Ryanodine Receptors Coupling Causes a Calcium Leak in Cardiac Cell

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Abstract

Here we introduce results of a mathematical modeling of calcium sparks in cardiac cells. We developed a model of the calcium release unit which includes a single sarcoplasmic reticulum (SR) lumen, a regular 9x9 cluster of RyRs and a dyadic space. 2D diffusion problem of Ca2+ ions across the dyadic space was solved thereby we reproduced Calcium-Induced-Calcium-Release (CICR) effect and domino-like RyRs activation in the cluster.

We take into account allosteric and Ca^{2+}-induced coupling between RyRs. We show, that coupling between RyRs leads to the stability of Ca^{2+} sparks in amplitude and frequency. However, a sudden stop of spontaneous Ca^{2+} releases can be a result of strong allosteric coupling between RyRs.

1. Introduction

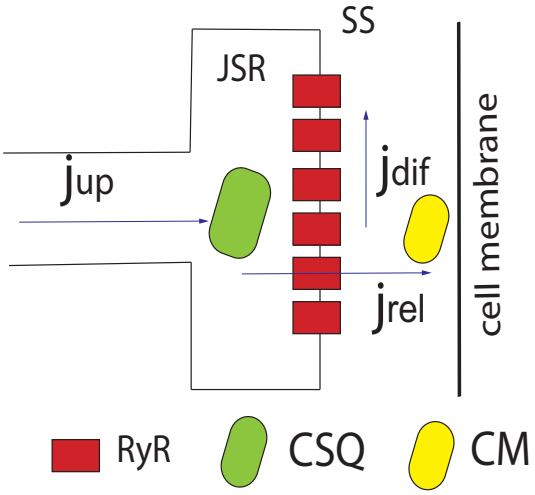

Figure 1. Schematic illustration of Ca^{2+} currents in the Ca^{2+}-release unit.

Local Ca^{2+} releases (so-called calcium sparks) from the sarcoplasmic reticulum (SR) are in the basis of a global Ca^{2+} release process which increases intracellular calcium level by an order of magnitude [1].

In the sinoatrial node cells (SANCs) spontaneousCa^{2+} releases play a major role in the action potential generation, thus, the study of the functioning of different parts of the Ca^{2+} release system is the important problem of the mathematical modeling. Traditionally calcium release system is described within the framework of the "Common Pool" model [2], which summarizes a complex Ca^{2+} release system to a single Ca^{2+} release unit (RU) (Fig. 1). The RU consists of a single lumen (junctial sarcoplasmic reticulum, jSR) and a subspace (SS). There is a compact cluster of Ca^{2+} releasing ryanodine receptors (RyRs). The regularity of the channel lattice is questionable [3,4]; however, the researchers cope with the conclusion that there is both an allosteric and conformational interaction between closely enough located channels [3,5].

Ca^{2+}-mediated, allosteric or conformational coupling between RyRs cause a cooperative effect of RyRs opening and closure and further spark formation. By means of computer modeling we tried to find out which mechanism of interaction can lead to Ca^{2+} leak from the SR.

Ca^{2+} ions released via RyRs can activate nearest neighbors in "domino-like" style (Ca^{2+}-mediated coupling), so this process also amplifies the Ca^{2+}-release. Thus, Ca^{2+} diffusion in the subspace attracts considerable interest due to the complex RyRs activation process as well as the spark initiation and spread.

In our computer simulations we adopted the formalism of the Maltsev-Lakatta model [6] of the rabbit SANC functioning, taking into account Ca^{2+}-mediated and allosteric coupling between RyRs in the cluster. Describing calcium dynamics we need to take into account Ca^{2+}-binding proteins (buffers): calmodulin and calsequestrin which cause a delay of Ca^{2+} dynamics in subspace and in jSR (Fig.1).

2. Methods

2.1. Model of calcium dynamics in the cardiac cell

In our model we take into account a single RU. Ca^{2+} dynamics is described by the system of reaction-diffusion

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equations:

$$\begin{array}{l} \frac{dCa_{SS}}{dt} = \frac{V_{jSR}}{V_{SS}} j_{rel} - CM_{tot} \cdot \frac{df_{CM}}{dt} \\ \frac{dCa_{jSR}}{dt} = j_{refill} - j_{rel} - CQ_{tot} \cdot \frac{df_{CQ}}{dt} \\ \frac{df_{CM}}{dt} = k_{fCM} Ca_{SS} (1 - f_{CM}) - k_{bCM} f_{CM} \\ \frac{df_{CQ}}{dt} = k_{fCQ} Ca_{jSR} (1 - f_{CQ}) - k_{bCQ} f_{CQ}, \end{array} \quad (1)$$

where j_{refill} is the lumen refill flux (constant in the current model), j_{rel} is a release flux via open RyRs, V_{SS} and V_{jSR} are volumes of the subspace and the lumen respectively, f_{CQ} and f_{CM} are current concentrations of a bound calsequestrin and calmodulin respectively, CQ_{tot} and CM_{tot} are total concentrations of calsequestrin and calmodulin respectively.

The release flux depends on the number of open RyRs: j_{rel}=$N_{open} k_{rel} (Ca_{jSR} - Ca_{SS})$, where k_{rel} is the release rate constant via a single RyR, N_{open} is the number of open RyRs .

2.2. Subspace Ca^{2+} diffusion model

In the current work we solve 2D Ca^{2+} diffusion problem across the subspace. In our model SR has a cluster of 9x9 RyRs.

$$\frac{\partial u}{\partial dt} = d \cdot \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right), \quad (2)$$

where u is the local (Ca_{SS} concentration in each node of the mesh. d is a diffusion constant.

We use an implicit finite-difference five-point stencil numerical scheme utilized for approximation of the diffusion equation. Parallel implementation on C++ with the use of PETSc makes it possible.

Our model describes Ca^{2+} fluxes between the RU compartments and the Ca^{2+} diffusion in the subspace.

2.3. RyRs stochastic dynamics model

Stochastic behavior of RyRs is described in our work in terms of previously developed Electron-Conformational model (ECM) [7,8]. This theory assumes that the RyR has only two degrees of freedom: slow conformational (refers to RyRs conformatioal opening/closure processes) and fast electronic (corresponds to Ca^{2+} ions effect on RyRs activational sites). RyRs states are described within the framework of elecrton-conformational potential formalism 1:

$$E_{\pm}(Q_m) = \frac{K}{2} Q_m^2 - pQ_m \pm \frac{1}{2} aQ_m + \frac{1}{2} k \sum_{n=1}^{4} Q_m Q_n, \quad (3)$$

where Q is a conformational coordinate, a is an electron-conformational coupling parameter, p is a parameter of an effective "pressure" of the lumen Ca^{2+}, K is the RyRs effective "elastic" constant. k is the conformational coupling parameter. Electron-conformational potential has two minima 2, left minimum corresponds to the closed state, right to the open. The probability of the interbranch transition between states depends on the Ca^{2+} concentration near each RyR:

$$P_{elect} = \alpha \cdot Ca_{SS}, \quad (4)$$

where α is a coefficient of proportionality.

The ECM introduce a novel approach of the description of the RyRs allosteric coupling with their nearest neighbours. In 3 last term describes this kind of interactions with the coupling parameter k. As can be seen from 2 the shape of the potential changes, the minimum corresponding to the closed state of the channel becomes more global (Fig.2).

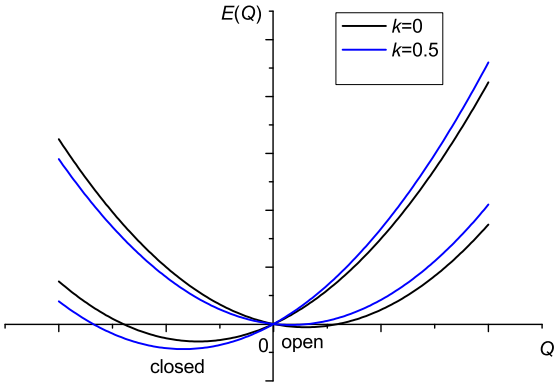

Figure 2. Electron-conformational potential of the RyR. Blue line corresponds to the electron-conformational potential with the allosteric coupling with the nearest closed neighbours.

3. Results

A series of computer experiments for the modeling of the Ca^{2+} release process and RyRs activation was performed. A standard set of the model parameters was taken from the Ca^{2+}-dynamics model in the rabbit pacemaker cell [6] to compare our previous simulation results [9] with the averaged Ca^{2+} and buffer concentrations in the current work: $k_{bCM} = 0.542$ ms^{-1}, $k_{bCQ} = 0.445$ ms^{-1}; $k_{fCM} = 227.7$ μM^{-1}ms^{-1}; $k_{fCQ} = 0.534$ μM^{-1}ms^{-1}; $CQ_{tot} = 10$ μM; $CM_{tot} = 0.045$ μM; $d = 10^{-10}$ m^2/s, $V_{jSR}/V_{SS} = 1.6$.

Parameters of the computational method. Number of mesh nodes $m_x = m_y = 240$; a single RyR width $L_{RyR} = 37$ nm, size of a single mesh node $L_{mesh} = 1$ nm, timestep $dt = 0.01$ ms. Ca^{2+} concentrations initial values $Ca_{jSR}(t = 0) = 1$ μM, $Ca_{SS}(t = 0) = 0$ μM, $N_{openrel}(t = 0) = 0$.

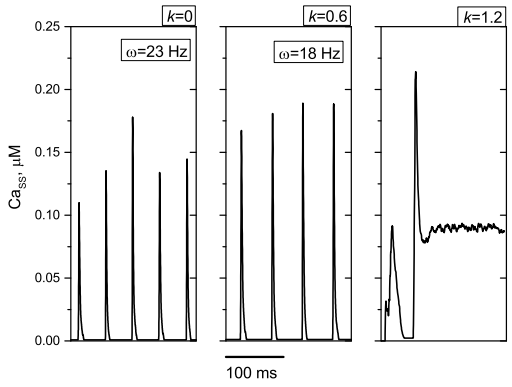

Figure 3. Timeseries of the mean subspace Ca^{2+} concentration Ca_{SS} for different values of the RyRs allosteric coupling k.

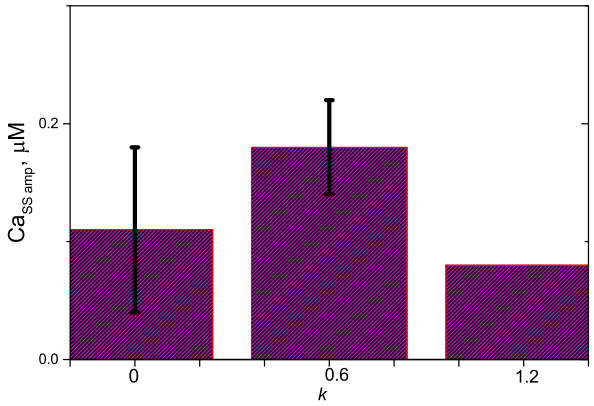

Figure 4. Calcium sparks amplitude Ca_{SSamp} for different values of the RyRs allosteric coupling k. Lines show deviations from the mean sparks amplitude.

Electron-conformational model parameters $a = 5$, $K = 12$, $K_{Ca} = 500$ μM, $Ca_{SS\_crit} = 100$ μM, $\alpha = 0.0012$ ms$^{-1}\mu$M^{-1}.

Without taking into account Ca^{2+} diffusion in the subspace, previously, it was shown [9] during computer simulations that the conformational coupling between RyRs in the RU can serve as a stabilizing factor. The strengthening of the conformational cooperativity (k=1) determines the stability of the Ca^{2+}-clock oscillatory dynamics, as well as fluctuations of the Ca_{SS} frequency and amplitude. The study of violations of the functioning of the Ca^{2+}-clock is especially important for studies of the arrhythmia. Extraordinary fluctuations of the internal Ca^{2+}-clock can disturb of self-oscillatory activity of the pacemaker cells, which can be an arrhythmogenic factor for the entire myocardium. In Fig.3 timeseries of Ca_{SS} for different values of k are presented. In case of the absence of coupling between RyRs a high variance of Ca^{2+} sparks is observed. Switching on coupling (k>0) leads to the increase of the sparks amplitude and to the decrease of Ca_{SSamp} range (Fig.4). The deviation from the mean sparks amplitude decreased with the increase of k value. It means that RyRs coupling leads to the stability of spontaneous sparks in the amplitude and in the frequency.

Further increase of the parameter k value (k>1.2) caused a sudden stop of Ca^{2+}-clock oscillations. It is manifested in the appearance of a steady cluster of opened RyRs.

4. Discussion

We have demonstrated that the simple biophysically reasonable Electron-Conformational model is a novel approach for RyRs stochastic dynamics description as well as allosteric/conformational coupling between RyRs. Integrated to the Ca^{2+} dynamics model, this theory also can describe Ca^{2+}-mediated RyRs coupling.

For sure, our model requires further developments like taking into account a complex structure of the Ca^{2+} release system as well as RyRs non-uniform spatial arrangement. However, on this stage we are able to describe Ca^{2+} sparks initiation-spread-termination process in a single RU and to determine the conditions for the periodic Ca^{2+} release disturbances.

We show that RyRs cooperativity is an important factor, which should be taken into account in Ca^{2+} sparks simulations in cardiac cells. RyRs coupling leads to sparks stability in the amplitude and frequency.

Also we observed a novel effect of the sudden stop of the periodic Ca^{2+} releases which can lead to Ca^{2+} leak and further cell functioning disturbances. both strong enough Ca^{2+}-mediated coupling and conformational coupling between RyRs can be a reason of Ca^{2+} leak from the SR. Special genetic mutations of RyRs can be a reason of diverse diseases (e.g. catecholaminergic polymorphic ventricular tachycardia (CPVT)) [5].

Acknowledgements

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