INCLUDING TITIN IN A SIMPLE MUSCLE CONTRACTION MODEL SUITABLE FOR A MYOCARDIAL CELL MODEL

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Abstract: Using the *simBio* software package [1] a new cardiac muscle contraction model has been developed that combines crossbridge mechanical elements with a seven-state model including Ca^{2+} activation and crossbridge kinetics. The model is designed for the usage in cardiac cell models and is to our knowledge the first myocyte contraction model that introduces experimental data of titin-based passive tension to account for the sarcomere length (SL) dependent Ca^{2+} sensitivity. Simulation results predict that interfilament lattice spacing modulation through titin-based radial force is sufficient to account for a steep length-tension relationship. Analysis of the force-$[Ca^{2+}]$ relationship elucidates a SL dependency of the Hill coefficient.

Introduction

The aim of this research is the development of a muscle contraction model based on guinea pig experimental data that can correctly reproduce important cardiac muscle characteristics such as cooperativity in myofilament activation, the length-tension and the force-velocity relation. The contraction model presented here evolved after a profound analysis of the molecular mechanisms underlying these characteristics. Several rate parameters are subject to dynamical changes. New experimental findings about the giant muscle protein titin have been included in the model (see [2] for a review on titin). Here the focus is on isometric contraction features.

Materials and Methods

The Ca^{2+} activation and crossbridge (Xb) kinetics shown in Figure 1 is given by a seven-state system of regulatory units (RU), each in a different activation state and/or conformation. [RUtotal] comprises all RUs:

$$[RUtotal]=[RUT]+[RUTCaoff]+[RUTCaon]+[RUTMon]+[RUAMADPPi]+[RUAM\_ADPPi]+[RUAM\_ADP] \quad (1)$$

One RU consists of seven actin (A) molecules, one troponin (Tn) complex (TnC, TnI, TnT) and one tropomyosin (Tm) molecule. It is assumed that only one myosin head can be bound per RU. To achieve a high cooperativity in myofilament activation positive and negative cooperativity mechanisms as described in [3] have been adapted for this seven-state model.

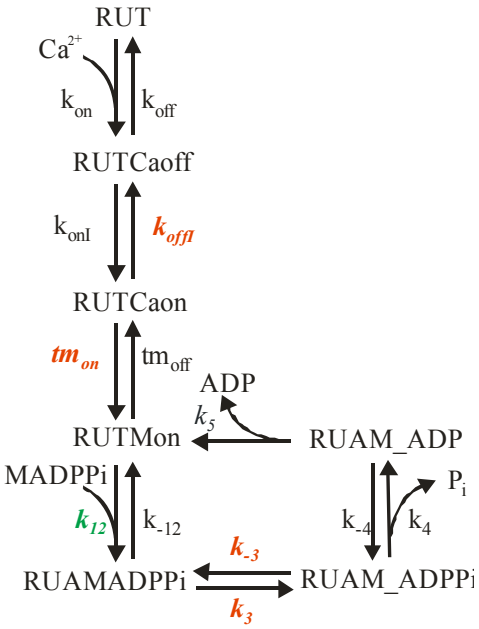

Figure 1: Model state diagram. The chemical part of the model consists of 7 different states of a RU (**RUT**: not activated RU; **RUTCaoff:** Ca^{2+} bound to TnC with TnI still bound to A ; **RUTCaon:** TnI released from A; **RUTMon:** Tm in the activated conformation; **RUAMADPPi:** M with a hydrolyzed ATP bound to A of an activated RU = weak Xb; M pocket for Pi closed; **RUAM_ADPPi:** strong Xb, M pocket for Pi open; **RUAM_ADP:** strong Xb, Pi released from the acto-myosin complex). k_{offI}, tm_{on}, k_3 and k_{-3} are subject to change due to cooperativity. k_{12} depends on titin-based radial force.

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In the first step Ca^{2+} binds to TnC of a not activated RU (RUT) with the following net rate (CaB):

$$CaB = k_{on} \cdot [RUT] \cdot [Ca^{2+}] - k_{off} \cdot [RUTCaoff] \quad (2)$$

This step is followed by a conformational change of TnI [4] with the net rate (TCaA) given by:

$$TCaA = k_{onI} \cdot [RUTCaoff] - k_{offI} \cdot [RUTCaon] \quad (3)$$

Unlike the Ca^{2+} binding step, TCaA is subject to cooperativity expressed in change of k_{offI} as follows:

$$k_{offI} = k_{offi} \cdot e^{k_{OFF} \cdot \frac{([RUT]+[RUTCaoff])}{[RUtotal]}} \cdot e^{k_{xboff} \cdot \frac{([RUAM\_ADPPi]+[RUAM\_ADP])}{[RUtotal]}} \quad (4)$$

To fully activate myofilaments a conformational change of Tm is necessary since it has to be moved away from the myosin binding site on actin leading to RUTMon. The net rate for this step (TMA) is given as:

$$TMA = tm_{on} \cdot [RUTCaon] - tm_{off} \cdot [RUTMon] \quad (5)$$

The rate parameter tm_{on} changes as follows due to positive and negative cooperativity:

$$tm_{on} = tm_{onc} \cdot \left(\frac{[RUTCaon]}{[RUtotal]}\right) \cdot \left[1 + k_{tmon} \cdot \left(\frac{[RUTMon]}{[RUtotal]}\right)\right]^2 \cdot e^{k_{tmxb} \cdot \left(\frac{[RUAM\_ADPPi]+[RUAM\_ADP]}{[RUtotal]}\right)} \quad (6)$$

The net rate of the myosin binding step (MB) which leads to the formation of weak Xbs is as follows:

$$MB = k_{12} \cdot KTitin \cdot [MADPPi] \cdot [RUTMon]_{eff} - k_{-12} \cdot [RUAMADPPi] \quad (7)$$

The concentration of activated RUs overlapping with the myosin filaments, $[RUTMon]_{eff}$, is SL dependent:

$$[RUTMon]_{eff} = \alpha \cdot [RUTMon] \quad (8)$$

α is the sarcomere overlap function that represents the fraction of activated RUs which is capable to interact with myosin heads to form Xbs [5]:

For $L < 0.001$ mm: $\alpha = 1.5 \cdot \left(\frac{L}{L_0}\right) - 0.5$

For $L \geq 0.001$ mm and $L \leq 0.0011$ mm: $\alpha = 1$ (9)

For $L > 0.0011$ mm: $\alpha = -1.6 \cdot \left(\frac{L}{L_0}\right) + 2.76$

where L is the half-sarcomere length and L_0 a normalization factor. [MADPPi] is the concentration of myosin (M) heads with bound and technically hydrolyzed ATP.
The following titin function (KTitin) has been designed:

For $L \leq 0.0011$ mm: (10)

$$KTitin = kTitin \cdot \frac{6.9299}{1 + e^{\frac{0.9798 - \left(\frac{L}{L_0}\right)}{0.0384}}}$$

For $L > 0.0011$ mm:

$$KTitin = kTitin \cdot \left(-33.153 \cdot \left(\frac{L}{L_0}\right) + 43.099\right)$$

For $L \leq 0.0011$ mm experimental data of mouse titin-based radial force [6] have been used, but slightly modified to account for the heart rate difference between mouse and guinea pig which is correlated with the titin isoform ratio [7]. Furthermore, the mouse data have been shifted to smaller SL because experiments were carried out using skinned fibers [8] which have a lower Ca^{2+} sensitivity than intact fibers. The equation for $L \geq 0.0011$ mm describes a linear relationship of titin damage with increasing SL which is based on experimental evidence of irreversible titin unfolding at a $SL \geq 0.0022$ mm [9].
The force generating step (Fgen) is a fast conformational change of the actin bound myosin to open the P_i binding pocket associated with the formation of a strong XB. The following equation gives the net rate with k_3 and k_{-3} changing due to cooperativity.

$$Fgen = k_3 \cdot [RUAMADPPi] - k_{-3} \cdot [RUAM\_ADPPi] \quad (11)$$

$$k_3 = k_{3c} \cdot e^{k_{3xb} \cdot \left(\frac{[RUAM\_ADPPi]+[RUAM\_ADP]}{[RUtotal]}\right)} \cdot \left[1 + k_{3f} \cdot \left(\frac{[RUTCaon]_{eff} + [RUTMon]_{eff} + [RUAMADPPi]}{[RUtotal]}\right)\right]^2 \quad (12)$$

$$k_{-3} = k_{-3c} \cdot e^{k_{-3xb} \cdot \left(\frac{[RUAM\_ADPPi]+[RUAM\_ADP]}{[RUtotal]}\right)} \quad (13)$$

The net rate for the P_i release (PiR) is given as:

$$PiR = k_4 \cdot [RUAM\_ADPPi] - k_{-4} \cdot [RUAM\_ADP] \quad (14)$$

The ADP release is the rate limiting step of the isometric contraction and the backward reaction is negligible [10]. The net rate (ADPR) is calculated as:

$$ADPR = k_5 \cdot [RUAM\_ADP] \quad (15)$$

The rate changes for the different RU species and $[Ca^{2+}]$ are determined as follows:

$$\frac{d[RUTCaoff]}{dt} = CaB - TCaA \quad (16)$$

$$\frac{d[RUTCaon]}{dt} = TCaA - TMA \quad (17)$$

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$$\frac{d[RUTMon]}{dt} = TMA - MB + ADPR \quad (18)$$

$$\frac{d[RUAMADPPi]}{dt} = MB - Fgen \quad (19)$$

$$\frac{d[RUAM\_ADPPi]}{dt} = Fgen - PiR \quad (20)$$

$$\frac{d[RUAM\_ADP]}{dt} = PiR - ADPR \quad (21)$$

$$\frac{d[Ca^{2+}]}{dt} = -CaB \quad (22)$$

Table 1: Numerical values of parameters

Parameter	Value	Source
B	1.2 /ms	[11]
h_c	5.0×10^{-6} mm	[11]
K	1.4×10^{20} mN/mm^7	[11]
L_{0P}	0.00097 mm	[11]
A	1.8×10^9 mN/mm^3/mM	[11]
k_{off}	0.2 /ms	[3]
k_{on}	17.3 /mM/ms	[3]
[RUtotal]	0.0792 mM	Calculated according to [13]
k_{onI}	0.2 /ms	Model fit
k_{offi}	0.03 /ms	Model fit
k_{OFF}	1	Model fit
k_{xboff}	-7	Model fit
tm_{off}	0.07 /ms	[14]; value doubled
tm_{onc}	0.012 /ms	[14]
k_{tmon}	10	Model fit
k_{tmxb}	-5	Model fit
L_0	0.001 mm	to normalize L
k_{12}	2 /mM/ms	Model fit according to [15] and [16]
k_{-12}	0.6 /ms	Model fit according to [15] and [16]
[MADPPi]	0.15 mM	Calculated according to [13]
kTitin	0.2	Model fit
k_{3c}	0.025 /ms	Model fit according to [16]
k_{3f}	50	Model fit
k_{3xb}	-3	Model fit
k_{-3c}	0.008 /ms	Model fit according to [16]
k_{-3xb}	-20	Model fit
k_4	0.077 /ms	[10]
k_{-4}	0.02 /ms	Model fit
k_5	0.03723 /ms	[10]; T adjusted to 37ºC

The part containing the mechanical element is the same as developed by [11]. The active force F_b is proportional to the number of developed Xbs with A being the force factor.

$$F_b = A \cdot (L - X) \cdot ([RUAM\_ADPPi] + [RUAM\_ADP]) \quad (23)$$

The sliding velocity of the Xbs is given by:

$$\frac{dX}{dt} = B \cdot (L - X - h_c) \quad (24)$$

where X is the length composed of half of the thick filament and the free portion of the thin filament and h_c the Xb elongation at steady L and B a proportionality parameter.
The passive elastic force (Fp) is calculated as follows [11]:

$$F_P = K \cdot (L - L_{0p})^5 \quad (25)$$

where K is the equation parameter and L_{0P} the unstressed L.
The developed total force F is determined by:

$$F = F_p + F_b \quad (26)$$

Table 1 shows the parameter values used in all simulations.

The model has been implemented in Java using the simBio package [1], a software for cell simulation and ODE solving. Steady state simulations have been carried out in an isolated model with constant $[Ca^{2+}]$. For twitch contractions the $[Ca^{2+}]$ kinetics part of the contraction model from [11] which is used in the Kyoto model ventricular cell [12] has been replaced by the chemical part of the contraction model described above.

Results

The newly developed contraction model has been tested for characteristics of isometric contractions.

An isometric twitch contraction time course simulated after inserting the contraction model into the myocardial cell model is depicted in Figure 2. The time course is similar to experimental data obtained from guinea pig trabeculae.

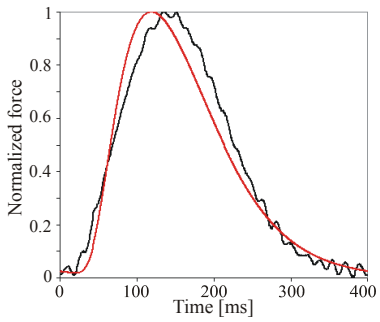

Figure 2: Simulated isometric contraction time course (red, SL=2.1µm) in comparison to experimental data (black, SL≈2.1 µm; guinea pig, left ventricular trabeculae, 36ºC; our unpublished data). In both cases the stimulation frequency was 2.5 Hz.

Figure 3 shows the simulated length-tension relationship (black) in comparison to experimental data

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(red and blue). The steep length-tension relationship is solely due to the titin-based lattice spacing changes affecting the ascending limb and the damage of titin in the descending limb of the graph. This is obvious since omitting the titin function (KTitin) from equation (7) (Figure 3, green) results in a broad length-tension relationship as found for skeletal muscle. In this case the SL-dependency is only caused by the sarcomere overlap function (equation 9).

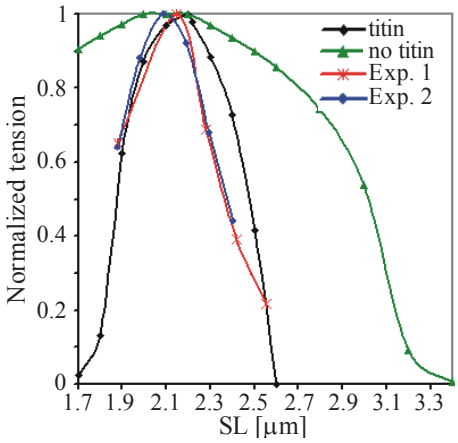

Figure 3: Length-tension relationship: Simulation results for the model including KTitin in equation (7) are shown in black and without KTitin in equation (7) in green. Two individual experimental data obtained from guinea pig, left ventricular trabeculae, 36ºC are depicted in red and blue (our unpublished data). The muscle length has been converted to SL assuming that the measured muscle slack length is equal to 1.88 µm SL (resting SL, [17]).

The force-[Ca^{2+}] relationship is characterized by the Hill coefficient (n) which indicates the level of cooperativity in filament activation and the EC_{50} value, the [Ca^{2+}] necessary for half maximum force. Simulation results for different SLs are shown in Figure 4. As elucidated in Table 2, n and EC_{50} values are SL dependent.

Table 2: Hill coefficient and EC_{50} values
The force-[Ca^{2+}] relationship for each SL has been fit to the Hill equation, $F=[Ca^{2+}]^n/(EC_{50}{}^n+[Ca^{2+}]^n)$, by nonlinear regression analysis using SigmaPlot (Version 8.02; SPSS Inc.) ± STD error (for all data $P<0.0001$).

SL [µm]	Hill coefficient	EC_{50} [µM]
2.2	8.54±1.27	0.67±0.01
2.1	7.63±1.07	0.69±0.01
2.0	6.31±0.78	0.82±0.02
1.9	4.37±0.32	1.27±0.03
1.8	3.91±0.37	1.98±0.05
1.7	5.07±0.62	2.57±0.06

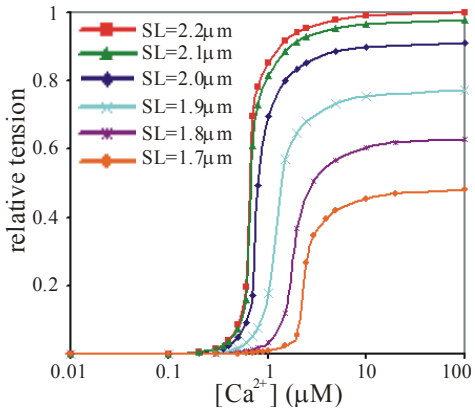

Figure 4: Simulation results for the force-[Ca^{2+}] relationship at different SLs.

Discussion

The new contraction model presented here displays the following unique features not found in other myocardial cell contraction models. All states are different activation and/or conformational states of RUs and are connected in contrast to e.g. Sachse Model [14] where the two-state Ca^{2+} activation is separated from a two-state Tm conformational change. Since the state with Ca^{2+} bound to Tn is the same as the state with TM in the 'off' conformation these states should rather be treated as one state.

The Ca^{2+} activation has been split into two steps (CaB (equation 2) and TCaA (equation 3)) to account for the conformational change of TnI which releases the inhibitory region of TnI from actin. Experimental data have shown that the TCaA step is an important regulatory switch [4] and therefore necessary for a correct modelling of cooperativity.

Positive and negative cooperativity mechanisms as described by [3] have been employed. Analysis of the force-[Ca^{2+}] relationship has revealed a high Hill coefficient for the new model as could be found experimentally (Table 2) [18,19]. The SL dependency of n is still an unsolved question among experimentalists. Most groups report n to be SL dependent [20,21]. However, an almost constant n for all SLs has also been described [18]. Insertion of the titin-based radial force in the contraction model results in a strong SL dependency of n comparable to [20].

Experimental results have shown that titin-based passive tension can change the Ca^{2+} sensitivity of myofilaments [21]. It has been suggested that interfilament lattice spacing modulation through titin-based radial force maybe the underlying mechanism for the SL dependent myofilament activation [21]. In the contraction model presented here a linear relationship of titin-based radial force (equation 10) and the change of the myosin binding rate parameter (k_{12}) has been applied in addition to the sarcomere overlap function (equation 9). As elucidated in Figure 3 the sarcomere

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overlap function alone (green) cannot account for a steep length-tension relationship as found for cardiac muscle [22]. However, the addition of the titin function (equation 10) is enough to reproduce cardiac muscle experimental data (black in Figure 3). It should be pointed out that in the new model described here the SL dependency of the Ca^{2+} activation is only caused by a change of the myosin binding rate (k_{12}). There is no SL dependency in the Ca^{2+} binding step in contrast to e.g. the Sachse Model [14]. A change of the binding affinity of Ca^{2+} to TnC would require a structural change of the Tn complex or Tm such as caused by phosphorylation and could probably not be achieved by a change in the SL. In the NL Model [11] the SL dependency is due to a steep sarcomere overlap function which determines the effective [TCa], a state comparable to the activated RU. However, the sarcomere overlap function, which gives the degree of overlap between thick and thin filaments for a given SL, should be the same for skeletal and cardiac muscle. Including titin-based radial force is certainly the approach getting closest to the underlying biological mechanism. The cardiac contraction model proposed here is unique in that it is to our knowledge the first such kind of model which accounts for titin.

Work is in progress to add mechano-chemical coupling in the model necessary for a correct simulation of the force-velocity relationship.

Conclusions

A new cardiac muscle contraction model is presented which includes titin-based radial force to modulate lattice spacing between thin and thick filaments according to SL changes. Simulation results for isometric contractions reveal a steep length-tension relationship. The Hill coefficient has been found to be SL dependent.

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