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COMMENTARY

REGULATION OF CONTRACTION BY MYOSIN PHOSPHORYLATION

A COMPARISON BETWEEN SMOOTH AND SKELETAL MUSCLES

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Phosphorylation and dephosphorylation of enzymes have been recognized as biochemical mechanisms by which many metabolic processes are regulated [1–5]. Recently, it was shown that specific myofibrillar proteins are also phosphorylated by protein kinases and that in some cases the contractile process may be regulated via protein phosphorylation [6]. This commentary will focus on the properties of phosphorylation of myosin by the Ca^{2+}-dependent enzyme, myosin light chain kinase. Although there are several general features that are shared by skeletal and smooth muscles, there are also important distinctions thay may explain, in part, the diverse physiological properties and pharmacological responses of these two different types of muscles.

The release of Ca^{2+} into the sarcoplasm is the primary event in excitation–contraction coupling in all types of muscle. The subsequent binding of Ca^{2+} to specific high-affinity sites on proteins associated with, or acting on, the contractile apparatus ultimately results in contraction. Although contraction in all types of muscle results from the interaction of the contractile proteins actin and myosin, the mechanism by which Ca^{2+} triggers actomyosin interactions is markedly different in smooth and skeletal muscles. These differences in regulation of actomyosin interactions can be ascribed to isozymic differences in the myosin molecule and to differences in the regulatory proteins which mediate the effects of Ca^{2+}.

Myosin is a hexameric molecule composed of two high molecular weight heavy chain subunits and four low molecular weight light chain subunits. The overall configuration of native myosin is that of a coiled-coil tail region and two protruding random coil head regions. The tail region of the molecule interacts with the tail regions of other myosin molecules to form thick filaments. The head regions project from the thick filaments to bind to filaments of actin (the thin filaments). The sliding filament theory of muscle contraction postulates that tension and shortening occurs as a result of actomyosin interactions causing thick and thin filaments to move past one another. The head region also has the ability to catalyze the hydrolysis of ATP (termed myosin ATPase activity), a process tightly linked to tension development. The molecular mechanism by which chemical energy (ATP hydrolysis) is converted to mechanical energy is not yet resolved, but represents an area under intense investigation.

The unique contractile properties of each muscle type may be due, in part, to the differences in myosin isozymes. On polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS), myosin from fast-twitch, white skeletal muscle and from smooth muscles has heavy chain subunits with apparent molecule weights of 200,000. Fast-twitch skeletal muscle myosin also has three light chain components with apparent molecular weights of 25,000, 18,500 and 17,000, respectively, while smooth muscle myosin contains two light chain components with apparent molecular weights of 17,000 and 20,000. Although the precise location of the light chain subunits within the myosin molecule is unknown, they appear to be associated with the head region and thus are in a potentially crucial position for involvement in actomyosin interactions. Recent reviews present more detailed discussions of the various physiological and biochemical properties of muscles [6–10].

Of particular interest to this discussion is the class of homologous light chains capable of being phosphorylated by myosin light chain kinases. The light chain subunits in this class are found in all types of vertebrate tissue and demonstrate many similar biochemical properties. For skeletal and smooth muscle myosins, these are the 18,500 dalton and 20,000 dalton light chains, respectively. In both smooth and skeletal muscles, there are myosin light chain kinases that catalyze the rapid incorporation of phosphate into a specific serine residue of this class of light chains (Fig. 1). The dephosphorylation of these light chains is catalyzed by another enzyme, myosin light chain phosphatase. Because they can exist as phos-

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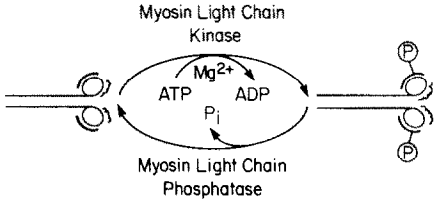

Fig. 1. Phosphorylation of myosin from skeletal and smooth muscles. A single type of light chain subunit is phosphorylated by myosin light chain kinase; the dephosphorylation is catalyzed by a different enzyme, myosin light chain phosphates.

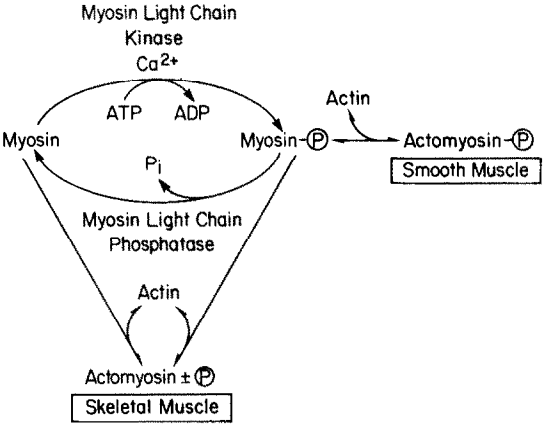

Fig. 2. Regulation of actomyosin by phosphorylation. Phosphorylation of smooth muscle myosin appears to be essential for activation of myosin ATPase activity by actin. Dephosphorylated smooth muscle myosin is not activated by actin. In skeletal muscle, both the nonphosphorylated and phosphorylated forms of myosin are readily activated by actin. Hence, the biochemical role for phosphorylation of skeletal muscle myosin remains unclear.

phoproteins, this class of light chains is referred to as the phosphorylatable or P-light chains [11].

Effect of phosphorylation on actomyosin

A number of *in vitro* studies have concluded that the phosphorylation of the myosin P-light chain is an effective mechanism for control of contraction in smooth muscle. Initial experiments using partially purified actomyosin isolated from chicken gizzard showed that the extent of ATPase activity correlated well with the degree of phosphorylation of the P-light chain [12–14]. When ATP-γS is used as a substrate for the kinase, the myosin P-light chain becomes thiophosphorylated and resistant to hydrolysis by myosin light chain phosphatase. Thiophosphorylation resulted in an irreversible activation of actomyosin ATPase activity [15]. Further investigations using myosin purified to remove endogenous kinase and phosphatase activities showed that actin was capable of activating the ATPase of phosphorylated myosin but not that of nonphosphorylated myosin [15–17]. Phosphorylation had no effect on the ATPase of myosin in the absence of actin [17]. Furthermore, Ca^{2+} was found to have no direct effect on the actin-activated ATPase of phosphorylated myosin purified from chicken gizzard [15], porcine stomach [16] or ovine uterus [17], although Ca^{2+} was required for actin activation of phosphorylated myosin purified from vas deferens [18]. Thus, the question of the direct effect of Ca^{2+} on the actomyosin interactions should be examined further in other types of smooth muscle.

Skinned muscle fiber preparations in which the sarcolemma is functionally removed by mechanical or chemical means have the advantage of allowing tension generation to be studied in a system where the contractile apparatus is presumed to be structurally intact. Kerrick and coworkers have used skinned fiber preparations from rabbit ileum and avian gizzard and observed results similar to those using purified contractile proteins [19, 20]. P-light chain phosphorylation and tension generation displayed identical Ca^{2+} sensitivities and both were reversed when Ca^{2+} concentrations were lowered to 0.01 μM. Ca^{2+}-dependent thiophosphorylation of the P-light chains resulted in irreversible activation of tension generation. It was also found that phosphorylation of a low proportion of the myosin heads resulted in maximal tension generation in the skinned fiber preparations, perhaps indicating cooperative interactions between the contractile elements. Thus, phosphorylation of smooth muscle myosin light chain appears to be essential or obligatory for actomyosin interactions in skinned fibers and is consistent with data obtained with purified contractile proteins (Fig. 2). Although this evidence suggests that phosphorylation is a primary control mechanism in smooth muscle, there is a possibility that other Ca^{2+} control mechanisms, which involve other proteins [21, 22], may also play a role in regulation of smooth muscle actomyosin interactions.

In contrast to the results obtained with smooth muscle myosin, phosphorylation of the P-light chain has no significant effects on actomyosin ATPase activity of purified skeletal muscle contractile proteins or tension generation of skinned skeletal muscle fiber preparations. The ATPase activity of myosin in the presence or absence of actin was found by Morgan *et al.* [23] to be unaffected by phosphorylation. These results have been confirmed over a range of Ca^{2+} and actin concentrations (W. Crooks, A. P. Toste and R. Cooke, unpublished observations). There is one preliminary report, however, that skeletal muscle actomyosin ATPase activity is enhanced by phosphorylation at micromolar Ca^{2+} concentrations [24]. The discrepancy between these results is unresolved but may be due to the use of an unpurified kinase in the latter experiments. As in the case of whole myosin, the ATPase activity of heavy meromyosin activated by actin was not apparently affected by phosphorylation; however, the published data do not allow an accurate extrapolation to V_{max} (the ATPase at infinite actin concentration) [23]. Phosphorylation also did not affect the rate or extent of actomyosin superprecipitation or the tension generated by actomyosin threads (W. Crooks and R. Cooke, unpublished observations). The conclusion drawn from these studies is that the *in vitro* properties of skeletal muscle actomyosin are largely

unaffected by myosin light chain phosphorylation, in sharp contrast to the properties of smooth muscle myosin (Fig. 2).

Further investigations of the role of myosin light chain phosphorylation have been carried out using skinned fiber preparations. Purified kinase and calcium-dependent regulator protein (CDR, also referred to as calmodulin) were diffused into glycerinated psoas fibers, and the effects on fiber function were assayed (C. J. Ritz-Gold, R. Cooke, D. K. Blumenthal and J. T. Stull, unpublished data). In the absence of kinase or of Ca^{2+}, the extent of myosin phosphorylation was less than 10 per cent of maximum, whereas in the presence of both it could be elevated to approximately 75 per cent as judged from densitometry of polyacrylamide gels after separating the light chains by electrophoresis in the presence of urea. Phosphorylation with [γ-^{32}P]ATP showed that only the P-light chain incorporated appreciable phosphate. Functional studies were carried out with fibers that were thiophosphorylated using ATP-γS. Thiophosphorylation of the P-light chain did not affect the isometric force or the force–velocity curves of the fibers. The isometric force, generated as a function of the Ca^{2+} concentration, was also unaffected. At low Mg–ATP concentrations, fibers produce tension in the absence of Ca^{2+}; this is thought to be a result of competition between the relaxation mechanism and rigor actomyosin bonds [25]. The isometric tension at low Mg–ATP concentration was not affected by thiophosphorylation of the P-light chain. A direct measurement of the binding constant of actin to the S1 fragment of myosin confirmed that it was not altered by phosphorylation of the P-light chain (K. E. Franks, R. Cooke, D. K. Blumenthal and J. T. Stull, unpublished data). These results indicate that light chain phosphorylation does not change either the strength of the actomyosin rigor bond or the effectiveness of the relaxation mechanism at low Mg–ATP concentrations.

From the above studies it is apparent that phosphorylation of the P-light chain of myosin is not required for contraction in striated muscle. Thus, the primary control of tension generation in striated muscle appears to consist of the binding of Ca^{2+} to troponin which, in turn, interacts with tropomyosin to activate actomyosin interactions. These *in vitro* findings, however, do not rule out the possibility that myosin light chain phosphorylation may modulate contractile activity in striated muscle, rather than being essential for contraction as in smooth muscle.

Several lines of biochemical evidence suggest that P-light chain phosphorylation plays a role in skeletal muscle contraction. First, the content of myosin light chain kinase in skeletal muscle is high (approximately 1 μM) [26, 27] and similar to that found in smooth muscle. Second, studies of the proteolytic digestion of phosphorylated and nonphosphorylated skeletal muscle myosin indicate that light chain phosphorylation causes significant changes in myosin structure [28]. Thus, the functional effects of conformational changes induced by phosphorylation might not be detectable by the *in vitro* techniques previously studied.

Regulation of myosin light chain kinase and phosphatase

The presence of Ca^{2+}-dependent myosin light chain kinase was first described by Perry and coworkers in skeletal muscle [29] and subsequently purified to homogeneity [26]. The enzyme was later identified in smooth [30] and cardiac [11] muscle, as well as several non-muscle tissues [31, 32]. More recently, it was shown that the Ca^{2+} sensitivity of the enzyme was mediated by a low-molecular protein identified as the Ca^{2+}-dependent regulator protein, also known as CDR or calmodulin [33, 34]. CDR has now been shown to confer Ca^{2+} sensitivity on a variety of enzymes including Ca^{2+}-dependent cyclic nucleotide phosphodiesterase, brain adenylate cyclase, erythrocyte membrane ATPase, and other enzymes [35, 36]. The proposed mechanism of activation of CDR-dependent enzymes, including myosin light chain kinase, is a sequential process (Fig. 3). Ca^{2+} first binds to CDR to form a $Ca_n^{2+}\cdot$CDR complex which subsequently binds to the inactive catalytic subunit resulting in a holoenzyme complex with enzymatic activity.

Studies of myosin light chain kinase activity from rabbit skeletal muscle with various Ca^{2+} and CDR concentrations support the proposed activation mechanism [37]. As would be expected from the law of mass action, lowering the CDR concentration decreases the Ca^{2+} sensitivity, and, conversely, lowering the Ca^{2+} concentration increases the concentration of CDR required for activation. At a fixed Ca^{2+} concentration, activation of myosin light chain kinase by CDR follows Michaelis–Menten kinetics with an activation constant of 1 nM at saturating (100 μM) Ca^{2+} concentrations. These data suggest that activation of the kinase involves a stoichiometry of one $Ca_n^{2+}\cdot$CDR complex per catalytic site. Ca^{2+}-activation of the kinase at fixed CDR concentrations displays non-Michaelis–Menten kinetics (Hill numbers = 3), indicating that at least three Ca^{2+}-binding sites interact in the activation process. Four divalent cation-binding sites per CDR molecule have been

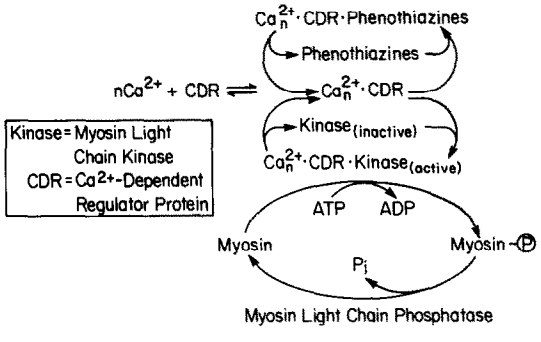

Fig. 3. Regulation of myosin light chain kinase by Ca^{2+}. Myosin light chain kinases from skeletal and smooth muscles are dependent upon Ca^{2+} and Ca^{2+}-dependent regulator protein (CDR) for activity. Ca^{2+} first binds to CDR to form a $Ca_n^{2+}\cdot$CDR complex which, in turn, binds to the inactive myosin light chain kinase (Kinase) to form a catalytically active phosphotransferase, $Ca_n^{2+}\cdot$CDR·Kinase. Phenothiazine antipsychotic drugs bind to the $Ca_n^{2+}\cdot$CDR complex, preventing it from activating myosin light chain kinase.

described [38, 39], but Ca^{2+} binding to these sites was not cooperative. In combination, these data indicate that Ca^{2+} binding to at least three of the divalent metal binding sites must occur before $Ca_n^{2+}\cdot$CDR can interact with the catalytic subunit of myosin light chain kinase. At saturating CDR concentrations (420 nM), half-maximal activation of the kinase is observed at 3 μM Ca^{2+} [37], a value consistent with the Ca^{2+} concentrations required for activation of the contractile apparatus in both smooth and skeletal muscle.

The Ca^{2+} concentration required for kinase activation in the intact muscle will depend on several factors. Although the CDR concentration in various smooth and skeletal muscle tissues has been estimated to be from 1 to 10 μM [33, 40], the total concentration of other CDR-binding proteins (including enzymes such as myosin light chain kinase) is also in the micromolar range [33, 40]. Since these proteins also have a high affinity for $Ca_n^{2+}\cdot$CDR, the free $Ca_n^{2+}\cdot$CDR concentrations, as compared to total $Ca_n^{2+}\cdot$CDR concentration, may be quite low. This would have the effect of decreasing the apparent Ca^{2+} sensitivity of myosin light chain kinase and, thus, may be a means by which the Ca^{2+} sensitivity of the enzyme activity is regulated.

The Ca^{2+} sensitivity of myosin light chain kinase activity can also be lowered by several pharmacologic agents. Previously, it was shown that the phenothiazines (trifluoperazine, promethazine and chlorpromazine) inhibited Ca^{2+}-dependent cyclic nucleotide phosphodiesterase by reversibly binding to $Ca_n^{2+}\cdot$CDR [41, 42]. These agents would decrease the $Ca_n^{2+}\cdot$CDR available for activation, resulting in a decreased Ca^{2+} sensitivity of $Ca_n^{2+}\cdot$CDR-dependent enzymes (Fig. 3). Indeed, phenothiazines inhibited phosphorylation and tension generation in skinned fiber preparations from rabbit ileum and pulmonary artery [19], but did not inhibit tension generation of skinned fibers in which the P-light chains were thiophosphorylated prior to phenothiazine exposure. Phenothiazines had no effect on tension generation in skinned skeletal muscle fibers [19] consistent with previous evidence that P-light chain phosphorylation is not required for skeletal muscle contractile activity.

The biochemical properties of myosin light chain kinase appear to differ between various muscle sources. Values reported for the molecular weight of the catalytic subunit of myosin light chain kinase indicate that the enzyme from smooth muscle (105,000–130,000 daltons) is larger than the enzyme from skeletal muscle (75,000–85,000 daltons) [26, 27, 34, 43–45]. Kinetic studies comparing the P-light chains from a variety of muscle sources as substrates for various light chain kinases indicate that a given P-light chain is preferentially phosphorylated by the homologous kinase [46, 47]. The kinases from different tissues also demonstrate significantly different isoelectric points [48]. These observations regarding the physical and enzymatic properties of myosin light chain kinases from smooth and skeletal muscles reflect tissue-specific, isozymic forms of the enzyme.

A major functional difference exists between the myosin light chain kinases from smooth and skeletal muscles in their capacities to be regulated via phosphorylation by cAMP-dependent protein kinase. Myosin light chain kinase from turkey gizzard was found to be rapidly phosphorylated to the extent of 1 mole phosphate/mole enzyme in the presence of cAMP and cAMP-dependent protein kinase [44]. The phosphorylation resulted in a 2-fold decrease in light chain kinase activity which was subsequently shown to be due to a decreased affinity of the enzyme for CDR [49]. More recently, Ca^{2+}-dependent phosphorylation of the P-light chains in bovine aortic actomyosin preparations was found to be markedly depressed in the presence of cAMP and cAMP-dependent protein kinase [50]. A concomitant decrease in actomyosin ATPase activity was also observed. Skeletal muscle myosin light chain kinase activity was unaltered when incubated with cAMP and cAMP-dependent protein kinase [26]. These results suggest a means by which β-adrenergic agents could effect relaxation in smooth muscle (Fig. 4).

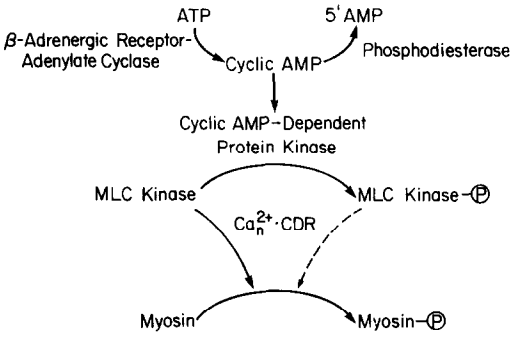

Fig. 4. Regulation of smooth muscle myosin light chain (MLC) kinase by cyclic AMP. Stimulation of cyclic AMP formation by β-adrenergic agents activates cyclic AMP-dependent protein kinase which, in turn, catalyzes the phosphorylation of myosin light chain kinase. Phosphorylated myosin light chain kinase has a 20-fold lower affinity for $Ca_n^{2+}\cdot$CDR and, therefore, will not be as readily activated as the nonphosphorylated myosin light chain kinase. The consequence of this phosphorylation is the inhibition of myosin phosphorylation and, hence, relaxation of the smooth muscle.

Another potential site of regulating the extent of myosin light chain phosphorylation *in vivo* is via the dephosphorylation reaction. Myosin light chain phosphatase has only been purified and characterized from rabbit fast-twitch skeletal muscle [23], although its activity has been demonstrated in smooth muscle [30]. The enzyme from skeletal muscle was highly specific for the phosphorylated P-light chain and was not inhibited by NaF. No evidence was presented to suggest regulation of skeletal muscle myosin light chain phosphatase, and the enzyme from smooth muscles has yet to be purified and characterized.

Studies of myosin light chain phosphorylation with intact smooth and skeletal muscle preparations

Although myosin light chain phosphorylation and its effects on the contractile elements have been fairly well studied *in vitro*, complementary studies with intact muscle preparations are rather limited, particularly in the case of smooth muscle. *In vitro* studies are useful for elucidating mechanisms of

regulation and function of biochemical events such as light chain phosphorylation, but hypotheses developed from such studies must be confirmed by experiments with intact living tissue. The intact tissue under study should represent a physiologically functional unit with all biochemical and contractile components present, structurally intact, and capable of responding to appropriate stimuli such as hormones and drugs. The following section will review attempts to correlate P-light chain phosphorylation in the intact muscle with various physiological and pharmacological phenomena.

Before light chain phosphorylation data from intact muscle studies can be interpreted unambiguously, certain critical control experiments must be performed (see Ref. 51 for recent review). These include: (1) demonstration that all pertinent enzymatic processes (i.e. kinase, phosphatase and protease activities) are arrested between the time when a given physiological or pharmacological event occurs and when the phosphate content of the P-light chain is determined; and (2) demonstration that the phosphate being measured is specifically and covalently associated with the P-light chain. In addition, careful time courses and dose–response curves are essential for correlating physiological and pharmacological events with biochemical phenomena. Finally, demonstration of activation or inactivation of the appropriate enzymes is useful in establishing causal relationships between a given manipulation and its consequent event.

Studies in intact skeletal muscle

P-light chain phosphorylation following tetanic stimulation has been studied with intact skeletal muscle preparations from frog [52] and rabbit [53]. More recently, Manning and Stull [54], using an intact, white, fast-twitch skeletal muscle preparation from rat, compared the time course of tension generation and P-light chain phosphorylation following a 1-sec tetanic contraction. Tension development and P-light chain phosphate content followed dissimilar time courses. Maximum tension generation was observed immediately following the onset of tetanic electrical stimulation, whereas relaxation occurred immediately following cessation of the electrical stimulation. P-light chain phosphate content did not change significantly during the 1-sec stimulation (0.14 mole P/mole light chain), but reached a maximum extent of phosphate incorporation (0.7 mole P/mole light chain) within 10–20 sec following relaxation. Dephosphorylation of the P-light chain was much slower ($T_{\frac{1}{2}} = 1.7$ min). These data clearly demonstrate a complete dissociation of contraction and P-light chain phosphorylation in skeletal muscle. P-light chain phosphorylation was, however, found to correlate very closely with post-tetanic potentiation of isometric twitches through the entire time course. These data indicate that, although P-light chain phosphorylation does not play an obligatory role in contractile element activation or force generation in skeletal muscle, it may function to modulate the contractile response, i.e. augment force generation. Recent work with frog skeletal muscle [52] has also demonstrated phosphorylation and dephosphorylation of the P-light chain in the intact muscle. Isotonic and isometric tetany and treatment with 10 mM caffeine all stimulated tension generation and an increase in the extent of P-light chain phosphorylation. Tension generation and P-light chain phosphorylation in frog skeletal muscle could also be temporally dissociated.

Thus, studies with intact skeletal muscles confirm data obtained with purified proteins, namely: (1) myosin light chain phosphorylation occurs in the intact tissue under circumstances of elevated Ca^{2+} concentrations; (2) when Ca^{2+} concentrations return to resting values, the P-light chain is dephosphorylated indicating the presence of a myosin light chain phosphatase; however, the rates of relaxation and myosin dephosphorylation are not the same, with the latter being a much slower process; (3) P-light chain phosphorylation can be clearly dissociated from tension responses during tetany, in agreement with experiments showing lack of an effect of phosphorylation on ATPase activity and tension in skinned fiber preparations; and (4) although light chain phosphorylation is not obligatory for contractile activity, it appears to augment isometric twitches following tetanic stimulation.

Studies in intact smooth muscle

Despite the abundance of data supporting the obligatory role of myosin light chain phosphorylation in actin activation of myosin ATPase activity and hence possibly in contraction, very little information regarding myosin phosphorylation in the intact smooth muscle exists. This may be largely due to the technical difficulties associated with obtaining sufficient quantities of pure P-light chain from various isolated smooth muscle preparations necessary for measurements of the extent of phosphate incorporation. Recent preliminary reports have provided some information in this area. After incubation of estrogen-dominated myometrial strips with [^{32}P]inorganic phosphate in order to label the γ-phosphate of intracellular ATP, Gualtieri and Janis [55] found that spontaneous contractions or those induced by oxytocin, carbachol or 127 mM KCl were associated with increases in ^{32}P-incorporation into a 20,000 dalton protein after polyacrylamide gel electrophoresis in SDS. Presumably, the protein was myosin P-light chain. Barron *et al.* [56], using pig carotid artery strips, found that ^{32}P was incorporated into the region of the P-light chain after polyacrylamide gel electrophoresis of partially purified myosin. In addition, they estimated the extent of phosphorylation by measuring the radiospecific activity of phosphocreatine and calculating the myosin content in the arterial strips. Under control conditions with passive tension, the phosphate content of arterial smooth muscle myosin was 0.59 mole phosphate/mole light chain which decreased to 0.34 mole phosphate/mole myosin with no passive tension. After a 9-min exposure to 50 μM norepinephrine, the value increased to 1.0.

Aksoy and Murphy [57] also used pig carotid artery but measured the extent of phosphorylation by densitometry after separating the nonphosphorylated and phosphorylated light chains by isoelectric focusing. Stimulation of contraction with high KCl concentrations resulted in an increase from 0.15 to 0.60

mole phosphate/mole light chain. The increase in the rate of phosphate incorporation preceded the increase in the rate of tension development and, upon relaxation, increase in the rate of dephosphorylation preceded the increase in the rate of decrease in tension. deLanerolle and Stull [58] used antibodies to purify myosin from tracheal smooth muscle and separated the nonphosphorylated and phosphorylated light chains by isoelectric focusing. After the addition of methacholine, the phosphate content of myosin P-light chain increased from 0.25 to 0.55 mole phosphate/mole myosin within 3 min and coincided temporally with the increase in isometric tension. In a Ca-free buffer, the phosphorylation reaction and tension response to methacholine were inhibited. Addition of Ca^{2+} to these muscles elicited an increase in isometric tension concomitant with phosphorylation of myosin.

In general, these results, obtained with different isolated smooth muscles, are consistent with the hypothesis that phosphorylation of smooth muscle myosin may be obligatory for contraction and that the reaction may be regulated intracellularly by Ca^{2+}. More information is required, however, to establish firmly a causal relationship between myosin phosphorylation and contraction of smooth muscles.

Pharmacological considerations pertaining to myosin phosphorylation

In skeletal muscle myosin phosphorylation is not essential for contraction, but it appears to modulate contractile activity via potentiation of isometric twitch tension. It should be noted that this modulation has been described in white, fast-twitch skeletal muscle fibers and no information is currently available in regard to this process in red, slow-twitch fibers. The regulation of myosin light chain kinase activity is relatively simple, involving formation of a $Ca_n^{2+} \cdot$CDR complex which binds to and activates the catalytic subunit of the myosin light chain kinase. The activation of myosin light chain kinase does not occur with a single twitch but requires a sustained elevation of cytoplasmic Ca^{2+} such as that produced during tetany or repetitive stimuli. The activation of myosin light chain kinase and myosin phosphorylation may play some role in enhancing contractile activity during physical exercise. Pharmacological agents capable of modifying the process of light chain phosphorylation in skeletal muscle would include those agents which have effects on Ca^{2+} release into, and sequestration in the sarcoplasmic reticulum. For example, caffeine has been shown to stimulate myosin phosphorylation in isolated frog skeletal muscle [52], presumably due to its ability to release Ca^{2+} from sarcoplasmic reticulum, causing contractures. These types of pharmacological agents are useful in exploring mechanisms of activation of skeletal muscle myosin light chain kinase, but their effects probably are of little consequence in living organisms. High concentrations of phenothiazine antipsychotic drugs should inhibit the activation of myosin light chain kinase in isolated muscles, but since the inhibition is competitive with respect to the kinase, it is not clear whether the concentration of these agents, under therapeutic situations, would effectively inhibit myosin light chain kinase activity.

The potential for affecting smooth muscle contraction by pharmacological interventions is much greater than for skeletal muscle due to differences in physiological as well as biochemical properties. Smooth muscles contract much more slowly than skeletal muscle and are highly dependent upon extracellular Ca^{2+} for the contraction process. Since myosin phosphorylation may be obligatory for smooth muscle contraction, the translocation and availability of Ca^{2+} for activation of myosin light chain kinase may be an important physiological determinant. Pharmacological agents which inhibit Ca^{2+} influx through slow Ca^{2+} channels, for example, would cause relaxation via decreasing Ca^{2+} availability. Such agents include verapamil, D-600 and other Ca^{2+} antagonists. For any particular type of smooth muscle, drugs and hormones which cause contractions may initiate the process of light chain phosphorylation by binding to their own specific receptors which, in turn, increase the permeability of receptor-modulated Ca^{2+} channels. In smooth muscles that normally generate action potentials, the potential-sensitive Ca^{2+} channels are responsive to a reduced membrane potential. Those agents that alter action potentials in these cells may modify the influx of Ca^{2+} and the subsequent activation of myosin light chain kinase and, thereby, cause a change in contractile tone. The antipsychotic drugs have been useful tools for investigating the role of myosin phosphorylation, particularly in smooth muscles. Although these drugs have been shown to inhibit the activation of smooth muscle myosin light chain kinase by binding to $Ca_n^{2+} \cdot$CDR, it is not clear whether the antipsychotic phenothiazines would cause relaxation of smooth muscle via this mechanism under therapeutic situations.

Other potential biochemical mechanisms for regulating smooth muscle myosin phosphorylation and contraction involve cyclic AMP. Stimulation of cyclic AMP formation may subsequently result in the conversion of myosin light chain kinase to a less active form via phosphorylation of myosin kinase by cyclic AMP-dependent protein kinase. This biochemical response would be associated with relaxation. This response should be associated not only with β-adrenergic receptor stimulation, but also with activation of other pharmacological receptors that result in stimulation of cyclic AMP formation. Similarly, agents which inhibit cyclic nucleotide phosphodiesterase activity would produce an increase in cyclic AMP content and thereby result in activation of cyclic AMP-dependent protein kinase. As discussed above, the net result would be inhibition of myosin phosphorylation and, hence, inhibition of contraction. The regulation of myosin phosphorylation by other cyclic nucleotides, such as cGMP, has not been investigated, but also presents a potential mechanism by which contractile activity may be regulated by drugs and hormones.

In summary, there are multiple sites at which myosin phosphorylation may be regulated in smooth muscles. The primary biochemical mechanisms involve Ca^{2+}, CDR, cyclic AMP, and cyclic AMP-dependent protein kinase. Since myosin phosphorylation may be essential for contraction, the contractile tone under any particular situation may be the result

of the summed effects of Ca^{2+} and cyclic AMP. Most of the information used to develop this hypothesis has relied upon results obtained with purified proteins. It will be essential to test critically each aspect of the biochemical processes involved in myosin phosphorylation with intact smooth muscle cells in order to unravel the regulatory mechanisms that are important *in vivo*.

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