ms)	--	60±6	87
APD_{90} (ms)	--	100±8	103

Table 1. Comparison between experimental biomarkers and the values obtained with the present model.

4. Discussion and Conclusions

In this study, based on experimental data, we have developed a single cell AP mouse model for AVN; the AP model is comparable to the APs recorded from the mouse AVN (Figure 2A, Table 1). The current formulations have been adapted according to densities and kinetics recorded in native AVN mouse cells [8].

The present model was validated by confirming some previous results in terms of the block of ionic currents and investigating their impact on the AP.

Despite the lower density of the "funny" current in AVN compared to the SAN, HCN-mediated I_f significantly contributed to AVN pacemaking. To test its contribution we performed simulations by blocking I_f of 50% or 90% (Figure 3). In the first case, a slower rate is obtained followed by the arrest of cell beating under the condition of the almost total current block.

Moreover, we tested the blockade of I_{CaD} (Figure 4). This current was found to be important for AVN pacemaking since its blockade arrests automaticity.

Finally, we have investigated the contribution of I_{Kr} to pacemaking. When I_{Kr} was reduced to half maximum conductance the cell slowed the beating (Figure 5), and increasing the percentage of blocking the AP stops, confirming that also I_{Kr} current affects AVN pacemaker activity.

In conclusion, our work proposes a new updated version of a mouse AVN single-cell AP. This model reproduces experimentally measured AP hallmarks and can be used to simulate the blockade of ionic currents to help understand the pacemaking mechanisms in mouse AVN.

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