Calcium Leak Induced Sinus Bradycardia

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Abstract

Bradycardia is found to be a complication during catecholaminergic polymorphic ventricular tachycardia in which calcium leak plays a pivotal role. In this computational study, we determined the effects of sarcoplasmic reticulum calcium leak on sino-atrial node and ventricular model cells function. A sarcoplasmic reticulum calcium leak current, J_{leak}, was increased in sino-atrial node and ventricle model cells. The J_{leak} current is determined by v_2, the calcium leak rate constant from the net sarcoplasmic reticulum. In the sino-atrial node cell model, the pacing cycle length increased steadily till v_2 values became 3.1×10^{-5} ms^{-1}. Further increase of v_2 made pacemaking give rise to long-short, big amplitude-small amplitude oscillations as well as arrest. The amplitude of subspace calcium, calcium diffusion, maximum upstroke velocity of the membrane potential, L-type calcium current and Na^+/Ca^{2+} exchanger current were decreased when v_2 was increased in sino-atrial node cell model. However, the effects of J_{leak} on ventricular action potential and ionic currents are small. The results show the significance of calcium leak as a major mechanism of sino-atrial node dysfunction.

1. Introduction

Bradycardia is a debilitating cardiac arrhythmia that present in many forms of cardiac disease [1, 2]. Clinical treatment of bradycardia is a significant burden on health care systems [2]. Therefore, it is crucial to understand the mechanisms of this disease to improve clinical therapies.

Ryanodine receptor channels located on the intracellular sarcoplasmic reticulum (SR) regulate intracellular calcium homeostasis [3]. Increased SR calcium (Ca^{2+}) leak increases cytosolic calcium content. The increased Ca^{2+} content has apparently paradoxical effects [4]. On one hand, it is known to lead to sino-atrial node (SAN) bradycardia. On the other hand, it gives rise to ectopic beats and delayed after depolarisations in atrial tissue [5, 6].

However, the mechanisms underlying Ca^{2+} leak and arrhythmias are poorly understood. The current study focused on the effects of SR calcium leak by using mouse SAN model [7] and ventricular cell model [8]. The results of simulation showed that increased Ca^{2+} leak from SR leading to bradycardia and cardiac arrest in SAN.

2. Methods

Detailed mathematical models of mouse sinoatrial node and ventricle electrophysiology were adopted in this study. The SAN cell model published in our laboratory [7] was adopted to reproduce SAN pacemaking. Under control conditions, the SAN model does not encompass a calcium leak from the SR to the cytosol. We therefore modified the model by adding a SR calcium leak current, J_{leak}, to the calcium dynamics of the model by using the following formulation (Eq.1). The Bondarenko model already has a J_{leak} component in the calcium dynamics. In the formulation, v_2 is Ca^{2+} leak rate constant from the net sarcoplasmic reticulum (NSR), Ca_{up} is the Ca^{2+} concentration in NSR, Ca_i is the Ca^{2+} concentration in the cytosol, J_{up} is Ca^{2+} uptake flux from the cytoplasm to the NSR, J_{tr} is Ca^{2+} transfer flux from the NSR to the junctional SR (JSR), v_{rel} is volume of the JSR, v_{up} is volume of the NSR, v_{sub} is volume of the subspace, J_{cadif} is Ca^{2+} diffusion flux from the subspace to the myoplasm, v_i is the myoplasmic volume available for Ca^{2+} diffusion, C_m is the capacitance, J_{trpn} is Ca^{2+} flux to troponin, I_{NaCa} is the Na^+/Ca^{2+} exchange current, I_{pCa} is the Ca^{2+} pump current, I_{Cab} is the background Ca^{2+} currents and v_{myo} is myoplasmic volume.

Eq.1.

Sino-atrial node

$$J_{leak} = \nu_2(Ca_{up} - Ca_i)$$

$$\frac{d}{dt}Ca_{up} = -J_{leak} + J_{up} - J_{tr}\nu_{rel}/\nu_{up}$$

$$\frac{d}{dt}Ca_i = (J_{leak} + J_{Cadif}\nu_{sub} - J_{up}\nu_{up})/vi$$

$$+ \text{ buffering terms.}$$

Ventricle

$$J_{leak} = \nu_2(Ca_{up} - Ca_i)$$

$$\frac{d}{dt}Ca_{up} = (J_{up} - J_{leak})\nu_{myo}/\nu_{up} - J_{tr}\nu_{rel}/\nu_{up}$$

$$\frac{d}{dt}Ca_i = bi(J_{leak} + J_{xfer} - J_{up} - J_{trpn} - (I_{Cab} - 2I_{NaCa} + I_{pCa})\frac{A\,C_m}{2\,\nu_{myo}\,F})$$

The model of Bondarenko et al. [8] that simulates ventricle APs was adopted to permit a simulation of mouse ventricle electrophysiology. During pacing of excitable cells by Bondarenko, a stimulus of -80 μA/μF is applied for a duration of 0.5 ms. The model is paced with a ion charge conservative current stimulus carried by potassium [10]. The SAN and ventricle model were encoded into the Beatbox simulation package as described previously[11].

The model behaviour was studied by increasing the maximum amount of J_{leak} (ν_2) in the two models. In a first set of simulations, J_{leak} was increased from basal values till either SAN pacemaking was affected, or when the excitable cell model demonstrated an increase of action potential duration at 90% of repolarization (APD_{90}). The J_{leak} was then increased and reduced in a large range beyond physiological limits to elicit model behaviour in an extensive range of J_{leak} conditions. To quantify the effects of J_{leak}, we observed the characteristics of SR Ca^{2+}, cytosolic Ca^{2+}, and AP properties. This included minimum and maximum values during an AP. The AP itself was characterised by minimum diastolic potential, take off potential, peak potential, and APD_{90}.

The ordinary differential equations of the two respective models were integrated using a simple forward Euler integration method. The time step for the SAN model was taken to be 0.0025 ms, whereas for the ventricle model was taken to be 0.0001 ms. In both models, measurements were performed on the 100th excitation which was assumed to be a steady oscillatory or resting response to the given value of J_{leak}. The simulations were performed on a standard PC using Ubuntu Linux and GNU C compiler. The figures were prepared using SigmaPlot.

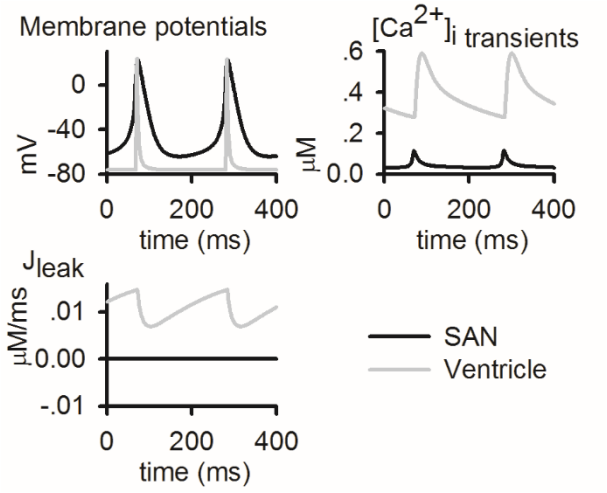

Figure 1. Membrane potential, calcium transient, and SR leak current in the control model. The SAN model has a cycle length of 233.3 ms. The ventricle model has an APD90 of 30 ms. B: The calcium transient in the SAN model is smaller than in the ventricle model. Ventricle has a J_{leak} at about 0.01μM/ms.

Table 1. Basal cell model properties.

	SAN	Ventricle
CL(SAN)/APD90(Vent), ms	233.3	30
dV/dtmax	8.2 to 32	147
Minimum potential, mV	-60	-75.9
Maximum potential, mV	25	23.7
Take off potential, mV	-48.45	/
Diatolic Ca_i, μM	0.03	0.25
Systolic Ca_i, μM	0.11	0.57
Diatolic Ca_{up}, μM	1734.3	1087.1
Systolic Ca_{up}, μM	1399.1	1069.0
Peak I_{CaL}, pA/pF	7.2	10.9
Peak I_{NaCa}, pA/pF	2.9	0.11
J_{leak}, μM/ms	0	0.01

3. Results

3.1. Basal model behavior

Control SAN and ventricle membrane potentials are shown in Figure 1. Table 1 shows basal model properties for APs, Ca^{2+} transients and J_{leak}. The simulated SAN and ventricle membrane potentials have the same features of isolated mouse SAN and ventricle cells. The SAN and ventricle model produces $[Ca^{2+}]_i$ oscillations with an amplitude of 0.1 μM and 0.6 μM, which are close to experimental data [12].

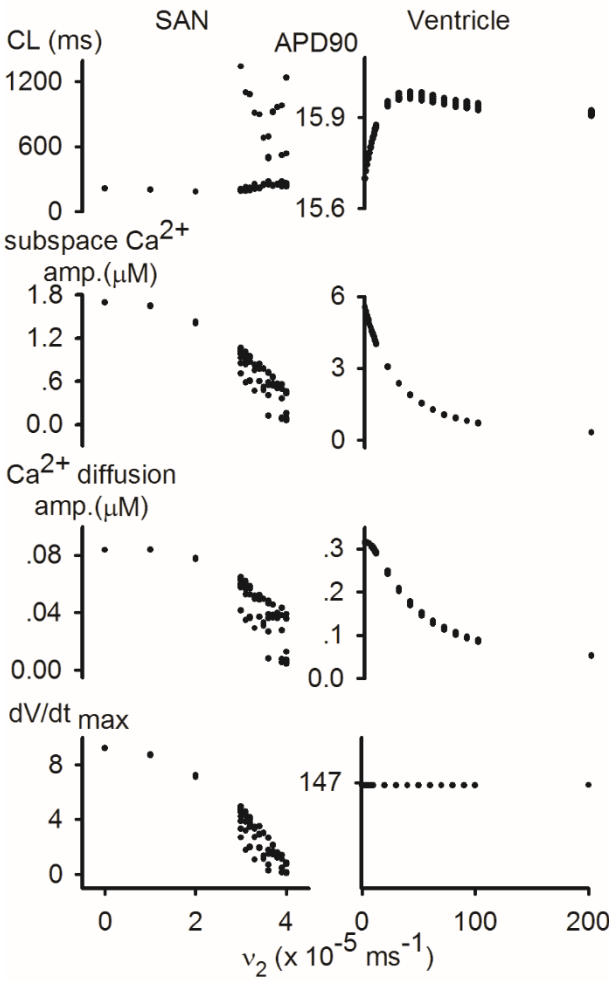

Figure 2. Cycle length (CL), action potential duration 90 (APD90), amplitude of subspace calcium, amplitude of calcium diffusion and dV/dt_{max} in SAN and/or ventricle model with different J_{leak}. CL in SAN model increased significantly following increased J_{leak}, accompanied with decrease of subspace calcium, calcium diffusion and dV/dt_{max}. In ventricle cell model, subspace calcium and calcium diffusion are also decreased due to increased J_{leak}. There are no significantly changes of APD90 and dV/dt_{max} in ventricle by increased J_{leak}.

3.2. AP and Ca^{2+} alterations because of a J_{leak}

APs in SAN and ventricle cell model were simulated at various values of J_{leak}. J_{leak} is mainly determined by Ca^{2+} leak rate constant from the NSR (ν_2) in both SAN and ventricle cell model as shown in Eq.1. As is showed in Figure 2, in the SAN cell, the cycle length (CL) was slightly shortened when ν_2 was increased from 0 to 2×10^{-5} ms^{-1}. CL was significantly increased when ν_2 is larger than $3\times10^{-5}ms^{-1}$ and AP was arrested when ν_2 is larger than $4\times10^{-5}ms^{-1}$. The amplitude of subspace $[Ca^{2+}]_i$, Ca^{2+} diffusion and dV/dt_{max} were decreased when ν_2 was increased. However, in ventricle cell model, the APD90 and dV/dt_{max} were not changed.

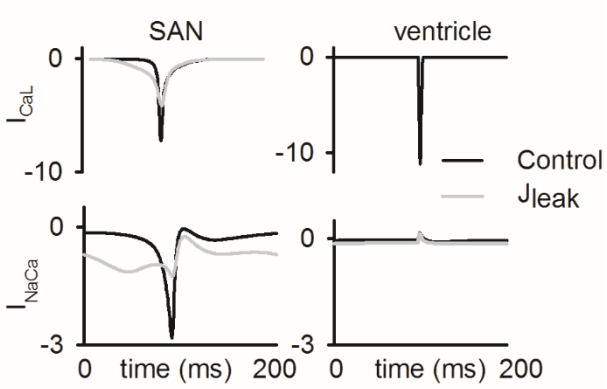

Figure 3. The effects of J_{leak} on ventricular ionic currents are small. Top panels show change of I_{CaL} due to increased J_{leak}. The change in I_{NaCa} is shown in the bottom panels. The ionic currents during APs are normalized to C_m (pA/pF).

In the SAN cell model, the pacing cycle length increased steadily till ν_2 values became 3.1 ms^{-1}. Upon further increase of ν_2, the pacemaking became complex and gave rise to long-short as well as big amplitude-small amplitude oscillations. This is reflected in our observation of several apparently random CLs in the SAN oscillations for $\nu_2 > 3.1\times10^{-5}$ ms^{-1}. In contrast, in the ventricle cell, as ν_2 was increased, the APD90 did not change significantly. It reduced from the basal 15.9 ms to 15.8 ms. However, as ν_2 was reduced, the APD90 reduced by 2%. As J_{leak} was increased, the SR Ca^{2+} content reduced in both cell types. It reached 0 at $\nu_2 = 4\times10^{-5}$ ms^{-1} in the SAN, and at $\nu_2 = 1\times10^{-3}$ ms^{-1} in the ventricle cell model. The Ca^{2+} amplitude followed a similar pattern to the SR Ca^{2+} behavior. An important observation that we saw was that the SAN upstroke velocity was very sensitive to the J_{leak} parameter, whereas in case of the ventricle model it was the contrary.

Figure 3 shows the effects of J_{leak} on L-type Ca^{2+} current (I_{CaL}) and Na^+/Ca^{2+} exchanger current (I_{NaCa}). Under basal conditions, the I_{CaL} has a peak of -10 pA/pF in both cell types. The I_{NaCa} is large in the SAN model (peak of -3 pA/pF), whereas it is small in the ventricle model. These are the two major membrane currents that are affected by cytosolic Ca^{2+}. In SAN model, both I_{CaL} and I_{NaCa} were decreased due to increased J_{leak} ($\nu_2=2\times10^{-5}ms^{-1}$). However, in ventricle cell model, I_{CaL} and I_{NaCa} were not affected by increased J_{leak} ($\nu_2=2\times10^{-3}ms^{-1}$).

4. Conclusions and discussion

This simulation study shows that SR Ca^{2+} leak affects several electrophysiological components in the cell

models. Overall, increase of Ca^{2+} leak was seen to cause arrhythmic alterations in the cytosolic Ca^{2+} concentrations which led to drastic alterations in the electrophysiological behavior. In contract, an increase of Ca^{2+} leak in the ventricle cell affected the intracellular content while the cell membrane electrophysiology remained unaltered. In the SAN cell, the cytosolic Ca^{2+} is strongly coupled to the cell membrane electrophysiology. This is due to the strong dependence of I_{CaL} inactivation, as well as I_{NaCa} function on $[Ca^{2+}]_i$. As these currents are major contributors to SAN cell upstroke (I_{CaL}) and to the slow depolarization phase (I_{NaCa}), In contrast, the $[Ca^{2+}]_i$ in the ventricle cell increases due to the an increasing leak. However, the coupling of the cytosolic calcium in this model is significantly smaller than in the SAN cell. Therefore, the membrane electrophysiology remains largely unaltered as seen in Figure 2. The results is useful to illustrate the mechanism of bradycardia during CPVT.

Previous studies have reported that patients with CPVT have SAN dysfunction [4]. SAN automaticity is regulated by the voltage clocks and Ca^{2+} clock [13]. The simulation results showed that SR Ca^{2+} leak causes decreased subspace Ca^{2+} concentration, reduces the state of Ca^{2+} overload, thus decreases the automaticity of SAN. Moreover, the downregulation of I_{CaL} caused by increased J_{leak} could reduce the slope of phase 0 and reduce the heart rate.

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