Institute of Physics Publishing Journal of Physics: Conference Series **21** (2005) 195–200
doi:10.1088/1742-6596/21/1/032 Second International Conference on Photo-induced Phase Transitions

Biophysical adaptation of the theory of photo-induced phase transition: model of cooperative gating of cardiac ryanodine receptors

A S Moskvin[1,2], M P Philipiev[1,2], O E Solovyova[1,2] and V S Markhasin[2]

[1]Ural State University, Ekaterinburg, 620083, Russia
[2]Institute of Immunology and Physiology, Ekaterinburg, 620219, Russia

E-mail: alexandr.moskvin@usu.ru

Abstract. Theory of photo-induced phase transitions has been adapted to describe the cooperative dynamics of the lattice of ryanodine receptors/channels (RyR) in cardiac muscle which regulate the release of the intracellular activator calcium from calcium stores in the sarcoplasmic reticulum (SR) by a process of Ca^{2+}-induced Ca^{2+} release (CICR). We introduce two main degrees of freedom for RyR channel, fast electronic and slow conformational ones. The RyR lattice response to the L-type channel triggering evolves due to a nucleation process with a step-by-step domino-like opening of RyR channels. Typical mode of RyR lattice functioning in a CICR process implies the fractional release with a robust termination due to the depletion of SR with a respective change in effective conformational strain. The SR overload leads to an unconventional auto-oscillation regime with a spontaneous calcium release. The model is believed to consistently describe the main features of CICR, that is its gradedness, coupled gating, irreversibility, inactivation/adaptation, and spark termination.

1. Introduction

In mammalian cardiac muscle, a large fraction of the intracellular activator calcium (Ca^{2+}) is released from Ca^{2+} stores in the sarcoplasmic reticulum (SR) via the so-called Ca^{2+} induced Ca^{2+} release (CICR) through ryanodine receptors/channels (RyR) named so due to their adhesiveness to the plant alkaloid ryanodine [1]. Ultra-structural studies suggest that the junctional SR contains nearly crystalline arrays of RyRs, organized in clusters with several hundred (100-300) RyRs. These form a nearly square lattice with ∼30 nm inter-spacing of RyR. It is understood that the key initiator of CICR is Ca^{2+} entry via voltage-dependent sarcolemmal dihydropyridine (DHPR)-sensitive L-type Ca^{2+} channels. At each junctional cleft, a cluster of individual RyRs is close to DHPRs forming a local SR Ca-release unit called a junction, or couplon[1](see figure 1). The main features of CICR that are usually characterized include stability, high gain, gradedness, and termination of Ca^{2+} release. Despite intense efforts, the processes involved in control of CICR remain poorly understood and we are still far from a comprehensive understanding of the underlying mechanisms. Recently [2, 3, 4] we have suggested a novel electron-conformational model for RyR channels, based on a biophysical adaptation of the well-known theory of photo-induced structural phase transitions, which has been applied to different solids [5].

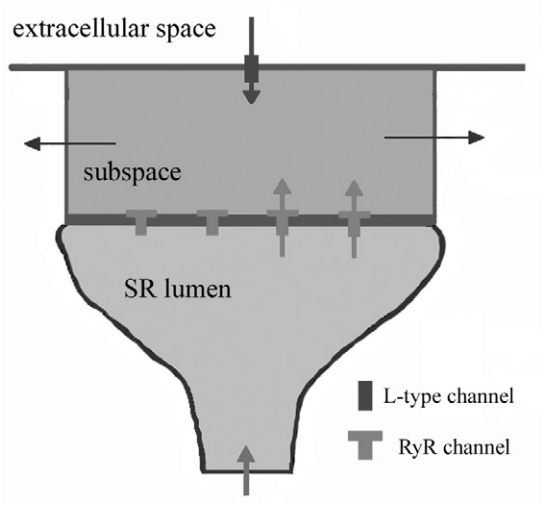

Figure 1. Scheme of SR Ca-release unit, or couplon. RyR channel is a giant (30×30 nm) macromolecular protein complex comprising 4 subunits of 565 000 Daltons each. Opposite to RyR, the DHPR channels are located more randomly and in the T-tubular membrane at an average distance of 10 nm from RyR channels. The RyR:DHPR ratio within T-tubules varies from 7:1 in rat, to 5.6:1 in human, and 5:1 in guinea pig, and may be variable even within a single species, cell type, cell, or T-tubule.

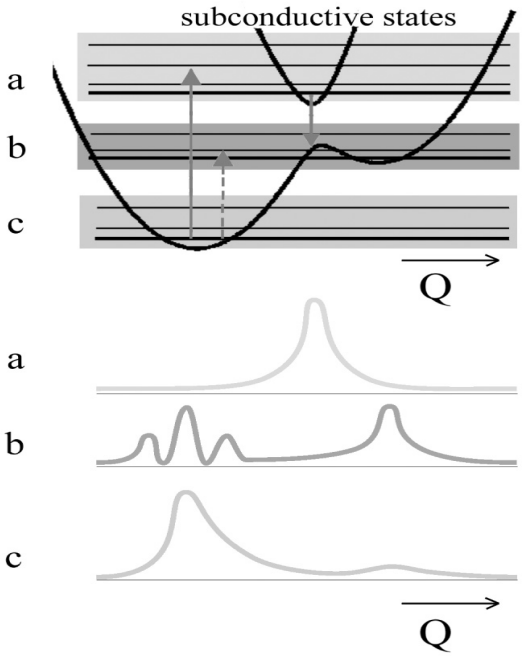

Figure 2. Conformational potential for low-open probability RyR state with schematic of quantum generalization of energy spectrum (top: a, b, c) with examples of conformational density distributions (bottom: a, b, c).

2. Introduction to the electron-conformational model for a single RyR channel

Our knowledge of molecular mechanisms of RyR channel functioning is limited; hence we are forced to start with the most general "physicists" approach, which is typical for protein biophysics. Such an approach to the modelling of biomolecular system implies its simplifying to bare essentials with guidance from experimental data. We start reducing a large variety of RyR degrees of freedom to only two: a fast and a slow one, conditionally termed electronic and conformational, respectively. Bearing in mind the main function of RyR channels, we assume only two actual electronic RyR states: "open" and "closed", and a single conformational degree of freedom, Q, to be described by a classical continuous variable. We shall assume a simple harmonic approximation of the conformational energy and use a formula similar to Hookes law for potential energy: $E = \frac{1}{2}KQ^2$, where K is the effective "elastic" constant and $Q = 0$ relates to a base state. The two electronic RyR states, open and closed, form a doublet and can be properly described within the framework of the s=1/2 pseudospin formalism, with "up" ($\uparrow$) and "down" ($\downarrow$) pseudospin states. Hereafter we assume that the conformational variable Q specifies the RyR channel permeability for Ca^{2+}, while electronic variable determines its opening and closure. This allows us to describe the Ca^{2+} flux through RyR as follows:$J_{RyR} = D(Q)(Ca_{lum} - Ca_{SS})$, if the channel is open, and $J_{RyR} = 0$, if it is closed. Here, the permeability coefficient $D(Q)$ reflects the ease with which Ca^{2+} passes through an open RyR (Ca_{lum}, Ca_{SS} are Ca^{2+} concentrations in the SR lumen and the subspace, respectively). Below we assume the $D(Q)$ is an increasing

function of conformational coordinate, varying from zero to some saturated value D_0, where Q oscillates from large negative to large positive magnitudes ($0 < D(Q) < D_0$), passing through some subconductive state at $Q = 0$ with a conductance $D(0) = \frac{1}{2}D_0$. As a starting point of the model we introduce the effective electron-conformational Hamiltonian for a single RyR channel as follows [5]

$$H_s = -\Delta\hat{s}_z - h\hat{s}_x - pQ + \frac{K}{2}Q^2 + aQ\hat{s}_z, \tag{1}$$

where $\hat{s}_z$ and $\hat{s}_x$ are Pauli matrices, and the first term describes the bare energy splitting of up and down states with an energy gap Δ, while the second term describes the mixing of up and down pseudospin states. The third and fourth terms in (1) describe the linear and quadratic contributions to the conformational energy. Here, the linear term formally corresponds to the energy of an external conformational strain, described by an effective strain parameter p. The last term describes the electron-conformational interaction where the parameter a is an electron-conformational coupling constant. Hereafter we make use of the dimensionless conformational variable Q, therefore all the model parameters are assigned energy units. The corresponding eigenvalues

$$E_{\pm}(Q) = \frac{K}{2}Q^2 - pQ \pm \frac{1}{2}\left[(\Delta - aQ)^2 + h^2\right]^{1/2} \tag{2}$$

define the upper and lower branches of the conformational potential (CP), respectively (see figure 2). The lower branch of the CP has an energy landscape with either a single minimum, or a two-well structure, leading to a bistability and a bifurcation point. The two wells of the CP may be associated with two important conformations of RyR channel: closed and open.

3. Ca^{2+} activation and regulation of RyR parameters

The results of numerous experiments suggest that the changes in Ca^{2+} concentration both on cytosolic (subspace) and luminal sides of the RyR receptor can modulate Ca^{2+} release [1]. Hereafter, we assume a purely electronic character of the interaction between cytosolic Ca^{2+} and RyR channels. The most general form of such an interaction is described by the effective pseudospin Hamiltonian

$$H_{L-RyR} = -\delta(t)\hat{s}_z - \kappa(t)\hat{s}_x \tag{3}$$

where $\delta(t)$, $\kappa(t)$ are two time-dependent parameters that are assumed to comprise all the information about the Ca^{2+} binding to RyR. At present we cannot distinctly specify the relative role of diagonal and off-diagonal terms in effective L-RyR coupling. However, it seems likely that namely the off-diagonal term plays a key role in the Ca^{2+} activation/regulation on the subspace-side of the RyR channel since namely the off-diagonal term induces the electron up-down transition stimulating the RyR channel. In contrast with purely electronic effect of Ca_{SS}, the effect of relatively slowly varying Ca_{lum} on the RyR channels is prone to be purely "mechanical" one, through the respective conformational stress applied to RyR channels. The effect can be incorporated to the total Hamiltonian, if we assume the stress parameter, p, to be a function of Ca_{lum}. The effective strain p we assume to rise with the luminal Ca^{2+} concentration in accordance with the Hill curve:

$$p = p(Ca_{lum}) = \frac{2[Ca_{lum}]^n}{[Ca_{lum}]^n + K_{Ca}^n} - 1\,, \tag{4}$$

where K_{Ca} is the half maximal value, or a luminal concentration at which the effective stress p turns into zero ($p(Ca_{lum} = K_{Ca}) = 0$), n is a Hill coefficient specifying the sensitivity to luminal calcium and the nonlinearity of the $p(Ca_{lum})$ dependence.

Above we assumed conformational coordinate to be a classical variable, however, one might address a quantum generalization with effects induced by a quantization of conformational

motion as it is done in the molecules and solids with core displacement modes. First, we arrive at an energy spectrum which may be grouped to two overlapping bands derived from bare electron "up" and "down" states and conditionally assigned to two branches of CP (see figure 2). Each quantum state is described by a certain conformational density distribution, or, in other words, by the probability to have a certain conformational coordinate. Traditional phenomenological approach to RyR channel implies a finite number of stable discrete RyR states, with transitions between states taking place instantaneously and at random. Quantum description of the RyR channel points to clear shortcoming of conventional models. First of all, stable gating schemes method implies only the variable transition probabilities and fails to account for the rearrangement of the RyR channel spectrum itself accompanying the nonequilibrium functioning of a single RyR channel under the CICR conditions.

3.1. Activation and relaxation effects for a single RyR channel

Under the adiabatic approximation, the dynamics of a single RyR channel consists of the adiabatic motion of the conformational subsystem and electronic up-down transitions. The classical dynamics of the conformational mode for RyR channel we assume to obey the conventional Langevin equation of motion

$$\ddot{Q} = -\frac{\partial}{\partial Q} E_{\pm}(Q) - \gamma_{\pm}\dot{Q} + \eta(\tau), \tag{5}$$

where first term describes a total systematic conformational force, γ is the effective dimensionless friction constant, and η is the thermal fluctuation force. The cytosolic Ca^{2+} coupling with the Ca-binding sites of the RyR channel can be considered in terms of inelastic scattering theory as a resonance scattering that implies a resonance dependence of electronic RyR excitation probability on the Ca^{2+} ion energy, which may be approximated by a Lorentzian spectral function

$$w(E) \propto \frac{\Gamma^2}{(E-\Delta)^2 - \Gamma^2} \tag{6}$$

(Breit-Wigner formula), where $\Delta = (E_+ - E_-)$ is an excitation energy, E is Ca^{2+} ion energy, Γ is a resonance half-width. Full electronic RyR excitation probability strongly depends on the Ca^{2+} concentration (Ca_{SS}). L-type channel stimulus results not only in a local rise in Ca_{SS}, but in a fast effective local heating (firing) of subspace with a proper variation in an energy distribution function for Ca^{2+} ions and a multiple rise in the RyR excitation probability. Hence, the trans-sarcolemmal Ca^{2+} from the L-type channel activates the RyR channel in a close apposition differently Ca^{2+} released from RyR channels.

4. Cooperative dynamics of the RyR lattice

The effective Hamiltonian of the RyR lattice can be written as a superposition of single channel Hamiltonians (1), and an inter-channel conformational coupling: $H_{QQ} = \frac{1}{2}\sum_{m,n} K_{mn} Q_m Q_n$, where m, n label different RyR channels, and K_{mn} is an inter-channel conformational coupling constant. To illustrate the cooperative dynamics of a RyR lattice in the framework of electron-conformational models, we simulated dynamics within 11×11 square RyR lattice after the momentary excitation of a single, centrally located RyR channel (m = 0). Their in-branch dynamics is assumed to be governed by equation (5) with no temperature fluctuations. We studied dynamics of the RyR-lattice as a function of two parameters: effective strain p and conformational cooperativity constants k, specifying nearest neighbour inter-channel coupling. The remaining parameters were as follows: $h = 0.1$, $\Delta = 0$, $a = 2.5$, $K = 2$, $\gamma = 5$. At an initial point $t_0 = 0$ all the channels (excluding the central one) were closed. The central channel m = 0 was driven to the upper CP branch to imitate opening due to a Ca^{2+} stimulus from

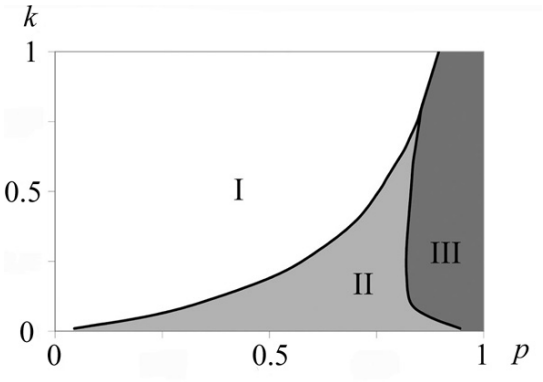

Figure 3. The (p,k)-phase diagram, showing three modes of 11×11 square RyR lattice gating: inactivation mode I, single RyR channel activation (Ca synapse, or quark) mode II, high gain multiple opening mode with a high degree of cooperativity, or cluster bomb mode III.

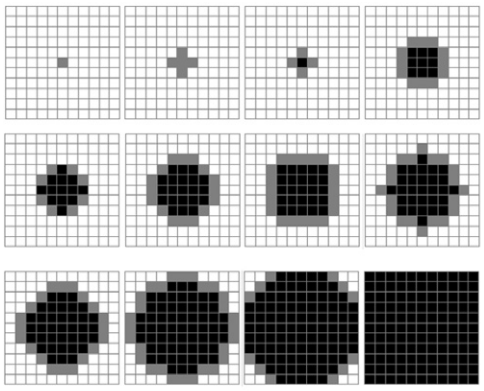

Figure 4. Illustration of the step-by-step (t = 10, 25, 41, 45, 47, 48, 49, 51, 52, 55, 58, 65 ms) domino-like opening of RyR channels. Black and white filling points to open and closed RyR channels, respectively, grey marks RyR channels which are only transferring to the open state.

an opposing L-type channel. After the relaxation to the minimum point at the instant $t = 20$ (all time steps in [ms]), the central channel was instantly moved to the lower CP branch that imitated spontaneous Franck-Condon transition. Subsequently, all channels relaxed within the lower CP branch. The lattice dynamics is described by means of a system of 121 ordinary differential equations such as (5) for $Q(m)$. The system was then solved by the Euler's method with an integration time step $\delta t = 0.01$. Depending on the parameter values, we arrive at three qualitatively different modes I-III of RyR lattice *in vitro* dynamics after triggered opening of the central channel (see figure 3). In phase III one deals with an avalanche-like process of a step-by-step opening of all the RyR channels triggered by a stimulus via L-type channel. Such a domino effect is illustrated in figure 4, where we show a step-by-step opening of RyR channels, induced by the opening of the central channel. Finally, one may conclude that mode III is associated with L-type channel stimulus induced non-equilibrium phase transitions from closed to open RyR channels, proceeding through the nucleation phenomenon.

Next we address the *in vivo*-like situation where the RyR lattice dynamics is incorporated to a complex process of CICR. In this case, we deal with Ca^{2+}-dependent effective strain p and positive/negative feedback effects. Electron-conformational dynamics of the RyR lattice is assumed to specify the flux from the SR to the subspace through RyR channels as a sum of individual channel fluxes. For simplicity, we assume a step-like Q-dependence of the RyR channel conductivity, so that the channel is open (closed) being located to right (left) of the maximum point on the lower CP branch. Because of our main goal was to uncover the role of RyR lattice gating, we neglected the inhomogeneity in Ca_{SS} and Ca_{lum} and made use of a simplified model of Ca^{2+} dynamics [6] where the Ca^{2+} fluxes Ca_{SS} and Ca_{lum} were assumed to obey a standard system of two differential equations with typical parameters used for Ca^{2+} dynamics modelling (see [4] for details). The system demonstrated four different modes of behaviour, depending on SR load. If $Ca_{SR} < 800$ μM, or at rather small SR load (heavily underload regime), it arrived at an inactivation mode I. With further increase in the SR load, up to a $Ca_{SR} = 900$ μM (underload), the system turns to a single channel activated mode II (Ca synapse, or quark mode). With further increase in the SR load, up to $Ca_{SR} = 1175$ μM (optimally load), the system turns to a domino-like firing-termination mode III with high degree of cooperativity

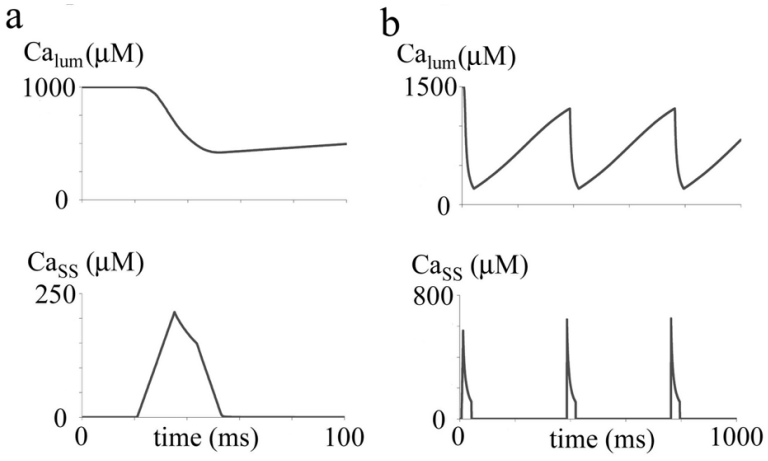

Figure 5. Simulation of the time course of Ca_{lum} (top panel), and Ca_{SS} (bottom panel) after single RyR channel triggering for two different Ca_{SR} load: A - Mode III ($Ca_{SR} = 1200$ μM); B - Mode IV, or auto-oscillation regime ($Ca_{SR} = 1500$ μM).

(cluster bomb mode), where stimulation causes step-by-step opening of the central channel and its neighbours, with formation of a cluster composed of ∼40% open RyRs. The process results in an effective high-gain Ca^{2+} release from the SR lumen which is usually termed as a *spark* (figure 5a). However, the SR depletion leads to lower effective strain and the shift of the system to preferable channel's closure. This negative feedback effect results in a slowing down of the nucleation process and in an evolution of the inverse domino-like effect with full collapse of the cluster of open RyR and termination of Ca^{2+} release, or spark. SR overload can result in the excitation of RyRs lattice auto-oscillations (figure 5b). Indeed, if $Ca_{SR} > 1175$ μM, stimulation of the central channel causes a domino-like opening of a cluster of RyR channels, and effective Ca^{2+} release that, in turn, causes a lowering of the effective strain and simultaneous closure of channels before the luminal concentration starts rising. However, until Ca_{lum} approaches the initial value, at a critical concentration all channels re-open simultaneously, and Ca^{2+} release is repeated spontaneously. This behaviour is repetitive, i.e. the system turned out to behave in an auto-oscillation mode IV with a spontaneous SR Ca^{2+} release which has been observed experimentally [1]. In conclusion, making use of the familiar photo-induced phase transition theory [5] we have developed the electron-conformational model for RyR channel and lattice which is believed to consistently describe the main features of CICR, that is its gradedness, coupled gating, irreversibility, inactivation/adaptation, and spark termination.

Acknowledgments

We acknowledge the support by CRDF Grant No. REC-005, RFBR Grant No. 03 04-48260, Wellcome Trust CRIG No. 074152, Young Scientist (M.P.P.) Grant (Ural Branch of RAS) and Grant of Russian Federal Agency for Education.

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