Journal of Molecular and Cellular Cardiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

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

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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 29 January 2015
Accepted 4 February 2015
Available online xxxx

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

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.

1. 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 (Fig. 1A) inorganic phosphate (Pi) release is required for transition from a loosely bound conformation (A_1) to strongly bound conformation (A_2). Rapid unbinding of MgADP and binding of MgATP precedes dissociation of myosin heads from actin. The release of myosin heads 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 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

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http://dx.doi.org/10.1016/j.yjmcc.2015.02.006
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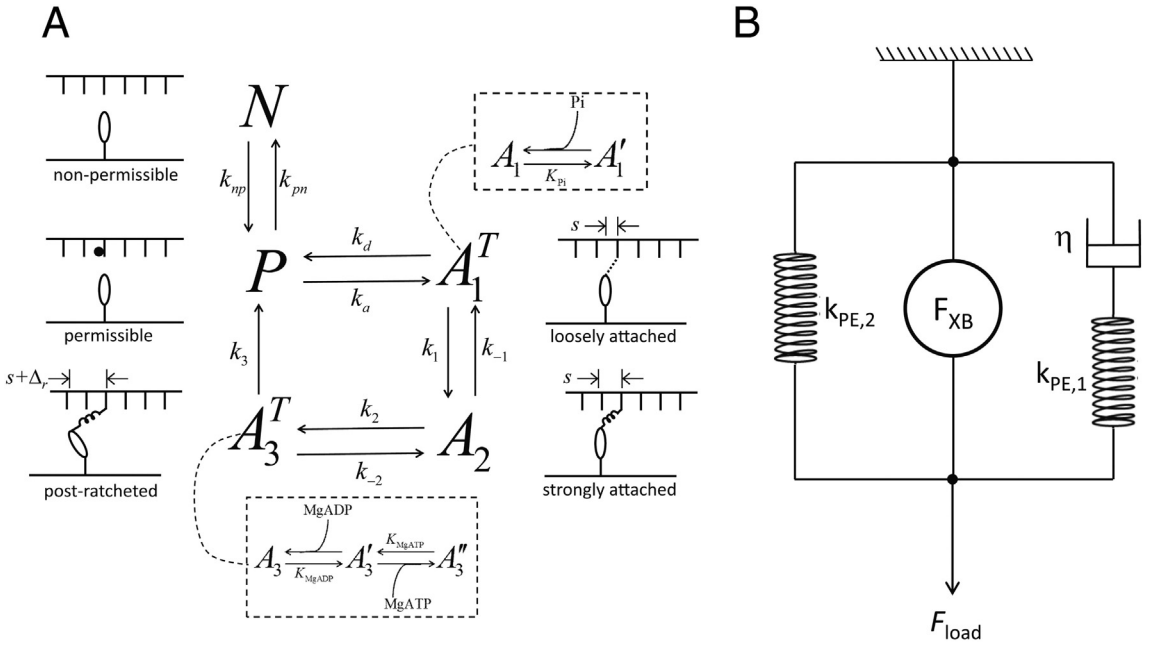

Fig. 1. A) The proposed model of cross-bridge (XB) cycling. *N* is a non-permissible XB state where myosin heads cannot bind with actin (as in the absence of cytosolic Ca^{2+}), *P* is a permissible XB state during which myosin heads can bind with actin (i.e., when cytosolic Ca^{2+} is present, represented 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.

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 viscoelastic 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 XBs.

2. Methods

2.1. Experimental data for model parameterization and corroboration

Data used to estimate parameters are obtained from published experimental studies [31,32]. In these studies sinusoidal length perturbation experiments were performed on myocardial strips excised from left ventricle of rat (150 μm diameter and 500 μm length) and mouse

Please cite this article as: Tewari SG, et al, Dynamics of cross-bridge cycling, ATP hydrolysis, force generation, and deformation in cardiac muscle, J Mol Cell Cardiol (2015), http://dx.doi.org/10.1016/j.yjmcc.2015.02.006