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The Latch-bridge Hypothesis of Smooth Muscle Contraction

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Abstract

In contrast to striated muscle, both normalized force and shortening velocities are regulated functions of cross-bridge phosphorylation in smooth muscle. Physiologically this is manifested as relatively fast rates of contraction associated with transiently high levels of cross-bridge phosphorylation. In sustained contractions, Ca^{2+} , cross-bridge phosphorylation, and ATP consumption rates fall, a phenomenon termed 'latch.' This review focuses on the Hai and Murphy (1988a) model that predicted the highly non-linear dependence of force on phosphorylation and a directly proportional dependence of shortening velocity on phosphorylation. This model hypothesized that (i) cross-bridge phosphorylation was obligatory for cross-bridge attachment, but that (ii) dephosphorylation of an attached cross-bridge reduced its detachment rate. The resulting variety of cross-bridge cycles as predicted by the model could explain the observed dependencies of force and velocity on cross-bridge phosphorylation. New evidence supports modifications for more general applicability. First, myosin light chain phosphatase activity is regulated. Activation of myosin phosphatase is best demonstrated with inhibitory regulatory mechanisms acting via nitric oxide. The second modification of the model incorporates cooperativity in cross-bridge attachment to predict improved data on the dependence of force on phosphorylation. The molecular basis for cooperativity is unknown, but may involve thin filament proteins absent in striated muscle.

Introduction

The objective of this review is to summarize the differences between smooth and striated muscle that led to the 'latch-bridge' hypothesis for the cross-bridge cycle in smooth muscle (Hai and Murphy, 1988a). We added a regulated myosin light chain phosphatase (Rembold, 1990; Walker *et al.*, 1994; Etter *et al.*, 2001) and cooperativity in attachment of phosphorylated cross-bridges to thin filaments (Rembold *et al.*, 2004) to generalize the model and account for later data

Functional Comparison of Vertebrate Striated and Smooth Muscle

The synthesis of biochemical, structural, and biophysical data that formed the sliding filament/cross-bridge hypothesis for vertebrate striated muscle remains valid after a half-century of testing and refinement. An allosteric Ca^{2+} - switch, described by the thin filament, troponin-based steric-blocking model, regulates cross-bridge attachment in striated muscle (reviewed in (Chalovich, 1992; Gordon *et al.*, 2000). Chemo-mechanical transduction manifested through the cross-bridge cycle and its regulation are independent processes (Fig. 1A). This independence facilitated the use of reductionist models such as permeabilized cells and isolated proteins to unravel the molecular events in ATP hydrolysis (Fig. 1B) and muscular contraction.

The quasi-crystalline organization of the contractile apparatus made cross-striated muscle an optimal model for research (Huxley, 1969).

Cross-bridge cycling rates are a function of the myosin isoforms expressed and the load on the contractile system in skeletal muscle (Bárány, 1967). Thus cycling rates manifested as unloaded shortening velocities are independent of the $[Ca^{2+}]$ and are not a regulated variable. Smooth muscles exhibit basic similarities to striated muscle that are consistent with the sliding filament/cross-bridge hypothesis (Murphy, 1980). These include a filamentous actin/myosin based contractile apparatus; an optimal length for force generation; a hyperbolic dependence of unloaded shortening velocity on load; and the ability to resist lengthening with the capacity to bear loads substantially exceeding those that can be isometrically developed (Murphy, 1980; Strauss and Murphy, 1996). However, smooth muscles exhibit properties not seen in striated muscle. These include variable force-length relationships implying that the number of force generating cross-bridges is regulated, and variable velocity-load relationships implying that cross-bridge cycling rates are regulated (Strauss and Murphy, 1996). Structural evidence for sliding filaments remains inconclusive. The continuing research challenge has been to explain the unique aspects of smooth muscle manifested by activation-dependent cross-bridge cycling rates and ATP consumption based on a molecular motor like that of striated muscle (Babu *et al.*, 2000).

Covalent Regulation

A fundamental development was the discovery that the ATPase activity of smooth muscle actomyosin was proportional to phosphorylation of Ser¹⁹ on the myosin regulatory light chains by myosin kinase (Gorecka *et al.*, 1976). ATPase activity was independent of Ca^{2+} , per se, although Ca^{2+} regulated phosphorylation by activating myosin kinase via calmodulin (Stull *et al.*, 1988; Stull *et al.*, 1983). This finding was the basis for the hypothesis that regulation of cross-bridge cycling in smooth muscle employed a covalent phosphorylation switch to turn cross-bridges on and off by phosphorylation and dephosphorylation (Fig. 2A, Murphy, 1994). Clearly regulation differed between smooth and striated muscle. However, covalent regulation would impose an ATP cost that seemed inconsistent with the extraordinary economy of force maintenance (Paul, 1989) unless myosin phosphatase relative to myosin kinase activity was very low (Hartshorne *et al.*, 2002).

An explanation for this puzzle was provided by estimates of phosphorylation in intact smooth muscle to test the hypothesis that cross-bridge phosphorylation, per se, determined activation. The results were inconsistent with the phosphorylation switch model. Isometric stress at the optimum length for force generation was not directly dependent on phosphorylation, but exhibited a highly non-linear dependence such that small supra-threshold increases markedly increased force with little further changes in force above some 30% phosphorylation (Dillon *et al.*, 1981; Rembold *et al.*, 2004). More strikingly, unloaded shortening velocities were directly dependent on phosphorylation accounting for regulation of this parameter (Dillon and Murphy, 1982).

Latch-bridge hypothesis

The first attempt to explain latch and variable cycling rates was based on a simple hypothesis. The scheme postulated that both free and attached cross-bridges were substrates for myosin phosphatase. Dephosphorylation of an attached cross-bridge was proposed to slow its detachment rate under cellular conditions (Hai and Murphy, 1988a, Fig. 2B). Such dephosphorylated cross-bridges were assumed capable of generating the same force as phosphorylated attached cross-bridges to explain near maximal values of force with some 30% phosphorylation. A slowed detachment rate (this is the normal rate limiting step in the cross-bridge cycle, Fig. 1B) would slow average cross-bridge turnover and thus shortening velocities

(Hai and Murphy, 1988b). In this scheme it was assumed that myosin phosphatase activities were constitutive (unregulated). Ca^{2+} -dependent myosin regulatory light chain phosphorylation was deemed both necessary and sufficient to explain contraction and relaxation in smooth muscles *activated by excitatory neurotransmitters, hormones, or depolarization*. This caveat was based on observations that nitrovasodilators could relax smooth muscle without corresponding changes in Ca^{2+} -dependent cross-bridge phosphorylation (McDaniel *et al.*, 1992). The physiological significance was uncertain for many years until the role of NO as an inhibitory neurotransmitter and paracrine hormone was recognized (McDonald and Murad, 1995).

The original Hai and Murphy latch-bridge hypothesis was tested by determining whether the dependences of isometric stress and shortening velocities experimentally observed in swine carotid medial strips were predicted by a simple mathematical model as described in the preceding paragraph and Fig. 2B. The fits between model and data were statistically robust (Hai and Murphy, 1988a; DiBlasi *et al.*, 1992) if basal values of phosphorylation was regarded as an experimental artifact. Numerous artifacts that could increase phosphorylation estimates were demonstrated (DiBlasi *et al.*, 1992), although several laboratories regarded basal phosphorylation values as real and challenged the validity of the Hai and Murphy latch-bridge model (Butler and Siegelman, 1998; Somlyo and Somlyo, 1994a).

The Hai and Murphy model (Fig. 2B) quantitatively predicted that the ATP cost of covalent regulation was significant and could approach that associated with cross-bridge cycling, per se (Hai and Murphy, 1989). This was counter-intuitive in a smooth muscle known to have an extraordinarily high economy of force maintenance (Paul, 1989). These energetics prediction further limited acceptance of the model. However, subsequent measures of oxygen consumption in protocols where ATP consumption could be partitioned into that used for phosphorylation and regulation and that required to power the cross-bridge cycle provided further support (Wingard *et al.*, 1994).

Regulation of myosin phosphatase activity

Cross-bridge phosphorylation is a function of the ratio of myosin kinase to myosin phosphatase activity. The key role of Ca^{2+} in activation-contraction coupling in smooth muscle was an early discovery (Filo *et al.*, 1965). However, recognition of the molecular mechanism of Ca^{2+} action via calmodulin and activation of myosin light chain kinase slowly developed (Stull *et al.*, 1988; Stull *et al.*, 1983). Phosphatases are difficult enzymes to characterize, and the identification of myosin light chain phosphatase as a trimeric protein with multiple potential regulatory pathways is quite recent (Hartshorne *et al.*, 2002; Somlyo and Somlyo, 2000). Biochemical studies have demonstrated changes in myosin phosphatase activity with binding or dissociation of a regulatory subunit with changes in its phosphorylation state as well as direct phosphorylation by several kinases (Eto *et al.*, 1995). This literature has led to the acceptance of the idea that changes in phosphatase activity as well as kinase activity modulate cross-bridge phosphorylation. However, changes in the activity of isolated enzymes or cell extracts do not establish a physiological regulatory role. Evidence for a potential physiological role of phosphatase regulation would be changes in the Ca^{2+} -dependence of myosin phosphorylation in experiments using permeabilized tissues where the $[\text{Ca}^{2+}]$ is experimentally set, or in the dependence of force on cross-bridge phosphorylation in intact smooth muscle preparations.

The functional questions are: (i) which of these potential mechanisms occur *in vivo* in smooth muscle; (ii) what are the physiological pathways mediating changes in phosphatase activity, and (iii) are changes in Ser^{19} -phosphorylation of the myosin regulatory light chains reflecting the overall kinase to phosphatase activity ratio necessary and sufficient to account for contraction or relaxation of smooth muscle?

Permeabilized smooth muscle retaining functional muscarinic receptors exhibit enhanced force at fixed $[Ca^{2+}]$, a phenomenon termed Ca^{2+} -sensitization (Nishimura *et al.*, 1988; Kitazawa *et al.*, 1989) confirming studies in intact muscle (Morgan and Morgan, 1984b; Rembold and Murphy, 1988a). Numerous subsequent studies have implicated inhibition of myosin phosphatase with enhanced cross-bridge phosphorylation as an explanation (Somlyo and Somlyo, 1994). Activators of protein kinase C such as phorbol esters can produce slow Ca^{2+} -independent 'contractures' in intact smooth muscle (Morgan and Morgan, 1984a; Rembold and Murphy, 1988b). Reports that protein kinase C isoforms can inhibit myosin phosphatase activity may explain these observations (Somlyo and Somlyo, 1994), although the physiological role of such pathways is not established.

Direct evidence for physiological regulation of myosin phosphatase requires measurement of changes in activity mediated by extracellular signals coupled with changes in the dependence of force on cross-bridge phosphorylation. Such measurements are technically difficult and there is a limited literature (Etter *et al.*, 2001; Gong *et al.*, 1992; Itoh *et al.*, 1993; Kubota *et al.*, 1992). As noted above, the dependence of force on cross-bridge phosphorylation in contracting intact smooth muscle is characteristically invariant (Rembold, 1992). Such results are not consistent with regulation of phosphatase activity. The clearest exceptions involve relaxation mediated by inhibitory pathways and nitric oxide (McDaniel *et al.*, 1992; Rembold *et al.*, 2000).

Relaxation and NO—cAMP-mediated changes in myosin phosphatase activity

Relaxation after withdrawal or pharmacological blockage of excitatory agonists is associated with proportional reductions in myoplasmic $[Ca^{2+}]$ and cross-bridge phosphorylation with no change in the dependence of force on phosphorylation (Rembold, 1991). While this inactivation is physiologically significant, it is now well established that inhibitory nerves or parenchymal cells that release NO and increase cellular cGMP are also an important element in most smooth muscle tissues (Andersson and Persson, 1995; Lincoln *et al.*, 1996). NO released by endothelial cells in blood vessels in response to circulating messengers acts similarly to reduce tone (Lincoln *et al.*, 1996). There is an enormous literature documenting protein kinase G mediated reductions in myoplasmic $[Ca^{2+}]$ by reductions in Ca^{2+} -mobilization and enhanced extrusion or sequestration by sarcoplasmic reticulum and plasma membranes (reviewed in Rembold, 1992). These were assumed to account for relaxation (Lincoln *et al.*, 1996). However, evidence of a lowered Ca^{2+} -sensitivity for cross-bridge phosphorylation value implied that there was a component of NO-induced relaxation that combined with lowered Ca^{2+} to enhance relaxation (Van Riper *et al.*, 1997). Tests of the hypothesis that this was caused by an elevated myosin phosphatase specific activity in swine carotid media were positive (Etter *et al.*, 2001). There is a transient nitrovasodilator induced increase in myosin phosphatase activity that correlates temporally with prior estimates when there was a reduction in the Ca^{2+} sensitivity of phosphorylation. These observations provide direct support for the hypothesis that myosin phosphatase activity is regulated by pathways increasingly recognized as important in determining force in smooth muscle. Extension of this experimental approach is an important research objective in view of the involvement of impaired smooth muscle relaxation in many diseases including asthma, hypertension, and vasospasm. For example, it is possible that the natriuretic peptides may also affect myosin phosphatase activity as well as cell Ca^{2+} .

The observations cited above reinforce the importance of the myosin kinase to phosphatase activity ratio in determining cross-bridge phosphorylation. Observed changes in kinase activity and phosphatase activity are reciprocal. Theoretical models (Walker *et al.*, 1994) and experimental measurements (Etter *et al.*, 2001) indicate that changes in phosphatase activity from a significant constitutive basal value are relatively small and would have modest impacts

on light chain phosphorylation if myosin kinase activity was constant. However, modest changes in phosphatase activity coupled with reciprocal changes in kinase activity could greatly enhance *changes in cross-bridge phosphorylation and the rates of contraction and relaxation*. Observed changes in myosin phosphatase activity were transient with time courses comparable to those of Ca^{2+} transients elicited by excitatory agonists (Etter *et al.*, 2001 and unpublished observations). This implies that phosphatase regulation occurs in a time frame that would not be detected in most experimental protocols.

We have argued that Ca^{2+} -dependent myosin regulatory light chain phosphorylation is necessary and sufficient to explain activation and inactivation of smooth muscle (Murphy, 1994), with the caveat that this applied only to excitatory physiological messengers. While the assertion remains true, it is clear that function of smooth muscle reflects a balance of excitatory and inhibitory signals. The latter can involve Ca^{2+} -independent mechanisms. So far these Ca^{2+} -independent changes in cross-bridge phosphorylation can be quantitatively explained by changes in myosin phosphatase activity. A generalized model for cross-bridge regulation by phosphorylation would incorporate Ca^{2+} -independent changes in myosin phosphatase activity (Fig. 3).

Cooperativity in cross-bridge attachment

The value of a model is critically dependent on the accuracy of the underlying data set. The original Hai and Murphy (1988a) model was based on a very large set of measurements in swine carotid medial strips stimulated by a wide variety of agents and combinations of excitatory stimuli (DiBlasi *et al.*, 1992). This data was best fit by a quasi-hyperbolic curve originating from a significant 'basal phosphorylation' estimate at zero force. Continuing efforts to improve the accuracy and sensitivity revealed that Western blots seriously underestimated low levels of phosphorylation (Walker *et al.*, 2000). A laborious dilution assay was optimized to minimize such errors, and the dependence of force on cross-bridge phosphorylation was re-examined in several types of smooth muscle. In all cases the data revealed a very steep sigmoid dependence of force on phosphorylation with a threshold value of some 15% required for measurable force development (Rembold *et al.*, 2004).

The experimental data sets suggested that cross-bridge phosphorylation might regulate force in a highly cooperative manner. Cooperativity was postulated to explain latch (Vyas *et al.*, 1992; Somlyo *et al.*, 1988; Arner *et al.*, 1987; Khromov *et al.*, 1995; Khromov *et al.*, 1998; Marston, 1988; Horiuchi and Chacko, 1989). We considered whether incorporating cooperative activation of cross-bridge cycling into the Hai and Murphy model (1988a, Fig. 2B) could predict the experimentally measured force-phosphorylation data (Rembold *et al.*, 2004, Fig. 4). We used the skeletal muscle paradigm where the attachment rate of cross-bridges is cooperative, but made this a function of phosphorylation (Fig. 4). The model described both the steady-state and the time course relationships between Ser^{19} -phosphorylation of the myosin regulatory light chains and force (Rembold *et al.*, 2004). Smooth muscle contains tropomyosin that links units of 7 actin monomers along a thin filament like striated muscle, where it is thought to participate in conferring cooperativity in cross-bridge attachment. The model was very sensitive to the number of myosin binding sites that are cooperatively activated. Thus the fact that only 7 assumed binding sites could predict the experimental data was an unexpected, but possibly significant outcome (Rembold *et al.*, 2004).

Concluding remarks

Biological systems are inherently complex. Relative simplicity is required for models to be useful in testing hypotheses. Models rarely capture all the important characteristics of a system that are often unknown. The value or success of a model ultimately resides in whether it can discriminate alternative explanations and identify new experimental directions that in turn

generate new hypotheses or alternative models. The latch-bridge hypothesis is a clear example. Our working model for cross-bridge regulation has evolved to incorporate a regulated phosphatase and cooperativity in attachment of phosphorylated cross-bridges to thin filaments in smooth muscle (Fig. 3). This was done to incorporate the results of new data involving inhibitory pathways mediating relaxation in many cases and to account for features revealed by improved estimates of phosphorylation. These are not trivial modifications, but the basic concepts to explain latch and variable cross-bridge cycling rates remain.

The core concept that chemo-mechanical transduction in smooth muscle is explicable in terms of the fundamental sliding filament/cross-bridge mechanism elucidated for striated muscle is rarely questioned. The novel suggestion to explain variable cross-bridge cycling rates in smooth muscle was the 'latch-bridge.' A latch-bridge is defined as all those molecular force-generating states of dephosphorylated, slowly detaching cross-bridges. This model still provides robust quantitative predictions of the contractile properties of smooth muscle. In retrospect, the use of the symbol AM to designate a latch-bridge was unfortunate as it engendered characterizations of latch in terms of a rigor state (Somlyo and Somlyo, 1994). The concept may have wider applicability to explain the classic 'catch' mechanism in specialized molluscan helically striated muscles (Siegman *et al.*, 1998).

Ser¹⁹ phosphorylation of smooth muscle myosin regulatory light chains remains a plausible mechanism to account for cross-bridge cycling and contraction in smooth muscle. However, it is clear that Ca²⁺-independent mechanisms involving regulation of myosin phosphatase activity participate in determining the rates of phosphorylation and dephosphorylation. There is an extensive literature, largely based on biochemical approaches for thin filament 'regulatory' proteins in smooth muscle such as caldesmon (Chalovich *et al.*, 1998; Lehman, 1986; Marston, 1986); calponin (Walsh, 1994; Gerthoffer, 1991; Small and Gimona, 1998; Winder *et al.*, 1998); HSP20 (Beall *et al.*, 1997; Brophy *et al.*, 1999; Rembold *et al.*, 2000; Rembold *et al.*, 2001; Flynn *et al.*, 2003; Tessier *et al.*, 2003); and/or tropomyosin (Chalovich, 1992; Morgan and Gangopadhyay, 2001). Evidence for cooperativity in attachment of phosphorylated cross-bridges may help identify the role(s) of such thin filament proteins.

Rapid scientific progress is an outcome of falsification of branches of trees of hypotheses to explain biological phenomena (Platt, 1964). This approach is facilitated by formalization of hypotheses in models.

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Figure 1.

Cross-bridge models in skeletal muscle. A. Simple two state model depicting force generation in terms of free (myosin, M) and attached (AM) force generating cross-bridges. Each cycle involves the hydrolysis of one ATP. Attachment and cycling depend on 4Ca^{2+} ions binding to troponin in the thin filaments (actin, A) to alter their confirmation (A^*) to allow myosin heads to attach, This is a highly cooperative process. B. The generally accepted steps in the hydrolysis of ATP by actin-activated myosin (ATP, T; ADP, D). The rate limiting step for all myosin isoforms involves product release between $\text{AM}\cdot\text{D}\cdot\text{P}_i$ and $\text{AM}\cdot\text{D}$. This model also applies to smooth muscle. Adapted from Adelstein and Sellers (1996).

Figure 2.

Models for cross-bridge cycling in smooth muscle. A. Two state (free and attached) cross-bridge model for smooth muscle illustrating regulation by a Ca^{2+} -dependent phosphorylation switch. Myosin light chain kinase (MLCK) is activated by Ca^{2+} -binding in a highly cooperative manner to four sites on calmodulin, thereby enabling it to bind to MLCK. Dephosphorylation is due to the action of myosin light chain phosphatase (MLCP). Note that ATP hydrolysis is required both for the cross-bridge cycle and for phosphorylation/dephosphorylation. B. The four state latch-bridge model of Hai and Murphy (1988a). Only phosphorylated crossbridges (M_p) can attach to actin, but attached phosphorylated cross-bridges are substrates for MLCP

and can become dephosphorylated to form a 'latch-bridge.' Latch-bridges are postulated to have unaltered force generating capacities, but a slowed detachment rate.

Figure 3.

Current working hypothesis for regulating contraction and relaxation in smooth muscle. The latch-bridge hypothesis of Hai and Murphy (1988a, Fig. 2B) is retained. Phosphorylation is a function of the ratio of Ca^{2+} -dependent MLCK activity and Ca^{2+} -independent MLCP activity. It is now postulated that MLCP activity is regulated physiologically at least during relaxation in response to inhibitory signals such as NO. This model also assumes that the attachment of M_p to thin filaments to form AM_p is cooperative (see Fig. 4).

Figure 4.

Steady-state predictions of the current latch-bridge model (Fig. 3) incorporating cooperativity in the attachment of phosphorylated cross-bridges. A. Model predictions for each cross-bridges species where active stress is the sum of AM + AM_p. B. Fit of model to experimental measurements of active stress as a function of myosin phosphorylation determined by a dilution assay procedure (Walker *et al.*, 2000). Adapted from Rembold *et al.*, (2004).