Published in final edited form as:

J Mol Cell Cardiol. 2016 July ; 96: 11–25. doi:10.1016/j.yjmcc.2015.02.006.

Dynamics of cross-bridge cycling, ATP hydrolysis, force generation, and deformation in cardiac muscle

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Abstract

Despite extensive study over the past six decades the coupling of chemical reaction and mechanical processes in muscle dynamics is not well understood. We lack a theoretical description of how chemical processes (metabolite binding, ATP hydrolysis) influence and are influenced by mechanical processes (deformation and force generation). To address this need, a mathematical model of the muscle cross-bridge (XB) cycle based on Huxley's sliding filament theory is developed that explicitly accounts for the chemical transformation events and the influence of strain on state transitions. The model is identified based on elastic and viscous moduli data from mouse and rat myocardial strips over a range of perturbation frequencies, and MgATP and inorganic phosphate (Pi) concentrations. Simulations of the identified model reproduce the observed effects of MgATP and MgADP on the rate of force development. Furthermore, simulations reveal that the rate of force re-development measured in slack-restretch experiments is not directly proportional to the rate of XB cycling. For these experiments, the model predicts that the observed increase in the rate of force generation with increased Pi concentration is due to inhibition of cycle turnover by Pi. Finally, the model captures the observed phenomena of *force yielding* suggesting that it is a result of rapid detachment of stretched attached myosin heads.

Keywords

Cross-bridge cycle; Viscoelasticity; Cardiac muscle; Force generation; Metabolites; Sinusoidal perturbation analysis

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Disclosures: none declared.

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Introduction

The coupling of chemical reaction and mechanical processes in muscle dynamics is not fully understood, despite the existence of a wealth of experimental observations and theoretical models [1–8]. We lack definitive knowledge of the sequence of biochemical steps in the cross-bridge (XB) cycle and how those steps are associated with mechanical processes. Specific questions that remain unresolved include: What biomolecular mechanical events are associated with which chemical events? How does deformation/strain influence the kinetics of the XB cycle? How do metabolite levels influence mechanical functions, such as the rate of force generation, rate of deformation, and peak force? These questions are addressed here using a model of XB cycling based on Huxley's sliding filament theory [2] that explicitly represents relationships between the biochemical energetics and mechanics. By explicitly linking mechanical and biochemical kinetic processes, the developed model provides a new means of interpreting measurements of muscle fiber dynamics.

Models that account for both strain as an independent variable and the influence of strain on chemical kinetics have their origins in Huxley's seminal model of sliding filament dynamics [2]. Often the computational expense of these distributed models can be circumvented using a moment distribution approach to reduce a system of partial differential equations (PDEs) to a system of ordinary differential equations (ODEs) [9, 10]. Previous studies have applied this approach [9–13], however none of these have accounted for the effect of metabolites on the kinetics. Similarly, a previously developed kinetic model [14] that does account for metabolite concentrations (energetic state) on XB cycling does not account for the coupling of deformation/strain and kinetics. One of the goals of the present study is to integrate the phenomena accounted for in these two classes of models to gain an improved understanding of muscle biomechanics and bioenergetics.

In the proposed model of XB cycle (Figure 1A) inorganic phosphate (Pi) release is required for transition from a loosely to strongly bound attachments state. Rapid unbinding of MgADP and binding of MgATP precedes dissociation of myosin from actin. The release of myosin from actin is associated with the hydrolysis of ATP forming the products ADP and Pi for the next cycle. In the model, the myosin head can bind either actin or MgATP but to both only transiently. To account for the effect of deformation on XB cycling, transition rates between different conformations are affected by stretch/strain as experienced by the myosin-actin complex during shortening or lengthening. The model is constructed around a four-state XB scheme [15, 16] which is less parsimonious than previous two-state or three-state XB models [3, 13, 17–19]. However, this formulation is the most economical that could fit all the sinusoidal perturbation experiments described below. Although we assume that the loosely-bound state (A_1) does not contribute to muscle force generation its inclusion (along with strain dependent transitions) was mandatory to fit all the data.

Model parameters are identified by fitting data on force from sinusoid length perturbation experiments, over a range of [MgATP] and [Pi], which characterize apparent elastic and viscous properties of myocardial strips at different frequencies. Using data from rat and mouse myocardial strips, model parameter values are estimated for both of these species. In these experiments, small amplitude sinusoidal length perturbations (below the unitary

myosin step-size) are applied to the myocardial strip and developed force is measured. The mechanical perturbations influence the elementary steps of XB cycle. The in-phase and out-of-phase components of the measured force at each frequency are in turn influenced by the kinetics of the XB cycle. Thus, these experiments can be used to identify a model capturing XB cycle kinetic and mechanical/chemical coupling of the cycle. Despite the fact that these observations depend on XB kinetics and elastic and viscous properties of striated muscle, there is no mathematical model of XB kinetics that quantitatively describes the effect of metabolites on the apparent viscoelastic properties of striated muscle. Attempts have been made to quantitatively describe A-, B-, and C-processes of sinusoidal perturbation experiments using empirical expressions [15, 20, 21] or qualitatively describe the elastic and viscous moduli obtained from such experiments [18, 22, 23]. Previous studies have either quantitatively described sinusoidal length perturbation experiments [17], or the effect of metabolites on XB kinetics [14, 24] but not both.

Elastic and viscous moduli are defined as the amplitude of the in-phase and out-of-phase force response to the sinusoidal length perturbation. Experiments on cardiac muscle from a variety of species exhibit negative viscous moduli at frequencies near the resting heart rate of the species studied. For example, cardiac muscle from human and mouse shows negative viscous moduli around 1.3 Hz and 10.9 Hz, respectively [25]. The developed model is able to capture this phenomenon as a consequence of the mean XB cycle turnover time.

The developed model is corroborated by simulating sarcomere quick-release, force development, slack-restretch, sarcomere lengthening, velocity-MgATP and force-velocity experiments. Simulations of slack-restretch experiments predict that the rate of force re-development is not proportional to the rate of XB cycling, suggesting that these experiments may have been misinterpreted in the past. Specifically, the model predicts an increase in the rate of force re-development with increasing [Pi] as observed in experiments [26, 27]. However the reason for faster rate of force redevelopment with increasing [Pi] in the model (and the reason the model captures the experimental observations) is that Pi inhibits and slows down cross bridge cycling. Simulations of sarcomere quick-release and lengthening (computational) experiments exhibit a phenomenon termed force-yielding observed in muscle lengthening experiments [28, 29]. Model analysis suggests that the amount of force-yielding depends upon the average amount a myosin-head is stretched before finally dissociating from actin. Simulations presented here also support the working stroke hypothesis proposed for force generation in skeletal muscles [30]. Specifically, the model suggests that force generated due to power-stroke is about an order of magnitude higher than the force due to intermittent attachment-detachment of myosin-actin.

Methods

Experimental data for model parameterization and corroboration

Data used to estimate parameters are obtained from published experimental studies [31, 32]. In these studies sinusoid perturbation length change experiments were performed on myocardial strips excised from left ventricle of rat (150 μm diameter and 500 μm length) and mouse (100 μm diameter and 300 μm length). Before application of the sinusoid perturbation myocardial strips were skinned and mounted on a piezoelectric motor and strain

gauge, and incrementally stretched to 2.2 μm sarcomere length (SL) measured by digital Fourier Transform [31, 32]. For experiments with various [MgATP] experimental buffer contained: pH 7.0, pCa 4.8, [Pi] 0 mM, [MgADP] 0 mM, and for experiments varying [Pi] buffer contained: pH 7.0, pCa 4.8, [Pi] 0 mM, [MgATP] 5 mM. These experiments were performed at 17 °C.

The temperature dependence of parameters is identified by estimating Q_{10} values from data collected by sinusoidal perturbation experiments on mouse myocardial strip with diameter 140 μm and length 800 μm [20]. For these experiments experimental buffer solution contained: pH 7.0, pCa 4.0, [Pi] 0.25 mM, [MgADP] 0 mM, [MgATP] 5 mM. These experiments were performed at both 17 °C and 37 °C.

The identified model is corroborated by simulating an independent force-velocity experiment [31]. In these experiments, the myocardial strip is held constant at a certain length and allowed to shorten with different mechanical loads (maintained over a period of 400 msec) which are expressed as fractions of maximum isometric force. Velocity is calculated as the slope of length change at the earliest steady-state force (~50 msec after release). Buffer solution contained: pH 7.0, pCa 4.8, [Pi] 0 mM, [MgADP] 0 mM, and [MgATP] 5 mM at 17 °C.

A (distributed) model for XB kinetics, stretch, and force generation

The basic XB model structure is illustrated in Figure 1A. We denote the state probabilities of the 5-state model by $N(t)$, $P(t)$, $p_1(t, s)$, $p_2(t, s)$ and $p_3(t, s)$, where the independent variable s represents the strain on an attached XB state. N is a non-permissible XB state when myosin heads cannot bind with actin i.e. when minimal or no Ca^{2+} is present and P is a permissible XB state when binding of myosin heads with actin is possible i.e. in the presence of sufficient Ca^{2+} . Here N is included in the proposed XB model scheme for the sake of completeness and is dropped later as all the sinusoidal length perturbation experiments were performed under saturating Ca^{2+} concentration. The probability densities $p_1(t, s)$, $p_2(t, s)$ and $p_3(t, s)$ represent the probability density of strain in the three attached states at time t . The

total fraction of XBs in A_1 state, for example, at time t is given by $\hat{p}_1(t) = \int_{-\infty}^{+\infty} p_1(t, s) ds$, and

the mean strain of XBs in A_1 state is computed by $\int_{-\infty}^{+\infty} s p_1(t, s) ds / \hat{p}_1(t)$. The state variables obey the overall conservation relation:

$$N(t) + P(t) + \int_{-\infty}^{+\infty} p_1(t, s) ds + \int_{-\infty}^{+\infty} p_2(t, s) ds + \int_{-\infty}^{+\infty} p_3(t, s) ds = 1. \quad (1)$$

The governing equations for the proposed XB kinetics model accounting for metabolites and strain are given by:

$$\frac{dN}{dt} = -k_{np}N(t) + k_{pn}P(t), \quad (2)$$

$$\frac{dP}{dt} = k_{np}N(t) - k_{pn}P(t) + \tilde{k}_d p_1(t, s) - k_a P(t) + \tilde{k}_3 e^{\alpha_3(s+s_3)^2} p_3(t, s), \quad (3)$$

$$\frac{\partial p_1}{\partial t} + \frac{dL}{dt} \frac{\partial p_1}{\partial s} = k_a \delta(s) P(t) - \tilde{k}_d p_1 - \tilde{k}_1 e^{-\alpha_1 s} p_1 + k_{-1} e^{+\alpha_1 s} p_2, \quad (4)$$

$$\frac{\partial p_2}{\partial t} + \frac{dL}{dt} \frac{\partial p_2}{\partial s} = \tilde{k}_1 e^{-\alpha_1 s} p_1 - k_{-1} e^{+\alpha_1 s} p_2 - k_2 e^{-\alpha_2 s} p_2 + \tilde{k}_{-2} p_3, \quad (5)$$

$$\frac{\partial p_3}{\partial t} + \frac{dL}{dt} \frac{\partial p_3}{\partial s} = k_2 e^{-\alpha_2 s} p_2 - \tilde{k}_{-2} p_3 - \tilde{k}_3 e^{\alpha_3(s+s_3)^2} p_3. \quad (6)$$

$$\begin{aligned} \tilde{k}_d &= k_d \frac{[Pi]/K_{Pi}}{1 + [Pi]/K_{Pi}}, \tilde{k}_1 \\ &= k_1 \frac{1}{1 + [Pi]/K_{Pi}}, \tilde{k}_{-2} \\ &= k_{-2} \frac{[MgADP]/K_{MgADP}}{1 + [MgADP]/K_{MgADP} + [MgATP]/K_{MgATP}}, \tilde{k}_3 \\ &= k_3 \frac{[MgATP]/K_{MgATP}}{1 + [MgADP]/K_{MgADP} + [MgATP]/K_{MgATP}} \end{aligned}$$

where . Following the Lymn-Taylor XB scheme [33], it is assumed that XB detachment is irreversible and therefore there is no k_{-3} parameter. The governing equations for $p_1(t, s)$, $p_2(t, s)$ and $p_3(t, s)$ are partial

differential equations that depend on muscle shortening velocity $\frac{dL}{dt}$ as initially suggested by Huxley [2]. The rate constants k_a and k_d govern attachment and detachment of myosin heads from actin. The Dirac delta function $\delta(s)$ appears in the equation for p_1 because $s = 0$ at the moment of attachment from P to A₁.

The model assumes that strain influences the rates of state transitions between A₁ and A₂, between A₂ and A₃, and the rate detachment from A₃ to P. Specifically, it is assumed that positive strain (stretching) inhibits the transition from loosely bound A₁ to strongly bound A₂. Positive strain also inhibits the ratcheting step from A₂ to A₃. Similarly, negative strain or (contraction) increases the rates of these transitions. The inhibition of XB cycling by positive strain and increase in cycling by negative strain are supported by experimental finding suggesting an increase in XB conformation changes during shortening as opposed to during lengthening [34]. Following previous models [18, 19], the rate of XB detachment is assumed to increase with positive or negative strain. Specifically XB detachment rate is proportional to $e^{\alpha_3(s+s_3)^2}$, where α_3 and s_3 are constants. These effects of strain on XB cycling rates were necessary to achieve negative viscous modulus seen in the sinusoidal perturbation experiments.

The exponential strain dependence accounts for stretch induced cooperativity effects on XB kinetics as previously suggested by Razumova et al. [18]. Moreover, it is assumed that the rate constants for transitions from A₁ to A₂ and A₂ to A₃ increases exponentially during contraction and decreases exponentially during lengthening.

Force due to XBs undergoing cycling and intermittent attachment-detachment of thick-thin filament is:

$$F_{XB}(t) = k_{\text{stiff},1} \left(\int_{-\infty}^{+\infty} s p_2(t, s) ds + \int_{-\infty}^{+\infty} s p_3(t, s) ds \right) + k_{\text{stiff},2} \Delta_r \int_{-\infty}^{+\infty} p_3(t, s) ds. \quad (7)$$

Here, $k_{\text{stiff},1}$ is the stiffness of the attached thick-thin filament, $k_{\text{stiff},2}$ represents ratcheted XB stiffness, and Δ_r represents the XB power-stroke size. The sign convention here is that a positive force opposes positive strain, as indicated in Figure 1. The two parameters Δ_r and $k_{\text{stiff},2}$ are not independently identifiable from the analyzed data. Estimated values of Δ_r range from 4 to 20 nm [35–46]. Here we fix XB stroke size at 10 nm, as there is some consensus regarding this value [40–46].

The total force generated by the muscle model (shown in Figure 1B) is given by:

$$F(t) = F_{XB}(t) + F_1(t) + F_2(t), \quad (8)$$

$$\frac{dF_1}{dt} = k_{\text{PE},1} \left(\frac{dL}{dt} - \frac{F_1}{\eta} \right), \quad F_2 = k_{\text{PE},2} L(t). \quad (9)$$

Where $k_{\text{PE},1}$ and $k_{\text{PE},2}$ are spring stiffness constants, η is the viscosity coefficient of the dashpot, $L(t)$ is the length of the sarcomere, and $\frac{dL}{dt}$ is the sarcomere shortening velocity. The complete muscle model invokes a total of 18 adjustable parameters (13 for XB kinetics and 5 for striated muscle force generation).

Model simplification

Sinusoidal length perturbation experiments were performed with fully activated myofilaments (under high Ca^{2+}) i.e. $k_{np} \gg k_{pr}$. Therefore, for these experiments Equation (2) can be ignored and Equation (3) is approximated as:

$$P(t) \approx 1 - \int_{-\infty}^{+\infty} p_1(t, s) ds - \int_{-\infty}^{+\infty} p_2(t, s) ds - \int_{-\infty}^{+\infty} p_3(t, s) ds. \quad (10)$$

Furthermore, the PDEs governing $p_1(t, s)$, $p_2(t, s)$ and $p_3(t, s)$ can be simplified to ODEs by expressing the governing equations in terms of the moments of the state variables. For a complete description of the model simplification approach please see Palmer [13]. Simplification is performed by expanding the exponentials as quadratic polynomials, multiplying Equations (4)–(6) by s^i , and integrating from $-\infty$ to $+\infty$ which yields:

$$\begin{aligned} \frac{d}{dt} p_1^i - i v p_1^{i-1} &= k_a \delta^i P(t) - \tilde{k}_d p_1^i(t) - \tilde{k}_1 \left(p_1^i - \alpha_1 p_1^{i+1} + \frac{1}{2} \alpha_1^2 p_1^{i+2} \right) + k_{-1} \left(p_2^i + \alpha_1 p_2^{i+1} + \frac{1}{2} \alpha_1^2 p_2^{i+2} \right), \\ \frac{d}{dt} p_2^i - i v p_2^{i-1} &= \tilde{k}_1 \left(p_1^i - \alpha_1 p_1^{i+1} + \frac{1}{2} \alpha_1^2 p_1^{i+2} \right) - k_{-1} \left(p_2^i + \alpha_1 p_2^{i+1} + \frac{1}{2} \alpha_1^2 p_2^{i+2} \right) - k_2 \left(p_2^i - \alpha_2 p_2^{i+1} + \frac{1}{2} \alpha_2^2 p_2^{i+2} \right) + k_{-2} p_3^i, \\ \frac{d}{dt} p_3^i - i v p_3^{i-1} &= k_2 \left(p_2^i - \alpha_2 p_2^{i+1} + \frac{1}{2} \alpha_2^2 p_2^{i+2} \right) - k_{-2} p_3^i - k_3 \left(p_3^i + \alpha_3 s_3^2 p_3^i + 2 \alpha_3 s_3 p_3^{i+1} + p_3^{i+2} \right). \end{aligned} \quad (11)$$

Where $p_k^i (k=1, 2, 3)$ is the i^{th} moment of $p_k(t, s)$ and $\delta^i = \int_{-\infty}^{+\infty} s^i \delta(s) ds$. With the parameter values estimated below for rat and mouse muscle, the full distributed model is effectively approximated using only the first, second order and third order moments, $i = 0$ (mean fraction of all attached XBs in k-state), $i = 1$ (mean length displacement of all attached XBs in k-state), $i = 2$ (variance in length displacement of all attached XBs in k-state) and ignoring all higher order moments ($i \geq 3$). The accuracy of the moment expansion of Equation (11) in capturing the behavior of the full distributed model is treated in Appendix A. The ODE approximation diverges from the PDE solution as strain on individual XBs increases. The error in ODE approximation is within 1.5% of the original distributed model as long as mean strain in the attached XBs is less than 20 nm.

For the sinusoidal length perturbation experiments SL is given by: $L(t) = L_0(1+0.00125\sin(2\pi\omega t))$, where $L_0 = 2.2 \mu\text{m}$ and ω is the frequency of length perturbation.

Parameter estimation

The complete model contains a total of 18 unknown parameters $\theta = \{k_a, k_d, k_1, k_{-1}, k_2, k_{-2}, k_3, K_{\text{MgATP}}, K_{\text{Pi}}, \alpha_1, \alpha_2, \alpha_3, s_3, k_{\text{stiff},1}, k_{\text{stiff},2}, k_{\text{PE},1}, \eta, k_{\text{PE},2}\}$ which are estimated by simultaneous least squares fitting of mice and rat cardiac muscle experiments. We do not have access to data from experiments sensitive to K_{MgADP} . (i.e., we have no data from experiments in which [MgADP] was varied.) Thus K_{MgADP} was held fixed at 0.194 mM, as reported by Yamashita, Sata [47]. Also, [Pi] was not perturbed for rat cardiac muscle experiments so K_{Pi} was assumed to be the same for both mouse and rat cardiac muscle. Thus, in total 18 unknown parameters are determined by simultaneous fitting data on mice cardiac muscle experiments and 17 unknown parameters are determined by simultaneous fitting data on rat cardiac muscle experiments:

$$\min_{\theta} E(\theta), \quad E(\theta) = \sum_i^{N_{\text{Exp}}} \sum_j^{N_{\text{freq}}} \frac{1}{N_{\text{freq}}} \frac{(X_{i,j}^{\text{data}} - X_{i,j}^{\text{model}}(\theta))^2}{\sigma(X)_{i,j}^2}.$$

N_{Exp} is the number of different [MgATP] and/or [Pi] experiments (rat: 0.25–5 mM [MgATP]; mouse: 0.4–5 [MgATP], 0–4 mM [Pi]), N_{freq} is the number of different frequencies at which sinusoidal length perturbation experiments are performed (rat: 0.125–100 Hz; mouse: 0.125–250 Hz), $X_{i,j}^{\text{data}}$ is the experimentally determined elastic (or viscous) modulus at j^{th} frequency and i^{th} experiment, $X_{i,j}^{\text{model}}$ is the model predicted elastic (or viscous) modulus at j^{th} frequency and i^{th} experiment, $\sigma(X)_{i,j}^2$ is the variance associated with the value of X at j^{th} frequency and i^{th} experiment. In experiments where [MgADP], [Pi] or both are absent k_{-2} , k_d or both are multiplied by zero.

Experimental data are illustrated in Figures 2 (rat) and 3 (mouse), which plot measured viscous and elastic modulus as function of frequency at different [MgATP], [MgADP], and [Pi]. Model parameter estimates (Table 1) were obtained by minimizing the difference between model predictions (solid lines) and experimental data (open circles). Temperature

dependence of parameters was determined by estimating Q_{10} values necessary to explain data from a sinusoidal perturbation experiment performed on a mouse myocardial strip at two different temperatures (17 and 37 °C). To simultaneously explain the 17 and 37 °C data for mouse required introducing six Q_{10} values. Specifically, it was determined that the temperature dependence of XB cycle rate constants k_a , k_d , k_1 , k_{-1} , k_{-2} , and k_3 could effectively be represented using a single shared Q_{10} value. It was necessary to allow k_2 to have its own unique Q_{10} value. In addition, temperature correction factors were required to be estimated for the force-generation parameters (Table 1). In general a parameter value at temperature T_2 , ($p(T_2)$), is computed from the parameter value $p(T_1)$ using the following relationship: $p(T_2) = p(T_1) Q_{10}^{\frac{(T_2 - T_1)}{10}}$. The minimization of the mean residual error, $E(\theta)$, for optimal estimation of the model parameter set was carried out using a MATLAB (The MathWorks, Natick, MA) implementation [48] of the PIKAIA Genetic Algorithm [49].

Muscle model

The muscle model used for simulating (computational) experiments is same as shown in Figure 1B except for the parallel spring component. As sinusoidal length perturbations are tiny the recorded passive muscle responses are (locally) linear as suggested by the linear spring formulation used during parameter estimation, see Equation (9), however under physiological conditions the passive forces are known to be non-linear in nature [50]. Thus in simulating the complete muscle model the parallel linear spring is replaced by a non-linear parallel spring from the Rice model [19]. The ODE governing the complete muscle model is given as:

$$\frac{dL}{dt} = \frac{-\dot{F}_{XB} - \dot{F}_{\text{passive}} + \dot{F}_{\text{pre-load}}}{k_{PE,1}} + \frac{-F_{XB} - F_{\text{passive}} + F_{\text{load}} + F_{\text{pre-load}}}{\eta}. \quad (12)$$

F_{passive} has the non-linear passive spring formulation from the Rice model [19], $F_{\text{pre-load}}$ corresponds to the constant force that will stretch the sarcomere to a length greater than the resting length and F_{load} is the constant load against which sarcomere contracts during quick-release experiments. The 'dot' represent the time-derivative of the respective forces. Details of the passive force and preload formulation are given in Appendix C.

Results

This section presents model fits to sinusoidal experiments from rat and mouse cardiac muscle [31, 32], and simulations of independent force-velocity [31], velocity-MgATP-MgADP [47], force redevelopment [51] and isometric force-MgADP [52, 53] experiments. All the model predictions and corroboration simulations presented here are performed using the parameters estimated from mouse cardiac muscle because those parameters were identified using more extensive data. Analogous predictions and corroborative simulations using the rat parameters are shown in Appendix B.

Effect of MgATP on rat cardiac muscle

Sinusoidal length perturbation experiments done with [MgATP] ranging from 0.025 to 5 mM were available for rat myocardial strips [32]. Here only data pertaining to [MgATP] concentration between 0.25 and 5 mM were used for model parameter estimation because concentration distributions of [MgATP] may become heterogeneous at lower concentrations due to diffusion limitation [52, 54]. Elastic and viscous moduli data at different frequencies over the analyzed range of [MgATP] are plotted in Figure 2, along with best-fit model simulations. At frequencies greater than 10 Hz elastic modulus steadily increases with frequency while the viscous modulus decreases. The model reproduces this phenomenon as well as the characteristic dip in elastic modulus that occurs in the frequency range of 1–8 Hz.

Effect of MgATP and Pi on mouse cardiac muscle

Figure 3 shows model fittings to data from sinusoidal length perturbation experiments performed on mouse myocardial strips with different [MgATP] (= 0.4, 1, 5 mM; n = 12) and [Pi] (= 0, 1.8, 4 mM; n = 5). Model fits to all other [MgATP] and [Pi] experiments used to identify parameters associated with mouse cardiac muscle is shown in Appendix E. Again, experiments with [MgATP] = 0.2 mM and below were excluded from parameter estimation. The model effectively reproduces the majority of the data, with deviations most pronounced at [MgATP] = 0.4 mM. Deviations could in part be explained by experimental variability. Note, for example, that the data sets labeled [MgATP] = 5 mM and [Pi] = 0 mM represent identical experimental conditions, while the model captures one of these data sets better than the other.

Model simulations of mouse cardiac muscle force-velocity relationship

Palmer et al. [31] performed force-velocity experiments on mouse cardiac muscle in which the muscle is held constant at a length for 100 msec and then allowed to contract against a constant afterload. The muscle shortening velocity was measured as the slope of percent change in muscle length ~45 msec after release and initiation of shortening. Normalized power was calculated as the product of shortening velocity and normalized force (against which the sarcomere contracts). Experimental data on velocity and power as function of tension from Palmer et al. [31] is shown in Figure 4 (open circles).

Simulations of these experiments assumed an initial sarcomere length of 2.2 μm , and predicted a maximum tension of $T_{\text{max}} = 28.55 \text{ mN mm}^{-2}$ assuming that 45% of the cardiac muscle cross-section is myofilaments [55], which is similar to the experimental observation of $27.76 \pm 1.21 \text{ mN mm}^{-2}$. Furthermore, the predicted relationships between velocity and tension matches the experimental data reasonably well (Figure 4A). Model simulations diverge from experimental data when afterloads equal to T_{max} are applied. In the experiments, velocity is near zero at an afterload of $0.9 \cdot T_{\text{max}}$ while the model simulated velocity reaches zero at an afterload equal to T_{max} . Given that these comparisons are based on predictions using the independently estimated parameter values, the agreement here is good. As observed experimentally, the maximum power output is obtained at a point where shortening velocity is approximately one third of its maximum, corresponding to a tension of approximately 40% of T_{max} [56].

Temperature dependence of XB cycling and muscle force generation

Changes in temperature affect XB cycling and muscle force generation [57–59]. In order to quantify the effects of temperature on XB cycling and cardiac muscle force generation, Q_{10} values were estimated for XB cycling parameters and muscle force generation parameters from sinusoidal length perturbation experiments performed on left ventricular myocardial strips from an individual mouse at two different temperatures [20]. Measured elastic and viscous moduli as function of frequency at 17 and 37 °C are plotted in Figure 5.

While frequency response characteristics of this data set at 17 °C resemble those reported in Figure 3, they are not identical. [Compare Figure 5 and Figure 3 ($P_i = 0$ mM) which correspond to similar experimental conditions.] To account for these differences force generation parameters were re-estimated for these 17 °C data while keeping the XB cycle parameters fixed. The estimated force generation parameter values are listed in the legend of Figure 5. Next, the six Q_{10} parameters (see *Parameter Estimation* above) were adjusted to fit the 37 °C data. Using these temperature correction values, reported in Table 1, the model is able to capture the rightward shift in characteristic dip of elastic modulus (indicative of faster XB kinetics) seen in the experiments at higher temperature.

Effect of temperature on sarcomere shortening velocity

Sarcomere quick-release experiments with constant afterload were simulated at three different temperatures to compare the predicted temperature dependence of the force-velocity relationships to that observed experimentally [57, 59]. The quick-release experiment simulation is illustrated in Figure 6A, in which the force applied to a muscle is instantaneously reduced from a fixed initial value to a reduced afterload. The initial value is the tension required to maintain an initial length of 2.2 μm . Simulations reveal the experimentally observed phenomenon known as force-yielding [28, 29], indicated by the arrow in Figure 6A. Analysis reveals that this phenomenon depends on the stretch sensitive parameter α_3 —the smaller its value the less apparent becomes the phenomenon. The mechanism underlying this phenomenon is discussed in detail in the Discussion section and Appendix D with sarcomere lengthening (computational) experiments. A direct effect of faster XB cycling and force generation with increasing temperature is faster shortening velocity. Shortening velocities are computed by taking average of slopes after the force yielding process. Predicted force-velocity curves are shown in Figure 6B for three different temperatures.

Effect of MgATP and MgADP on sarcomere shortening velocity

Metabolites not only affect isometric force but also the rate of isometric force development [51–53], consequently affecting sarcomere shortening velocity [47]. Using a motility assay Yamashita, Sata [47] determined the effect of MgATP and MgADP competition on sarcomere shortening velocity. To simulate these experiments, the sarcomere is held constant at a length of 2.2 μm and released with no load. Shortening velocity is computed as the average of sarcomere slopes between 30 and 50 msec after release. Model predicted velocities as function of [MgADP] and [MgATP] are compared to data from Yamashita, Sata [47] in Figure 7 (Data: closed circles and squares; Model: open circles and squares). For these simulations all the parameters are same as listed in Table 1 for mouse cardiac muscle,

[Pi] set to zero, and temperature was set to 30 °C. As with the force-velocity predictions of Figure 4, the agreement here is good given that these comparisons are model predictions, not fits based on parameter adjustment.

Effect of [MgATP] on force development

The effects of [MgATP] on maximum isometric force and the rate of force development were simulated and compared with the experiments reported by Ebus, Papp [51]. In these experiments, fully activated trabeculae are stretched to 2.1 μm SL [51]. After isometric force reaches steady state, the trabecula is allowed to shorten by 1% of the initial length and force development is recorded. The recordings in Figure 8A correspond to the phase of force re-development reported by Ebus, Papp [51]. Force recordings are made at three different [MgATP] and normalized such that minimum and maximum force at each individual [MgATP] is scaled between 0 and 1. To simulate rapid detachment of all the XBs, due to sudden length change, we simulated the muscle model with initial SL equal to 2.1 μm (starting with all the XBs in permissible state) and computed total force. Figure 8B shows model simulated rate of force development [simulated force (F) minus minimum force ($F_{\min}$) is normalized to maximum force ($F_{\max}$) minus $F_{\min}$ at individual each MgATP concentration]. Simulations use parameter values listed in Table 1 for mouse and metabolite concentrations [MgADP] = 0 mM, [Pi] = 0 mM and temperature 20 °C, to match the experimental conditions. In Figure 8C model simulated relative force is normalized with respect to the maximum isometric force at [MgATP] = 0.5 mM so that model output and experimental data are comparable. The model effectively predicts the general trend in peak isometric force versus [MgATP]. The model also captures the effect of [MgATP] on the rate of force development (Figure 8A–B).

Effect of [MgADP] and [Pi] on isometric force

Similar simulations of peak isometric force and rate of force development were conducted at various MgADP and Pi concentrations to investigate the effects of these metabolites on muscle dynamics. Figure 9A illustrates that the model predicts a monotonic increase in isometric force with increasing [MgADP], similar to the experiments of Cooke and Pate [52]. Increasing [MgADP] leads to a decrease in the rate of force development (Figure 9B). Lu, Moss [53] reported a similar decrease in rate of force development with increasing in [MgADP] using photo-generation of [MgADP] in skinned rabbit psoas muscles.

Simulations of the effects of [Pi] on force and rate of force generation are shown in Figure 10. Similar to observations of Godt and Nosek [60], model predictions shown a decrease in maximum isometric force with an increasing [Pi]. Model simulations predict a 70% decrease in peak isometric force at [Pi] = 30 mM for mouse cardiac muscle, comparable to the 60% reduction reported by Godt and Nosek [60] in rabbit cardiac muscle. Model simulations also predict a decrease in rate of force generation (Figure 10B) and unloaded shortening velocity (Figure 10C) with increasing [Pi]. The decrease in unloaded shortening velocity is in agreement with the effect of [Pi] on slow-twitch skeletal muscles [61]. However, the decrease in rate of force development with [Pi] is in disagreement with slack-restretch experiments which is discussed in the discussion section.

XB cycle turnover rates in rat versus mouse

Since the model includes an irreversible step, the rate of turnover of the XB cycle is equal to the net probability flux [62]:

$$J = \tilde{k}_1 \hat{p}_1(\infty) - k_{-1} \hat{p}_2(\infty) = k_2 \hat{p}_2(\infty) - \tilde{k}_{-2} \hat{p}_3(\infty) = \tilde{k}_3 \hat{p}_3(\infty). \quad (13)$$

Here ∞ is used to denote steady-state distribution. Applying Equation (13) to the XB model developed here, for isometric conditions at 37 °C, we obtain cycle times of 898 cycles/min for rat (mouse Q_{10} values were used for this calculation) and 3020 cycles/min for mouse. These calculations assume metabolite concentrations of: [Pi] = 0.25 mM, [MgADP] = 36 μ M, and [MgATP] = 5 mM.

Discussion

The apparent viscoelastic properties of cardiac muscle tissue are significantly affected by metabolite concentrations [31, 32, 52] that vary as a function of cardiac work rate [63, 64] and are altered in disease conditions such as cardiac hypertrophy and heart failure [65, 66]. Although sinusoidal length perturbation experiments of myocardial strips have been used to study viscoelastic properties of cardiac muscle [15, 21, 25, 32, 67], model-based analysis of data from these experiments have not attempted to describe these data in terms of a metabolite sensitive sliding filament model. The presented muscle model is the first such model that quantitatively describes the effects of metabolite concentrations on the apparent elastic and viscous moduli of cardiac muscle. The developed model effectively describes the effect of [MgATP], [MgADP] and [Pi] on maximum force, sliding velocity, and rate of force development (all of which in-turn describes the rate of pressure development in the heart).

One important prediction that our model makes is a reduction in rate of force development and sarcomere shortening velocity with increasing [Pi] (Figure 10). This result seems intuitive as both Pi and MgADP are released during the forward cycling of XBs and an increase in these concentrations will inhibit forward turnover of the XB cycle via simple product inhibition [68]. However, counter-intuitively, slack-restretch experiments done on skeletal muscle myofibrils [26] and cardiac myocytes [27] have suggested an increase in the rate of force development with an increase in [Pi]. Figure 4A from Hinken and McDonald [27] is reproduced here in Figure 11A. In this experiment, myocyte length is initially held at constant value, L_o . At time 100 msec L_o is reduced by 15% and allowed to shorten for ~20 msec; afterwards muscle is re-stretched to 105% of L_o for ~2 msec and then returned to L_o . Figure 11A reports measurements of force following this protocol for different values of [Pi]. To understand this phenomenon, we simulated the experiment using the muscle model developed here. The predicted force response to the slack-restretch maneuver, plotted in Figure 11B, reproduces the Pi-dependence observed by Hinken and McDonald [27]. However, in the model, force generation is faster at higher [Pi] because at high [Pi] XBs tend to stay attached during the 100-msec slack period. As a result, the muscle tends to act like an elastic material with a fast response. In contrast, at lower [Pi] the XBs tend to un-attach and re-attach/reset at zero strain during the slack period. At low [Pi] the observed recovery in force is slower because the system has been perturbed farther from its initial (and final)

steady state. The concept that high [Pi] causes some XBs to remain in a potentiated (attached) state that facilitates rapid binding is also suggested by measurements of the residual force immediately after slack-restretch, which is elevated with increasing [Pi] (see [69, 70]). Thus, these experimental observations should not be interpreted to indicate that increasing Pi concentration increases the rate of XB cycle turnover.

The interplay between XB kinetics and muscle dynamics is also manifest in the poorly understood phenomenon of force yielding, which is the sudden drop in force development during constant stretching of muscles above a certain velocity [28, 29]. Model simulations showing sarcomere lengthening experiments exhibit this force yielding effect as a drop in force seen during sarcomere lengthening (Figure D1 in Appendix D). Similar to the muscle stretch experiments [28, 29], this force yielding effect depends upon the velocity of sarcomere shortening (more at higher velocities and vice-versa). As detailed in Appendix D, force yielding in the model arises from the coordinated detachment of myosin heads, as suggested by [28]. This coordinated detachment occurs through the strain dependence of the detachment rates in the model. A sudden change in strain rate (as in a muscle stretch or quick-release experiment) causes attached myosin heads to undergo sudden coordinated strain, resulting in a coordinated tendency to detach, reducing force. This finding is similar to that reported by Mitov et al. [72], who analyzed the effect of stretching of cardiac muscle using a simpler Huxley-type model and suggested that force yielding (which they refer to as short-range force response) is a result of strain induced rapid detachment of myosin heads.

The identified XB model may be used to estimate effective single-molecule mechanical properties of cardiac muscle fibers. Assuming that XBs in the sarcomere are arranged in a parallel manner and that the number of myosin heads working in parallel per mm² of mouse cardiac muscle cross-section area is $\zeta = 40 \times 10^{15}$ (i.e. 2.5 times smaller [73] than skeletal muscle cross-section area [74]), the individual XB stiffness (k_{uni}) can be estimated: $k_{\text{uni}} = k_{\text{stiff}}/2/\zeta = 0.48 \text{ pN nm}^{-1}$. The work done on a single XB at the single-molecule level during a cycle in an unloaded contraction will be [75]: $E = \frac{1}{2} \cdot k_{\text{uni}} \cdot r^2 = 24 \text{ zJ}$, a value that is remarkably close to the reported value of $22 \pm 3 \text{ zJ}$ [76]. Since in cardiac muscles under aerobic conditions the free energy released during hydrolysis of one ATP molecule is approximately 100 zJ [77] the estimated mechanical efficiency is 24%.

The ratcheted XB stiffness constant $k_{\text{stiff},2}$ for rat cardiac muscle is estimated to be almost 2.5 times higher than that for mouse, resulting in an estimate of $k_{\text{uni}} = 1.3 \text{ pN nm}^{-1}$ which is within the range of $1\text{--}5 \text{ pN nm}^{-1}$ reported for intact muscle fibers under isometric conditions [78–80]. The higher estimate of stiffness is associated with a lower estimated XB cycling rate in rat compared to mouse myocardium. Specifically, the estimated rates of XB cycle turnover are 898 cycles/min for rat and 3020 cycles/min for mouse, even though the intrinsic heart rates for these species are not substantially different. Thus it is possible that the lower ratchet stiffness in mouse is compensated for by a higher cycling rate. However, there is significant uncertainty in these estimates. The estimates of k_{uni} are associated with an assumed value of the ratchet displacement $r = 10 \text{ nm}$. The two parameters k_{uni} and r cannot be independently estimated from the data we have analyzed. Furthermore, since we have data obtained at 37° C for rat cardiac muscle, we have used the Q_{10} values estimated from mouse to estimate the cycle turnover rate for rat.

Finally, our model may provide some insight into potential mechanisms of force generation in striated muscle, for which there exist two main hypotheses: (1.) the attachment/detachment hypothesis [81] and (2.) the working-stroke hypothesis [30]. The attachment/detachment hypothesis states that the primary event of force generation is rapid attachment-detachment of myosin-actin filaments occurring at a much faster scale than the rate of XB cycling [81]. In our model formulation, deformation of attached states results in a passive force proportional to and in the opposite direction of the strain. The working stroke contribution—second terms in Equation (7)—represents the working stroke hypothesis in which force generation is associated with the working stroke (power stroke) of the actin-bound myosin head. Thus our force model includes contributions from both a stress-strain relationship for attached states as well as an active ratcheting associated with turnover of the cross-bridge cycle. Active force generation is associated only with the working stroke of the cross bridge.

Summary

We have developed and identified a mathematical model of the cardiac muscle XB cycle that explicitly accounts for the chemical transformation events and the influence of strain on state transitions. The model provides a coherent synthesis for a number of previously observed phenomena, including the effects of [MgATP], [MgADP] and [Pi] on muscle dynamic properties, such as the rate of force generation, sliding velocity, and apparent viscoelastic properties. Furthermore, the model predicts a novel mechanistic explanation for the experimentally observed phenomenon of force yielding in striated muscle. Finally, the model resolves the paradox related to the apparent effect of inorganic phosphate on the rate of force generation in slack-restretch experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Computer model development was supported by the National Institutes of Health grants HL072011 and GM094503; experiments were supported by National Institutes of Health grant HL59408.

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Appendix

Appendix A: Comparison of the moment-based approach with the PDE model

To demonstrate that the moment-based approximation of Equation (11) effectively captures the behavior of the full distributed model of Equation (2–6), here we simulate both systems of equations, demonstrating that the difference in numerical solution is negligible for a test initial value problem. Numerical results are obtained using a converged first-order upwind method for the hyperbolic PDE system [82] and the MATLAB built-in integrator `ode15s` for the ODE system.

Figure A1.

Comparison of numerical simulations of distributed PDE model and ODE system for moments. A. Simulated time courses of fraction in each state for the distributed model. B. Mean strain of each attached state for the distributed model. C. Simulated time courses of fraction in each state for (0-order moments) for the ODE approximation. Mean strain of each attached state for the ODE approximation. Initial conditions and definitions are provided in the text. Metabolite concentrations are: [MgATP] = 5 mM; [MgADP] = 0 mM; [Pi] = 0 mM and temperature is 17 °C. Parameters used for simulations are as listed in Table 1 for rat.

Initial values for the PDE system of Equation (3–6) are: $P = 1$, $p_1(x, s) = p_2(x, s) = p_3(x, s) = 0$. Note that there is no non-permissible state as the developed model is for a fully activated myofilament. The sarcomere length is given by $L(t) = 2.2 \cdot (1 + 0.00125 \sin(2\pi \cdot 100t))$. For Equation (11), first three moments are simulated resulting in a system of nine ODEs. The initial conditions are: $p_i^m = 0 \forall i, m = 1, 2, 3$ (here, index i indicates state and m indicates moment order) with $P = 1$ (using the law of conservation). Parameter values used for simulating PDEs and ODEs are listed in Table 1 for rat at 17 °C.

Figure A1 (panel A and B) shows results from the PDE model, where the mean fractions in

states A_1 , A_2 , and A_3 state are denoted: $\hat{p}_k(t) = \int_{-\infty}^{+\infty} p_k(t, s) ds$ ($k=1, 2, 3$). The mean strain in

each bound state is calculated by $\langle s_k \rangle = \int_{-\infty}^{+\infty} s p_k(t, s) ds / \hat{p}_k(t)$. Panels C and D show corresponding results from integrating the ODE system, where the 0th order moment for each state represents the fraction in that state: $\hat{p}_k(t) = p_k^0(t) \forall t=1, 2, 3$ and the mean strain in

an attached XB state is computed using: $\langle s_k \rangle = p_k^1(t) / p_k^0(t)$ $k=1, 2, 3$. Results from both simulations are nearly identical with the maximum deviation between predicted state fractions is equal to 0.06% and the maximum deviation in predicted strains is equal to 0.7%.

Appendix B: Effects of ATP, ADP and Pi on force development in rat cardiac muscle

Simulations of the effect of [MgATP], [MgADP] and [Pi] on rate of force development are shown in Figure B1. For these simulations, the sarcomere is initially stretched to a length of $2.2\ \mu\text{m}$ and total force (due to XBs and passive spring) is computed using the muscle model shown in Equation (12). Since the developed model is for a fully activated myofilament, all the XBs are assumed to be in permissible state ($P = 1$). Under these conditions, there is no XB strain and rate of force development reflects the time taken to attain a steady state.

Figure B1.

Effect of [MgATP], [MgADP] and [Pi] on absolute isometric force and rate of force development. A. Absolute isometric force relative to peak isometric force at [MgATP] = 0.25 mM. B. Rate of force development at three different [MgATP]. Force is normalized using maximum and minimum at each [MgATP]. For both A and B, [MgADP] = 0 mM, [Pi] = 0 mM and temperature is $17\ ^\circ\text{C}$. C. Absolute isometric force relative to peak isometric force at [MgADP] = 0.25 mM. D. Rate of force development at three different [MgADP]. Force is normalized using maximum and minimum at each [MgADP]. For both C and D, [MgATP] = 1 mM, [Pi] = 0 mM and temperature is $17\ ^\circ\text{C}$. E. Absolute isometric force

relative to peak isometric force at $[Pi] = 0$ mM. F. Rate of force development at three different $[Pi]$. Force is normalized using maximum and minimum at each $[Pi]$. $[MgATP] = 5$ mM, $[MgADP] = 0$ mM and temperature is 17°C . Parameters used for simulations are as listed in Table 1 for rat.

Appendix C: Nonlinear passive force formulation

The passive force formulation used during all the (computational) experiments is adapted from Rice, Wang [19]:

$$F_{\text{passive}}(x) = \begin{cases} \beta \cdot k_{\text{PE},2} \times (\exp(\text{PE}x_{\text{titin}} \times (x - SL_{\text{rest}})) - 1) & \text{if } x \geq SL_{\text{rest}} \\ -\beta \cdot k_{\text{PE},2} \times (\exp(\text{PE}x_{\text{titin}} \times (SL_{\text{rest}} - x)) - 1) & \text{if } x < SL_{\text{rest}} \end{cases}$$

Here, $k_{\text{PE},2}$ corresponds to the estimated stiffness of the linear passive spring, β is a scaling factor to give units of force, $\text{PE}x_{\text{titin}}$ is the expression level of titin, x is sarcomere length, and SL_{rest} is the resting sarcomere length. In addition, for quick-release experiments:

$$F_{\text{pre-load}} = \begin{cases} F_{\text{passive}}(L_0) & \text{if } L_0 \neq SL_{\text{rest}} \\ 0 & \text{if } L_0 = SL_{\text{rest}} \end{cases}$$

Where, L_0 is the initial length of the sarcomere before release.

Table C1

Parameters associated with passive force generation

Parameter	Value	Unit
SL_{rest}	1.9	μm
β	0.007	μm
$\text{PE}x_{\text{titin}}$	10	μm^{-1}

Appendix D: Mechanism of force yielding

To investigate the phenomenon of force yielding, we simulated a constant-velocity stretching experiment, such as that reported by Josephson and Stokes [29]. In this experiment, the sarcomere is initially held in a constant steady state with length ($L_0 = 2 \mu\text{m}$). At time $t = 200$ msec, a constant velocity lengthening is applied ($dL/dt = 0.1L_0/\text{sec}$) until 500 msec resulting in the force transient illustrated in Figure D1. The predicted force transient shows increasing force for the first 15 msec followed by a decreasing yield phase where force diminishes, lasting approximately 30 msec. This yielding characteristic is strikingly similar to that observed by [29]. Panels C and D of Figure D1 plot the model predicted mean fractions of XB distributions and mean strains of attached XB states for the simulation of this experiment. Model analysis suggests that the extent of force yielding depends on the rate of XB detachment which in turn depends on the rate of muscle deformation, as previously

suggested by Nichols [28]. Detachment of XBs results in a rapid decrease in the strongly bound post-ratcheted XBs (marked by vertical dashed line in figure D1).

Figure D1.

Force yielding during muscle lengthening (computational) experiment. A) Muscle lengthening protocol begins at 200 msec and continues for 300 msec after which the lengthening is stopped (at 500 msec). B) Steady-state force before 200 msec (solid vertical line) reflects the maximum isometric force. After 200 msec there is a sudden increase in force in response to lengthening of the sarcomere, however there is a sudden drop seen after few msec (dashed vertical line) indicative of the force yielding process. The drop in force continues till a new steady-state of strongly bound post-ratcheted XBs is reached (vertical dashed line). Soon after the force starts to increase till lengthening is stopped (at 500 msec). Soon after the lengthening is stopped there is a drop in force due to a drop in mean strain (D) seen after 500 msec. During this drop in strain the XBs continue to cycle to reach isometric force set by the new muscle length. Model parameters used for simulation are listed in Table 1 under rat column. Temperature is set to 17 °C and metabolites are: [MgATP] = 5 mM, [MgADP] = 0 mM, and [Pi] = 0.25 mM.

Appendix E: Model fits to other [MgATP] and [Pi] for mouse cardiac muscle data

Figure E1.

Model fits to remainder of elastic and viscous moduli data from mouse cardiac muscle experiments and model fittings not shown in Figure 3.

HIGHLIGHTS

- A new kinetic-dynamic model of muscle contraction is developed.
- The model describes apparent elastic and viscous properties of cardiac muscle.
- A synthesis for a number of previously observed phenomena is developed.
- The effects of [Pi] in slack-restretch experiments are elucidated.
- The phenomenon of force yielding during muscle lengthening is elucidated.

Figure 1.

A) The proposed model of cross-bridge (XB) cycling. *N* is a non-permissible XB state where myosin heads cannot bind with actin (as in the absence of cytosolic Ca²⁺), *P* is a permissible XB state during which myosin heads can bind with actin molecules (i.e. when cytosolic Ca²⁺ is present, represented by black dot), *A*₁^T is a loosely attached XB state (with [MgADP] bound). Pi, bound with *A*₁ sub-state from the previous XB cycle, rapidly dissociates to form *A*₁' (shown with the attached dotted box); Pi binding/release step is assumed to be in rapid-equilibrium and has a dissociation constant *K*_{Pi}. *A*₂ is a strongly bound (pre-ratcheted or pre-powerstroke) XB state. *A*₃^T is a strongly bound (post-ratcheted or post-powerstroke) XB state. It is assumed that the release of MgADP and binding of MgATP is in rapid-equilibrium (shown with the dotted box attached with *A*₃^T) and occurs after the force generation step (power-stroke); dissociation constants of MgADP and MgATP are represented by *K*_{MgADP} and *K*_{MgATP}, respectively. B) Proposed mechanism of force generation in cardiac muscle. F_{XB} is the force generated due to XBs undergoing cycling and stretching. Springs (*k*₁ and *k*₂) and dashpot (η) in parallel represent the passive cardiac muscle force response, which is independent of the XBs and dominates at very low and high frequencies. F_{load} represents the afterload against which the muscle contracts during sarcomere (computational) shortening experiments.

Figure 2.

Frequency response of rat cardiac muscle. Measured elastic and viscous moduli are plotted as functions of frequency as open circles (with error bars; $n = 8$). Solid lines are model fits to data obtained at $[MgATP] = 0.25\text{--}5\text{ mM}$. With increasing $[MgATP]$ there is an increase in the 'dip' observed in elastic modulus between 1 – 8 Hz which is accurately captured by the computational model. Likewise, there is shift (towards the right side) in the peak viscous modulus with increasing $[MgATP]$ that the computational model captures reasonably well.

Figure 3.

Frequency response of mouse cardiac muscle. Measured elastic and viscous moduli are plotted as functions of frequency as open circles. Model fits to data are obtained with different $[MgATP]$ ($= 0.4, 0.6, 0.8, 1, 2, 5$ mM; $n = 12$) and $[Pi]$ ($= 0, 0.4, 0.8, 1.25, 1.8, 2.4, 3, 3.5, 4$ mM; $n = 5$). For clarity fits to data for $[MgATP] = 0.4, 1.0$ and 5 mM (A) and $[Pi] = 0, 1.8$ and 4 mM (B) are shown here. Model fits to all other experimental data are shown in Appendix E.

Figure 4.

Force-velocity relationship in mouse cardiac muscle. A) Open circles are force-velocity data from Palmer, Schmitt [31] for control mouse myocardial strips. Solid line represents model simulations reproducing the experiments. Model based force-velocity data are computed by performing (computational) sarcomere quick-release experiments by holding the (virtual) sarcomere constant at a fixed length ($2.2\ \mu\text{m}$) till maximum isometric tension (T_{max}) is developed and velocity was computed from the slope of percent SL change after ~ 45 millisecond (msec) of release. After release the sarcomere was made to contract against different loads (T) less than equal to T_{max} . For simulating the sarcomere release experiments the parallel linear spring is replaced by a non-linear spring as described by [19] and force due to this spring is set to be $\beta k_{\text{PE},2}$, where β is a scaling factor chosen such that simulated unloaded shortening velocity matches experimentally reported value. (Details of passive force formulation are given in Appendix C.) For all the (computational) experiments, force due to this Rice-type spring is $\beta k_{\text{PE},2}$. All other parameters are as reported in Table 1 for mouse. B) Normalized power is the product of $(T/T_{\text{max}}) \times \text{Velocity (L}\cdot\text{s}^{-1})$.

Figure 5.

Temperature dependence of myocardial mechanics. Data are from mouse myocardial strip undergoing sinusoid length perturbation experiments at 17 °C (A) and 37 °C (B). Open circles are experiments data and solid lines are model fits. Force generation parameters were optimized to explain the data obtained at 17 °C; all parameters associated with XB cycling were kept fixed. A total of 6 Q_{10} parameters are identified to explain data obtained at 37 °C. Notice the shift of characteristic ‘dip’ in elastic modulus towards higher frequencies with increase in temperature (17 °C: 8 Hz; 37 °C: 20 Hz), which the model captures accurately. The model also exhibits the negative viscous modulus seen at 37 °C. The force generation parameters estimated for the data at 17 °C are: $k_{stiff,1} = 1423.3 \text{ mN mm}^{-2} \mu\text{m}^{-1}$, $k_{stiff,2} = 26348 \text{ mN mm}^{-2} \mu\text{m}^{-1}$, $k_{PE,2} = 1256.6 \text{ mN mm}^{-2} \mu\text{m}^{-1}$, $\eta = 0.86 \text{ mN sec mm}^{-2} \mu\text{m}^{-1}$, $k_{PE,1} = 222.3 \text{ mN mm}^{-2} \mu\text{m}^{-1}$.

Figure 6.

A. Representative sarcomere quick-release (computational) experiment with constant afterload. Increasing value of afterload decreases the amount sarcomere can contract. The arrow signifies time-point around which slopes were computed and an average of those slopes is reported as the shortening velocity (B). Velocity is computed after the end of ‘yielding’ process (slight increase in sarcomere length visible before the arrow). B. Model predicted increase in sarcomere shortening velocity with increasing temperature. Metabolite concentrations are: $[\text{MgATP}] = 5 \text{ mM}$, $[\text{MgADP}] = 36 \mu\text{M}$, $[\text{Pi}] = 0.25 \text{ mM}$. Metabolites conditions are same for all temperatures.

Figure 7.

Effect of ATP and ADP on shortening velocity. Filament velocity data from Yamashita, Sata [47] obtained in the absence MgADP (closed circles) and presence of MgADP (closed squares) is compared with model predictions (open circles and open squares). To perform these predictions, sarcomere release computational experiments were performed using the muscle model with mouse parameters shown in Table 1. The (virtual) sarcomere is held constant at a length of 2.2 μm and released after 100 msec to contract against no load with different [MgATP]. Shortening velocity is computed by taking an average of the sarcomere slope between 30–50 msec of release. K_{MgADP} is kept fixed at the value reported by Yamashita, Sata [47].

Figure 8.

Effect of ATP on force development. A. Experimentally observed rate of isometric force development at different [MgATP], from Ebus, Papp [51]. B. Model-predicted rate of isometric force development at different [MgATP]. C. Experimentally measured changes in maximum isometric force (open circles) as a function of [MgATP] with [MgATP] = 10 μM, 100 μM, and 5 mM. Data are normalized to maximum isometric force at [MgATP] = 5 mM). Model predicted changes in maximum isometric force under the same conditions (solid line). Simulations use parameter values listed in Table 1 for mouse and metabolite concentrations [MgADP] = 0 mM, [Pi] = 0 mM and temperature 20 °C, to match the experimental conditions. Figure in panel A reproduced from Ebus, Papp [51] (pending permission).

Figure 9.

Model predicted changes to peak isometric force and rate of force development at different [MgADP]. Simulation conditions are [MgATP] = 1 mM, [Pi] = 0 mM, and temperature 30 °C. A. Model suggests an increase in isometric force with increasing [MgADP], similar to experimental observations [52]. Force is normalized to peak isometric force at [MgADP] = 0.25 mM. B. Rate of isometric force development with increasing [MgADP]. The rate of force development decreases with increasing [MgADP], similar to experimental observations of Lu, Moss [53].

Figure 10.

A. Model predicted decrease in peak isometric force with increasing [Pi]. Force is normalized for the maximum isometric force at [Pi] = 25 μM. Other metabolite concentrations: [MgATP] = 5 mM, [MgADP] = 0 mM, and temperature 30 °C. B. Model predicts a decrease in rate of force generation with increasing [Pi]. Metabolite concentrations and temperature are same as A. C. Model predicts a reduction in sarcomere shortening velocity with increase in [Pi] (solid line: 0 mM [Pi]; broken line: 20 mM [Pi]). The method for computing shortening velocity is as in Figure 7B. [MgADP] = 0 mM, [MgATP] as shown in the figure.

Figure 11.

Effect of P_i on rate of force development. A. Slack-restretch maneuver reproduced from Hinken and McDonald [27] (pending permission). B. Simulated slack-restretch experiment. Following the slack-restretch experimental protocol [71], SL is initially held fixed at length of $2.2 \mu\text{m}$ (L_o). At time 100 msec L_o is reduced by 15% and allowed to shorten for 20 msec; afterwards muscle is re-stretched to 105% of L_o for 2 msec and then returned to L_o . Metabolites concentrations: $[\text{MgATP}] = 4 \text{ mM}$, $[\text{MgADP}] = 0 \text{ mM}$; temperature is 13°C .

Table 1

Parameters estimated from mouse and rat cardiac muscle experiments

Parameter	Description	Value at 17 °C		Unit	Q ₁₀
		Mouse ^d	Rat ^d		
XB Cycling parameters					
k_a	Myosin-actin rate of attachment	454±3.3	294.0±18	sec ⁻¹	1.44
k_d	Myosin-actin rate of un-attachment	1.25±3.7	35.5±3	sec ⁻¹	1.44
k_1	Rate of partially-bound conformation to strongly-bound conformation	41.2±0.9	10.2±0.1	sec ⁻¹	1.44
k_{-1}	Rate of strongly-bound conformation to partially-bound conformation	17.6±0.1	10.3±0.8	sec ⁻¹	1.44
k_2	Rate of ratcheting	159.3±12.9	88.6±3.8	sec ⁻¹	3.03
k_{-2}	Rate of un-ratcheting	153.7±0.04	2.1±0.2	sec ⁻¹	1.44
k_3	Myosin-actin detachment rate	87.7±5.3	35.6±0.8	sec ⁻¹	1.44
a_1	Stretch-sensing parameter for k_1 and k_{-1}	15.1±0.01	10.0±0.1	µm ⁻¹	-
a_2	Stretch-sensing parameter for k_2	10.1±0.04	9.1±0.9	µm ⁻¹	-
a_3	Stretch-sensing parameter for k_3	50.2±0.3	59.3±0.9	µm ⁻¹	-
s_3	Stretch in state A_3^T at which k_3 is minimum	9.9±0.1	9.9±0.1	nm	-
K_{MgATP}	[MgATP] dissociation constant	597±55	489.7±13.2	µM	-
K_{MgADP}	[MgADP] dissociation constant	0.194 ^b	0.194 ^b	mM	-
K_{Pi}	[Pi] dissociation constant	4.0±0.1	4.0 ^c	mM	-
Force generation parameters					
$k_{siff,1}$	Stiffness constant of frictional forces arising due to myosin-actin interaction	1137±4	2827.1±54	mN mm ⁻² µm ⁻¹	1.34
$k_{siff,2}$	Stiffness constant of forces arising due to working stroke of XBs	19066±11	51871±526	mN mm ⁻² µm ⁻¹	1.44
$k_{pe,1}$	Stiffness constant of spring in series with dashpot	729±5	944.9±10.3	mN mm ⁻² µm ⁻¹	1.07
η	Viscosity coefficient of the dashpot	0.5±0.01	2.4±0.1	mN sec mm ⁻² µm ⁻¹	1.07
$k_{pe,2}$	Stiffness constant of spring in parallel	254.6±1	203.3±2.8	mN mm ⁻² µm ⁻¹	1.48

^aParameter values are displayed as mean ± standard deviation of independent GA runs (N=21).^bValue is from [47] and was fixed during parameter estimation.

^cValue determined from mouse data and is fixed for rat data.

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