Mathematical Modeling of the Role of Cooperativity between Contractile and Regulatory Proteins in the Mechano-Calcium Feedbacks in Myocardium

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Abstract

There is a complicated interplay between different factors contributing to the calcium regulation of the heart muscle contraction. Mathematical modeling is an efficient approach to analyze this compound system and recognize the key links. In this work the modeling is applied to the analysis of mechano-calcium feedback mechanisms and their role in the cardiomyocyte calcium activation.

1. Introduction

Mechano-Calcium Feedback (MCF) and Mechano-Electric Feedback (MEF) are important regulatory links for the Excitation-Contraction Coupling (ECC) in the myocardium. They reveal themselves e.g. in load-dependent relaxation in isotonic twitches, in inactivating effects of quick deformations during isometric twitches, both accompanied with specific responses of calcium transients and action potentials [1]. In general, MCFs provide a fine tuning of electrical and calcium activation to the mechanical conditions of myocardium contractions. There is much experimental and theoretical evidence that cooperative dependence of CaTnC kinetics on the cross-bridge concentration ([Xb]) is one of the key mechanisms underlying MCFs in the intact cardiomyocytes [2-4]. Nevertheless, a discrepancy seems to arise between these arguments for role of cooperativity and experimental data obtained on skinned (demembranized) heart muscles showing that the muscle length significantly affects only calcium sensitivity of the 'pCa-force' relationship but practically does not affect its Hill coefficient of cooperativity [5]. These findings have prompted the authors of the cited work to doubt that cooperativity contributes to the mechano-calcium feedbacks in muscles. Trying to overcome this discrepancy we suggest and verify by means of mathematical modeling a refined concept of cooperativity developing our previous approach. The concept makes the cooperativity behave differently in steady-state and transitional processes and helps to explain both mechano-dependence of calcium activation in the intact myocardium and the data obtained on the skinned muscles.

2. Methods

We have earlier developed and published mathematical model of the ECC in the cardiomyocyte [6-10]. The model allowed us to simulate and explain (*ibidem*) a wide range of MCF effects and a number of MEF effects observed in the twitches (i.e. contraction-relaxation cycles) of the intact cardiac muscles *in vitro*. Mechanisms of cooperativity of contractile and regulatory proteins (cross-bridges (Xb) and CaTnC complexes) underlie these effects within the model.

The following differential equations describe cross-bridge and CaTnC kinetics in the model:

$$\frac{dN}{dt} = k_+(v)\cdot M(A)\cdot n_1(l_1)\cdot L_{oz}(l_1)\cdot(1-N) \;-\; k_-(v)\cdot N \quad (1)$$

$$\frac{dA}{dt} = a_{on}\cdot(A_{tot}-A)\cdot Ca(t) - a_{off}\cdot A \quad (2)$$

Here l_1 is a sarcomere length, $v = dl_1/dt$, N = [Xb], A = [CaTnC], and $a_{off} = \bar{a}_{off}\cdot\pi(N)\cdot e^{-k_A\cdot A}$, where $\pi(N)$ is an explicit function defining Xb-CaTnC cooperativity (see below).

The model also includes equations linking N with myocardium mechanics and equations describing Ca^{2+} handling and action potential development during myocardium twitches [9].

$\pi(N)$, $k_+(v)$, $k_-(v)$, $M(A)$, $n_1(l_1)$, $L_{oz}(l_1)$ are explicit functions defined in detail elsewhere [9]. In particular, function $n_1(l_1)$ in the equation (1) indirectly prescribes dependence of the cross-bridge attachment probability on the distance between thick and thin filaments of the sarcomere, bearing in mind that the distance depends on the sarcomere/muscle length due to the constancy of the cardiomyocyte volume during its contraction. It is $n_1(l_1)$ that together with Xb-CaTnC cooperativity underlies mechano-sensitivity of the calcium activation of myocardium contractions.

A few types of cooperativity are included in the kinetic equations. Two of them enter into the term a_{off} of the equation (2):

- Xb-CaTnC cooperativity (monotonically decreasing function $\pi(N)$ in the formula describing a_{off}): CaTnC

affinity increases with the average number of strongly attached cross-bridges around each CaTnC complex, and thereby with N = [Xb];

- CaTnC-CaTnC cooperativity (function $e^{-k_A \cdot A}$ in the formula describing a_{off}): CaTnC affinity increases with concentration of other CaTnC complexes as a result of troponin-tropomyosin conformations after Ca^{2+} binding to TnC.

The above definition of both Xb-CaTnC and CaTnC-CaTnC cooperativity based on the explicit functions $\pi(N)$ and $e^{-k_A \cdot A}$ led to the equivalent occurrence of the cooperativity in numerical experiments simulating two different types of the real experimental conditions: (i) steady-state conditions typical for the experiments on skinned muscle preparations to furnish 'pCa-force' relationship, (ii) transients typical, in particular, for the twitches of the intact heart muscle.

As we have already noted, such cooperativity used in previous versions of our model allowed us to reproduce adequately and explain the key MCF and a MEF effects in the intact myocardium. At the same time, when simulating 'pCa-force' we obtained correct features of this relationship (including length-dependence of its calcium sensitivity rather than of its Hill's coefficient) in the model only for a quite low degree of the cooperativity, e.g. for small values of the parameter k_A, which was not sufficient for reproducing MCF and a MEF effects in intact myocardium twitches.

The novelty we have recently introduced in the model allowed us to avoid this inconsistency and thus reconcile the seemingly opposing concepts suggested by different teams of muscle physiologists regarding the role of cooperativity in the MCF mechanisms, as those concepts had been produced alternatively from the analysis of experimental data obtained either on the intact muscle preparations or on the skinned ones.

Now both types of cooperitivity (Xb-CaTnC and CaTnC-CaTnC) reveal themselves in distinct ways for steady-state and transitional processes. This is done by means of the modified definition of the CaTnC dissociation rate "constant" a_{off} in the equation (2):

$$a_{off} = \alpha \cdot \underbrace{[\bar{a}_{off} \cdot \pi(N) \cdot e^{-k_A \cdot A}]}_{a_{off1}} + \\ +(1-\alpha) \cdot \underbrace{[a_{on} \cdot \bar{a}_{eq\_limit}]}_{a_{off2}} \quad (3)$$

Here a_{off1} is a dynamic component of the CaTnC dissociation rate "constant" a_{off}, and a_{off2} is its stable component. The dynamic component coincides with the total rate "constant" a_{off} used in the former definition of the cooperativity described above and implemented in the previous versions of our model [6 -10].

$$\alpha = \begin{cases} 1, \quad \text{for } [\bar{a}_{off} \cdot \pi(N) \cdot e^{-k_A \cdot A}] > a_{on} \cdot \bar{a}_{eq\_limit} \\ \text{the solution of the eq. (5),} \\ \text{for } [\bar{a}_{off} \cdot \pi(N) \cdot e^{-k_A \cdot A}] \le a_{on} \cdot \bar{a}_{eq\_limit} \end{cases} \quad (4)$$

$$\frac{d\alpha}{dt} = -\frac{1}{\tau_\infty} \cdot \alpha \quad (5)$$

The initial condition for the solution is: $\alpha(t_0) = 1$, where t_0 is a moment, when the inequality $[\bar{a}_{off} \cdot \pi(N(t_0)) \cdot e^{-k_A \cdot A(t_0)}] > a_{on} \cdot \bar{a}_{eq\_limit}$ turns out violated. Thus $\alpha(t) = exp((t_0 - t)/\tau_\infty)$.

In other words, contribution of the dynamic component a_{off1} to the CaTnC dissociation arises when this component becomes lower than the stable border a_{off2} due to an increase in the [Xb]. After that a_{off1} initially dominates in the total sum a_{off}, but gradually a_{off} returns to the border value. Particular rate of the a_{off} restitution depends on the time constant τ_∞ in the equation (5). This constant is a model parameter.

Modified equations rearrange the cooperative dependence of CaTnC on [Xb] so that the cooperativity appears quite differently in steady-state (where [Ca^{2+}], and consequently [CaTnC] and [Xb] are fixed) and during transients in response to a change in [Xb]. Specifically, attachment of cross-bridges amplifies TnC affinity to Ca^{2+} due to an instant conformational impact of the attachment on the CaTnC decay, but this impact then relaxes, and in steady-state the constant of the affinity can't exceed some maximum value AL (i.e. Affinity Limit) determined by the parameter $\bar{a}_{eq\_limit}$. Thus, significant changes in the affinity occur only immediately in response to a change (increase/decrease) in [Xb] and some time after that. In particular, after attachment of new cross-bridges the affinity constant may surpass AL considerably, while then it gradually lowers to the AL level. Therefore, innovate cooperativity is less pronounced in steady-state than immediately in response to changes in [Xb] and at the beginning of the transient process initiated by such changes.

3. Results

Characteristics of the 'pCa-force' relationship are determined in the model by the steady-state conditions of the cooperativity. Specifically, at low [Ca^{2+}] (and low [Xb], respectively), where TnC affinity to Ca^{2+} is less than AL, the steady-state cooperativity is quite sensitive to the difference in [Xb]-s, unlike higher [Ca^{2+}]. Hence, Ca^{2+} sensitivity of the 'pCa-force' (i.e. shift of the curve along the 'pCa' axis) depends on the [Xb] (and therefore on the muscle lengthening via the inter-filament distance) much stronger than Hill's coefficient (i.e. the slope of the

curve). Respective numerical data represented in Figure 1 are in a good concordance with experimental results obtained on skinned trabeculas [5]. Now we are being verifying these data involving *in vitro* motility assay experimental method that enables us to assess 'pCa-force' relationship for fast and slow cardiac isomyosins [11].

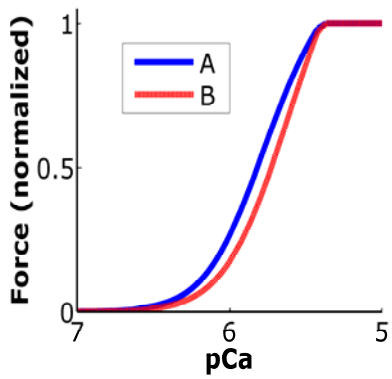

Figure 1. Simulation of the length-dependence of 'pCa-Force' relationship in skinned cardiac muscle: $L_1 = 2.2$ µm/sarc. (line A), $L_2 = 2.0$ µm/sarc. (line B).

On the other hand, the same cooperativity during twitches of the intact cardiac muscles reveals itself in the transient processes initiated by Calcium-transients. As a result the cooperativity is pronounced quite strongly during contraction/relaxation phases of the twitches. For sufficiently big values of the time constant τ_∞ the modified cooperativity manifests itself in twitches in the same manner as the non-modified variant underlying a number of the mechano-dependence effects observed in the intact myocardium and simulated in our previous works [6-10].

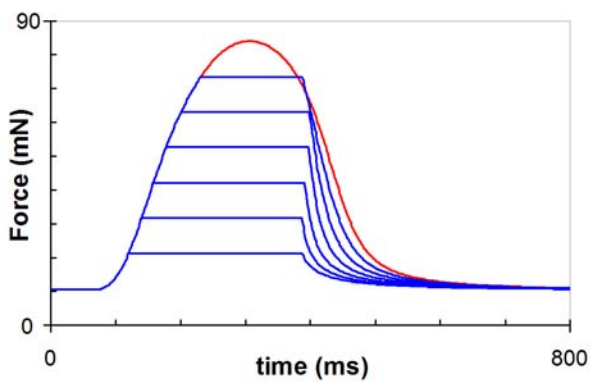

Figure 2. Simulation of the phenomenon of Load-Dependence of Relaxation (LDR) in the intact cardiac muscle. Isometric twitch and 6 isotonic twitches (under afterloads F = 21 mN, F = 31 mN, F = 42 mN, F = 52 mN, F = 63 mN and F = 73 mN) are represented.

Numerical simulation of the most important phenomenon of LOAD-DEPENDENCE is shown in Figures 2 and 3. For τ_∞ = 1000 s the shown data totally coincide with the none-modified case and thus correctly reproduce experimental data.

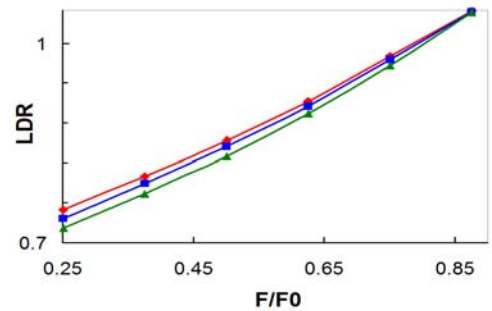

Figure 3. Index of LDR estimated for 3 time constants τ_∞ (top to bottom: 60 s, 100 s, 1000 s). For each afterload F/F_0 (where F_0 is a peak isometric force) the LDR index value is a ratio between durations of the isotonic contraction–relaxation phase (under this afterload) and of the time interval within the fully isometric twitch where the force was higher than this afterload. Such LDR index is commonly used [8], [12].

Importantly that both types of simulations - that of twitches and of steady-state are performed with the same degree of the cooperativity.

At last but not at least, there is experimental evidence obtained on skinned muscle preparation that the placement of the muscle in the Ca^{2+} containing solution may cause nonmonotonic transient changes in the muscle force until it reaches a steady-state for this particular $[Ca^{2+}]$ [13]. The non-monotonicity arose for the temperatures of the solution equal 22°C or higher, but did not arise at lower ones: e.g. at 5°C [13] or at 15°C [14].

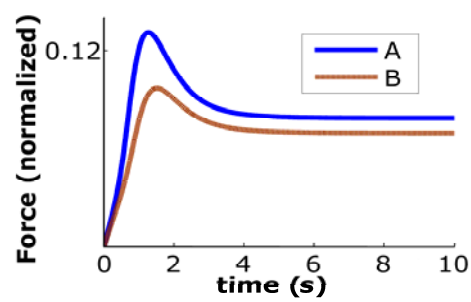

Figure 4. Nonmonotonic force transient arising in the model during simulated response of a skinned muscle to the Ca^{2+} addition ($[Ca^{2+}]$ = 0.39 µM (line A), $[Ca^{2+}]$ = 0.37 (line B)) in the calcium-free solution. Xb kinetic constants in this case were k_-=0.024 s^{-1}; k_+=0.045 s^{-1} (k_+/k_-=1.8). Force is normalized to the value corresponding to saturating $[Ca^{2+}]$ (see Figure 1).

Modified concept of cooperativity allowed us to reproduce these results in the model: both non-monotonic pattern of the force development in a skinned muscle

(Figure 4), and monotonic one (Figure 5). Lower temperature (Figure 5) was simulated by means of the tenfold decrease in the rate constants (k_+ and k_-) of the cross-bridge kinetics.

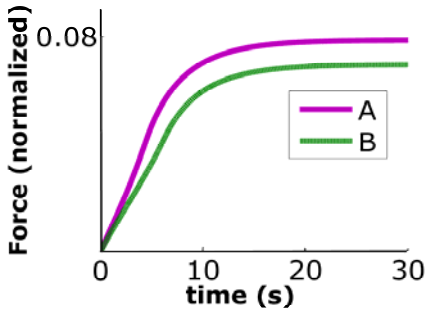

Figure 5. Simulation distinct from that shown in Figure 4 only by the tenfold decrease in the Xb kinetic constants (k_+ =0.0045 s^{-1}; k_-=0.0024 s^{-1} (the same k_+ / k_-=1.8 as in Figure 4)). Monotonic force transient occurs.

4. Discussion

The refined concept of the Xb-CaTnC cooperativity was verified in mathematical model where the cooperativity turned out to reveal itself differently in steady-state and transitional processes:

- it affected noticeably calcium sensitivity of the 'pCa-Force' relationship, but had no essential effect on its Hill's coefficient;

- at the same time just this cooperativity in the model contributed to the mechano-calcium feedback in the intact heart muscle.

Thus, the refined concept may help to overcome a seeming discrepancy between significant contribution of the cooperativity to the performance of the intact myocardium and experimental data on the 'pCa-Force' relationship obtained on skinned muscle preparations.

Moreover, the refined cooperativity concept allowed us to reproduce within the model experiments revealing non-monotonic pattern of the force transient that arose in skinned muscle preparations at sufficiently high temperatures [13]. It seems to be a first attempt to explain (and simulate) such a pattern of transients.

Acknowledgements

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