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ForceLAB simulator update: a study on the positive cooperativity of cross-bridge formation in ventricular myocyte

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Abstract— **Cardiovascular diseases are one of the leading causes of death in the world. Understanding the mechanisms that control heart contraction is essential to deal with this problem. As auxiliary tools, mathematical models have been used to describe these phenomena at the cellular level. Electrical and mechanical models of ventricular myocytes have been implemented in computer simulators for analysis. One of them is ForceLAB, in which two of the main models of contraction force were implemented: Rice et al. (1999) (RWH) and Negroni & Lascano (2008) (NL). The objective of this paper is to present an updated version of the ForceLAB tool, to which additional features were added, among which the values of important variables, as one of the outputs. The new version of ForceLAB was implemented using App Designer for the development of the new interfaces. As an application, the influence of the positive crossbridge cooperativity on the unusual behavior of the Ca^{2+}-force relationship simulated with the RWH was tested. The waveforms of twitch transient of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), developed force and the force vs. pCa curve (where pCa = -log $[Ca^{2+}]_i$) were simulated. The simulation results showed that the non-paralel shift of the force vs. pCa curve upon variation of myofilament Ca^{2+} was not corrected by the absence of the positive cooperativity mechanism did in the RWH model formulation.**

Keywords— **mathematical model, contraction, cardiac myocyte, crossbridge cooperativity, *in silico* experiments.**

I. Introduction

Cardiovascular diseases are the leading cause of death in the world. In 2019, they caused about 17.9 million deaths, accounting for 32% of global death count, 85% of which were from myocardial infarction and stroke [1].

Understanding the mechanisms of cardiac contraction is a crucial task to elucidate the genesis of several heart diseases. This understanding involves the description of the dynamics of Ca^{2+} ions in the ventricular myocyte, which plays a key role in contraction development [2]. Ca^{2+} entry into the cell occurs through the L-type channel during the action potential (AP), triggering Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum when Ca^{2+} binds to the Ca^{2+} channels of the organelle, the ryanodine receptors. The increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) favors Ca^{2+} binding to troponin C (TnC), located in the thin filament of the sarcomeres, triggering the contraction process [3-5]. The Ca^{2+}-TnC binding starts a sequence of conformational changes in proteins of the myofibril thin filament, leading to actin-myosin interaction (crossbridge attachment). Different approaches have been used to model crossbridge formation, for example, taking into account the cooperativity between the formation of neighboring crossbridges and/or the size of the sarcomere [6].

Mathematical and computational modeling is an important tool for understanding biological phenomena [7]. It is possible to investigate, for instance, the mechanisms of cardiac arrhythmias by simulation of myocardial cell electrical activity [8-12]. For the description of the phenomenon of cardiac contraction in ventricular myocytes, two models have been widely used: the 1999 model of Rice et al. (the Rice, Winslow and Hunter model, RWH) [13] and the 2008 model of Negroni & Lascano (NL) [14]. These models were implemented in a simulator recently developed by Silva et al. [15], the ForceLAB. In that paper, the simulator functions and comparison of the two models were presented. The simulator was initially developed using the 2017a version of MATLAB, which has limitations regarding the development of applications. One of the pending issues of the tests performed in that study was the atypical result observed in the force vs. pCa curve simulated with the RWH model when the affinity of the Ca^{2+}-TnC interaction was varied. One of the hypotheses raised was that this unusual behavior would be due to the positive cooperativity of crossbridge attachment implemented by the authors in the RWH model.

The aim of the present article was to report an update of the ForceLAB simulator, presenting the new functions and the main differences between the two versions of the tool. As an application of the new version of the simulator, the test of the previous hypothesis of the involvement of the positive crossbridge cooperativity in the unusual curve simulated with the RWH model was carried out.

II. Materials and Methods

A. Rice et al. 1999 model (RWH)

The model to estimate the myocyte contraction force proposed by Rice et al. (RWH) [13] is based on the formation of crossbridges between myosin and actin. The binding of these proteins occurs after exposure of the myosin binding site in actin filament after tropomyosin displacement triggered by Ca^{2+}-TnC association. The RWH model describes 6 permissivity states for crossbridge formation: 2 non-permissive (N0 and N1), and 4 permissive (P0, P1, P2 and P3). All these states are described in the model as coupled differential equations:

$$N0 + N1 + P0 + P1 + P2 + P3 = 1 \qquad (1)$$

The transition between the N0 and N1 states to the permissive states depends on the Ca^{2+}-TnC association, which is modeled with basis on the association and dissociation rate constants of these ligands, k_{on} and k_{off}, respectively. In the permissive states, the transition occurs via parameters that predict positive cooperativity of crossbridge attachment. The peak developed contraction force (F_{contr}), expressed in mN per mm^2 of cell cross sectional area, is formulated in the RWH model as:

$$F_{contr} = \alpha . \frac{P1 + N1 + 2.(P2) + 3.(P3)}{P1_{max} + 2.(P2_{max}) + 3.(P3_{max})} \qquad (2)$$

where α is a model fit factor.

Fig. 1A illustrates the transition scheme among the states of crossbridge formation in the RWH model. In the study of Mullins & Bondarenko [16], the RWH model was coupled to the electrophysiological model of the mouse ventricular myocyte, previously developed by Bondarenko et al. [17]. These coupled models were used also in the present study.

B. Negroni & Lascano 2008 model (NL)

In the NL model [14], the force of contraction is directly related to the Ca^{2+}-TnC interaction. This binding is described in 6 states of the troponin system (TS):

(a) [TS]: Ca^{2+}-free state (b) [TSCa]: Ca^{2+} binds to TnC without crossbridge formation; (c) [TSCa~]: Ca^{2+} binds to TnC leading to the formation a weak crossbridge; (d) [TSCa*]: Ca^{2+} binds to TnC leading to the formation of a strong crossbridge; (e) [TS~]: maintainance of weak crossbridge interaction without Ca^{2+}-TnC binding; (f) [TS*]: maintainance of strong crossbridge interaction without Ca^{2+}-TnC binding. Fig. 1B illustrates the transition among these states.

In the NL model, the contraction force (F, in mN/mm^2) is given by:

$$F = A_w.([TSCa\sim] + [TS\sim]).h_w + A_p.([TSCa^*] + [TS^*]).h_p \qquad (3)$$

where A_w and A_p are elastic constants for the weak and strong crossbridge states, respectively, and h_w and h_p indicate the crossbridge elongation in these states. In this study, the NL model was also coupled to the electrophysiological model of Bondarenko et al. [17].

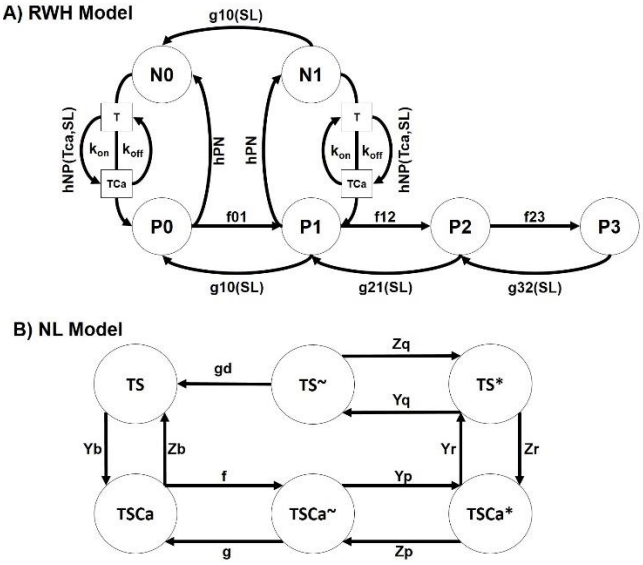

Fig. 1. State diagrams of the models implemented in ForceLAB. A) RWH model state diagram. B) State diagram of the NL model

C. The ForceLAB 2.0 simulator

To allow solving the system of 51 coupled differential equations and presenting the results of the simulations graphically, an upgrade was made in the previously published ForceLAB simulator, [15]. The new version was developed using MATLAB 2019a, with the interfaces created with the App Designer.

D. Simulation protocols

Using the new version of the ForceLAB tool, sustained for values in response to variation in extra myofibrillar Ca^{2+} concentration and twitch Ca^{2+} transients and contractions were evoked by suprathreshold electric stimuli were simulated with the RWH and NL models using protocols previously employed [15]: (a) total simulation duration: 20 s; (b) duration of electric stimulation pulse: 1 ms; (c) electric stimulation frequency: 1 Hz; (d) stimulation current: 20 pA/pF; (e) initial sarcomere length: 2.1 µm; (f) cell length: 100 µm. For

the RWH model, two simulations were performed, one including the positive cooperativity for crossbridge formation, and one excluding it. This change was accomplished by modifying the values assumed for the parameters f01, f12 and f23 that control the transition between the states P0 and P1, P1 and P2, and P2 and P3, respectively, as shown in Fig. 2.

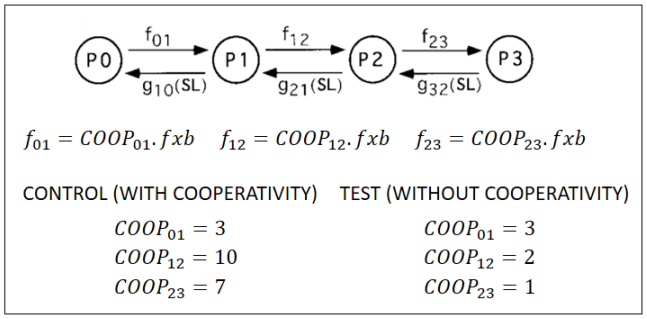

Fig. 2. Illustration of the steps that involved and assumed values of the parameters changed to test the influence of positive cooperativity of crossbridge formation on contraction simulated with the RWH model

From the twitch $[Ca^{2+}]_i$ and force waveforms evoked in response to electric stimulation, the following variables were obtained directly in the ForceLAB simulator: the diastolic (Dia, i.e., just before stimulus delivery) and peak values; amplitude (Δ, i.e., difference of peak and diastolic values); and times to peak (t_{peak}) and half decay ($t_{0.5}$). The force vs. pCa relationship was determined using the steady-state force corresponding to each value of pCa (i.e., the negative logarithm of the molar Ca^{2+} concentration).

The force vs. pCa curve was simulated following the same changes in TnC affinity for Ca^{2+} as tested in [15], namely, increase and decrease of the apparent dissociation constant of Ca^{2+} at TnC (K_d) by dividing and multiplying k_{on} by 3, respectively. Using the ForceLAB simulator, the values of pCa_{50} (pCa value that corresponds to 50% of the maximum contraction force recorded at saturating Ca^{2+} concentration) and the Hill coefficient N_H (slope constant of the curve) were calculated from the force vs. pCa curve.

III. Results

A. ForceLAB simulator update

The main result of this work was the updated version of the ForceLAB simulator, in which the major functions of the original version [15] were recreated by adding new features. The main and implemented features of ForceLAB 2.0 were:

Start screen: It starts the tool and gives access to the "ABOUT" section, where information on the tool, the collaborating institutions and the main authors is presented.

Simulation: The first tab shown when starting the tool (Fig. 3) offers the user the possibility to run simulations of the electrical model [16] coupled to the RWH and NL contraction models. The user can also change the simulation protocols, the general parameters of the electrical model and the main parameters of the two contraction force models.

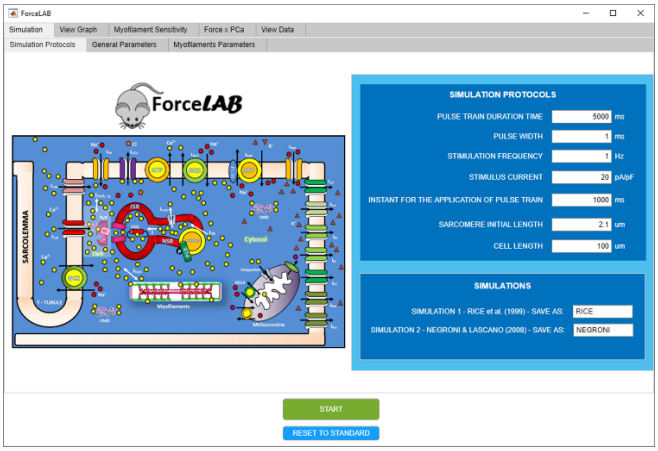

Fig. 3. ForceLAB simulation tab: Access to general and electrophysiologic parameters, and myofilament-related parameters of the RWH and NL models

View graph: It makes it possible to graphically display up to 3 of the simulations performed in each of two graphs. The waveforms that can be plotted are: AP, $[Ca^{2+}]_i$, TnC-bound $[Ca^{2+}]$ ($[Ca^{2+}]_b$) and contraction force. One of the new features implemented was the automated calculation of important values extracted from the curves: Dia and peak values, Δ, t_{peak}, $t_{0.5}$ and AP duration at 20%, 50% and 90% of repolarization (APD20, APD50 and APD90, respectively). These values are shown in tables below the graphics, and can be exported in the ".xlsx" format.

Myofilament sensitivity: In this part of the simulator, one can perform tests by changing Ca^{2+}-TnC k_{on} and k_{off}, and choose the simulation protocols. Simulations are performed for the two implemented models of contraction force, and the Ca^{2+} transient and contraction traces are plotted along with the calculated Dia, peak, Δ, t_{peak} and $t_{0.5}$ values, which can be exported as ".xlsx" files.

Force vs. pCa: In this tab the user can vary the pCa and obtain the corresponding steady-state contraction force. The user can choose the contraction model and the value of K_d by changing k_{on} and/or k_{off} ($K_d = k_{off} / k_{on}$). Other available options are the initial and final pCa values, pCa step, and general simulation protocols. The K_d, pCa_{50} and N_H values are calculated and can also be exported as ".xlsx" files.

View data: Area where the user can access the exported data in the other tabs as a table. It is also possible to open the generated ".xlsx" files.

B. Influence of positive cooperativity on cross-bridge formation

Simulations were performed to investigate if and how the positive cooperativity mechanism of crossbridge formation, present in the RWH model, affects the simulation results. In Fig. 4, which shows the "View Graph" screen generated by the ForceLAB simulator itself, the comparison of the twitch Ca^{2+} transients and contractions simulated with the NL and RWH models, the latter with and without cooperativity, is illustrated. Tables 1 and 2 show the values of the twitch $[Ca^{2+}]_i$ and contraction, respectively, extracted from the traces depicted in Fig. 4.

Table 1. Twitch $[Ca^{2+}]_i$ variables obtained with the NL and RWH models, the latter also without including the positive cooperativity for crossbridge formation (*no coop*). The values of diastolic (*Dia*) and peak $[Ca^{2+}]_i$, transient amplitude (Δ) and times to peak (t_{peak}), and to half-decay ($t_{0.5}$) are presented

Variables	NL	RWH	RWH (no coop)
Dia (μM)	0.13	0.14	0.14
Peak (μM)	0.51	0.53	0.53
Δ (μM)	0.38	0.38	0.38
t_{peak} (ms)	31.09	18.03	17.90
$t_{0.5}$ (ms)	199.37	157.31	157.41

Table 2. Contraction variables obtained with the NL and RWH models, the latter also without including the positive cooperativity for crossbridge formation (*no coop*). The values of diastolic (*Dia*) and peak force, developed tension (Δ) and times to peak (t_{peak}), and to half-decay/relaxation ($t_{0.5}$) are presented

Variables	NL	RWH	RWH (no coop)
Dia (mN/mm^2)	0.27	0.23	0.05
Peak (mN/mm^2)	9.01	9.10	4.74
Δ (mN/mm^2)	8.74	8.87	4.69
t_{peak} (ms)	149.12	127.50	87.84
$t_{0.5}$ (ms)	169	187	97

Fig. 5 shows the force vs. pCa curves obtained with the RWH model without cooperativity considering the original K_d value (control) and the situations of increase and decrease in K_d by a factor of 3. The corresponding variables are shown in Table 3.

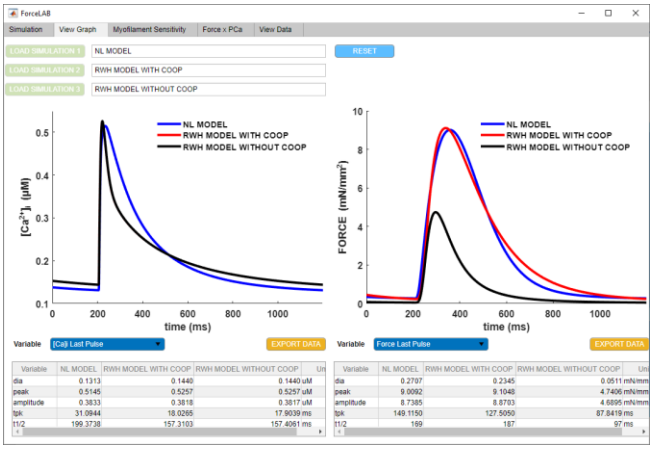

Fig. 4. Timecourse of twitch $[Ca^{2+}]_i$ transients and contractions shown in ForceLAB graph view tab. They correspond to the waveforms simulated with the NL and RWH models, the latter including or not positive cooperativity for crossbridge formation

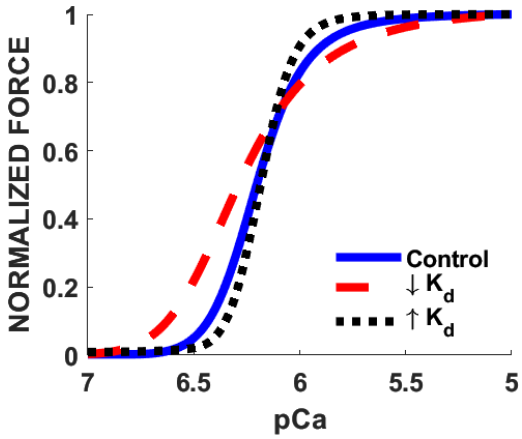

Fig. 5. Force vs. pCa relationship simulated with the RWH model without the cooperativity mechanism using the original K_d value (control), and after it was increased (upward arrow) and decreased (downward arrow) by 3 times.

Table 3. Parameters of the force vs. pCa curves shown in Fig. 5, without the crossbridge positive cooperativity, using the original (control), increased and decreased values of the apparent dissociation constant (K_d) by a factor of 3. The values of the pCa at which force is half-maximal (pCa_{50}) and the Hill coefficient (N_H) and (K_d) are shown

Variables	Control	K_d↑	K_d↓
pCa_{50}	6.21	6.19	6.29
N_H	3.17	3.79	2.31
K_d (μM)	0.60	1.80	0.20

IV. Discussion

The present update of ForceLAB aimed to use the new features of the MATLAB 2019 version, especially the App

Designer. Regarding usability, the simulator has now its functions grouped in a single screen where the user accesses the resources by means of tabs. This change makes the simulator easier to use, as it provides a single environment for simulations. One of the main advantages over the previous version [15] is the automatic calculation of values of the main variables used for analysis, such as Dia and peak values, Δ, t_{peak} and $t_{0.5}$ of the twitch waveforms. Thus, the analysis of the simulation results has become much easier, and the function of data export as ".xlsx" files ensures the prompt saving and storage of the data.

The removal of the positive crossbridge cooperativity from the formulation of the RWH model did not affect the $[Ca^{2+}]_i$ values or the transient time-course during a twitch. However, diastolic tension was almost null and the developed force was decreased by almost 50%, with marked acceleration of contraction development and relaxation. Thus introduction of positive cooperativity, which reflects a physiological phenomenon [18], is required for a realistic simulation of a twitch contraction. Nevertheless, alterations were not observed in the force vs. pCa relationship upon removal of the cooperativity from the RWH model formulation.

Symmetrical (increase and decrease by the same factor), moderate changes in K_d are expected to result in symmetrical and parallel shifts (to the right and the left, respectively) in this relationship, as observed with the NL model. However, this was not the case with the RWH model: decreasing K_d also decreased the slope of the force vs. pCa curve, whereas the opposite was observed when K_d was enhanced [15]. Unfortunately, model instability prevented us to test a possible role of the myosin positive cooperativity mechanism in this unusual result at that time. With the 2.0 ForceLAB version, software limitations of the former version could be overcome, and the observed model instability could be eliminated, which has made it possible to test the previously raised hypothesis. Nevertheless, the present results (Table 3 and Fig. 5) show that variation of n_H resulting from variations in the K_d value for the Ca^{2+}-TnC interaction is still present, also with very similar n_H values as when the cooperativity was included in the model [15]. Thus, other aspect of the formulation should be responsible for the odd pattern of dependence of the slope of the force vs. pCa relationship on the affinity of Ca^{2+} for TnC.

V. Conclusions

The new features of the updated ForceLAB simulator have made it more user-friendly, and make it possible to gather quantitative data as simulation proceeds, allowing their prompt recording and storage. ForceLAB version 2.0 is available for download after a brief registration at the following link: https://forms.gle/9nneEyX9NKDcjiah8.

With this improved version of the tool, it was possible to rule out the hypothesis that the formulation of the crossbridge positive cooperativity mechanism could be responsible for the non-parallel shifts in response to variations in the Ca^{2+}-TnC affinity in the force vs. pCa relationship simulated with the RWH model. Thus, more studies are required to clarify the origin of this odd result.

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Conflict of interest

The authors declare that they have no conflict of interest.

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