Calcium-Independent Contraction in Lysed Cell Models of Teleost Retinal Cones: Activation by Unregulated Myosin Light Chain Kinase or High Magnesium and Loss of cAMP Inhibition

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Abstract. The retinal cones of teleost fish contract at dawn and elongate at dusk. We have previously reported that we can selectively induce detergent-lysed models of cones to undergo either reactivated contraction or reactivated elongation, with rates and morphology comparable to those observed in vivo. Reactivated contraction is ATP dependent, activated by Ca^{2+}, and inhibited by cAMP. In addition, reactivated cone contraction exhibits several properties that suggest that myosin phosphorylation plays a role in mediating Ca^{2+}-activation (Porrello, K., and B. Burnside, 1984, *J. Cell Biol.*, 98:2230–2238).

We report here that lysed cone models can be induced to contract in the absence of Ca^{2+} by incubation with trypsin-digested, unregulated myosin light chain kinase (MLCK) obtained from smooth muscle. This observation provides further evidence that MLCK plays a role in regulating cone contraction. We also report here that lysed cone models can be induced to contract in the absence of Ca^{2+} by incubation with high concentrations of $MgCl_2$ (10–20 mM). Mg^{2+}-induced reactivated contraction is supported by inosine triphosphate (ITP) just as well as by ATP. Because ITP will not serve as a substrate for MLCK, this finding suggests that Mg^{2+}-activation of contraction does not require myosin phosphorylation.

Although Ca^{2+}-induced contraction is completely blocked by cAMP at concentrations <10 µM, cAMP has no effect on cone contraction activated by unregulated MLCK or by high Mg^{2+} in the absence of Ca^{2+}. Because trypsin digestion of MLCK cleaves off not only the Ca^{2+}/calmodulin-binding site but also the site phosphorylated by cAMP-dependent protein kinase, and because Mg^{2+} activation of cone contraction circumvents MLCK action altogether, both these observations would be expected if cAMP inhibits reactivated cone contraction by catalyzing the phosphorylation of MLCK and thus reducing its affinity for Ca^{2+}, as has been described for smooth muscle.

Together our results suggest that in lysed cone models, myosin phosphorylation is sufficient for activating cone contraction, even in the absence of other Ca^{2+}-mediated events, that cAMP inhibition of contraction is mediated by cAMP-dependent phosphorylation of MLCK, and that 10–20 mM Mg^{2+} can activate actin–myosin interaction to produce contraction in the absence of myosin phosphorylation.

ALTHOUGH it is generally accepted that the phosphorylation–dephosphorylation of the 20,000-D light chains of myosin forms an important component of contraction regulation in smooth muscle and nonmuscle cells (1, 2, 19, 21, 28, 44, 48, 50), our understanding of this regulatory process is still incomplete. In its simplest version, the myosin phosphorylation theory holds that contraction is initiated by Ca^{2+}/calmodulin activation of myosin light chain kinase (MLCK)[1] to produce phosphorylation of two myosin light chains, thereby converting myosin from an inactive form into one that can be actin activated, and that dephosphorylation returns myosin to the inactive form. Inhibition of myosin phosphorylation (and contraction) by cAMP has been shown to result at least in part from cAMP-dependent phosphoryla-

tion of MLCK, thereby reducing the affinity of MLCK for Ca^{2+}/calmodulin (4).

Although numerous observations are consistent with this theory, others suggest that the situation may be more complex. In fact, several other regulatory mechanisms have been proposed, either as alternative or complementary to the myosin phosphorylation theory: (a) the thin-filament–linked leiotonin system (36), (b) thin filament phosphorylation (49), (c) thin filament regulation by the actin- and calmodulin-binding protein caldesmon (5, 17, 35, 41), and (d) phosphorylation-independent regulators of actomyosin ATPase activity (13, 37). Furthermore, the details of contraction regulation in nonmuscle cells are far less well characterized than in smooth muscle. For example, there remains considerable controversy concerning not only the possible roles of thin filament regulation (5, 17, 41), but also the effect of light-

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1. *Abbreviation used in this paper:* MLCK, myosin light chain kinase.

chain phosphorylation on the assembly of myosin into filaments (6, 14, 22, 27) and the association of myosin with the cytoskeleton (6, 20, 27, 34).

To extend our understanding of the regulation of nonmuscle contractility, we have been investigating reactivated contraction in lysed cell models of teleost retinal cones (10, 39, 40). These long, slender cells contract in the light and elongate in the dark as a part of the retina's diurnal adjustment to changing light intensities. Cones undergo dramatic actin-dependent contraction (up to 100 μm) each day at dawn (9). Using models of these cells that have been lysed in 1% Brij-58, we have previously shown that reactivated cone contraction is ATP dependent and inhibited by myosin subfragment 1 that has been inactivated by N-ethylmaleimide, indicating that cone contraction depends on actin–myosin interaction (10, 39, 40). Several observations from our previous model studies further indicate that cone contraction is regulated by myosin phosphorylation in a manner similar to that in smooth muscle: (a) reactivated cone contraction is activated by Ca^{2+} concentrations above pCa 7.5 and inhibited by calmodulin inhibitors; (b) contraction is not supported by incubation with ATPγs or ITP alone but is supported by ATPγs followed by ITP; and (c) contraction is strongly inhibited by cAMP (39).

In the present study we have further examined the possible roles of myosin phosphorylation and other Ca^{2+}-mediated regulatory processes in controlling cone contractions with experiments designed to bypass the Ca^{2+} activation step. We investigated whether lysed cone models would contract in the absence of Ca^{2+} when incubated with "unregulated" MLCK or with high concentrations of magnesium.

MLCK can be treated with trypsin in the presence of bound calmodulin to cleave away the portion of the molecule which bears the Ca^{2+}/calmodulin-binding site from the catalytic region, leaving the enzyme permanently activated (3, 47). This "unregulated" MLCK has been shown to induce contraction in the absence of Ca^{2+} in both glycerinated and skinned smooth muscle fibers (23, 47). These observations indicate that in smooth muscle, contraction can be activated by myosin phosphorylation in the absence of any other Ca^{2+}-dependent activation process. Protease digestion also cleaves off the portion of the MLCK molecule which is phosphorylated by cAMP-dependent protein kinase (46). If cAMP inhibits contraction by catalyzing the phosphorylation of MLCK, it would not be expected to affect contraction activated by unregulated MLCK.

Recently Ikebe and co-workers have shown that high concentrations of $MgCl_2$ induce increased actin-activatable Mg ATPase activity in smooth muscle myosin and also stimulate the development of tension in glycerinated smooth muscle fibers in the absence of light-chain phosphorylation (23, 26). In the present study we have extended these investigations of high Mg^{2+} effects to a nonmuscle system by testing whether high Mg^{2+} will induce contraction in the absence of Ca^{2+} in lysed cone models and whether cAMP has any effect on Mg^{2+}-induced contraction.

We report here that lysed cone models, like smooth muscle cell models, can be induced to contract in the absence of Ca^{2+} after incubation in media containing unregulated MLCK or high Mg^{2+}, and that cAMP has no effect on contraction under either of these conditions.

Materials and Methods

Animals

Green sunfish (*Lepomis cyanellus*) were obtained from Fenders Fish Hatchery (Baltic, OH) or local suppliers and maintained in Berkeley in outdoor ponds. In order to obtain retinas with cone myoids ∼40 μm long and free of retinal pigment epithelium, we placed fish in dark, aerated tanks 30 min before the start of each experiment. All experiments were done at the same time of day to minimize effects of circadian rhythm on cone length.

Preparation of Retinas

Dissections and incubations were carried out under infrared illumination using an infrared converter. Fish were killed by spinal cord section and eye-cups were prepared. The retinas were detached with a gentle stream of gassed (95% O_2/5% CO_2) modified Earle's balanced salt solution containing <10^{-8} M Ca^{2+}, 5 mM EGTA, 1 mM $MgSO_4$, 24 mM $NaHCO_3$, 20 mM glucose, 3 mM Hepes, 1 mM ascorbic acid, pH 7.4. The retinas were bisected along the choroid fissure, providing four comparable pieces from each fish. One half retina was fixed immediately to provide initial cone lengths (L_0), and the remaining halves were lysed in reactivation medium (see below) containing 1% Brij-58, a nonionic detergent, for 3 min and then transferred to detergent-free medium for 15 min. For the unregulated MLCK studies, 0.1 ml of medium was used in 6-mm wells of Linbro tissue culture trays (Flow Laboratories, Inc., McLean, VA), and for the Mg^{2+} studies 0.5 ml of medium was used in 17-mm tissue culture trays. The trays were agitated gently on a vibrator during both incubations. At the end of the 15-min incubation, half retinas were placed in fixative (6% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0) and left overnight.

Measurements

Rectangular pieces from the fundic region of the retina were removed and chopped in 50-μm slices using a hand tissue chopper as previously described (10). Sections were examined using Nomarski optics on an Olympus microscope (Olympus Corporation of America, New Hyde Park, NY) with a ×40 objective and an ocular micrometer. Cone myoid length was determined by measuring the distance between the base of the cone ellipsoid and the outer limiting membrane. Changes in cone myoid length (L) were calculated by subtracting the final cone length of the three experimental half retinas from the initial length (L_0) of the same fish. 20 representative cones were measured for each half retina. In all cases n refers to the number of retinas examined. In each set of experiments described in the figures and text, the observations for contraction media were obtained from the same group of fish used for the other treatments in that set of experiments; therefore the extents of contraction in contraction media vary slightly from one set of experiments to another.

Solutions

Reactivation media contained 0.1 M Pipes buffer, pH 6.94, 10 mM EGTA, 1 mM free $MgSO_4$, and 1 mM Mg-ATP unless otherwise indicated. For relaxation solution, no calcium was added (yielding a free calcium concentration of <10^{-8} M), and for contraction solution $CaCl_2$ was added to produce a free calcium concentration of 10^{-5} M. Calcium/EGTA buffer concentrations were calculated according to formulas from Steinhardt et al. (42).

Smooth muscle MLCK was kindly donated by Dr. Zac Cande (University of California, Berkeley, CA). It was prepared from chicken gizzard as previously described (3, 29). Unregulated kinase was prepared by tryptic digestion of MLCK in the presence of bound calmodulin (3). MLCK at 15 μg/ml in 100 mM NaCl, 15 mM Tris HCl, pH 7.5, 0.2 mM $CaCl_2$, 2 mM dithiothreitol with excess calmodulin was digested with trypsin at 250:1 for 1 h on ice. The digestion was terminated by the addition of 40-fold excess of trypsin inhibitor. The resulting "unregulated" kinase was then diluted 1:10 when added to relaxation medium. Calculations indicate that because the relaxation medium contained 10 mM EGTA, the resulting free calcium concentration was still <10^{-8} M. For control experiments, no MLCK was added at the digestion step, but all other components were present. Cyclic AMP (Boehringer-Mannheim Diagnostics, Houston, TX) was dissolved in 0.4 M Pipes buffer, pH 6.94, and then added to reactivation medium to give the required concentration.
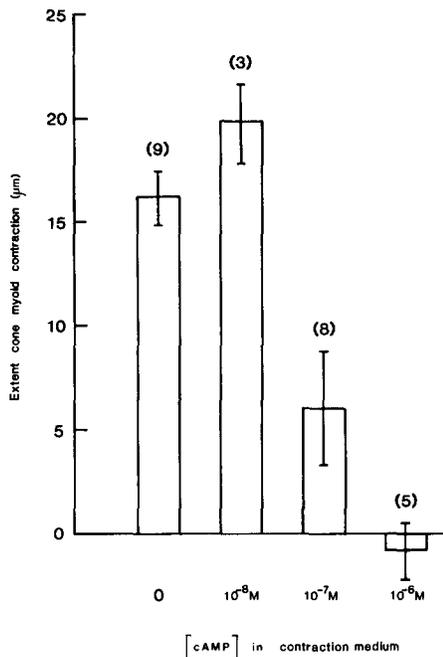
Figure 1. Inhibition by cAMP of Ca^{2+}-induced reactivated contraction in lysed cell models of teleost retinal cones. Contraction medium contained 10^{-5} M free Ca^{2+}, 1 mM free Mg^{2+}, and 1 mM Mg^{2+}-ATP. Error bars indicate SEM and parentheses denote number of retinas examined.

Figure 2. Dose-response curve for effects of free Mg^{2+} concentration on cone contraction in Ca^{2+}-free relaxation medium. All preparations contained 1 mM Mg^{2+}-ATP and $<10^{-8}$ M Ca^{2+}. Values below the x-axis indicate extents of elongation rather than contraction. Open circle illustrates cone contraction in contraction medium containing 1 mM free Mg^{2+} and 10^{-5} M free Ca^{2+}. Error bars indicate SEM and parentheses denote number of retinas examined.

Results

Effects of Ca^{2+} and cAMP

When dark-adapted retinas were lysed in 1% Brij-58 and incubated in contraction medium (1 mM Mg^{2+}/ATP, 1 mM free Mg^{2+}, and 10^{-5} M free Ca^{2+}) for 18 min, the lysed cone models contracted 20–25 μm (Fig. 1). No contraction was observed with free Ca^{2+} $<10^{-8}$ M (relaxation medium). When cAMP was added to the contraction medium, reactivated contraction was inhibited in a dose-dependent fashion between 10^{-8} and 10^{-6} M (Fig. 2). Complete inhibition was observed at 10^{-6} M cAMP; no inhibition was observed at 10^{-8} M cAMP.

Unregulated MLCK

When lysed cone models were incubated in relaxation medium with unregulated MLCK from smooth muscle, cones were reactivated to contract in the absence of Ca^{2+} (Table I). The extent of contraction induced by unregulated MLCK in the absence of Ca^{2+} was 80% of that obtained in contraction medium (Table I). No contraction was observed in the control medium which contained all components used in the preparation of unregulated MLCK except the MLCK itself.

Although incubation with cAMP (10^{-6} M) completely inhibited Ca^{2+}-induced cone contraction (Fig. 1), cAMP failed to inhibit contraction induced by unregulated MLCK in the absence of Ca^{2+}, even when present at relatively high concentrations (10^{-4} M) (Table I).
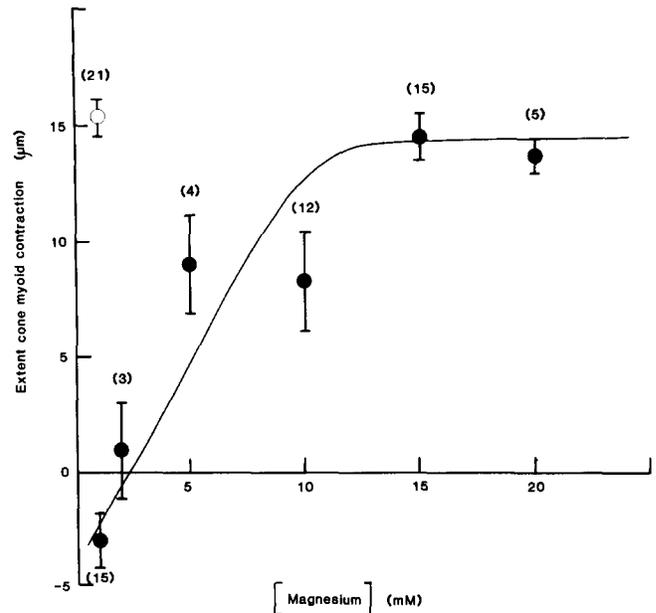
High Magnesium

Reactivated cone contraction was induced in the absence of Ca^{2+} by incubating lysed cone models in relaxation medium supplemented with high concentrations of Mg^{2+} (Table II, Fig. 2). In relaxation medium containing 15 mM $MgCl_2$, lysed cone models contracted to an extent not significantly different from that observed in contraction medium. When ITP was substituted for ATP in relaxation medium containing 15 mM $MgCl_2$, the extent of contraction was 85% that obtained in similar medium containing ATP and 95% that obtained in contraction medium containing ATP and 10^{-5} M Ca^{2+}. Previous studies have shown that in lysed cone models no reactivated contraction is observed when ITP is substituted for ATP in contraction medium (10^{-5} M Ca^{2+}) (39). The effect of Mg^{2+} ion contraction was dose dependent between 2 and 15 mM.

The activation of cone contraction by high Mg^{2+} was not due to ionic strength alone. When 30 mM KCl was added to relaxation medium, cones contracted only 3.6 ± 2.3 μm (n = 3) as compared with 20.1 ± 4.3 μm (n = 3) in relaxation medium with 15 mM $MgCl_2$. KCl did not have an inhibitory effect in that cones contracted 22.4 ± 6.0 μm (n = 3) in relaxation medium containing both 15 mM $MgCl_2$ and 30 mM KCl. Adding cAMP (10^{-4} M) to relaxation medium containing 15 mM Mg^{2+} failed to inhibit Mg^{2+}-induced reactivated contraction. The extent of cone model contraction in relaxation medium containing 20 mM $MgCl_2$ plus 100 mM cAMP was 14.4 ± 2.2 μm (n = 3), compared with 12.6 ± 0.6 μm (n = 3) in relaxation medium containing 20 mM $MgCl_2$ alone and 14.6 ± 2.3 μm (n = 3) in contraction medium containing 10^{-5} M Ca^{2+} and 1 mM Mg^{2+}.

Table I. Effects of Unregulated MLCK and cAMP on Reactivated Contraction in Lysed Cell Models of Teleost Retinal Cones

Treatment	Extent cone myoid contraction
	μm
Contraction medium	22.7 ± 2.7 (9)
Relaxation medium	-2.7 ± 2.5 (9)
Relaxation medium +unregulated MLCK	18.7 ± 1.2 (13)
Control	-1.5 ± 3.5 (4)
Contraction medium $+10^{-4}$ M cAMP	1.1 ± 1.0 (10)
Relaxation medium +unregulated MLCK $+10^{-4}$ M cAMP	16.3 ± 1.2 (7)

All reactivation media contained 1 mM Mg^{2+}-ATP and 1 mM free Mg^{2+}. Free calcium concentrations were 10^{-5} M in contraction medium and $<10^{-8}$ M in relaxation medium. The preparation labeled "control" lacked MLCK but contained all the remaining components used in the preparation of unregulated MLCK (trypsin and trypsin inhibitor). Negative values indicate extents of elongation rather than contraction. Values are given as mean $\pm$ SEM. Numbers in parentheses denote number of retinas examined; 20 cones were measured per retina.

Discussion

We have shown that lysed cone models are induced to contract in the absence of Ca^{2+} by unregulated smooth muscle MLCK. This response of cone models resembles that observed with glycerinated and skinned smooth muscle fibers (11, 47). It demonstrates that myosin phosphorylation alone, in the absence of other Ca^{2+}-mediated processes, is sufficient to induce contraction in lysed cone models. These observations thus provide strong evidence that other regulatory systems, such as Ca^{2+}-activated changes in thin filaments (5, 17, 35, 36, 41), are not required to reactivate cone contraction. These observations do not, of course, eliminate the possibility that other Ca^{2+}-mediated processes contribute to regulating cone contraction in vivo. Our findings add to the growing list which support a role for myosin phosphorylation in regulating contraction in nonmuscle systems (1, 6, 18, 27, 30, 32, 33).

Previous studies have indicated that cAMP plays an important role in regulating cone movement. In living fishes and

Table II. Induction of Reactivated Contraction by High Mg^{2+} in Lysed Cone Models in the Absence of Ca^{2+}

Treatment	Extent cone myoid contraction
	μm
Contraction medium (with ATP)	16.2 ± 1.7 (7)
Relaxation medium (with ATP)	-4.0 ± 2.4 (7)
Relaxation medium + 15 mM $MgCl_2$ (with ATP)	18.3 ± 3.8 (5)
Relaxation medium + 15 mM $MgCl_2$ (with ITP)	14.9 ± 1.2 (3)
Relaxation medium + 15 mM $MgCl_2$ (no ATP or ITP)	-1.1 ± 2.1 (3)

Free Ca^{2+} concentrations were 10^{-5} M in contraction medium and $<10^{-8}$ M in relaxation medium. All preparations contained 1 mM Mg^{2+}-ATP except the one in which 1 mM Mg^{2+}-ITP was substituted for Mg^{2+}-ATP and the last with no ATP. Free Mg^{2+} concentration was 1 mM unless otherwise indicated. Values are given as mean $\pm$ SEM. Numbers in parentheses denote number of retinas examined; 20 cones were measured per retina.

in cultured retinas, cones contract in response to onset of light and elongate in response to onset of darkness (9). We have previously reported that treating intact retinas with agents that elevate cAMP will block light-induced contraction in intact cones (15), and will induce cone elongation in continuous light (8). In this report, we have shown that also in lysed cone models, cAMP inhibits Ca^{2+}-induced cone contraction in a dose-dependent fashion, with complete inhibition observed at 10^{-6} M cAMP.

Because smooth muscle unregulated MLCK is effective in activating cone contraction, it has been possible to consider in more detail the regulatory role of cAMP in this process. One probable locus of action for cAMP in inhibiting cone contraction is cAMP-dependent phosphorylation of MLCK. Adelstein and co-workers (4) have shown that phosphorylation of both smooth muscle and platelet MLCK by a cAMP-dependent protein kinase inhibits the formation of the Ca^{2+}-calmodulin-MLCK complex which is responsible for phosphorylating the 20-kD myosin light chains. Kerrick and co-workers (31) have further shown that addition of exogenous catalytic subunit of the cAMP-dependent protein kinase causes an inhibition of Ca^{2+}-activated tension in skinned smooth muscle preparations.

Our results with unregulated MLCK are consistent with the suggestion that in the lysed cone models cAMP inhibition of contraction results from an inhibition of MLCK activity similar to that observed in smooth muscle. Because the trypsin digestion used to prepare unregulated MLCK cleaves off the cAMP-dependent kinase phosphorylation sites along with the Ca^{2+}/calmodulin-binding sites (3, 47, 48), one would expect cAMP to be ineffective in inhibiting the activity of unregulated MLCK. In that cAMP produced no inhibition in cone models under these circumstances, it seems clear that cAMP does not interfere with any steps subsequent to myosin phosphorylation in the activation cascade for cone contraction.

In this study we have also shown that high concentrations of Mg^{2+} (15-20 mM) induced maximal contraction of cone models in the absence of Ca^{2+}. This response resembles that of glycerinated smooth muscle fibers reported by Ikebe and co-workers (23); there also maximal tension was achieved at Mg^{2+} concentrations between 12 and 20 mM. In glycerinated smooth muscle fibers Mg^{2+}-induced contraction occurred in the absence of myosin phosphorylation (23). Though we cannot analyze the effects of high Mg^{2+} on myosin phosphorylation in cone models directly (because of the large excess of other retinal neurons in the whole retina preparations), indirect experiments strongly suggest that myosin phosphorylation is not necessary for Mg^{2+}-induced cone contraction; in high Mg^{2+} both ITP and ATP were equally effective in supporting contraction in lysed cone models. In 10^{-5} M Ca^{2+}, reactivated cone contraction was supported by ATP but not at all by ITP (39). Though ITP will serve as a substrate for myosin ATPase, it will not serve as a substrate for MLCK (38). In models of smooth muscle fibers similar results have been reported: ITP did not support Ca^{2+}-activated contraction in the absence of myosin phosphorylation at lower levels of Mg^{2+} (1 mM) (12), but fully supported Ca^{2+}-independent contraction in high Mg^{2+} (23).

These results suggest that in both nonmuscle cells and smooth muscle, high Mg^{2+} concentrations produce conformational changes in myosin similar to those produced by

phosphorylation of the 20-kD light chains, thus increasing the actin-activatable ATPase activity of the myosin and permitting force development (23, 26). Both smooth muscle and nonmuscle myosins can assume two conformational states, an elongated state with the tail relatively straight (6S), and a folded state in which the tail curls back toward the head (10S) (14, 23, 24, 43, 45). Under physiological ionic conditions, phosphorylation of myosin light chains tends to favor the straight (6S) state (23). However, the 6S state can also be induced in dephosphorylated myosin by a variety of conditions, including high magnesium concentrations (10–30 mM) (23). The 6S state is associated with increased actin-activated myosin ATPase activity and with filament self-assembly whether or not the myosin light chains are phosphorylated.

It has been suggested that in nonmuscle cells, the folding of myosin into a compact form (the 10S) might facilitate its transport to the intracellular site where motile activity is required (14). Phosphorylation of the light chains might then induce the formation of filaments which could act in combination with actin filaments to produce force. However, it is possible that in the higher viscosity of the living cell, the changes in myosin conformation associated with phosphorylation might be less dramatic than observed in vitro. For example the 10S–6S transition might reflect some conformational change in the myosin heads that is usually correlated with phosphorylation and activation, rather than with the curling of the tail per se. This suggestion is supported by the observations that smooth muscle heavy meromyosin is activated by phosphorylation (when no tail is present) (24). Recent evidence suggests that the conformation change effected by phosphorylation concerns an interaction between S-1 and S-2 portions of the myosin molecule rather than the tail: light-chain phosphorylation increases ATPase and actin-binding activity in heavy meromyosin but not in S-1 (25).

The effects of phosphorylation and ATP on the state of assembly of myosin and the extent of its association with the cytoskeleton have been examined in several nonmuscle systems with varying results (6, 14, 20, 22, 27, 34). In lysed cone models we have previously shown that Ca^{2+} induces maximal contraction in the models even after the lysed models have been incubated for 90 min in $<10^{-8}$ M Ca^{2+} (10 mM EGTA) with 1 mM ATP (39). This result contrasts with observations from glycerinated smooth muscle, where myosin is rapidly lost in Ca^{2+}-free media (11). In isolated intestinal brush border 80% of the myosin is lost on incubation with ATP in Ca^{2+}-free media; however, maximal contraction is still possible upon addition of Ca^{2+} (28). We cannot rule out the possibility that similar partial extraction occurs in lysed cones but does not compromise the cone's ability to contract. Our earlier studies using myosin subfragment 1 and heavy meromyosin indicate that in 18 min sufficient amounts of these agents diffused into the models to decorate the cone actin filaments, and when N-ethylmaleimide-modified S1 and heavy meromyosin were used, to block Ca^{2+}-induced contraction (40). Thus one might expect that if cone myosin were present as soluble monomers in the folded configuration, some of it might be lost during 90 min of incubation with ATP and EGTA. Further work is clearly necessary before we fully understand this component of regulation.

The failure of cAMP to inhibit Mg^{2+}-induced cone contraction is consistent with its failure to inhibit contraction

induced by unregulated MLCK. In both cases the step involving binding of Ca^{2+}/calmodulin to MLCK to activate myosin light-chain phosphorylation was circumvented, in the first case by using permanently activated MLCK and in the second by circumventing myosin light-chain phosphorylation altogether. Together these observations strongly argue that in the lysed cone models, the mechanism by which cAMP interferes with contraction entails activation of a kinase that phosphorylates MLCK and inhibits its activity. These observations do not rule out the possibility that in intact cones, cAMP might have other modes of inhibition in addition to phosphorylation of MLCK. For example, cAMP might act indirectly to inhibit cone contraction by influencing the cytoplasmic Ca^{2+} concentration, as has been reported for cardiac and smooth muscle (16).

In conclusion, we have shown that lysed cone models can be induced to contract in the absence of Ca^{2+} by incubating them with unregulated MLCK or high Mg^{2+}. These observations argue that other Ca^{2+}-mediated events such as thin filament regulation, are not required for activating contraction in cone models. The failure of cAMP to inhibit contraction under these conditions suggests that in lysed cone models, cAMP acts by catalyzing the phosphorylation of MLCK and thus inhibiting its activation by Ca^{2+}/calmodulin.

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