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NUMERICAL STUDIES ON THE ACTIVITY OF THE MUSCLE CALCIUM CHANNEL*

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Abstract. In the skeletal or heart muscle, the excitation-contraction coupling is mediated by release of Ca^{2+} ions from the sarcoplasmic reticulum through the ryanodine receptor. The activity of the ryanodine receptor is modulated by a number of cytosolic ligands, such as Ca^{2+}, caffeine or quercetin. However, the mechanisms of pore opening/closing by ligand binding to the receptor remain largely unknown at the moment. We found that a series of published experimental data on the activity of the ryanodine receptor can be accurately explained by a model with two functional independent gates of the channel that are driven by Ca^{2+} binding to antagonist sites on the receptor, and allosterically regulated by caffeine and quercetin. The open probability of the channel in steady state is calculated by considering first-order kinetics for reactions of binding to and dissociation from the receptor, mass balance equations, and conditions for thermodynamical equilibrium. Our model can provide an excellent agreement with the data only if four different but equally probable kinetic modes of the activation gate are assumed. We calculate the open probability of the channel at various concentrations of the three ligands, and compare stochastic simulations of channel opening with the deterministic solution of the system.

Key words: ionic channel, open probability, ligand affinity, steady state, mathematical model.

1. INTRODUCTION

The excitation-contraction coupling in the skeletal or heart muscle is mediated by release of Ca^{2+} ions from the sarcoplasmic reticulum. This step is crucial in relating membrane depolarisation to the mechanical muscle contraction [1, 2]. There are two classes of Ca^{2+} channels of the sarcoplasmic reticulum (SR): the inositol 1,4,5-trisphosphate receptor (IP_3R) and the ryanodine receptor (RyR). The first receptor type predominates in non-excitable cells, whereas the second type appears to be mostly abundant in excitable cells.

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A number of pathological states are associated with mutations in the skeletal or cardiac ryanodine receptors, such as in malignant hyperthermia or the central core disease [3]. Therefore, theoretical models can provide valuable insights into the molecular mechanisms of Ca^{2+} sensing by RyR, and offer important clues regarding the action of important pharmacological agents.

The activity of the ryanodine receptor is regulated by a number of cytosolic ligands, such as Ca^{2+}, ATP and Mg^{2+} [4]. Numerous experimental studies have proved that Ca^{2+} has a bimodal effect on the conducting activity of both IP_3R and RyR channels [5-7], and it is generally accepted that this behaviour is due to the existence of two antagonist classes (activating/inhibitory) of Ca^{2+} binding sites, presumably located on the large cytosolic region of the RyR receptor protein [4]. Recent studies have shown that two exogenous modulators of the RyR1 calcium channel (a skeletal muscle specific RyR), namely caffeine and the bioflavonoid quercetin, can act as potent activators of this channel [7].

We have analyzed the data presented by Lee *et al.* [7] and developed a model for the RyR1 activity [8], derived from our previous work on a similar receptor, IP_3R [9]. We consider that RyR1 activity is determined by two independent gates, each operated by a specific module of the receptor. In order for the channel to be in an open conformation, both gates must be activated. Ca^{2+} bound to the activation module opens the channel, whereas Ca^{2+} bound to the inhibition module shuts the channel. We assume two classes of RyR binding sites for each of the three ligands (Ca^{2+}, caffeine and quercetin), with one class of sites belonging to the activation module, and the other class of sites belonging to the inhibition module. This model provides an excellent agreement with the data and proposes several hypotheses regarding the functioning of the skeletal muscle calcium release channel.

2. METHODS

In the presence of caffeine (Caf) or quercetin (Que) alone, each X–type module (X ≡ A for the activation module, X ≡ I for the inactivation module) may display four possible states (Fig. 1), denoted X_1, X_2, X_3 and X_4.

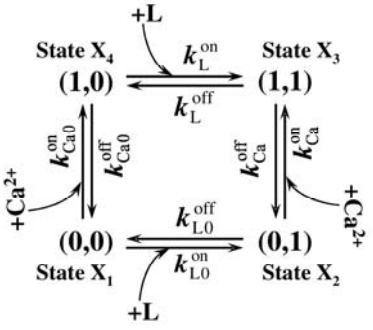

Fig. 1 – States and transitions within activation/inhibition modules of the RyR calcium channel. This scheme is representative for both modules. L represents the exogenous ligand (Caf or Que). The four possible states in the cycle are characterized by different combinations (n_{Ca}, n_L) of Ca^{2+}, and L-site occupancies; n equals 1 (or 0) if the site is (or is not) bound.

By analytical calculation one can find that the four states are occupied in steady state with the probabilities (see also the Appendix):

$$P_{\mathrm{X}_1} = 1 - P_{\mathrm{X}_2} - P_{\mathrm{X}_3} - P_{\mathrm{X}_4}, \tag{1}$$

$$P_{\mathrm{X}_2} = (\gamma_4^{\mathrm{X}} - \gamma_2^{\mathrm{X}})/\delta^{\mathrm{X}}, \tag{2}$$

$$P_{\mathrm{X}_4} = (\gamma_1^{\mathrm{X}} - \gamma_3^{\mathrm{X}})/\delta^{\mathrm{X}}, \tag{3}$$

$$P_{\mathrm{X}_3} = (P_{\mathrm{X}_2}\lambda_{23}^{\mathrm{X}} + P_{\mathrm{X}_4}\lambda_{43}^{\mathrm{X}})/\lambda_3^{\mathrm{X}}, \tag{4}$$

where

$$\gamma_1^{\mathrm{X}} = 1 + \frac{\lambda_{21}^{\mathrm{X}}}{\lambda_1^{\mathrm{X}}} + \frac{\lambda_{23}^{\mathrm{X}}}{\lambda_3^{\mathrm{X}}}, \tag{5}$$

$$\gamma_2^{\mathrm{X}} = 1 + \frac{\lambda_{41}^{\mathrm{X}}}{\lambda_1^{\mathrm{X}}} + \frac{\lambda_{43}^{\mathrm{X}}}{\lambda_3^{\mathrm{X}}}, \tag{6}$$

$$\gamma_3^{\mathrm{X}} = 1 + \frac{\lambda_{23}^{\mathrm{X}}}{\lambda_3^{\mathrm{X}}}\left(1 - \frac{\lambda_{34}^{\mathrm{X}}}{\lambda_{14}^{\mathrm{X}}}\right), \tag{7}$$

$$\gamma_4^{\mathrm{X}} = 1 + \frac{\lambda_{43}^{\mathrm{X}}}{\lambda_3^{\mathrm{X}}}\left(1 - \frac{\lambda_{34}^{\mathrm{X}}}{\lambda_{14}^{\mathrm{X}}}\right) + \frac{\lambda_4^{\mathrm{X}}}{\lambda_{14}^{\mathrm{X}}}, \tag{8}$$

$$\delta^{\mathrm{X}} = \gamma_1^{\mathrm{X}}\gamma_4^{\mathrm{X}} - \gamma_2^{\mathrm{X}}\gamma_3^{\mathrm{X}}. \tag{9}$$

This formalism can be used provided that $\delta^{\mathrm{X}} \neq 0$. Here $\lambda_{ij}^{\mathrm{X}}$ is the rate of the transition $X_i \rightarrow X_j$, ($i, j = 1, \ldots, 4$; $i \neq j$), and $\lambda_1^{\mathrm{X}} = \lambda_{12}^{\mathrm{X}} + \lambda_{14}^{\mathrm{X}}$, $\lambda_3^{\mathrm{X}} = \lambda_{32}^{\mathrm{X}} + \lambda_{34}^{\mathrm{X}}$, and $\lambda_4^{\mathrm{X}} = \lambda_{41}^{\mathrm{X}} + \lambda_{42}^{\mathrm{X}}$. Binding rate constants are assumed of the general form $k^{\mathrm{on}} = k^{\mathrm{off}} \times ([\mathrm{BF}]/K)^{\mathrm{h}}$, where [BF] is the concentration of the binding factor (Ca^{2+}, Caf or Que). Here and all over the paper k^{on} denotes binding rate constant, k^{off} – dissociation rate constant, K – dissociation constant, and h – Hill coefficient. The open probability P_{o} of the channel in steady state is calculated by considering first-order kinetics for the reactions of association to, or dissociation from the receptor, mass balance equations, and the conditions for thermodynamical equilibrium [9]:

$$E_{\mathrm{L}}^{\mathrm{X}} \times E_{\mathrm{Ca}0}^{\mathrm{X}} = E_{\mathrm{L}0}^{\mathrm{X}} \times E_{\mathrm{Ca}}^{\mathrm{X}}, \tag{10}$$

where X = A or I, L = Caf or Que, and the generic notation $E = k^{off}/k^{on}$ denotes the equilibrium constant of the forward and backward transitions between the unoccupied and the occupied state of the respective binding site.

The activation module has an open conformation when it is either in state A_3 or A_4, whereas the inhibition module is in open conformation in either state I_1 or I_2. The open probability of the channel in steady state in the presence of Caf or Que is calculated as the product of the two gate open probabilities:

$$P_o = P_A \times P_I, \tag{11}$$

where

$$P_A = P_{A_3} + P_{A_4}, \tag{12}$$

$$P_I = P_{I_1} + P_{I_2}. \tag{13}$$

In order to perform stochastic simulations of ligand binding to or dissociation from a certain site, a random number was generated at every time step of a discrete time sequence, and compared to the product between the time step value Δt and the corresponding transition rate constant. This procedure was applied for a total number of six regulatory sites. All the off-rate constants (k^{off}) were assumed to be equal to 50 s^{-1}. If the occupancy of a certain binding site is 0 and the generated random number is $< k^{on} \times \Delta t$, the site occupancy is switched to value 1. If the site occupancy is 1 and the generated random number is $< k^{off} \times \Delta t$, the site occupancy is switched back to 0 (all rate constants k^{on} and k^{off} are calculated for each state as described above). The time step was optimised to give consistent results under the restrictions $k^{on} \times \Delta t \le 1$ and $k^{off} \times \Delta t \le 1$.

3. RESULTS

Our model could provide an excellent agreement (Figs. 2, 3) with published experimental data on the activity of RyR1 [7] only when four different but equally probable kinetic modes of the activation gate were assumed. Consequently, the overall open probability of the RyR1 channel can be written as:

$$P_o = \frac{1}{4} \sum_{j=1}^{4} P_A^j \times P_I, \tag{14}$$

where P_A^j is the open probability of gate A in mode j (j = 1, …, 4), and is calculated according to Eq. 12 with a specific set of binding constants.

By fitting the model to the data one can derive the dissociation constants and Hill coefficients describing binding of Ca^{2+}, caffeine or quercetin to the RyR1 receptor [8]. It follows that quercetin decreases the affinity of Ca^{2+} ions toward the

activation site in all four configurations of the receptor, whereas caffeine increases the affinity of activating Ca^{2+} ions in three of the four configurations. Within the activation module, Ca^{2+} binding presents negative cooperativity (h = 0.65) in one configuration, and strong cooperativity in the other three configurations (h = 3, h = 3.5). In the inactivation module there seems to be no differences in affinity between different configurations of the receptor. However, both caffeine and quercetin consistently decrease the affinity of Ca^{2+} ions towards the inactivation module. Calcium ions bind with negative cooperativity (h = 0.55) to the inactivation sites of the RyR1 receptor.

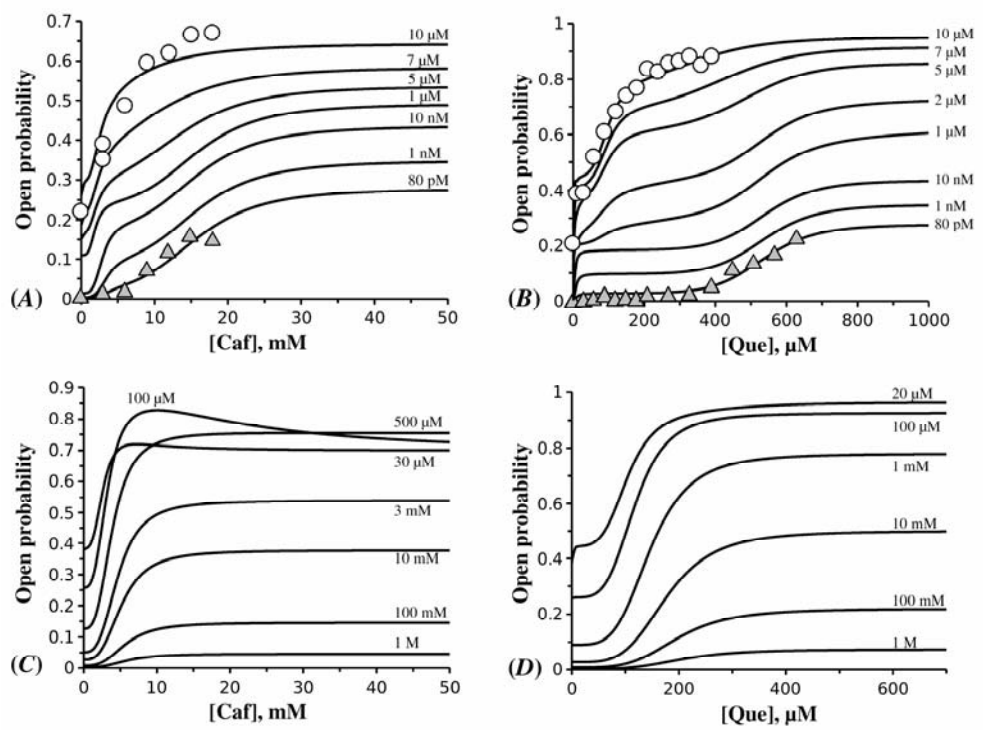

Fig. 2 – The dependence of the RyR open probability on the concentration of the exogenous ligand caffeine (***A***, ***C***) or quercetin (***B***, ***D***) at various Ca^{2+} levels, indicated on each curve. Curves are obtained by analytical calculation (Eqs. 1-14).The experimental data [7] are obtained with 10 μM Ca^{2+} (circles) or 80 pM Ca^{2+} (triangles).

We also performed a series of stochastic simulations of channel opening at various Ca^{2+} concentrations, using the parameters obtained from the best fit to the data, and the results appeared to be consistent with the analytical formalism presented above (Fig. 3). There is also a close similarity between mean values obtained from four different simulations and the experimental data [7].

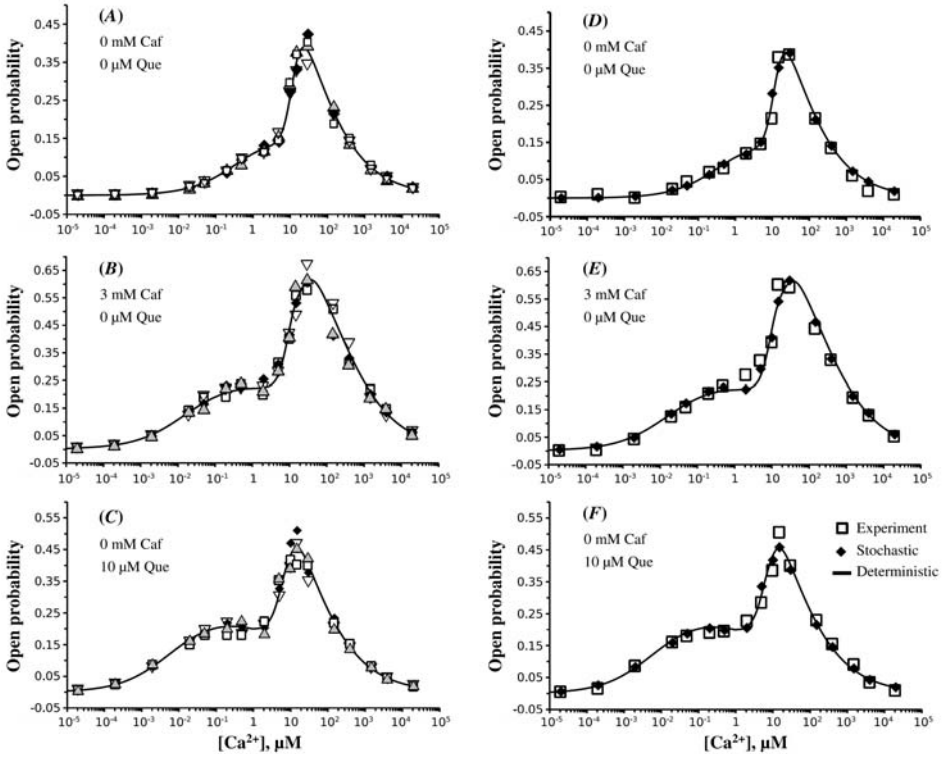

Fig. 3 – Deterministic approach and stochastic simulation of RyR opening in the absence of exogenous ligands (***A***, ***D***) or in the presence of 3 mM caffeine (***B***, ***E***) or 10 μM quercetin (***C***, ***F***). RyR open probability is obtained either by analytical calculation (Eqs. 1-14) – continuous curves – or by stochastic simulation of ligand binding to the receptor – symbols in *A*-*C* (generated by four different simulations). In ***D***-***F***, experimental data (squares) are from [7] and stochastic data (diamonds) represent the average from the simulations shown in *A*-*C*.

4. DISCUSSION

In agreement with the conclusions of other previous studies [7], our model predicts that quercetin is a stronger activator of the RyR1 calcium channel than caffeine (Fig. 4). High values of the open probability P_o (> 0.5) can be obtained in a calcium domain which is about 4 times larger in the presence of quercetin (between 0.29 μM and 10.3 mM Ca^{2+}) than in the presence of caffeine (between 0.29 μM and 4.0 mM Ca^{2+}). Moreover, according to our model, caffeine or quercetin in large concentrations can open the RyR1 channel even at extremely low concentrations of calcium (> 5 pM). In addition, the channel activation phase in the presence of saturating quercetin (~1 mM) or caffeine (~100 mM) should present four distinct domains when the cytosolic calcium level is increased progressively (Fig. 4), corresponding to the four different modes of channel activation. In the

RyR1 activation range (< 10 μM Ca^{2+}) the effect of caffeine and quercetin saturates at about > 30 mM (Figs. 2A, 4A) and about > 800 μM (Figs. 2B, 4B, respectively, whereas in the inhibition domain (> 500 μM Ca^{2+}), the agonist effect becomes almost saturated at about > 15 mM caffeine (Figs. 2C, 4A) and > 300 μM quercetin (Figs. 2D, 4B). This reflects different sensitivities of the activation and inhibition modules to each one of the two agonists.

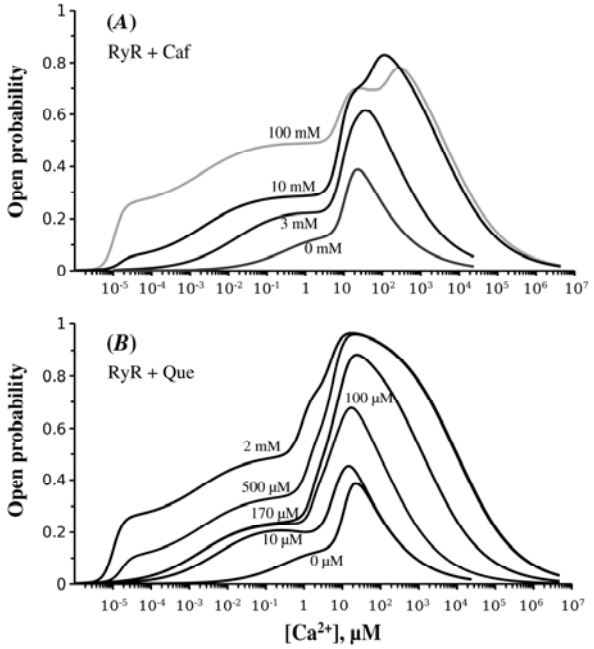

Fig. 4 – Predicted RyR1 open probability in function of the cytosolic [Ca^{2+}], at different concentrations of caffeine (***A***) or quercetin (**B**). Agonist concentration is specified on each curve.

In conclusion, our model considers that the RyR1 calcium channel has two independent gates, activated and inhibited by Ca^{2+}, respectively, which can be associated with an activation and an inhibition module of the receptor, respectively. Each module has one equivalent class of binding sites for Ca^{2+} ions, which are allosterically regulated by binding of caffeine and quercetin to their sites in the respective module. There are four different, but equally probable, configurations of the receptor, which affect ligand binding to the activation module, but not to the inhibition module. Our results can help understanding the behaviour of an important ionic channel involved in muscle contraction. Moreover, it can be adapted and combined with detailed simulations of calcium release from the sarcoplasmic reticulum [10,11], which could offer valuable insights into the involvement of calcium signals in cardiac activity or skeletal muscle contraction.

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APPENDIX

According to the scheme in Fig. 1, the following kinetic equations are obtained, which are then solved in steady state ($\mathrm{d}P_{\mathrm{X}_i}/\mathrm{d}t = 0;\ \ i = \overline{1,4}$):

$$\mathrm{d}P_{\mathrm{X}_1}/\mathrm{d}t = \lambda_{21}P_{\mathrm{X}_2} + \lambda_{41}P_{\mathrm{X}_4} - \lambda_{12}P_{\mathrm{X}_1} - \lambda_{14}P_{\mathrm{X}_1} \tag{16}$$

$$\mathrm{d}P_{\mathrm{X}_2}/\mathrm{d}t = \lambda_{12}P_{\mathrm{X}_1} + \lambda_{31}P_{\mathrm{X}_3} - \lambda_{21}P_{\mathrm{X}_2} - \lambda_{23}P_{\mathrm{X}_2} \tag{17}$$

$$\mathrm{d}P_{\mathrm{X}_3}/\mathrm{d}t = \lambda_{23}P_{\mathrm{X}_2} + \lambda_{43}P_{\mathrm{X}_4} - \lambda_{32}P_{\mathrm{X}_3} - \lambda_{34}P_{\mathrm{X}_3} \tag{18}$$

$$\mathrm{d}P_{\mathrm{X}_4}/\mathrm{d}t = \lambda_{14}P_{\mathrm{X}_1} + \lambda_{34}P_{\mathrm{X}_3} - \lambda_{41}P_{\mathrm{X}_4} - \lambda_{43}P_{\mathrm{X}_4} \tag{19}$$

where λ_{ij}^{X} is the rate constant of the transition $X_i \rightarrow X_j$, ($i, j = \overline{1,4}$; $i \neq j$). In steady state one obtains a linear system with three unknown independent variables, with the solution described by Eqs. 1-9.

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