Published in final edited form as:

Biophys Chem. 2016 April ; 211: 9–18. doi:10.1016/j.bpc.2016.01.001.

The sensitivity of fast muscle contractile function to the major components of the sarcomere Ca^{2+} -cycling system

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Abstract

A reaction–diffusion model of a muscle sarcomere was developed to evaluate the sensitivity of force characteristics to diffusion and Ca^{2+} -cycling components. The model compared well to experimental force measurements. Diffusion led to Ca^{2+} gradients that enhanced maximal force and accelerated relaxation compared to when diffusion was infinitely fast. However, a modest increase in sarcomere length or radius led to a decrease in maximal force. Lowering the Ca^{2+} release rate caused a lower maximal force, but increasing the rate led to only modest gains in maximal force while incurring much greater ATP costs associated with reuptake. Greater parvalbumin binding rates decreased maximal force but enhanced relaxation, and this effect was magnified when Ca^{2+} uptake rates were lowered as may occur during fatigue. These results show a physiological set of parameters that lead to a functional sarcomere of known dimensions and contractile function, and the effects of parameter variation on muscle function.

GRAPHICAL ABSTRACT

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Keywords

Skeletal muscle; Calcium; Diffusion; Sarcomere; Contraction; Sarcoplasmic reticulum

1. Introduction

Muscle contraction begins when a wave of depolarization radiates from the neuromuscular junction to the t-tubules activating voltage-gated Ca^{2+} channels that release Ca^{2+} from the sarcoplasmic reticulum (SR). Ca^{2+} then diffuses across the sarcoplasm and binds to troponin C (Tn) in the sarcomeres, which facilitates cross-bridge attachment and thus contraction. Relaxation of muscle is induced by the reuptake of Ca^{2+} via the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), as well as by sequestration of Ca^{2+} by the soluble Ca^{2+} binding protein parvalbumin (Fig. 1A) [4,6,12,16]. Maximal contractile frequency is therefore largely dictated by the rate at which Ca^{2+} cycling allows muscle to switch between phases of contraction and relaxation.

Several prior studies have developed reaction–diffusion models of sarcomere Ca^{2+} cycling in skeletal muscle. Cannell and Allen [5] demonstrated steep Ca^{2+} concentration gradients along the sarcomere, and their model compared well to Ca^{2+} transients measured experimentally in frog muscle. Baylor and Hollingworth [1] expanded on this approach and confirmed the presence of Ca^{2+} gradients and also showed that ATP had a Ca^{2+} buffering role in frog muscle. These authors further adapted their model to mouse fast-twitch muscle, and evaluated the effects of the location of the Ca^{2+} release site, sarcomere length, the Ca^{2+} binding protein, parvalbumin (Par), as well as mitochondrial uptake of Ca^{2+} [2,3]. Similarly, Novo et al. [13] and Groenendaal et al. [7] found that the location of the Ca^{2+} release site from the SR was an important contributor to the Ca^{2+} dynamics during contraction/relaxation cycles. More recently, Liu and Olson [11] developed a model for frog muscle that incorporated voltage sensitivity of Ca^{2+} release.

The present study integrated reaction and diffusion components of Ca^{2+} cycling with force production, allowing comparison of the model output to experimental contractile data. In addition, we evaluated the role of Ca^{2+} diffusion by comparing normal diffusion results to those with infinitely fast diffusion rates, and we conducted a sensitivity analysis of the major components of the Ca^{2+} cycling system. A model base case was generated using experimental contractile data from fish white muscle. This experimental system was selected because fish white muscle has a uniform fiber type, thus eliminating confounding effects of mixed fiber muscles with different contractile properties, and because it has a high maximal contractile frequency, which allows the evaluation of a relatively high performance muscle that may be near functional limits of Ca^{2+} cycling. This analysis sheds insight into the parameters that influence muscle contractile function, and it will have application to future modeling efforts as well as tissue engineering studies.

2. Methods

2.1. Model species balance

The species continuity equation for all components (Ca^{2+} (Ca), Mg^{2+} (Mg), troponin-C (Tn), parvalbumin (Par), calcium bound troponin (CaTn), calcium bound parvalbumin (CaPar), and magnesium bound parvalbumin (MgPar)) is given by

$$\frac{\partial C_i}{\partial t} = D_i \left(\frac{\partial^2 C_i}{\partial x^2} + \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C_i}{\partial r} \right) \right) + R_i \quad (1)$$

where C_i is the concentration of the i th component and R_i is the net rate of formation of the i th component by chemical reaction. The boundary conditions are given by Eqs. (2)–(5). (The uptake and release functions are given by Eqs. (15)–(18).) It should be noted that calcium is the only component that has a non-zero flux. In Eqs. (2)–(5), where not specified, i is for all species.

$$-D_i \frac{\partial C_i}{\partial r} \Big|_0 = 0 \quad (2)$$

$$-D_i \frac{\partial C_i}{\partial r} \Big|_R = 0 \text{ where } i \neq [\text{Ca}] \quad (3)$$

$$-D_{[\text{Ca}]} \frac{\partial [\text{Ca}]}{\partial r} \Big|_R = \sum J_i(t, z, [\text{Ca}]) \quad (4)$$

$$-D_i \frac{\partial C_i}{\partial x} \Big|_{0 \text{ and } L} = 0 \quad (5)$$

Reaction rates, R_i are given in Eqs. (6)–(9). The overall model has 3 specified reactions with 7 species: $\text{Ca} + \text{Tn} \rightleftharpoons \text{CaTn}$, $\text{Ca} + \text{Par} \rightleftharpoons \text{CaPar}$, and $\text{Mg} + \text{Par} \rightleftharpoons \text{MgPar}$. In Eqs. (6)–(9) $R'_i = R_i/S_i$ where S_i is a scaling factor for sensitivity analysis (see Eq. (25)).

$$R'_{\text{Ca}} = \sum_l R_{\text{Ca},l} \quad (6)$$

$$R'_{\text{Ca},\text{TnC}} = (k_{r,\text{CaTn}} [\text{CaTn}] - k_{f,\text{CaTn}} [\text{Ca}][\text{Tn}]) \quad (7)$$

$$R'_{Ca,Par} = (k_{r,CaPar} [CaPar] - k_{f,CaPar} [Ca][Par]) \quad (8)$$

$$R'_{Mg,Par} = (k_{r,MgPar} [MgCaParATP] - k_{f,MgPar} [Mg][Par]) \quad (9)$$

$$R_{Ca} = R_{Ca,Tn} + R_{Ca,Par} \quad (10)$$

The initial conditions for all species were obtained by using the equilibrium of the reaction equations and the known species concentrations at rest of Ca and Mg. Diffusion of each species in the radial direction is very fast and leads to small concentration gradients in the radial direction as depicted by Cannel and Allen[5]. For small gradients in the radial direction, an average in the radial direction (Eq. (11)) can be applied to Eq. (1) and this yields Eq. (12).

$$\langle \text{Average of } f(r) \rangle = \frac{\int_0^R f(r) r dr}{\int_0^R dr} \quad (11)$$

$$\frac{\partial \langle C_i \rangle}{\partial t} = D_i \frac{\partial^2 \langle C_i \rangle}{\partial x^2} + \frac{2}{R} D_i \left[\frac{\partial \langle C_i \rangle}{\partial r} \right]_R + \langle R'_i \rangle \quad (12)$$

The radially averaged model, Eq. (12), is used to analyze the diffusion limitations in the axial direction of the myofibril and is termed here the *Diffusion* model. Averaging again over the axial direction produces a volume averaged model (removing diffusion gradients in all dimensions). There are 3 distinct regions, L_k along the length of the sarcomere which are accounted for by the sum over the index k in Eq. (14).

$$\langle \text{Average of } f(x) \rangle = \frac{\int_0^L f(x) dx}{\int_0^L dx} \quad (13)$$

$$\frac{d\{C_i\}}{dt} = \sum_k \frac{L_k}{L} \left(\frac{2}{R} D_i \left[\frac{\partial \{C_i\}}{\partial r} \right]_R + \{R'_i\} \right) \quad (14)$$

Eq. (14) represents the infinitely fast diffusion model (IFD). Since the troponin-C complex is wrapped around the actin filaments and the length of the filaments does not span the entire length of the sarcomere, then the troponin-C reaction only occurs in the specified area,

which is 0.86 μm in length. Similarly the release region is approximately 22% of the total length of the fiber. The length of the uptake region spans the entire length of the sarcomere. L_1 denotes where release, uptake and troponin-C are located, L_2 denotes where uptake and troponin-C are located, and L_3 denotes where uptake occurs.

2.2. Model Ca^{2+} release and uptake equations

A variety of uptake and release functions have been reported in the literature [2,5,7,13,14]. Raeymaekers et al. [14] tested these release equations and found no differences in calcium concentrations as long as the total amount released was the same and the concentration difference was included in the release equation, because the release occurred over a very small time frame compared to the uptake. The release function used in the present work is given by Eq. (15) [7].

$$J_{\text{release}} = P \left(1 - e^{-t/\tau_{\text{on}}} \right) e^{-t/\tau_{\text{off}}} ([\text{Ca}^{2+}] - [\text{Ca}^{2+}]_{\text{SR}}) \quad (15)$$

The uptake function used here is given by Eq. (16) [2,13].

$$J_{\text{uptake}} = - \frac{V_{\text{max}} \cdot [\text{Ca}^{2+}]^2}{k_d^2 + [\text{Ca}^{2+}]^2} \quad (16)$$

A calcium leak term was also included as given by Eq. (17) where the parameter, L_e is given by Eq. (18). At rest the leak is balanced with the uptake rate of the SERCA pumps.

$$J_{\text{Leak}} = L_e ([\text{Ca}^{2+}] - [\text{Ca}^{2+}]_{\text{SR}}) \quad (17)$$

$$L_e = \frac{V_{\text{max}} \cdot [\text{Ca}^{2+}]_{\text{rest}}^2}{(k_d^2 + [\text{Ca}^{2+}]_{\text{rest}}^2) ([\text{Ca}^{2+}]_{\text{rest}} - [\text{Ca}^{2+}]_{\text{SR}})} \quad (18)$$

2.3. Model force production

The force model utilizes a spring–motor–damper equation developed by Wexler et al. [21] and given by Eqs. (19), (20), and (21), respectively, which are combined into the final force model shown in Eq. (22).

$$F = Kx \quad (19)$$

$$F = b \left(V - \frac{dx}{dt} \right) \quad (20)$$

$$V = B[Tn_B] \left(1 - \frac{F}{F_{\max}} \right) \quad (21)$$

$$\frac{dF}{dt} = KB[Tn_B] \left(1 - \frac{F}{F_{\max}} \right) - \frac{F}{\tau_1 + \tau_2 \frac{[Tn_B]}{[Tn_{\text{Total}}]}} \quad (22)$$

K is the spring constant, b is the dampening coefficient, B is the maximum force production of the motor, τ_1 is the dynamic relaxation term and τ_2 is the static relaxation term (as defined by Wexler et al. [21]). To simplify Eq. 22, τ_1 is set to zero because the experimental force measurements were conducted isometrically, and combining terms and removing τ_1 yields Eq. (23).

$$\frac{dF}{dt} = A_1 \frac{[Tn_B]}{[Tn_{\text{Total}}]} \left(1 - \frac{F}{F_{\max}} \right) - A_2 \frac{F}{[Tn_B] / [Tn_{\text{Total}}]} \quad (23)$$

A_1 and A_2 are the force production and relaxation parameters, respectively. The force used in this model is a relative force without units and has a maximum of 1 ($F_{\max}$). When the force model is solved the maximum and minimum forces were obtained from the second peak and the second valley of the force vs time output, respectively. This allows an analysis of frequency effects, since the first force peak is always the same height because the initial conditions and parameters for the model are the same for all frequencies. The fractional relaxation is defined by Eq. (24).

$$\text{Fractional Relaxation} = 1 - \frac{\text{Minimum Force}}{\text{Maximum Force}} \quad (24)$$

2.4. Model parametric study

The parametric study shows the effect of each parameter when adjusted. The following parameters were varied: L , R , J , $V_{\max}$, P , $R_{Ca,Tn}$, and $R_{Ca,Par}$. The parameters were varied by using a scaling parameter, S_i , that is multiplied by the base parameter given in Table 1, where i indicates the parameter that is varied. For all reported cases where a scaler is not shown it can be assumed to be set to the value of 1.

$$L = L' \cdot S_{\text{Length}}$$

$$R=R' \cdot S_{\text{Radius}}$$

$$J=J' \cdot S_{\text{Release/Uptake}}$$

$$P=P' \cdot S_{\text{Release}} \quad (25)$$

$$V_{\text{max}}=V'_{\text{max}} \cdot S_{\text{Uptake}}$$

$$R_{Ca,Tn}=R'_{Ca,Tn} \cdot S_{CaTn}$$

$$R_{Ca,Par}=R'_{Ca,Par} \cdot S_{CaPar}$$

$$[\text{Par}_{\text{Total}}]=[\text{Par}'_{\text{Total}}] \cdot S_{\text{ParTotal}}$$

S_{Length} is the length scalar, S_{Radius} is the radius scalar, $S_{\text{Release/Uptake}}$ is the uptake/release scalar, S_{Release} is the release scalar, S_{Uptake} is the uptake scalar, S_{CaTn} is the CaTn reaction scalar, S_{CaPar} is the CaPar reaction scalar, and S_{ParTotal} is the Par concentration scalar. When scaling S_{Length} it should be noted that L_2 and L_3 were scaled, while L_1 (the release zone) was not.

2.5. Experimental measurements of muscle contraction

Black sea bass (*Centropristis striata*) were anesthetized prior to dissection using MS-222 (500 mg/L) followed by cervical dislocation. A section of skin was reflected posteriorly and a strip of anterior epaxial white muscle was cut parallel to the fiber orientation using a razor blade. The muscle sample was transferred to a Petri dish containing a physiological saline solution (132.2 mM NaCl, 10 mM sodium pyruvate, 2.6 mM KCl, 2.7 mM CaCl₂, 1 mM MgCl₂, 18.5 mM NaHCO₃, 3.2 mM NaH₂PO₄, pH 7.4) aerated with 99.5% O₂–0.5% CO₂. Muscle samples were trimmed to 1.0 mm thick bundles and 6.0 surgical silk was used to fasten the muscle bundle to custom-made plastic rings that were placed on glass perfusion hooks. The muscle tissue was maintained at 20 °C in a water jacketed superfusion chamber with built-in field stimulation electrodes. The muscle tissue was stretched to a length that achieved maximal force production. Stimulation was applied using a Grass S88 physiological stimulator. The muscle was stimulated at 70 V for 3 ms duration with a train rate of 1 per s and a train duration of 200 ms. The frequency was increased from 10 Hz to 50

Hz in increments of 5 Hz with 4min between each stimulation in order to allow the muscle to recover.

3. Results and discussion

In our base case, which used the parameter values given in Table 1, rapid transients in free $[Ca^{2+}]$ led to a return to near resting concentrations between each sarcomere activation at 20 Hz (Fig. 2A) and 30 Hz (Fig. 2D). This was associated with more protracted changes in the $[CaTn]$ (Fig. 2B,E), and even slower transients in $[CaPar]$ that led to its gradual saturation during repeated muscle activation (Fig. 2C,F). Spatial concentration gradients were observed along the length of the half-sarcomere, consistent with prior results [1,5], indicating the effects of Ca^{2+} and parvalbumin diffusion, with high concentrations of free $[Ca^{2+}]$, $[CaTn]$, and $[CaPar]$ near the sites of Ca^{2+} release at the end of the sarcomere (Fig. 2). The model was also run with ATP present as an additional Ca^{2+} buffer as originally described in Baylor and Hollingworth [1]. While the presence of ATP required adjustment of the amount of Ca^{2+} released, it had little impact on either Ca^{2+} diffusive flux or the sensitivity analysis described below, so our simulations were run in the absence of ATP for computational simplicity. However, the sensitivity analysis of Par described below also serves as a proxy to capture the effects of ATP Ca^{2+} buffering.

To further evaluate the role of diffusion, volume averaged concentrations of free $[Ca^{2+}]$, $[CaTn]$ and $[CaPar]$, as well as force production, were examined in the presence of normal diffusion and infinitely fast diffusion (IFD) at 20 and 30 Hz (Fig. 3). IFD led to a lower peak free $[Ca^{2+}]$ (Fig. 3A,B), which results from the immediate equilibration of Ca^{2+} throughout the sarcomere (no gradients) giving all of the SR Ca^{2+} pumps along the sarcomere length access to free Ca^{2+} . This accelerates uptake causing the lower peak $[Ca^{2+}]$. There is also a lower peak $[CaTn]$, due to the lower peak free $[Ca^{2+}]$ (Fig. 3C,D). The somewhat slower decrease in $[CaTn]$ in the IFD case (during the recovery phase) occurs because Tn is uniformly bound to Ca^{2+} at low levels along the sarcomere length, whereas in the diffusion case the $[CaTn]$ is high near the Ca^{2+} release site. Thus, in the IFD case when free $[Ca^{2+}]$ decreases as a result of reuptake, there is a relatively small difference between the concentration of free Ca^{2+} and Ca^{2+} that is bound to Tn, leading to a slower release from Tn. The slower release of Ca^{2+} from Tn in the IFD case was paralleled by a higher Ca^{2+} -bound fraction of the slow-binding parvalbumin during the recovery phase. The gradual saturation of parvalbumin during repeated contractions is consistent with its role as a temporal Ca^{2+} buffer [4,8,15,17] (Fig. 3E,F). The force production, follows the $[CaTn]$ for both the diffusion and IFD case, as would be expected (Fig. 3G,H).

In many reaction–diffusion processes the IFD cases have greater rates of overall fluxes than cases with normal diffusion, since diffusion can limit reaction rates, and the IFD cases therefore represent the reaction-controlled maximal capacity for flux. This view is central to the evaluation of reaction–diffusion processes in both engineered and biological systems [9,19]. However, the present study reveals that the effect of diffusion on Ca^{2+} cycling and force production is more complex than in many systems, since the ultimate output (force) is dependent not on a simple one-way diffusion of Ca^{2+} from source to sink, but on Ca^{2+} release, binding, and reuptake, as well as on the peak $[Ca^{2+}]$. That is, the efficacy of force

production is dependent on the rates of rise and fall in force, as well as on the peak force during contraction and the minimal force between contractions. Evaluation of these properties indicates that the sarcomere actually functions better in our base case in the presence of normal diffusion than with IFD. Thus, while diffusion may not play a sizable role in constraining sarcomere contractile function, our results indicate that the sarcomere has evolved its contractile properties to accommodate diffusion.

Evaluation of the force production also revealed that 20 Hz is near the upper functional range for complete relaxation of the modeled sarcomere, whereas 30 Hz is beyond the functional range and leads to incomplete relaxation (Fig. 4G,H). This is consistent with the upper contractile frequency in fishes in vivo [18]. Therefore, we have evaluated these two frequencies in other analyses described below. The effect of diffusion on force production described above is consistent across a broad range of contractile frequencies (Fig. 4). Here and in subsequent figures, the fractional relaxation (Eq. (24)), minimum force and maximum force were evaluated using the second contraction in the series. Again, the decline in fractional relaxation (Fig. 4A) and increase in minimum force (Fig. 4B) at higher frequencies illustrate a maximal contractile frequency of about 20 Hz for this sarcomere, and the increase in maximal force at higher frequencies is due to an accumulation of Ca^{2+} during repeated contractions (Fig. 4C).

The sensitivity to sarcomere size was evaluated at different sarcomere lengths and radii. The $[\text{CaTn}]$ (Fig. 5A) and force transients (Fig. 5B) demonstrate that as sarcomere length increases there is a reduced Tn Ca^{2+} binding and a subsequent decrease in force. This can be seen for both length and radius in the force characteristics at 20 and 30 Hz (Fig. 5C–H). The maximum force was most sensitive to changes in sarcomere size, where at 20 Hz a 50% increase in sarcomere length (Fig. 5G) or radius (Fig. 5H) led to a 15% or 22% decrease in maximum force, respectively. The observed effects of increasing sarcomere size reflect both the longer Ca^{2+} diffusion distances and the greater sarcomere volume over which Ca^{2+} must equilibrate.

Contractile properties were highly sensitive to rates of release and uptake of Ca^{2+} (Fig. 6). The range of release rates that led to a high fractional relaxation within the functional range of the sarcomere at 20 Hz were centered around that used for our base case (release scalar of 1) (Fig. 6A). Lower release rates led to a steep decline in maximum force, and higher values led to more Ca^{2+} release and therefore slightly more maximum force (Fig. 6G), but this was accompanied by a higher minimum force and a reduced fractional relaxation (Fig. 6A,D). The fractional relaxation had a hyperbolic dependence on Ca^{2+} uptake rate such that a rate less than in our base case led to a rapid decline in this contractile property (Fig. 6B). This was due to the fact that the persistently high Ca^{2+} at lower uptake rates led to a steep rise in minimum force (Fig. 6E), but only a modest increase in maximum force (Fig. 6H). There were also hyperbolic dependences of fractional relaxation, minimum force and maximum force on the uptake/release scalar when both properties were adjusted in concert (Fig. 6C,F,I). Again, Ca^{2+} cycling rates that were less than our base case led to a reduction in all 3 measures of contractile function. However, increases in the Ca^{2+} cycling rate led to only slight increases in any property, including maximum force (Fig. 6I). Thus, a higher rate of Ca^{2+} cycling during contraction would lead to little or no improvement in function but

would consume more ATP in proportion to the increase in rate due to an increase in SERCA pump activity.

Contractile properties were insensitive to the rate of Tn binding to Ca^{2+} when binding rates exceeded our base case (Fig. 7). However, at lower rates of Tn binding there was a large drop in fractional relaxation (Fig. 7A). This was because the slow binding kinetics caused Tn to remain bound to Ca^{2+} longer, leading to a modestly higher maximum force (Fig. 7C), but a much greater increase in minimum force (Fig. 7B).

Parvalbumin is thought to be a slow-binding temporal Ca^{2+} buffer that helps facilitate rapid relaxation in fast-contracting muscle [4,8,15,17]. An increase in parvalbumin concentration leads to a decrease in peak $\langle \text{Ca} \rangle$, peak $\langle \text{CaTn} \rangle$ and maximal relative force, but all of these properties return to baseline faster at higher concentrations of parvalbumin (Fig. 8). The sensitivity of contractile properties to the parvalbumin concentration and Ca^{2+} binding rate was evaluated at 20 and 30 Hz (Fig. 9). At 20 Hz the fractional relaxation and minimum force were largely insensitive to elevations above our base case of both the [Par] over a four-fold range (Fig. 9A,C) and the binding kinetics over a range of several orders of magnitude (Fig. 9B,D), whereas maximum force was inversely related to [Par] (Fig. 9E). In contrast, when [Par] or the Par reaction scalar was reduced below our base case, there was a noticeable effect on muscle performance. Specifically, the fractional relaxation was reduced and minimum force elevated, albeit slightly, as parvalbumin's effects were reduced (Fig. 9A,B,C,D). The sensitivity of contractile properties to reduced [Par] or Par reaction scalar followed the same pattern at 30 Hz but the effect was greater than at 20 Hz, which is consistent with the notion that parvalbumin is more important at aiding relaxation at high contractile frequencies. Thus, while the presence of parvalbumin has subtle effects, the relatively large investment made by fast-twitch muscle fibers in this protein suggests that this seemingly modest role is biologically meaningful in temporally buffering Ca^{2+} (Fig. 9). However, increases of Par concentration or reaction scalar well above our base case have little effect on this function.

Since the role of parvalbumin may become greater during fatigue in fast-twitch muscle, we simulated one potential source of fatigue by reducing the rate of Ca^{2+} reuptake, which is dependent on the SERCA pump [20] (Fig. 10). The absence of parvalbumin had a greater effect on fractional relaxation (Fig. 10A) and minimum force (Fig. 10B) at lower rates of Ca^{2+} uptake, consistent with a greater role for parvalbumin as muscle fatigues.

4. Conclusions

The major findings of the present study were that (1) steep Ca^{2+} concentration gradients were observed, and in our base case the sarcomere contractile performance was better in the presence of diffusion than when diffusion was infinitely fast, and (2) contractile function is particularly sensitive to sarcomere size, Ca^{2+} uptake and release rate, and Tn binding kinetics, but it is only modestly sensitive to parvalbumin binding rate and concentration. These results provide a range of parameters that yield a functional sarcomere as well as the sensitivity of contraction to these parameters for fast-twitch muscle.

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HIGHLIGHTS

- Reaction–diffusion model of a sarcomere matched experimental force measurements.
- Steep concentration gradients were present, but diffusion did not limit contraction.
- Increases in Ca^{2+} release rate enhance force slightly, but are energetically costly.
- Effects of variation in the parameter set that defines a sarcomere are described.

Fig. 1.

Model schematic of a half-sarcomere, where the L_1 region is the site of Ca^{2+} release, and also sites of Ca^{2+} uptake and the troponin-C reaction. The L_2 region contains Ca^{2+} uptake and troponin-C, while the L_3 region has only Ca^{2+} uptake. R is the sarcomere radius and Z is the length of the half sarcomere, and diffusion occurs between all 3 regions.

Fig. 2. Spatial and temporal variation in the concentration of Ca^{2+} (A, D), Ca^{2+} -bound Tn (B, E), and Ca^{2+} -bound Par (C, F) during consecutive muscle stimulations at 2 different frequencies. The top set of panels (A, B, C) is at 20 Hz while the bottom panels (D, E, F) are at 30 Hz. Color scale bars are in μM .

Fig. 3.

The effect of diffusion during consecutive muscle stimulations on the volume averaged concentration of Ca^{2+} (A, B), Ca^{2+} -bound Tn (C, D), and Ca^{2+} -bound Par (E, F), as well as relative force (G, H). The base case with diffusion is compared to the case with infinitely fast diffusion (IFD). For the relative force panel, the experimental measurements of isometric force are shown for comparison to the model (G, H). The left panels (A, C, E, G) are at 20 Hz and the right panels (B, D, F, H) are at 30 Hz.

Fig. 4. The effect of contractile frequency on fractional relaxation (A), minimum relative force (B), and maximum relative force (C) for the base case with normal diffusion and the case with infinitely fast diffusion (IFD).

Fig. 5.

The effect of sarcomere size on contractile properties at stimulation frequencies of 20 and 30 Hz. The volume averaged concentration in Ca^{2+} -bound Tn (A) and relative force (B) over time during consecutive stimulations at 20 Hz are shown as a function of length scalar, where a value of 1 is our base case. Changes in sarcomere dimensions alter fractional relaxation (C, D), minimum relative force (E, F) and maximum relative force (G, H) at both 20 and 30 Hz. Panels C, E and G show the effect of variation in length, and panels D, F and H show the effect of variation in radius.

Fig. 6. The effect of the Ca²⁺ release rate (A, D, G), Ca²⁺ uptake rate (B, E, H) and Ca²⁺ uptake and release combined (C, F, I) on contractile properties at stimulation frequencies of 20 and 30 Hz. A reaction scalar of 1 is our base case.

Fig. 7. The effect of the Tn-Ca²⁺ reaction rate on the fractional relaxation (A), minimum relative force (B), and maximum relative force (C) at stimulation frequencies of 20 and 30 Hz. A reaction scalar of 1 is our base case.

Fig. 8. The effect of [Par] on the volume averaged concentration of Ca^{2+} (A) and Ca^{2+} -bound Tn (B), as well as relative force (C) over time at a stimulation frequency of 20 Hz. A [Par] scalar of 1 is our base case.

Fig. 9.

The effect of [Par] and Par- Ca^{2+} reaction rate on fractional relaxation (A, B), minimum relative force (C, D) and maximum relative force (E, F) at stimulation frequencies of 20 and 30 Hz. Panels A, C and E show the effect of variation in [Par] and panels B, D and F show the effects of variation in Par- Ca^{2+} reaction rate. A scalar of 1 (10^0 for Par reaction scalar) is our base case.

Fig. 10.

The effect of Par on fractional relaxation (A), minimum relative force (B), and maximum relative force (C) as a function of Ca^{2+} uptake rate, which may be reduced during fatigue, at a stimulation frequency of 20 and 30 Hz. A Ca^{2+} uptake scalar of 1 is our base case.

Table 1

Model parameters used in the present study.

Parameter	Value used	Source of parameter
L'	1.10 μm	Available lab data
L_1	22% of total length	[7]
L_2	0.86 μm	Available lab data
R'	0.73 μm	Available lab data
P	Set to total increase of $\langle \text{Ca} \rangle$ in the fiber to 181 μM	Fit to literature based on the max increase in calcium with no reaction or uptake present
V_{max}'	Set max $\langle \text{Ca} \rangle$ during a single pulse to 15 μM	Fit to literature based on the max average calcium peak
$k_{r,\text{CaTnC}}$	1.20×10^5 1/ms	[5]
$k_{f,\text{CaTnC}}$	8.85×10^7 1/ μM /ms	[5]
$k_{r,\text{CaPar}}$	500 1/ μM /ms	[2]
$k_{f,\text{CaPar}}$	0.160 1/ μM /ms	[2]
$k_{r,\text{Mg,Par}}$	3000 1/ms	[2]
$k_{f,\text{Mg,Par}}$	33,000 1/ μM /ms	[2]
A_1	206 1/s	Approximate
A_2	60 1/s	Approximate
k_d	1 μM	[7]
τ_{on}	5 ms	[7]
τ_{off}	1.6 ms	[7]
$[\text{Ca}^{2+}]_{\text{rest}}$	0.1 μM	[7]
$[\text{Mg}^{2+}]_{\text{rest}}$	1 mM	[7]
$[\text{Ca}^{2+}]_{\text{SR}}$	1.1 mM	[7]
$[\text{Tn}_{\text{Total}}]$	140 μM	[5]
$[\text{Par}_{\text{Total}}]$	940 μM	[5]
D_{Ca}	3×10^{-6} cm^2/s	[10]
D_{Mg}	3×10^{-6} cm^2/s	Approximately the same as for Ca
D_{Par}	1.5×10^{-6} cm^2/s	[13]
D_{Tn}	0 cm^2/s	Assumed stationary
D_{ParCa}	1.5×10^{-6} cm^2/s	Approx. same as Par
D_{ParMg}	1.5×10^{-6} cm^2/s	Approx. same as Par
D_{CaTn}	0 cm^2/s	Assumed stationary