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Phosphorylation of myosin light chain kinase: a cellular mechanism for Ca^{2+} desensitization

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Abstract

Phosphorylation of the regulatory light chain of myosin by the Ca^{2+}/calmodulin-dependent myosin light chain kinase plays an important role in smooth muscle contraction, nonmuscle cell shape changes, platelet contraction, secretion, and other cellular processes. Smooth muscle myosin light chain kinase is also phosphorylated, and recent results from experiments designed to satisfy the criteria of Krebs and Beavo for establishing the physiological significance of enzyme phosphorylation have provided insights into the cellular regulation and function of this phosphorylation in smooth muscle. The multifunctional Ca^{2+}/calmodulin-dependent protein kinase II phosphorylates myosin light chain kinase at a regulatory site near the calmodulin-binding domain. This phosphorylation increases the concentration of Ca^{2+}/calmodulin required for activation and hence increases the Ca^{2+} concentrations required for myosin light chain kinase activity in cells. However, the concentration of cytosolic Ca^{2+} required to effect myosin light chain kinase phosphorylation is greater than that required for myosin light chain phosphorylation. Phosphorylation of myosin light chain kinase is only one of a number of mechanisms used by the cell to down regulate the Ca^{2+} signal in smooth muscle. Since both smooth and nonmuscle cells express the same form of myosin light chain kinase, this phosphorylation may play a regulatory role in cellular processes that are dependent on myosin light chain phosphorylation. (Mol Cell Biochem **127/128:** 229–237, 1993)

Key words: calcium, contraction, smooth muscle, myosin, protein phosphorylation, second messengers

Introduction

Many signal transduction pathways are involved in the regulation of the cytosolic Ca^{2+} concentrations. Increases in free cytosolic [Ca^{2+}] may be affected by Ca^{2+} influx across the cell membrane and/or release from internal storage sites leading to a variety of biological responses. In smooth muscle Ca^{2+} is the primary activator of the contractile elements [1, 2]. Ca^{2+} binds to calmodulin, and the Ca^{2+}/calmodulin complex binds to and activates myosin light chain kinase (Fig. 1). Phosphorylation of the regulatory light chain subunit of myosin at serine 19 by the activated kinase results in a marked increase in actin-activated myosin MgATPase activity. This phosphorylation is associated with attachment of myosin cross bridges to actin, development of force, and an increase in maximal shortening velocity. A return of cytosolic Ca^{2+} concentrations to resting values *via* cellular

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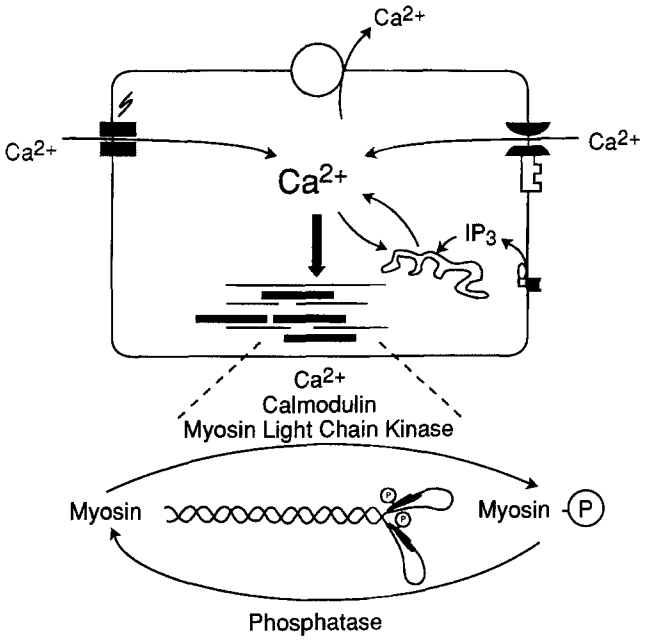

Fig. 1. Calcium-dependent myosin light chain phosphorylation. Cytosolic Ca^{2+} concentrations in a cell may be increased by influx through voltage-dependent or receptor-operated channels or by Ca^{2+} release from intracellular storage sites. Ca^{2+} binds to calmodulin which binds to myosin light chain kinase in the contractile protein system. The activated kinase phosphorylates the regulatory light chain subunit of myosin. In smooth and nonmuscle cells this phosphorylation leads to an increase in the actin-activated myosin MgATPase activity.

uptake into the storage site or extrusion from the cell leads to inactivation of myosin light chain kinase, dephosphorylation of the myosin light chain by protein phosphatase type 1_M, and smooth muscle relaxation. The Ca^{2+}/calmodulin-dependent phosphorylation of the regulatory subunit of myosin from a variety of nonmuscle cells also leads to a marked increase in the actin-activated MgATPase activity. This phosphorylation reaction has been proposed to play functional roles in cell shape changes, receptor capping, platelet contraction, secretion, and other biological processes [3, 4].

Because there is considerable crosstalk among different signal transduction pathways, it has been considered that the cascade of reactions leading to myosin light chain phosphorylation may be affected by other protein kinases. For example, the regulatory light chain in purified smooth muscle myosin is also phosphorylated by protein kinase C at sites different from serine 19 [5, 6]. However, stimulation of smooth muscle with a variety of agonists that lead to activation of protein kinase C does not result in phosphorylation of these sites in smooth muscle tissues [7–10]. Although phosphorylation of serine 19 by myosin light chain kinase plays the primary role in initiating smooth muscle contraction, it should be noted that in some nonmuscle systems myosin light chain phosphorylation by protein kinase C may be functionally significant [11–13].

Ultimately, the Ca^{2+} concentrations required for myosin light chain phosphorylation depend upon the relative activities of myosin light chain kinase and phosphatase. One potential site for regulation of this Ca^{2+}-dependence involves myosin light chain kinase. It was originally observed that phosphorylation of smooth muscle myosin light chain kinase by cyclic AMP-dependent protein kinase leads to an increase in the concentration of Ca^{2+}/calmodulin required for half-maximal activation (K_{CaM}) [14]. If this phosphorylation occurred in a smooth muscle cell, it would desensitize myosin light chain kinase to activation by Ca^{2+}/calmodulin and hence would require higher cytosolic Ca^{2+} concentrations for myosin light chain phosphorylation. A proposal was made that β-adrenergic receptor stimulation and the consequent increases in cyclic AMP formation would result in phosphorylation of myosin light chain kinase and relaxation of smooth muscle [14]. This was an attractive hypothesis, since it is well known that β-adrenergic receptor stimulation results in relaxation of smooth muscle. However, the conclusions derived from a variety of physiological experiments were controversial with an unresolved central set of observations: stimuli leading to increases in cyclic AMP formation did not significantly change the Ca^{2+}/calmodulin activation properties of the kinase in intact muscle [15] but did increase the extent of myosin light chain kinase phosphorylation [16].

Krebs and Beavo (1979) [17] presented criteria to establish the physiological importance of enzyme phosphorylation (Table 1). The application of these criteria has been useful in elucidating the cellular mechanism

Table 1. Criteria for establishing that an enzyme is phosphorylated physiologically

1. Demonstration *in vitro* that the enzyme can be phosphorylated stoichiometrically at a significant rate in a reaction(s) catalyzed by an appropriate protein kinase(s) and dephosphorylated by a phosphoprotein phosphatase(s).
2. Demonstration that functional properties of the enzyme undergo meaningful changes that correlate with the degree of phosphorylation.
3. Demonstration that the enzyme can be phosphorylated and dephosphorylated *in vivo* or in an intact cell system with accompanying functional changes.
4. Correlation of cellular levels of protein kinase and/or phosphoprotein phosphatase effectors and the extent of phosphorylation of the enzyme.

From: Krebs and Beavo [17].

and functional consequence of myosin light chain kinase phosphorylation in smooth muscle. This paper will review the more recent experiments that have provided insights into the physiological role of myosin light chain kinase phosphorylation. We first provide a brief overview of the biochemical properties of myosin light chain kinase phosphorylation *in vitro*.

Myosin light chain kinase phosphorylation in vitro

Cyclic AMP-dependent protein kinase phosphorylates myosin light chain kinase purified from smooth muscles (Fig. 2). In the absence of Ca^{2+}/calmodulin, 2 sites (sites A and B) are phosphorylated, leading to a 10-fold increase in the K_{CaM} value [14]. In the presence of Ca^{2+}/calmodulin, phosphate is incorporated into site B with no effect on myosin light chain kinase activation properties. The serine phosphorylated in site A is in the calmodulin binding domain [18], whereas the serine phosphorylated in site B is more toward the C-terminus [18, 19].

Myosin light chain kinase purified from smooth muscle is also phosphorylated by the cyclic GMP-dependent protein kinase at 1 site [20]. However, phosphopeptide mapping shows that it is not site A; furthermore, there is no change in Ca^{2+}/calmodulin activation or catalytic properties associated with this phosphorylation. Thus phosphorylation by cyclic GMP-dependent protein kinase, if it occurred *in vivo,* would not appear to regulate myosin light chain kinase activity.

Protein kinase C phosphorylates smooth muscle myosin light chain kinase with a maximal incorporation of 2 mol phosphate per mol kinase in the absence of Ca^{2+}/calmodulin [21]. This phosphorylation also leads to an increase in K_{CaM} similar to the effect produced by phosphorylation with cyclic AMP-dependent protein kinase. When calmodulin is bound to myosin light chain kinase the single site phosphorylated by protein kinase C is different than that phosphorylated by the cyclic AMP-dependent protein kinase; however, the additional site that is phosphorylated by protein kinase C when calmodulin is not bound appears to be the same site phosphorylated by the AMP-dependent protein kinase.

The multifunctional Ca^{2+}/calmodulin-dependent protein kinase II phosphorylates smooth muscle myosin light chain kinase to a molar stoichiometry of 2.7 [22, 23]. Peptide mapping and sequence analysis show that both calmodulin-dependent protein kinase II and cyclic AMP-dependent protein kinase phosphorylate the same serine in phosphopeptide A. Because phosphorylation of site A is blocked when calmodulin is bound to myosin light chain kinase, the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II has to be converted to a Ca^{2+}/calmodulin-independent form *via* autophosphorylation so that site A can be phosphorylated in the absence of Ca^{2+}. Similar to results obtained with other potein kinases, phosphorylation of site A increases the K_{CaM} value for the smooth muscle kinase.

Myosin light chain kinase is dephosphorylated by different protein phosphatases. Each of the three different

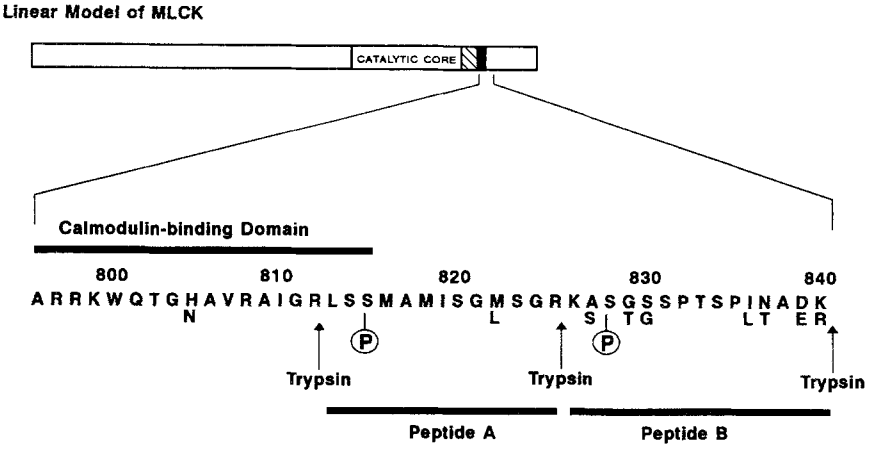

Fig. 2. Phosphorylation of the smooth muscle myosin light chain kinase. The domain organization of the smooth muscle myosin light chain kinase is shown in the upper portion of the figure [2]. The kinase has a catalytic core in the center which is similar in primary structure to other protein kinases. On the C-terminal side of the catalytic core is an autoinhibitory region that overlaps with the calmodulin-binding domain. The more extended portion of the kinase labelled telokin is expressed as an independent protein in some but not all smooth muscle cells. The continuous sequence of a portion of the chicken smooth muscle myosin light chain kinase is shown with serine residues phosphorylated by cyclic AMP-dependent protein kinase identified. The letters below identify residues that are different in the rabbit smooth muscle myosin light chain kinase [35]. Phosphopeptides derived from tryptic digestion are labelled A and B.

oligomeric forms of protein phosphatase 2A from cardiac muscle, including the free catalytic subunit, specifically dephosphorylated site A and not site B [24]. This dephosphorylation pattern is not altered by the presence or absence of Ca^{2+}/calmodulin. These observations are different from those reported with smooth muscle phosphatase I, which has been classified as a heterotrimeric form of protein phosphatase 2A [25]. Both sites A and B are dephosphorylated by this phosphatase in the absence of Ca^{2+}/calmodulin; however, in the presence of Ca^{2+}/calmodulin, it dephosphorylates only site A. This result suggests that dephosphorylation of site B is blocked by the presence of bound calmodulin. It is unlikely these differences are due to the catalytic subunits of the 2 phosphatases, since the primary sequence is highly conserved among species. The A and B subunits of protein phosphatase 2A play important roles in regulating the specificity of the catalytic subunit, and it is possible that a distinct B subunit present in the smooth muscle protein phosphatase allows dephosphorylation of site B in the absence of Ca^{2+}/calmodulin. The catalytic subunit of protein phosphatase 1 also dephosphorylates both sites A and B in the absence of Ca^{2+}/calmodulin; however, only site A is dephosphorylated in the presence of Ca^{2+}/calmodulin [24]. Finally, protein phosphatase activity extracted from smooth muscle actomyosin dephosphorylates both sites A and B in the absence of Ca^{2+}/calmodulin; however, when calmodulin is bound to the enzyme neither site is dephosphorylated.

In summary, it has been demonstrated that 3 different protein kinases, including cyclic AMP-dependent protein kinase, protein kinase C, and Ca^{2+}/calmodulin-dependent protein kinase II, phosphorylate a common serine residue in the calmodulin-binding domain of smooth muscle myosin light chain kinase *in vitro* (Fig. 2). Phosphorylation of this serine (which is blocked when calmodulin binds to myosin light chain kinase) leads to an increase in K_{CaM}, which would be predicted to desensitize myosin light chain kinase to activation by Ca^{2+} in a smooth muscle cell. Different protein phosphatases dephosphorylate site A, and the effect of Ca^{2+}/calmodulin on site A dephosphorylation is dependent upon the type of protein phosphatase.

Myosin light chain kinase phosphorylation in vivo

It was originally proposed that cyclic AMP-dependent phosphorylation of myosin light chain kinase *in vivo* could decrease catalytic activity via a reduction in the affinity of myosin light chain kinase for Ca^{2+}/calmodulin [14]. This decrease in activity would result in a decrease in myosin light chain phosphorylation and hence would contribute to relaxation of smooth muscle. Miller *et al.* (1983) [15] examined the calmodulin activation properties of myosin light chain kinase from tracheal smooth muscle tissues treated with the β-adrenergic agonist isoproterenol which increases cyclic AMP formation. Incubation of smooth muscle tissue at concentrations of isoproterenol sufficient to affect relaxation did not re-

Table 2. Phosphorylation of myosin light chain kinase in ^{32}P-labelled tracheal smooth muscle

Treatment	Force fractional	Light chain phosphorylation mol P/LC	MLCK[a] phosphorylation mol P/MLCK	Peptide A phosphorylation mol P/peptide A
Control	0.00	0.09	0.90	0.00
Carbachol				
1 min	0.78	0.46	1.28	0.42
5 min	1.00	0.70	1.66	0.71
25 min	1.00	0.42	1.94	0.39
KCl				
5 min	0.82	0.45	1.60	0.80
Phorbol dibutyrate				
25 min	0.06	0.22	2.73	0.08
Isoproterenol				
1 min	0.00	0.08	1.28	0.15
CCh (5 min)				
+Iso (1 min)	0.10	0.16	1.43	0.33

From: Stull *et al.* [26].

[a] Abbreviations include: MLCK, myosin light chain kinase; CCh, carbachol; Iso, isoproterenol; P, phosphate; LC, regulatory light chain of myosin.

sult in significant changes in the calmodulin activation properties of myosin light chain kinase. The curious observation was made that when smooth muscle tissue was stimulated to contract with either a muscarinic agonist that releases Ca^{2+} from the sarcoplasmic reticulum or with KCl to bring about membrane depolarization thereby causing an influx of Ca^{2+}, there was an increase in K_{CaM} values. Although phosphorylation of myosin light chain kinase was not measured directly, these results indicated that the kinase was phosphorylated in regulatory site A when the smooth muscle contracted. (This observation has been explained by recent experiments showing phosphorylation of myosin light chain kinase by Ca^{2+}/calmodulin-dependent kinase II [see below].) de Lanerolle *et al.* (1984) [16] subsequently measured ^{32}P incorporation into myosin light chain kinase in tracheal smooth muscle tissue. Under control conditions the extent of phosphorylation was 1.1 mol phosphate per mol myosin light chain kinase. The addition of forskolin increased cyclic AMP formation 17-fold and the net phosphate incorporated into myosin light chain kinase by 0.7 mol phosphate per mol kinase. The phosphorylation sites and the calmodulin activation properties of the kinase were not measured. Therefore, it was difficult to draw conclusions about the specific sites of phosphorylation and effects on myosin light chain kinase activity.

In a more recent study, phosphorylation of site A and the functional consequences on the calmodulin activation properties of myosin light chain kinase were examined after stimulation of different protein kinase cascades [26]. Under control conditions, the extent of myosin light chain kinase phosphorylation was 0.90 mol phosphate per mol kinase in untreated ^{32}P-labelled tracheal smooth muscle tissues (Table 2). Treatment with isoproterenol, which stimulates cyclic AMP formation and activates cyclic AMP-dependent protein kinase, increased the extent of phosphorylation of myosin light chain kinase to 1.28 mol phosphate per mol myosin light chain kinase. Phorbol dibutyrate, an activator of protein kinase C, also stimulated myosin light chain kinase phosphorylation to 2.73 mol phosphate per mol myosin light chain kinase. Carbachol and KCl, which increase the cytosolic Ca^{2+} concentration and thereby initiate smooth muscle contraction, resulted in an increase in myosin light chain kinase phosphorylation to about 1.60 mol phosphate per mol myosin light chain kinase. These results show that treatment of tracheal smooth muscle with agents that activate different protein kinases stimulated myosin light chain kinase phosphorylation; however, there was no simple correlation between the extent of kinase phosphorylation and the contractile state of the muscle (Table 2).

Since myosin light chain kinase was obviously phosphorylated at multiple sites *in vivo,* phosphopeptide mapping was used to identify and quantitate the extent phosphorylation of specific sites [26]. Six phosphopeptides were consistently identified after treatment with various agents. A phosphopeptide containing the serine phosphorylated at site A near the calmodulin-binding domain showed little phosphorylation in tissues treated with nothing, isoproterenol or phorbol dibutyrate; however, treatment with the contractile agents carbachol and KCl resulted in a high extent of phosphorylation at site A (Table 2). Furthermore, the phosphorylation of peptide A appeared to be transient with the maximal extent of phosphorylation obtained by 5 min and subsequently declining to a lower extent by 25 min. This pattern of peptide A phosphorylation was similar to the temporal pattern obtained with myosin light chain phosphorylation (Table 2). Since myosin light chain phosphorylation is Ca^{2+}-dependent, it was noted that phosphorylation of myosin light chain kinase at site A may also be Ca^{2+}-dependent. Tissues depleted of Ca^{2+} by incubation in the presence of EGTA showed no myosin light chain kinase phosphorylation with the addition of carbachol [26]. Phosphorylation of site A *in vivo* increased the K_{CaM} values as measured in a kinase assay *in vitro.* The quantitative relationship between the extent of site A phosphorylation and the increase in K_{CaM} was predicted from biochemical studies with purified enzymes [26]. It is not clear at this time what role, if any, phosphorylation at the other 5 sites may play.

Stimulation of tracheal smooth muscle cells in culture with the Ca^{2+} ionophore ionomycin resulted in a rapid increase in the cytosolic Ca^{2+} concentrations and an increase in the extent of phosphorylation in both myosin light chain kinase and myosin light chain [27]. Most of the ^{32}P incorporated into myosin light chain kinase was in site A. These responses were markedly inhibited in the absence of extracellular Ca^{2+}. Pretreatment of cells with KN-62, an inhibitor of the multifunctional calmodulin-dependent protein kinase II, did not have an effect on the increase in cytosolic Ca^{2+} concentrations in response to ionomycin. However, KN-62 acted to inhibit phosphorylation of myosin light chain kinase at the regulatory site and potentiated myosin light chain phosphorylation in response to ionomycin [27]. In cell lysates phosphorylation of myosin light chain kinase decreased its sensitivity to activation by Ca^{2+}. Thus, evidence was obtained that Ca^{2+}/calmodulin-dependent protein ki-

nase II phosphorylated myosin light chain kinase in a Ca^{2+}-dependent manner. Furthermore, inhibition of this phosphorylation increased the Ca^{2+} sensitivity of myosin light chain phosphorylation. These observations in intact cells were extended to studies within smooth muscle cells made permeable with β-escin [28]. The Ca^{2+} dependencies of myosin light chain kinase and myosin light chain phosphorylation were measured quantitatively with a Ca^{2+}-EGTA buffer system. The concentrations of Ca^{2+} required for half-maximal phosphorylation of myosin light chain kinase and myosin light chain were 500 and 250nM, respectively. The latter value is very similar to that reported in intact cells under steady-state conditions indicating calmodulin was not lost from the cells. Preincubation of the permeable cells with either of two inhibitors of CaM kinase II that act by distinct mechanisms resulted in inhibition of myosin light chain kinase phosphorylation. These inhibitors included KN-62, which prevents calmodulin activation, and a peptide inhibitor derived from the autoinhibitory sequence of the Ca^{2+}/calmodulin-dependent protein kinase II, which inhibits the kinase activity directly. The inhibition of myosin light chain kinase phosphorylation by both inhibitors supports the hypothesis that Ca^{2+}/calmodulin-dependent protein kinase II phosphorylates myosin light chain kinase in smooth muscle cells. Furthermore, inhibition of myosin light chain kinase phosphorylation by both inhibitors decreased the concentration of Ca^{2+} required for half-maximal phosphorylation of myosin light chain to 170nM. These results demonstrate directly that phosphorylation of myosin light chain kinase by Ca^{2+}/calmodulin-dependent protein kinase II decreases the Ca^{2+} sensitivity of myosin light chain phosphorylation with smooth muscle cells.

The criteria of Krebs and Beavo (1979) [17] for establishing the physiological importance for an enzyme phosphorylation have been satisfied in respect to the phosphorylation of myosin light chain kinase by the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (Table 3). This is a complex cascade, because it involves the Ca^{2+}/calmodulin-dependent phosphorylation of an enzyme (myosin light chain kinase) that is itself dependent upon Ca^{2+}/calmodulin for activity (Fig. 3); however, a key feature of the regulation of this cascade appears to be related to the higher Ca^{2+} concentrations required for myosin light chain kinase phosphorylation relative to those required for myosin light chain phosphorylation. Thus, it is predicted that the Ca^{2+} desensitization due to myosin light chain kinase phosphorylation would occur physiologically under situations where cytosolic Ca^{2+} concentrations are greater than those necessary to initiate myosin light chain phosphorylation.

What conclusions can be derived about the phosphorylation of myosin light chain kinase by other protein kinases? Activation of protein kinase C by phorbol ester results in a high extent of myosin light chain kinase phosphorylation in intact smooth muscle; however, no significant phosphate is incorporated into site A. It is not clear whether myosin light chain kinase is directly phosphorylated by protein kinase C in intact cells, or whether it is the result of the activation of another kinase that phosphorylates myosin light chain kinase. The functional importance of the phosphorylation in sites other than site A is not known. Stimulation of cyclic AMP formation does result in phosphorylation of myosin light chain kinase, but there is little incorporation into site A. The small extent of phosphorylation may be due to the fact that myosin light chain kinase is a poor substrate for the cyclic AMP-dependent protein kinase with a V_{max} value less than 1% that of physiological substrates [20]. In addition, the cell's protein phosphatase activity may be sufficient to prevent extensive phosphorylation. Physiologically, the most important response to cyclic AMP formation is the inhibition of agonist-induced increases in cytosolic Ca^{2+} concentration [29, 30]. Thus, the addition of isoproterenol to an agonist-contracted smooth muscle results in inhibition of myosin light chain kinase phosphorylation due to the decrease in cytosolic Ca^{2+} concentration (Table 2) [29].

One puzzling aspect of myosin light chain kinase phosphorylation at site A is related to the high extent of phosphorylation found in contracting smooth muscle when the cytosolic Ca^{2+} concentration has been increased. As noted by Hashimoto and Soderling (1990), when calmodulin is bound to myosin light chain kinase,

Table 3. Criteria satisfied for establishing physiological significance of smooth muscle myosin light chain kinase phosphorylation

1. Purified myosin light chain kinase can be rapidly and stoichiometrically phosphorylated at a regulatory site in the calmodulin binding domain by Ca^{2+}/calmodulin-dependent protein kinase II and dephosphorylated by protein phosphatases.
2. Phosphorylation at the regulatory site increases the concentration of Ca^{2+}/calmodulin required for activation.
3. Myosin light chain kinase is phosphorylated and dephosphorylated at the regulatory site *in vivo*. Phosphorylation leads to a decrease in the Ca^{2+} sensitivity of myosin light chain phosphorylation.
4. The extent of myosin light chain kinase phosphorylation correlates to the cytosolic Ca^{2+} concentrations.

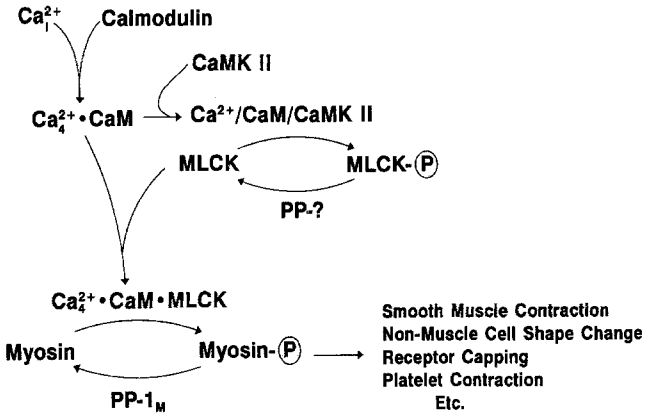

Fig. 3. Biochemical scheme for the regulation of myosin light chain phosphorylation. Phosphorylation of myosin light chain kinase by the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II results in desensitization of myosin light chain kinase to activation by Ca^{2+}/calmodulin. Myosin light chain kinase phosphorylation requires Ca^{2+} concentrations greater than those required for myosin light chain phosphorylation in smooth muscle cells.

phosphorylation by the Ca^{2+}/calmodulin-dependent protein kinase II at site A is blocked. Thus, it is not clear why there is such a large extent of phosphorylation in a contracting muscle. One possibility is that myosin light chain kinase could be phosphorylated if a large fraction of the enzyme did not have calmodulin bound. Alternatively, a small population of nonbound kinase may be rapidly phosphorylated and during the time required for the development of force due to light chain phosphorylation, Ca^{2+}/calmodulin associates and dissociates from myosin light chain kinase. While in the dissociated state the kinase is phosphorylated so that over some period of time substantial phosphorylation is obtained. However, other possibilities could also be considered.

Physiological importance of myosin light chain kinase phosphorylation

The experiments described above demonstrate that myosin light chain kinase is phosphorylated to a high extent in smooth muscle cells by Ca^{2+}/calmodulin-dependent protein kinase II (Fig. 3). Furthermore, this phosphorylation desensitizes myosin light chain kinase to activation by Ca^{2+}/calmodulin and hence to Ca^{2z} in cells. The physiological circumstances by which this mechanism would be recruited to modulate smooth muscle contraction have been explored recently [31]. The temporal relationships of cytosolic Ca^{2+} concentrations, myosin light chain phosphorylation, and force development were examined in neurotransmitter-induced contractions of tracheal smooth muscle and in spontaneously depolarized contractions of human myometrium. In the tonic trachealis smooth muscle electrical stimulation of neurotransmitter release resulted in the immediate and rapid increase in cytosolic Ca^{2+} concentrations with a maximal increase obtained by 9 sec. Myosin light chain phosphorylation and contraction did not increase for 230 ms. After this latency period, light chain phosphorylation increased from 5 to 65% in 1 sec. Thus, the maximal extent of light chain phosphorylation was obtained prior to the maximal increase in cytosolic Ca^{2+} concentration. During spontaneous depolarization of phasic myometrial smooth muscle, the maximal extent of light chain phosphorylation was also obtained prior to maximal increases in cytosolic Ca^{2+} concentrations, indicating a high sensitivity of myosin light chain kinase activation to Ca^{2+}. In both types of smooth muscle cytosolic Ca^{2+} concentrations started to increase before myosin light chain phosphorylation; however, the rate of increase in light chain phosphorylation was significantly greater than the rate of increase in cytosolic Ca^{2+} concentrations. In both types of preparations myosin light chain kinase was phosphorylated as myosin light chain reached its maximal extent of phosphorylation in the smooth muscle cells. The phosphorylation and desensitization of myosin light chain kinase may account in part for the partial phosphorylation (65%) of myosin light chain, since this mechanism is activated at the higher Ca^{2+} concentrations.

Phosphorylation of myosin light chain kinase is one of

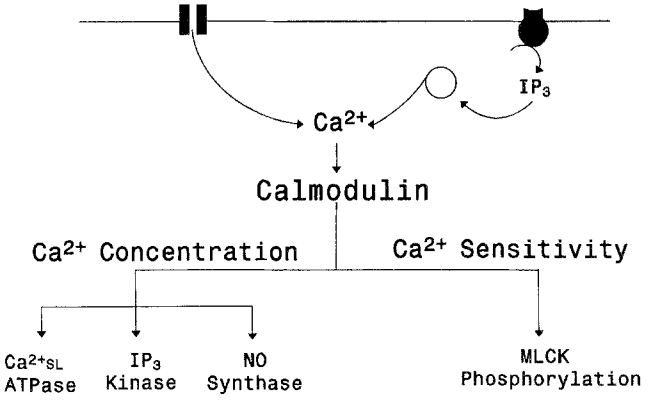

Fig. 4. Ca^{2+}/calmodulin-dependent mechanisms that down-regulate the Ca^{2+} signal. Increases in cytosolic Ca^{2+} concentrations and subsequent formation of Ca^{2+}/calmodulin activates multiple mechanisms for decreasing Ca^{2+} concentrations, including activation of sarcolemmal Ca^{2+} ATPase, activation of IP_3 kinase, and activation of nitric oxide synthase. Another mechanism for decreasing contractile responses involves phosphorylation of myosin light chain kinase by Ca^{2+}/calmodulin-dependent protein kinase II, which desensitizes myosin light chain kinase to activation by Ca^{2+}/calmodulin.

several mechanisms that a cell may use to downregulate the Ca^{2+} signal (Fig. 4). Increases in cytosolic Ca^{2+} with the resultant formation of Ca^{2+}/calmodulin activates a sarcolemmal Ca^{2+} ATPase that pumps Ca^{2+} out of the cell, thereby decreasing cytosolic Ca^{2+} concentrations [32]. Ca^{2+}/calmodulin activation of the IP_3 kinase results in the conversion of this second messenger to an inactive metabolite incapable of releasing Ca^{2+} from the sarcoplasmic reticulum [33]. An additional pathway involves the Ca^{2+}/calmodulin activation of nitric oxide synthase and the resultant product, nitric oxide, stimulates cyclic GMP, which acts to decrease cytosolic Ca^{2+} concentrations *via* activation of the cyclic GMP-dependent protein kinase [34]. Phosphorylation of myosin light chain kinase provides a unique aspect to the downregulation of the Ca^{2+} signal whereby it results in desensitization of light chain phosphorylation to Ca^{2+}. Although this mechanism has been elucidated in smooth muscle, it should be recognized that this contractile protein system is similar to that found in nonmuscle cells where myosin light chain phosphorylation has been proposed to play various roles (Fig. 3). The mechanism described for the Ca^{2+} desensitization of the contractile protein system in smooth muscle cells would be expected to play a role as well in nonmuscle functions that are dependent upon myosin light chain phosphorylation, since both smooth and nonmuscle cells express the same enzyme [35]. However, there will undoubtedly be unique regulatory schemes dependent upon specific cell types.

Acknowledgements

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