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

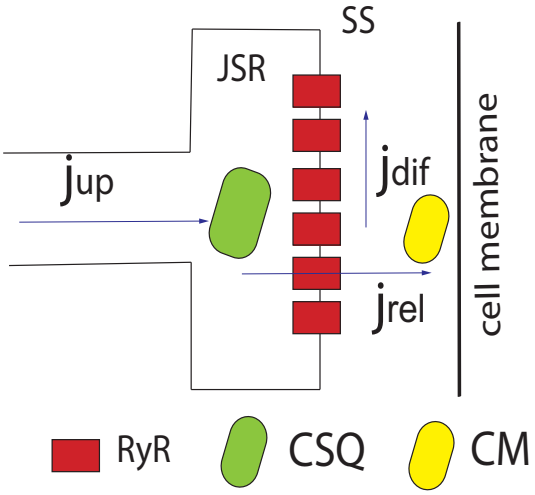

Figure 1. Schematic illustration of Ca^{2+} currents in the Ca^{2+}-release unit.

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

Talking about a single release unit (RU), which consists of a single jSR and a subspace, 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. 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.

As it was argued recently [2] isolated from a sarcolemmal voltage oscillator (membrane "clock") RU can operate as a self-sustained oscillator (SR Ca^{2+} "clock"), described by a simple "release-pumping-delay" mechanism when a small spontaneous Ca^{2+} release from jSR to the subspace occurs as the primary or initiating event. When Ca_{SS} increases to a sufficient level, it amplifies the Ca^{2+} release via the mechanism of the CICR [3]; this relatively strong, secondary Ca^{2+} release simultaneously depletes (i.e., resets) jSR. The released Ca^{2+} is pumped into the nSR. The delay between releases is determined by the Ca^{2+} pumping rate and Ca^{2+} diffusion from the subspace to cytosol as well as diffusion from nSR to jSR. As Ca_{jSR} slowly increases, RyRs are restituted, and the next release is ultimately initiated, etc. However, disturbances in the periodicity of Ca^{2+} release may cause undesirable consequences for the automaticity of the pacemaker cells.

Calcium leak is caused by SERCA disturbances and RyRs dynamics violations. The Ca^{2+} leak is frequently found to be arrhythmogenic and contribute to Ca^{2+} waves and alternance [4]. Special genetic mutations of RyRs can be a reason of diverse diseases (e.g. catecholaminergic polymorphic ventricular tachycardia (CPVT)) [5]. Thus, RyRs opening-closing process should be described in details in the Ca^{2+} dynamics model. The regularity of the channel lattice is questionable [6, 7]; however, the researchers cope with the conclusion that there is both an allosteric and conformational interaction between closely enough located channels [5, 6]. 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.

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 equations:

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

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.

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) [8,9]. 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.

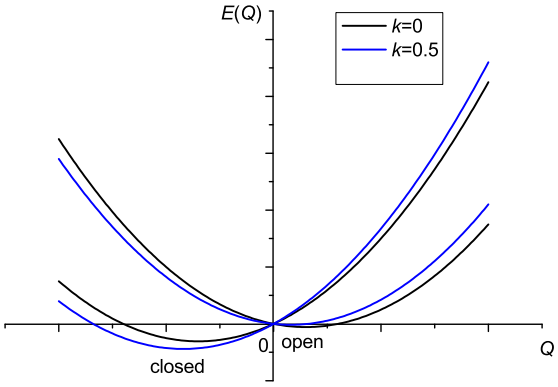

Figure 2. Electron-conformational potential of the RyR. Blue line corresponds to the electron-conformational potential with the allosteric coupling with the nearest closed neighbours.

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 [2] to compare our previous simulation results [10] with the averaged Ca^{2+} and buffer concentrations in the current work: $k_{bCM} = 0.542\ \mathrm{ms}^{-1}$, $k_{bCQ} = 0.445\ \mathrm{ms}^{-1}$;

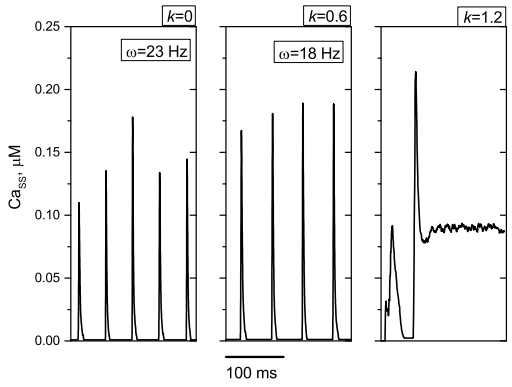

Figure 3. Timeseries of the mean subspace Ca^{2+} concentration Ca_{SS} for different values of the RyRs allosteric coupling k.

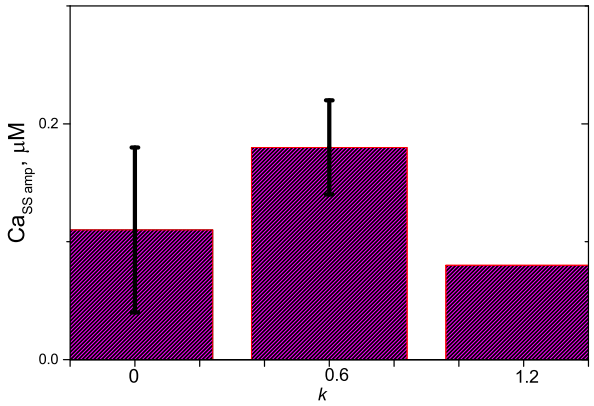

Figure 4. Calcium sparks amplitude Ca_{SSamp} for different values of the RyRs allosteric coupling k.

$k_{fCM} = 227.7\ \mu\mathrm{M}^{-1}\mathrm{ms}^{-1}$; $k_{fCQ} = 0.534\ \mu\mathrm{M}^{-1}\mathrm{ms}^{-1}$; $CQ_{tot} = 10\ \mu$M; $CM_{tot} = 0.045\ \mu$M; $d = 10^{-10}\ \mathrm{m}^2/\mathrm{s}$, $V_{jSR}/V_{SS} = 1.6$.

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 $Ca_{jSR}(t = 0) = 1\ \mu$M, $Ca_{SS}(t = 0) = 0\ \mu$M, $N_{openrel}(t = 0) = 0$.

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

Without taking into account Ca^{2+} diffusion in the subspace, previously, it was shown [10] 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 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).

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

In summary, we have demonstrated that the simple biophysically reasonable Electron-Conformational model is useful for the description of RyRs stochastic dynamics during sparks initiation-spread-termination process. Integrated to the Ca^{2+} dynamics model, this theory also can describe conformational and Ca^{2+}-mediated RyRs coupling.

Clearly, our model has a large number of simplifications and approximations. For example we do not take into account yet a complex structure of the Ca^{2+} release system as well as RyRs non-uniform spatial arrangement. Solving this problem is already underway, 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.

In this paper we found out 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. We have shown that both strong enough Ca^{2+}-mediated coupling and conformational coupling between RyRs can be a reason of Ca^{2+} leak from the SR. Further studies should aim at the effect of sudden stop of the whole heart cell functioning taking into account extracellular ion currents.

Acknowledgements

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