Sarcoplasmic Reticulum Luminal [Ca^{2+}] Regulates the Spontaneous Ca^{2+} Release Events and Consequently Arrhythmia

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Abstract

The role that sarcoplasmic reticulum (SR) luminal Ca^{2+} concentration plays in regulating ryanodine receptors (RyRs) is considered to be very important in process of cardiac excitation-contraction coupling. Here we developed a multi-scale mathematical model by coupling a two-dimensional spatio-temporal Ca^{2+} reaction-diffusion model with an action potential model of the ventricular myocyte. The simulation results showed that 1) the frequency of spontaneous sparks increased when SR Ca^{2+} content was elevated; 2) when SR Ca^{2+} was overloaded, spontaneous Ca^{2+} waves might occur without any external stimulus, and 3) once formed, the propagation of Ca^{2+} waves was accelerated as the SR Ca^{2+} content increased. Moreover, those spontaneously occurred Ca^{2+} release events elevated cytoplasmic $[Ca^{2+}]_i$ and then activated inward currents which might cause a delayed afterdepolarization (DAD).

1. Introduction

Ca^{2+} handling is considered to be the key process in cardiac excitation-contraction coupling (E-C coupling). The intracellular Ca^{2+} release from sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs) as the form of Ca^{2+} sparks [1] provides the major Ca^{2+} source required in E-C coupling. In a diastolic ventricular myocyte, sporadic Ca^{2+} sparks occur spontaneously at very low frequency. Upon depolarization, Ca^{2+} influx via L-type Ca^{2+} channels will trigger synchronously occurrence of sparks, summating into a global rise of Ca^{2+} concentration named Ca^{2+} transient. However in some diseased hearts, successive recruitment of Ca^{2+} sparks often evolves into propagating Ca^{2+} waves which might trigger ventricular arrhythmias [2]. Besides RyRs activation and inhibition by cytoplasmic [Ca^{2+}], the important role that SR luminal Ca^{2+} concentration plays in regulating RyRs has been demonstrated by many experimental observations [3-5]. It is generally accepted that overloaded SR Ca^{2+} store enhances RyR sensitivity to induce abnormal SR Ca^{2+} release [6]. However, the precise relationships between the Ca^{2+} loading status of SR and intracellular Ca^{2+} dynamics as well as electrophysiological properties are not completely clear.

In this paper, we developed a multi-scale mathematical model including Ca^{2+} cycling processes from subcellular to cellular level and membrane action potential of the ventricular myocyte. The proposed multi-scale model was applied to study the effects of changes in SR Ca^{2+} content on subcellular spatiotemporal Ca^{2+} cycling, and on the possible membrane potential changes caused by aberrant Ca^{2+} release events.

2. Methods

The multi-scale model consists of two parts: a two-dimensional spatial Ca^{2+} reaction-diffusion model and an action potential model of the ventricular myocyte.

2.1. A subcellular Ca^{2+} reaction-diffusion model

The two-dimensional spatio-temporal Ca^{2+} cycling model is described based on a reaction-diffusion system proposed by Izu et al [7]. Figure 1 shows the subcellular structural representation of RyRs network. The *x*-axis denotes the cell's longitudinal direction and the *y*-axis is along the Z-line. The dots represent clusters of RyRs opening of which elicit sparks. In this study, the simulation was performed on the longitudinal section of a cardiac myocyte with the size of 100 μm× 20 μm along the cellular longitudinal direction (*x*-axis) and Z-line (*y*-axis), respectively.

The free Ca^{2+} concentration $[Ca^{2+}]_i$ in the reaction-diffusion is described as follows:

$$\frac{\partial [Ca^{2+}]_i}{\partial t} = D_x \frac{\partial^2 [Ca^{2+}]_i}{\partial x^2} + D_y \frac{\partial^2 [Ca^{2+}]_i}{\partial y^2} + J_{dye} + J_{buffers} + J_{pump} + J_{leak} + J_{RyR} \quad (1)$$

where D_x and D_y are the diffusion coefficients, J_{dye} and $J_{buffers}$ is due to fluorescent indicator dye and endogenous Ca^{2+} buffer, respectively, J_{pump} is pumping rate of SR Ca^{2+} ATPase, J_{leak} is a leak flux, and J_{RYR} is RyR channel release flux.

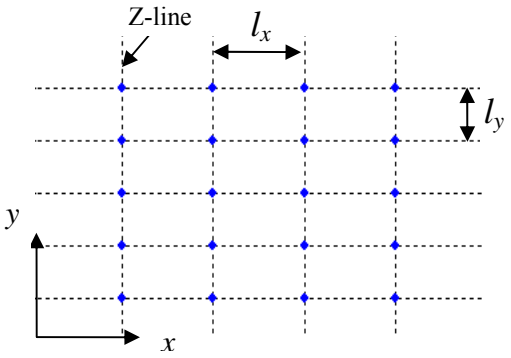

Figure 1. Geometry of RyRs distribution. The blue dots represent Ca^{2+} release units consisting of clusters of RyRs. l_x = 2.0 μm, and l_y = 1.0 μm.

Firings of RyR channels are considered to be stochastic processes and treated by the Monte Carlo simulation in our work. To evaluate the effects of luminal SR Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) on Ca^{2+} cycling, we integrate a new parameter k_{CaSR} into the probability of firing of Ca^{2+} sparks (P) as follows:

$$k_{CaSR} = \frac{k_{\max}}{1+(D_{SR}/[Ca]_{SR})^{nSR}} \quad (2)$$

$$P = \frac{P_{\max}}{1+(K_m/[Ca^{2+}]_i)^n} k_{CaSR} \quad (3)$$

2.2. A cellular electrophysiological model

The electrophysiological properties of myocardial cell is modelled based on a cardiac action potential model proposed by ten Tusscher et al [8]. The voltage across the cell membrane is described using the following differential equation:

$$\frac{dV_m}{dt} = -\frac{1}{C_m}(I_{Na}+I_{CaL}+I_{to}+I_{Kr}+I_{Ks}+I_{K1}+I_{NaK} + I_{NaCa}+I_{pCa}+I_{pK}+I_{bCa}+I_{bNa}+I_{stim}) \quad (4)$$

where C_m is the membrane capacitance, I_{stim} is a stimulus current, and I_x denotes all kinds of transmembrane ionic currents.

However, different from the Ca^{2+} dynamical system by ten Tusscher et al, SR Ca^{2+} release current I_{rel} was replaced by the sum of RyR channel currents i_{RyR} due to the firing of sparks in the spatio-temporal Ca^{2+} model described above.

Because of the stochasticity of Ca^{2+} sparks, some properties of Ca^{2+} signalling were described by statistical results by carrying out repeated Monte Carlo simulations. All averaged data were expressed as mean±SEM. One-way analysis of variance (ANOVA) was used for comparison and P<0.05 was taken to indicate statistical significance.

3. Results

3.1. Spontaneous sparks and $[Ca^{2+}]_{SR}$

The SR Ca^{2+} load of the myocyte was varied by different cycle lengths. Figure 2 shows the recorded $[Ca^{2+}]_{SR}$ after 20 beats at a fixed cycle length. $[Ca^{2+}]_{SR}$ decreased when the cycle length was elevated, due to diastolic SR Ca^{2+} leak [9]. Then no stimulus was applied for duration of 200ms, and the number of spontaneously occurred sparks was counted. When cycle length was reduced which caused increase of SR Ca^{2+} content in a diastolic myocyte, the frequency of spontaneous sparks increased (Figure 3), which suggested a positive regulating effect of SR luminal $[Ca^{2+}]$ on Ca^{2+} release.

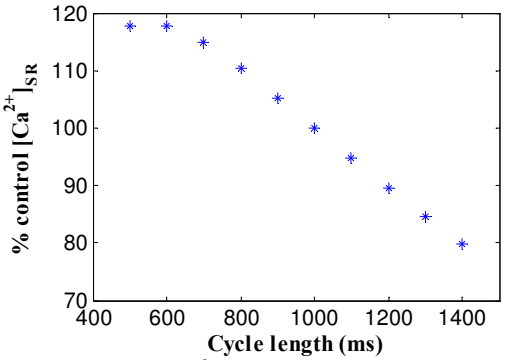

Figure 2. Relative $[Ca^{2+}]_{SR}$ after 20 beats at a fixed cycle length. Control $[Ca^{2+}]_{SR}$ was recorded at the cycle length of 1000 ms.

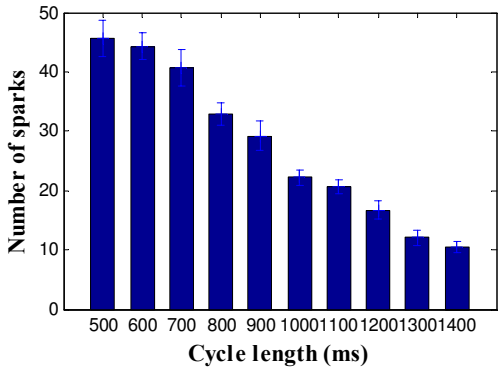

Figure 3. Relationship between cycle length and number of spontaneous sparks during a period of 200 ms.

3.2. Ca^{2+} waves and propagating velocity

The cell was paced rapidly to elicit overloaded SR Ca^{2+} content. Besides the increased spontaneous sparks, spontaneous Ca^{2+} waves might occur without any external stimulus when cycle length was reduced to 800 ms or shorter. Figure 4 shows one group of snapshots of the simulation result after 20 beats at the cycle length of 600

ms. A spontaneous Ca^{2+} wave emerged at 260 ms, and then another small wave occurred at 300 ms which fused with the former wave to form a big one after 380 ms.

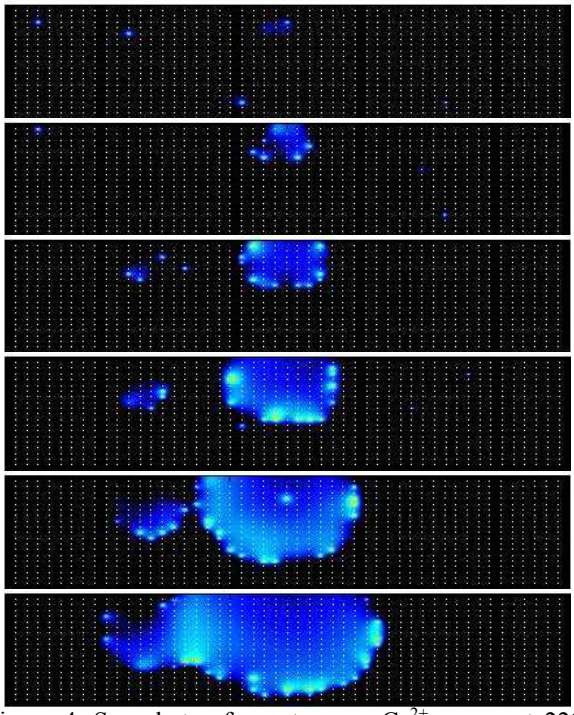

Figure 4. Snapshots of spontaneous Ca^{2+} waves at 220, 260, 300, 340, 380 and 420 ms (top to bottom).

Furthermore, SR luminal Ca^{2+} concentration also influenced the propagating velocity of Ca^{2+} waves (V_{pro}). Ca^{2+} waves were triggered after 20 beats at a fixed cycle length, and the average velocity along *x*-direction was calculated by repeated simulations. Figure 5 shows the effect of SR luminal $[Ca^{2+}]$ on longitudinal propagating velocity of triggered Ca^{2+} waves. As SR Ca^{2+} content increased, propagation of Ca^{2+} waves was accelerated significantly.

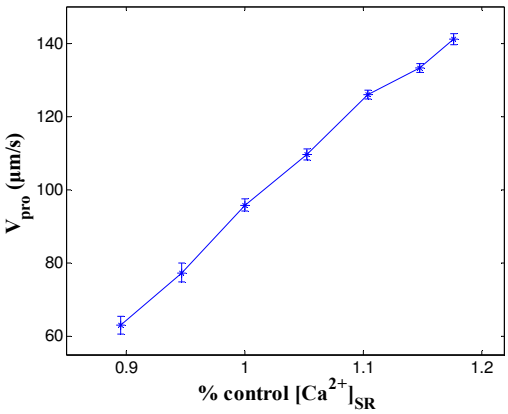

Figure 5. Dependence of longitudinal propagating velocity of Ca^{2+} waves on $[Ca^{2+}]_{SR}$. Each V_{pro} was calculated from ten repeated simulations.

3.3. DAD

By utilizing our multi-scale model, subcellular Ca^{2+} dynamics and global cellular electrophysiology could be traced simultaneously. In a cardiac myocyte, occurrence of propagating Ca^{2+} waves could elicit obvious amplitude of cytoplasmic Ca^{2+} transient which caused a DAD. Figure 6 shows one group of simulation results indicating a paced action potential and subsequently a DAD when SR Ca^{2+} was overloaded.

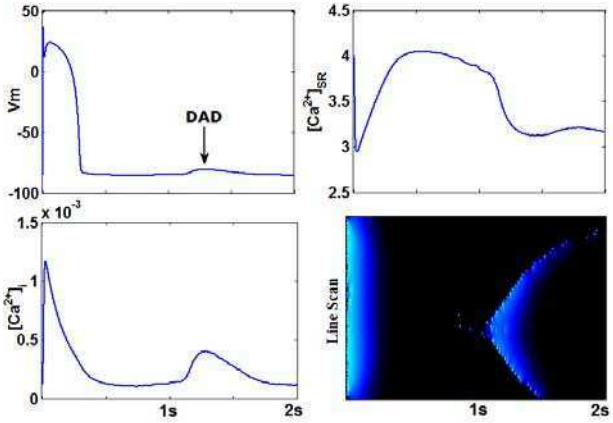

Figure 6. Simulation results of membrane potential (V_m), luminal $[Ca^{2+}]_{SR}$, cytoplasmic $[Ca^{2+}]_i$ and linescan image along y = 10 μm, showing the 21st paced action potential after 20 beats at the cycle length of 600 ms, followed by spontaneously occurred Ca^{2+} waves which caused a DAD when SR Ca^{2+} was overloaded.

4. Discussion

Aberrant Ca^{2+} release from SR may contribute to arrhythmias and even sudden death [10]. With normal SR Ca^{2+} content, sporadic Ca^{2+} sparks are unlikely to induce visible Ca^{2+} transient, as well as DADs, due to potent buffer system. However, when SR Ca^{2+} store is overloaded by rapid pacing or due to other diseases, RyRs tend to be more sensitive, and current flow through RyR channels would become bigger [11]. Our simulation results suggest that these two factors disturb cytoplasmic Ca^{2+} homeostasis. The increased frequency of spontaneous Ca^{2+} sparks together with the bigger currents make it easier to initiate Ca^{2+} waves without any external stimulus. Occurrence of propagating Ca^{2+} waves could elevate cytoplasmic Ca^{2+} concentration obviously, and then activate Ca^{2+}-dependent inward currents which depolarize the sarcolemma, causing a DAD. As spontaneous Ca^{2+} waves propagate in the myocardial cell, SR luminal Ca^{2+} concentration declines gradually and the regulating effect on the gating of RyRs changes correspondingly which suppresses firing of Ca^{2+} sparks on the later stage of Ca^{2+} waves.

Once formed, propagating velocity of Ca^{2+} waves changes remarkably at different $[Ca^{2+}]_{SR}$ due to the regulation of RyRs by luminal $[Ca^{2+}]$. At high $[Ca^{2+}]_{SR}$, RyRs are more likely to be activated by Ca^{2+} waves, and the bigger release currents elicit higher local $[Ca^{2+}]_i$, both of which facilitate propagation of Ca^{2+} waves. On the contrary, in the simulations when cycle length was longer than 1200 ms, triggered Ca^{2+} waves could not propagate stably due to the lower probability of firing of Ca^{2+} sparks and smaller channel current at the low $[Ca^{2+}]_{SR}$, and the V_{pro} was different to calculate.

In the further work, our multi-scale model will be improved and other pathological factors will be incorporated into the regulation of SR luminal Ca^{2+} on the gating of RyRs and consequently arrhythmia.

5. Summary

By integrating the spatio-temporal Ca^{2+} reaction-diffusion model into the cellular electrophysiological model, appearance of subcellular Ca^{2+} sparks and evolution of waves together with dynamic of ionic concentration and membrane potential on the cellular level could been monitored simultaneously. The simulation results suggest that regulation of RyRs by SR luminal $[Ca^{2+}]$ plays an important role in formation and propagation of Ca^{2+} waves and consequently heart arrhythmia with overloaded SR Ca^{2+} content.

Acknowledgements

This project is supported by the 973 National Basic Research & Development Program (2007CB512100) and the National Natural Science Foundation of China (30570484).

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