Regulation of Reactivated Elongation in Lysed Cell Models of Teleost Retinal Cones by cAMP and Calcium

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Abstract. Teleost retinal cones elongate in the dark and contract in the light. In isolated retinas of the green sunfish *Lepomis cyanellus*, cone myoids undergo microtubule-dependent elongation from 5 to 45 μm. We have previously shown that cone contraction can be reactivated in motile models of cones lysed with Brij-58. Reactivated contraction is both actin and ATP dependent, activated by calcium, and inhibited by cAMP. We report here that we have obtained reactivated cone elongation in lysed models prepared by the same procedures. Reactivated elongation is ATP dependent, activated by cAMP, and inhibited by calcium. The rate of reactivated elongation is proportional to the cAMP concentration between 10 μM and 0.5 mM, but is constant between 10 μM and 1.0 mM Mg-ATP. No elongation occurs if cAMP or Mg-ATP concentration is ≤5 μM. Mg-ATP is required for both cAMP-dependent and cAMP-independent processes, suggesting that Mg-ATP is required both for a regulatory process entailing cAMP-dependent phosphorylation and for a force-producing process. Free calcium concentrations $\geq 10^{-7}$ reduce the elongation rate by 78% or more, completely inhibiting elongation at 10^{-5} M. This inhibition is not due to competition from calcium-activated contraction. Cytochalasin D blocks reactivated contraction, but does not abolish calcium inhibition of reactivated elongation. Thus calcium directly affects the elongation mechanism. Calcium inhibition is calmodulin dependent. The calmodulin inhibitor trifluoperazine abolishes calcium inhibition of elongation. Furthermore, calcium blocks elongation only if present during the lysis step; subsequent calcium addition has no effect. However, if calcium plus exogenous calmodulin are subsequently added, elongation is again inhibited. Thus calcium inhibition appears to require a soluble calmodulin which is lost shortly after lysis.

CELL elongation plays an important role in the life of many cell types. Although it is now generally accepted that microtubules are required for many cell shape changes involving elongation of the cell or of cellular projections, the mechanism and regulation of cell elongation are still not fully understood. Two mechanisms have been suggested for motive force production during elongation: microtubule sliding by means of cross-bridges containing the ATPase dynein (12, 13, 62, 71), and microtubule elongation by the assembly of soluble tubulin subunits (29, 60, 67). Neither of these mechanisms has been ruled out for any example of cell elongation.

In our laboratory we have been using teleost retinal cones to investigate the mechanisms and regulation of elongation and contraction in nonmuscle cells (8–11, 16, 17, 50, 51, 71). In teleosts, photoreceptors change shape in response to changes in light intensity. Cones elongate in the dark and contract in the light. Cone contraction and elongation are mediated by the necklike myoid region of the cell (Fig. 1). The extent of myoid length change is dramatic, changing in vivo from 5 μm in the light to as much as 90 μm in the dark. Complications resulting from cell–cell or cell–substrate attachments are minimized since the motile myoid regions of the cones project freely into the subretinal space (Fig. 1).

We have previously reported that cone elongation is microtubule dependent, and that cAMP and calcium appear to play roles in its regulation (8, 10, 16, 71). Cone elongation can be induced in isolated intact retinas by treatments which elevate cytoplasmic cAMP and inhibited by those which elevate calcium (8, 10, 16). Opposite cAMP and calcium conditions are required for cone contraction. In isolated intact retinas, cone contraction is activated by treatments that elevate cytoplasmic calcium and inhibited by those that elevate cAMP (16, 17). We have also reported extensive studies of reactivated cone contraction in motile models lysed with 1% Brij-58 (11, 31, 50, 51). Reactivated cone contraction is actin and ATP dependent, activated by calcium, and inhibited by cAMP. Thus, cone myoid length in the green sunfish appears to be regulated by antagonistic actions of cAMP and calcium.

To examine the regulation of cone elongation in more detail we have developed lysed cell models of teleost cones which will undergo reactivated elongation. We have obtained reactivated elongation in cone models prepared by the same lysis procedures as those used previously for contraction models. Thus we can directly contrast conditions required for elongation with those required for contraction. In this report,

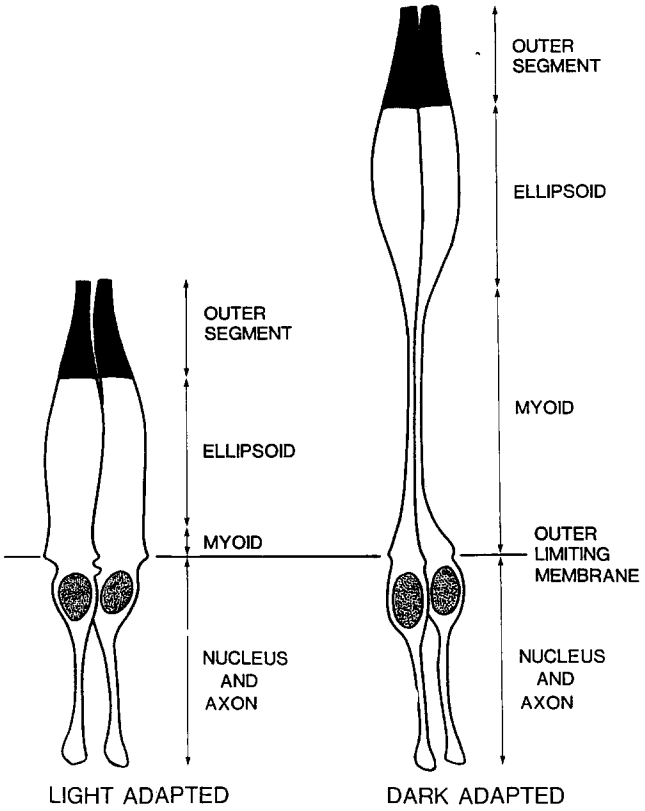

Figure 1. Schematic illustration of light-adapted (contracted) and dark-adapted (elongated) double cones indicating cell landmarks.

we have used lysed cone models to investigate the roles of cAMP, calcium, and calmodulin in regulating cone elongation.

Materials and Methods

Animals

All experiments were carried out on retinas from the green sunfish, *Lepomis cyanellus*. Fish were obtained from local venders and maintained in outdoor ponds prior to use. To minimize the effects of circadian rhythms on cone myoid length (8, 40) all experiments were performed between 10 A.M. and 2 P.M. To facilitate the removal of retinas free of pigment epithelium, fish were dark adapted in an aerated dark box for 20–40 min before they were killed. Thus cones were partially elongated at the time of dissection.

Preparation of Retinas

All procedures were performed under room light unless otherwise noted. Eyes of dark adapted fish were enucleated, hemisected, and the retinas isolated using a stream of oxygenated (95% O_2, 5% CO_2) modified Earle's balanced salt solution containing 24 mM sodium bicarbonate, 25 mM glucose, 3 mM Hepes buffer, and 1 mM ascorbic acid, pH 7.4 (EBSS).[1] Each retina was bisected along the choriod fissure to obtain four comparable half-retinas per fish.

In initial experiments half-retinas were isolated from dark-adapted fish and incubated in EBSS for 30–60 min to produce fully light-adapted, short cones of uniform length. Isolated half-retinas were placed vitreous down in 0.5 ml of medium in wells of a 24-well tissue culture tray (Costar, Cambridge, MA). Medium volume was immediately decreased to 0.2 ml, and the dish placed in a clear plastic box on a rotating platform and gassed with 95% O_2, 5% CO_2. Fully contracted cones did not lyse, so it was necessary to develop methods which produced slight cone elongation while maintaining a uniform cone myoid length. Three different methods were used. Each of these methods produced cones of 15 to 20 μm myoid length that were more consistently lysed. The behavior of lysed cone models obtained from retinas prepared using these three procedures was identical.

In the first method, half-retinas were isolated from dark-adapted fish and incubated in the light for 30–60 min in EBSS containing 10^{-4} M free Ca^{++}, 1 mM free Mg^{++}, and 1 mM EGTA to induce full cone contraction. These light-adapted half-retinas were then incubated in Ca^{++}-free ($<10^{-8}$ M free Ca^{++}) EBSS containing 1 mM free Mg^{++} and 1 mM EGTA for 30 min. Incubation of retinas in Ca^{++}-free medium induced a slight cone elongation (16).

In the second method, half-retinas were isolated from dark-adapted fish and incubated in the light for 30–60 min in EBSS containing 1.8 mM Ca^{++} and 0.8 mM Mg^{++} to induce full cone contraction. These light-adapted half-retinas were then incubated in EBSS containing 0.2 to 1.0 mM 3-isobutyl-1-methylxanthine (IBMX) for 30–60 min. IBMX, a phosphodiesterase inhibitor, induced slight cone elongation at these concentrations. To prevent the transfer of Ca^{++} to the lysis medium, half-retinas were washed in Ca^{++}-free EBSS for ~2 min.

In the third method, fish were sacrificed after 18–25 min of dark adaptation during which time cones elongated to the appropriate myoid length. Of the first two methods, the second was more consistent in producing cones of the desired length. The third method was also effective and had the advantage of being less time consuming. Since it was discovered late in the project, all three methods have been used to obtain the data presented in this paper.

Reactivation of Elongation in Lysed Cone Models

Just before lysis, one half-retina from each fish was fixed for determination of initial cone length (L_o). The remaining three half-retinas were incubated for 3 min in reactivation media containing 1% Brij-58 (polyoxethylene 20 cetyl ether; Sigma Chemical Co., St. Louis, MO), a non-ionic detergent. Half-retinas were then transferred to detergent-free reactivation media and incubated for an additional 15 min. Reactivation media contain 0.1 M Pipes buffer, pH 6.94, 10^{-8} M free Ca^{++}, 1 mM free Mg^{++}, 10 mM EGTA, 1 mM Mg-ATP, and 1 mM cAMP (Table I). Reactivation incubations were carried out in 0.5 ml of medium on a platform vibrator. Reactivation media were modified from media used previously for reactivation of cone contraction (11) by the addition of cAMP and the lowering of the free Ca^{++} concentration. Otherwise the protocols for reactivation of elongation and contraction are identical.

Half-retinas were fixed in 6% glutaraldehyde (Ted Pella, Inc., Tustin, CA) in 0.1 M phosphate buffer, pH 7.0, for 1 h or more. Small blocks were cut from the fundic region of each half-retina and chopped with a manual tissue chopper for examination with Nomarski optics as described elsewhere (11). Cone myoid length was measured as the distance from the base of the cone ellipsoid to the outer limiting membrane (Fig. 2). Extent of cone elongation ($L_f - L_o$) was determined by subtracting the initial cone myoid length (L_o) from the final cone myoid length after reactivation (L_f) measured from half-retinas from the same fish. Data are presented as the mean ± the standard error of the mean (SEM), where n is the number of half-retinas examined. The significance of the difference between control and experimental group means was determined using a two-tailed Student's t test.

Cone Elongation Rate Studies

Rates of reactivated cone elongation were compared to those for elongation in unlysed retinas in vitro, and elongation in vivo in response to dark onset. For reactivated cone elongation, the rate was determined by fixation of retinas at indicated time intervals after the initiation of lysis in elongation medium. Indicated times include the 3-min step in detergent plus subsequent detergent-free incubation periods.

Cone elongation was induced in unlysed retinas in vitro by incubation in dibutyryl cAMP plus IBMX. It has previously been shown that treatments which elevate cAMP levels induce cone elongation in light-adapted retinas (10).

Table I. Ca^{++}/EGTA Buffer Compositions

EGTA	Added $MgSO_4$	Added $CaCl_2$	Calculated free Mg^{++}	Calculated free Ca^{++}
mM	*mM*	*mM*	*mM*	*M*
10.0	1.33	—	1.0	$<10^{-8}$
10.0	1.33	0.17	1.0	10^{-8}
10.0	1.28	1.51	1.0	10^{-7}
10.0	1.12	6.40	1.0	10^{-6}
10.0	1.02	9.48	1.0	10^{-5}

Calculated free Ca^{++} and Mg^{++}concentrations are based on Steinhardt et al. (61) assuming an association constant for EGTA and Ca^{++} at $10^{10.7}$. pH = 6.94.

[1] *Abbreviations used in this paper:* EBSS, modified Earle's balanced salt solution containing 24 mM sodium bicarbonate, 25 mM glucose, 3 mM Hepes buffer, and 1 mM ascorbic acid, pH 7.4; IBMX, 3-isobutyl-1-methylxanthine; TFP, trifluoperazine.

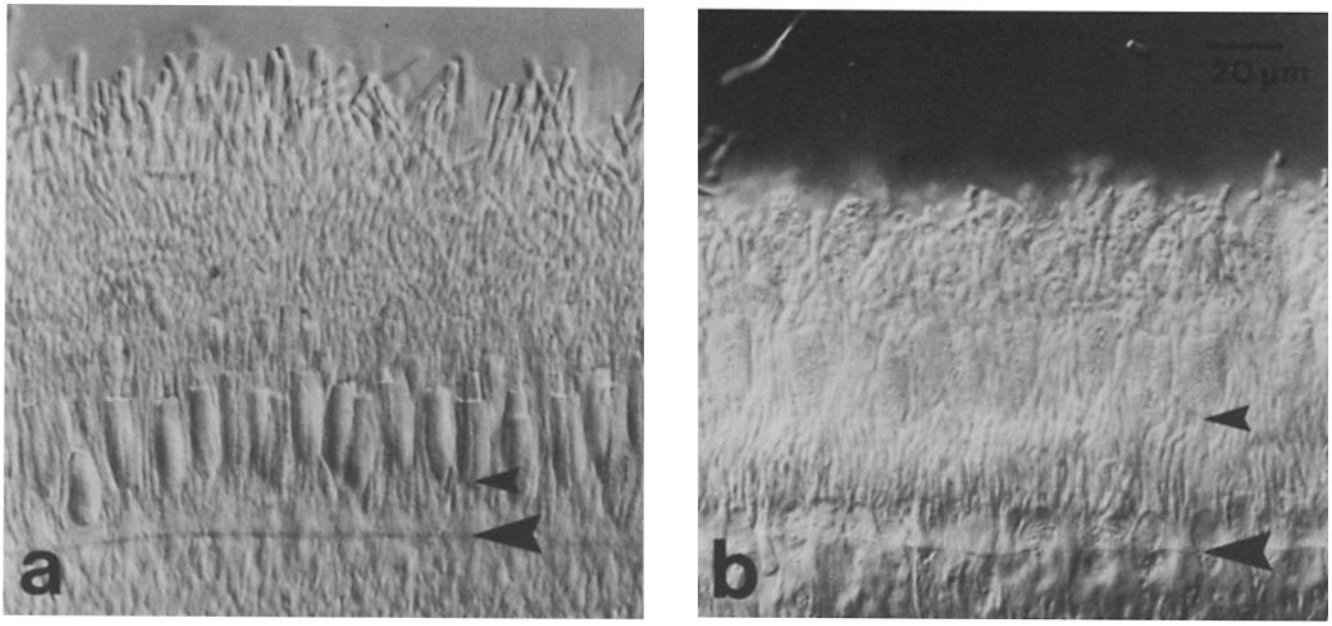

Figure 2. Light micrographs of retinal slices observed with Nomarski optics. (*a*) Half-retinas fixed before lysis to determine initial cone myoid length (L_o). (*b*) Half-retinas fixed after the 3-min lysis/15-min reactivation protocol to determine final cone myoid length after reactivated cone elongation (L_f). Cone myoid length was measured as the distance from the base of the ellipsoid (small arrows) to the outer limiting membrane (large arrows). Bar, 20 μm.

Half-retinas were light adapted as described in method two above. Half-retinas were then incubated in EBSS containing 2.5 mM dibutyryl cAMP, 0.1 mM IBMX, 10^{-6} M free Ca^{++}, 1 mM free Mg^{++}, and 1 mM EGTA for indicated time intervals before fixation.

For analyzing cone elongation rates in vivo, fish were killed after dark adaptation for indicated time intervals. Retinas were isolated using Ca^{++}-free EBSS and fixed immediately, or were fixed in the eyecup after dissection under dim red light. Rates were calculated using linear regression on the mean values for each time point.

cAMP and ATP Dose–Response Studies

cAMP and ATP were purchased from Sigma Chemical Co. (St. Louis, MO) and from Boehringer-Mannheim Biochemicals (Indianapolis, IN). For all dose–response studies, the specified concentrations were present in both the 3-min lysis and the 15-min reactivation steps. In each case, the ATP concentration was matched by equimolar concentrations of $MgSO_4$, in excess of the buffered free Mg^{++} concentration of 1 mM.

Ca^{++} Dose–Response Studies

Ca^{++}/EGTA buffers were prepared with 10 mM EGTA according to formulae from Steinhardt et al. (61) (Table I). The specified concentrations were present during both the 3-min lysis and the 15-min reactivation steps.

Reactivation of Contraction

Experiments were performed under infrared light with an infrared converter as previously described (11, 50, 51). Fish were dark adapted for 30–50 min and retinas isolated using Ca^{++}-free EBSS. One half-retina was fixed immediately for determination of initial cone myoid length before lysis (L_o). A 3-min lysis/15-min reactivation protocol as described for reactivated elongation was used. Contraction media contain 0.1 M Pipes buffer, pH 6.94, 10^{-5} M free Ca^{++}, 1 mM free Mg^{++}, 10 mM EGTA, 1 mM Mg-ATP, and no cAMP (Table I). Extent of contraction ($L_o - L_f$) was determined by subtracting the final cone myoid length after reactivation (L_f) from the initial cone myoid length (L_o) measured from the same fish.

Cytochalasin D Studies

Cytochalasin D was purchased from Sigma Chemical Co. and prepared as a 1 mg/ml stock solution in dimethyl sulfoxide. Cytochalasin D was added to reactivation media to a final concentration of 5 μg/ml (0.5% dimethyl sulfoxide) for both the 3-min lysis and the 15-min reactivation steps.

Calmodulin Inhibitor Studies

Trifluoperazine (TFP) was a gift from Smith, Kline, and French in Philadelphia, PA. TFP was prepared as a 5-mM stock solution in distilled water and added to reactivation media to a final concentration of 10 μM for both the 3-min lysis step and the 15-min reactivation step.

Calmodulin Studies

Calmodulin was purchased from Calbiochem-Behring Corp. (San Diego, CA) and prepared as a 1 mg/ml stock solution in distilled water and stored at −80°C. Calmodulin was added to the elongation medium to a final concentration of 10^{-6} M for the 15-min reactivation step only.

Electron Microscopy

The fixation procedure used for ultrastructural studies was adapted from a protocol by McDonald (42). Half-retinas were fixed in elongation media containing 2% glutaraldehyde (TAAB; Redding, England) for 30 min following a 3-min lysis and 10-min reactivation protocol. Small blocks from the fundic region of each half-retina were postfixed in 0.8% $K_3Fe(CN)_6$ in 0.1 M Pipes buffer, pH 6.94, for 30 min, and in 0.5% OsO_4 added to the same solution for an additional 30 min at room temperature. Retinas were en bloc stained in 5% aqueous uranyl acetate for 2 h in the dark, dehydrated in graded acetones, and embedded in Polybed 812. Blocks were sectioned perpendicular to cone long axis, and the thin sections stained with uranyl acetate and lead citrate and viewed with a JEOL 100S electron microscope.

Results

Reactivation of Elongation in Lysed Cone Models

Lysed cell models of teleost cones were induced to undergo a 16–20-μm elongation when lysed with Brij-58 and incubated with cAMP and ATP (Fig. 3). When the detergent was deleted from the media, cones were not lysed and elongated only ~4 μm. When either cAMP or ATP was deleted from the medium, cones elongated only 4–5 μm as observed in the absence of lysis (Fig. 3). Thus lysed cell models of cones exhibit >10 μm of cAMP- and ATP-dependent reactivated elongation.

We observed some range in the maximum extent of reactivated cone elongation, depending both on the source of the

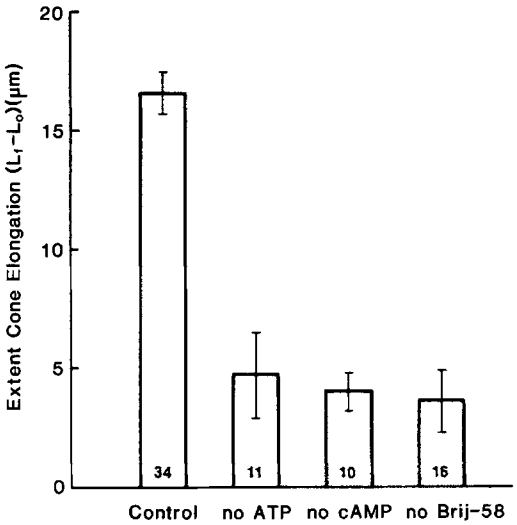

Figure 3. Requirements for reactivated cone elongation. If ATP, cAMP, or the detergent Brij-58 is omitted from the elongation media, cones elongate <5 μm. If cones are lysed with 1% Brij-58 in elongation medium containing 1 mM cAMP, 1 mM Mg-ATP, and 10^{-8} M free Ca^{++}, cones elongate >15 μm.

fish and on seasonal variability. For this reason, all experimental protocols include a positive control for each fish in which elongation media contained 1 mM cAMP and 1 mM Mg-ATP. Only those fish whose cones elongated >10 μm in the positive control sample were used to compile the data presented in this paper. Maximum extents of cone elongation in the positive controls ranged from 16–20 μm in the different experimental groups.

Ultrastructural examination of lysed cone models showed that after detergent lysis, the extent of cytoplasmic extraction was extensive (Fig. 4). Although the plasma membranes appeared relatively intact, we have previously shown that membranes were sufficiently permeabilized by this lysis protocol to allow entry of myosin subfragment-1 (molecular weight 100,000) (51). Microtubules and actin filaments were still present in similar number and distribution to those seen in unlysed preparations. Microtubules are longitudinally oriented in the motile myoid region of the cone models. Cross-sections revealed that cones contain between 100 and 300 microtubules per myoid. The microtubules of these cone models are stable in the presence of calcium. They were not disrupted after an 18- or 30-min incubation in medium containing 10^{-5} M free Ca^{++} (11; Porrello, K., and B. Burnside, unpublished data).

Rates of Cone Elongation

The rates of cone elongation are illustrated in Fig. 5. Reactivated cone elongation was initiated at lysis and proceeded at a constant rate of 2.0 μm/min. This rate was faster than the 0.9-μm/min rate observed in unlysed retinas in vitro. In this study, the rate of cone elongation observed in vivo (1.6 μm/min) fell between these two rates. However, in other studies in this laboratory, in vivo rates have been observed to range between 1 and 2 μm/min depending on the season and the source of the fish (8; and Gilson, C. A., unpublished data). Thus under our reactivation conditions, lysed cone models elongate at the fastest rates that we have observed in vivo.

Both lysed cone models and unlysed cones of isolated retinas in vitro elongated to similar maximal lengths (40 to 50 μm) (Fig. 5). This cone myoid length is less than that observed in vivo where cone myoid length can reach 70–90 μm under optimal conditions (i.e., in the dark in the middle of the night). Although we do not know why final cone myoid lengths are shorter in vitro, we have consistently observed this 40–50-μm maximum length under all conditions that we have used to induce cone elongation in vitro (10, 16).

There is a lag period of ~10 min before the onset of cone elongation in unlysed retinas in vitro and in retinas in vivo. However, elongation starts immediately upon lysis in the cone models.

cAMP Requirement for Reactivated Cone Elongation

Figs. 6 and 7 illustrate the effect of cAMP concentration on reactivated cone elongation. Results indicate that cAMP concentration affects the rate, rather than the final extent of reactivated cone elongation. Although cones have not fully elongated within 18 min in media containing 10 μM cAMP (Fig. 6), cones are still able to maximally elongate in this medium if allowed sufficient time (17.1 ± 1.9 μm, $n = 6$, in 33 min). If cAMP concentration was ≤5 μm, reactivated elongation did not occur. The maximum rate of cone elongation (2.0 μm/min) was observed at cAMP concentrations ≥0.5 mM, while at concentrations between 5 μM and 0.5 mM, the rate of elongation was proportional to the cAMP concentration.

Transient exposure to cAMP was sufficient to support the maximal subsequent elongation response. When 1 mM cAMP was present only during the initial 3-min lysis step, followed by 15 min in cAMP-free reactivation medium, cones elongated 19.4 ± 1.8 μm ($n = 5$). In control samples in which cAMP was present during both the 3-min lysis step and the 15-min reactivation step, cone elongation was 18.8 ± 1.7 μm ($n = 4$). These extents of elongation are not significantly different ($P > 0.8$).

Extended incubation of lysed cone models in the absence of cAMP inhibits the subsequent elongation response upon the addition of cAMP. When cones were lysed for 3 min in medium containing 1 mM Mg-ATP but no cAMP, followed by a 30-min incubation in similar, detergent-free medium, cones elongated only 5.6 ± 3.2 μm ($n = 6$). When cones were incubated an additional 18 min in medium containing 1 mM cAMP plus 1 mM Mg-ATP after the 3-min lysis/30-min incubation described above, cones elongated 11.0 ± 3.1 μm ($n = 7$). This elongation extent is significantly reduced from that observed following a 3-min lysis/15-min incubation protocol in media containing cAMP plus Mg-ATP (18.2 ± 2.1 μm; $n = 7$) ($P = <0.02$). However, when cones were lysed for 3 min in medium containing no cAMP, followed by 15 min in medium containing cAMP, the full elongation response was still observed (16.4 ± 1.5 μm; $n = 8$). Thus it appears that cAMP need not be present at the time of lysis to induce full elongation, but that the ability to respond to cAMP has diminished after 30 min.

cGMP was significantly less effective than cAMP for inducing reactivation of cone elongation. In reactivation media containing 1 mM cGMP (no cAMP), cones elongated 8.3 ± 1.7 μm ($n = 6$) in 18 min. This elongation is significantly less ($P < 0.001$) than that observed in media containing 1 mM cAMP (18.5 ± 0.9 μm; $n = 6$). However, it does represent a

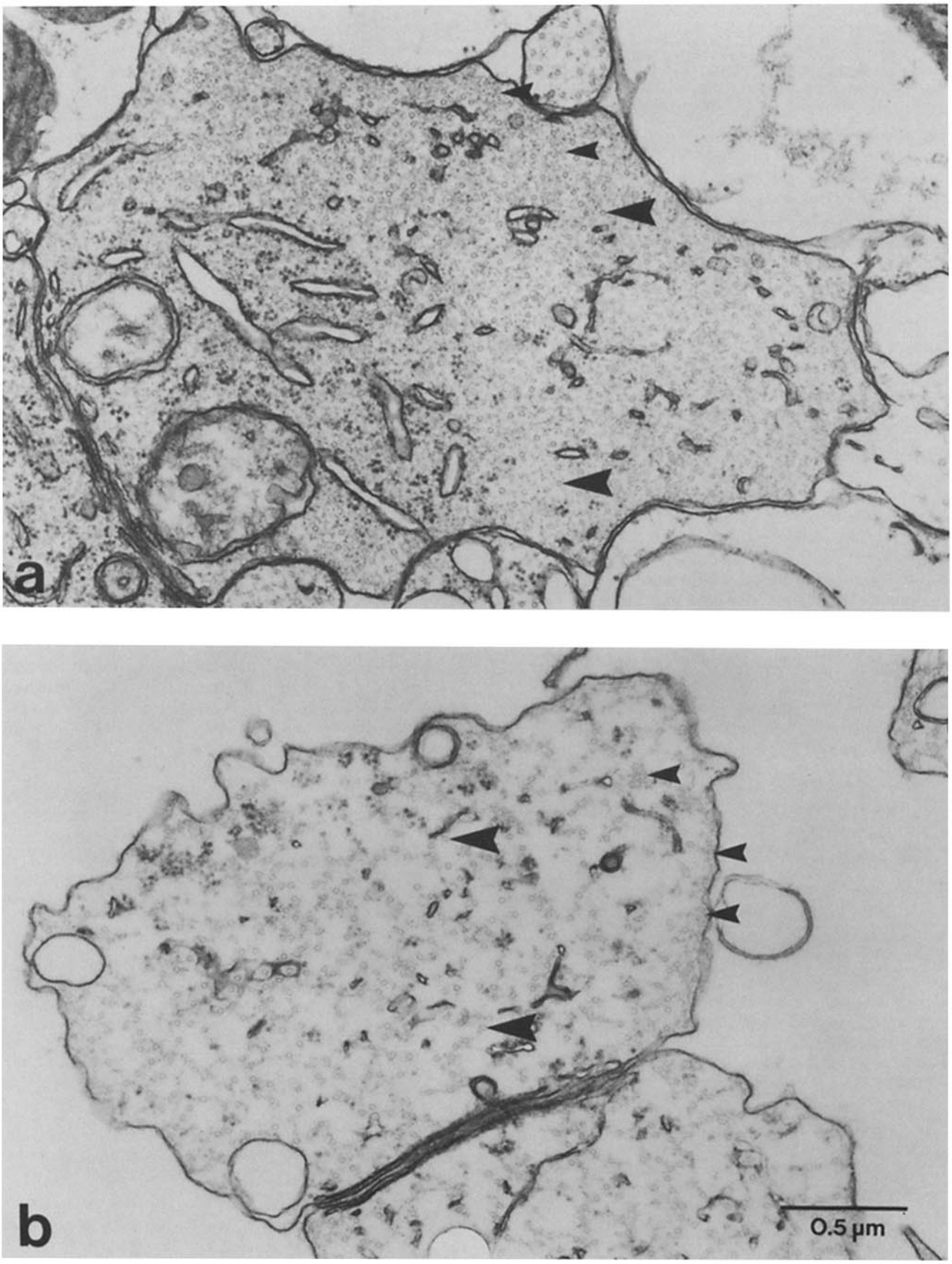

Figure 4. Electron micrographs of green sunfish cone myoids fixed (*a*) during dark-induced cone elongation in vivo and (*b*) during reactivated cone elongation after a 3-min lysis and a 10-min reactivation protocol. Cone myoids are shown in cross section. Extensive cytoplasmic extraction is evident after cell lysis. Numerous microtubules (large arrows) and actin filaments (small arrows) are present in both the lysed and unlysed preparations. Bar, 0.5 μm.

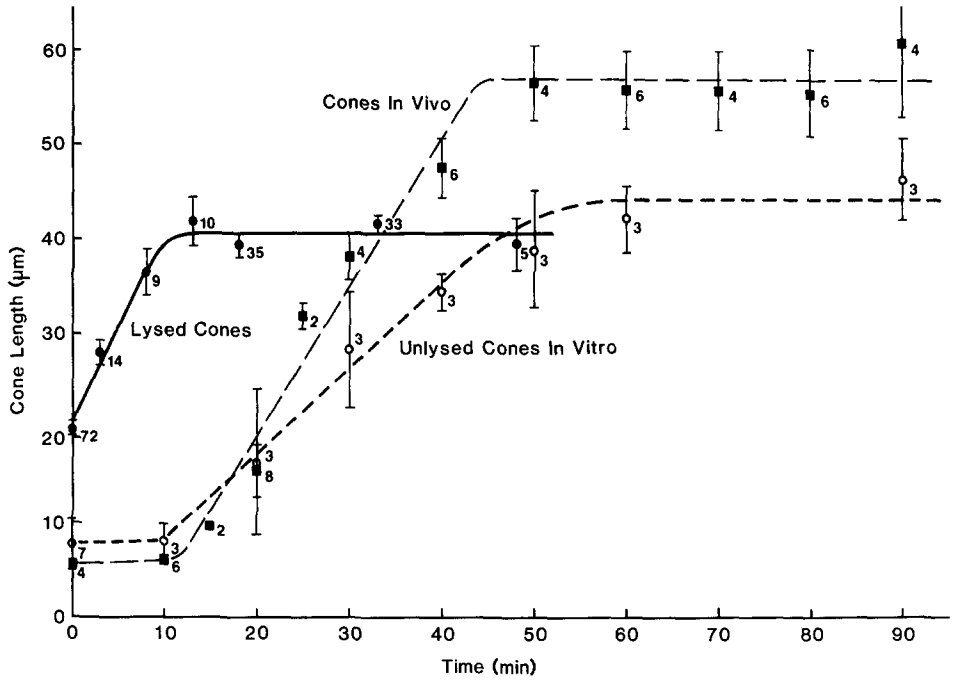

Figure 5. Rates of cone elongation observed for reactivated cone elongation (●), and for cone elongation in unlysed retinas in vitro (○), and in retinas in vivo (■). For cone models reactivation media contained 1 mM cAMP, 1 mM Mg-ATP, and 10^{-8} M free Ca^{++}. Cone elongation was induced in unlysed retinas in vitro by incubation in EBSS containing 2.5 mM dibutyryl cAMP plus 0.1 mM IBMX, and in vivo by exposure to darkness. Rates were calculated using linear regression on the mean values for each time point. Elongation rates were 2.0 μm/min (r = 0.99), 0.9 μm/min (r = 0.99), and 1.6 μm/min (r = 0.97), respectively.

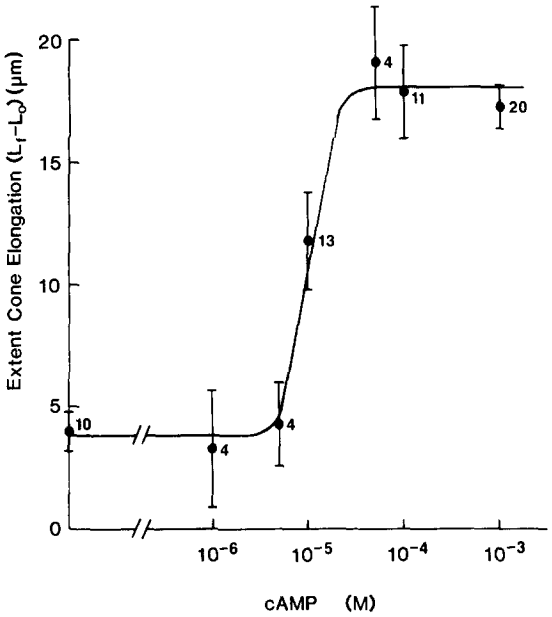

Figure 6. The effect of cAMP concentration on the extent of reactivated cone elongation in 18 min. Reactivation media contained 1 mM Mg-ATP and 10^{-8} M free Ca^{++}.

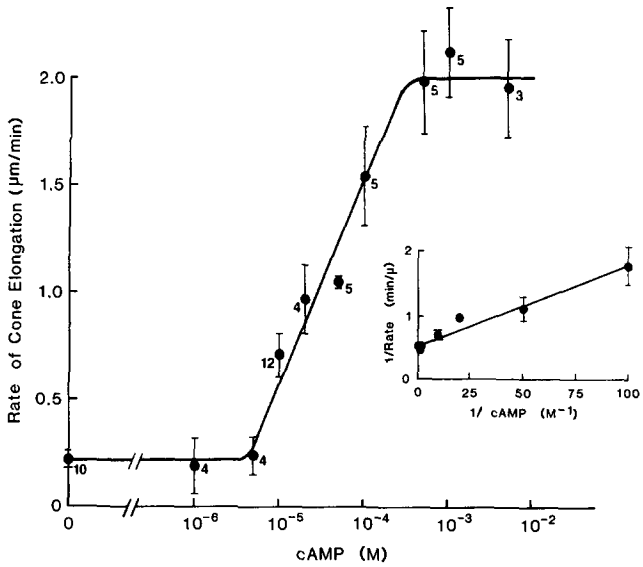

Figure 7. The effect of cAMP concentration on the rate of reactivated cone elongation. The maximum rate of elongation (2.0 μm/min) was observed at cAMP concentrations ≥0.5 mM, while at concentrations between 5 μM and 0.5 mM the rate of elongation was proportional to the cAMP concentration. Although rates are indicated for cAMP concentrations ≤5 μM, no reactivated elongation occurred at these concentrations. The values indicated represent the 5-μm movement seen under all experimental protocols. The cAMP concentration required to obtain one-half the maximum rate of cone elongation was calculated to be 23 μM using linear regression on the double reciprocal plot (r = 0.99). Elongation media contained 1 mM Mg-ATP and 10^{-8} M free Ca^{++}.

partial elongation over that observed in the absence of cAMP or cGMP (3.1 ± 2.4 μm; n = 5).

The concentrations of cAMP which induced reactivated cone elongation (≥10 μM) have previously been shown to inhibit reactivated cone contraction (50). In fact, cAMP concentrations as low as 0.1 μM have been found to inhibit reactivated cone contraction (9).

ATP Requirement for Reactivated Cone Elongation

Fig. 8 illustrates the effects of Mg-ATP concentration on the extent of reactivated cone elongation observed in 18 min. While maximum reactivated elongation occurred if the Mg-ATP concentration was ≥50 μM, only partial elongation was observed in 10 μM Mg-ATP.

To determine whether 10 μM Mg-ATP could support maximal elongation if allowed sufficient time, we measured the extent of cone elongation after a 33-min incubation in media

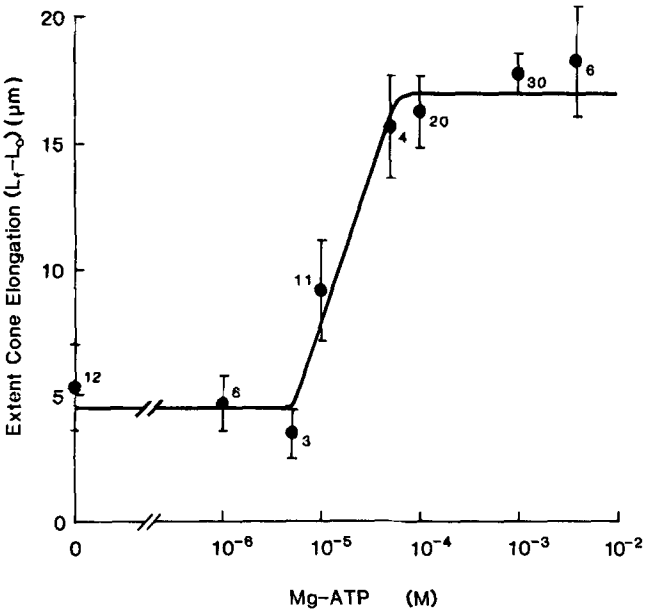

Figure 8. The effect of Mg-ATP concentration on the extent of reactivated cone elongation in 18 min. Reactivation media contained 1 mM cAMP and 10^{-8} M free Ca^{++}.

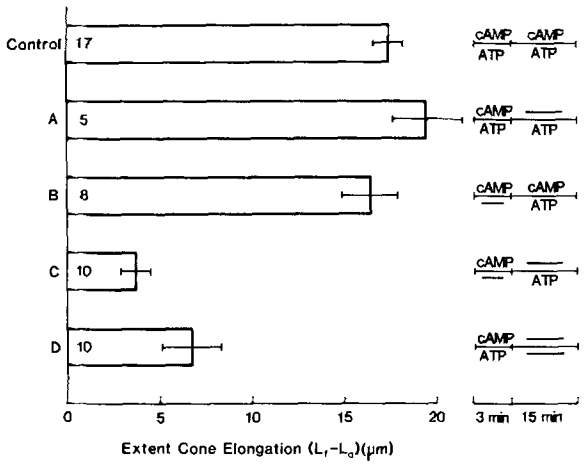

Figure 9. The effects of eliminating Mg-ATP from either the 3-min lysis step or the 15-min reactivation step. (*A*) Cones were lysed for 3 min in medium containing cAMP plus Mg-ATP, followed by 15 min in medium containing Mg-ATP only. (*B*) Clones were lysed for 3 min in medium containing cAMP only, followed by 15 min in medium containing both cAMP and Mg-ATP. (*C*) Cones were lysed for 3 min in medium containing cAMP only, followed by 15 min in medium containing Mg-ATP only. (*D*) Cones were lysed for 3 min in medium containing cAMP plus Mg-ATP, followed by 15 min in medium containing neither cAMP nor Mg-ATP. In each case, cAMP and Mg-ATP concentrations were 1 mM when present.

containing 10 μM Mg-ATP. Results indicate that Mg-ATP concentration limits the extent of reactivated cone elongation. After 33 min in 10 μM Mg-ATP, cones were no longer than they were after 18 min (9.1 ± 1.5 μm, n = 6; and 9.2 ± 2.0 μm, n = 11, respectively).

To determine whether the limit on the extent of cone elongation in 10 μM Mg-ATP was due to depletion of Mg-ATP prior to completion of cone elongation, we measured the extent of cone elongation after 18 min in medium containing 10 μM Mg-ATP plus an additional 15 min in fresh medium also containing 10 μM Mg-ATP. The extent of elongation seen after this additional 15 min was double that seen in the initial 18 min (24.5 ± 0.3 μm, n = 3; and 12.5 ± 1.9 μm, n = 3; respectively). Thus the limitation on the extent of cone elongation by 10 μM Mg-ATP observed in 18 min was due to a depletion of Mg-ATP, and cone elongation was able to proceed to the maximum extent if Mg-ATP was replenished.

To determine whether Mg-ATP concentration affects the rate of reactivated cone elongation, we compared the rates of elongation in media containing either 10 μM or 1 mM Mg-ATP. The same rate of cone elongation was observed in both media (n = 4). Since no reactivated elongation occurs if Mg-ATP concentration is ≤5 μM it appears that varying the Mg-ATP concentration does not affect the rate of reactivated cone elongation.

Since cAMP is required for reactivated cone elongation, Mg-ATP is likely to be required for a cAMP-dependent phosphorylation regulatory step. Thus it is not clear from the observations reported so far that Mg-ATP is required as an energy source for force production. To further clarify the role of Mg-ATP, we eliminated Mg-ATP from the cAMP-dependent 3-min lysis step, or from the subsequent cAMP-independent 15-min reactivation step (Fig. 9). When cAMP was present during the 3-min lysis step but deleted from the 15-min incubation, the full elongation response was observed (Fig. 9*a*). Thus cAMP need not be present during the 15-min reactivation step to induce full cone elongation. When Mg-ATP was deleted from the 3-min lysis step but present during the 15-min incubation the full elongation response was observed (Fig. 9*b*). Thus the absence of Mg-ATP from the first 3-min incubation step does not prevent cone elongation. However, when Mg-ATP was deleted from the 3-min lysis step and cAMP was deleted from the 15-min incubation, no reactivated elongation was observed (Fig. 9*c*). Thus cAMP and Mg-ATP must be present at the same time to induce cone elongation. Therefore, Mg-ATP is required for a cAMP-dependent role.

When both cAMP plus Mg-ATP were present during the 3-min lysis step but deleted from the 15-min incubation, no reactivated elongation was observed past that expected to occur during the first 3 min when both cAMP and Mg-ATP were present (Fig. 9*d*). Since the full elongation response is observed if the second, 15-min incubation contains Mg-ATP only (no cAMP) (Fig. 9*a*), Mg-ATP must be required to induce full elongation even in the absence of cAMP. Thus Mg-ATP is also required for a cAMP-independent role.

Calcium Inhibition of Reactivated Cone Elongation

Figs. 10 and 11 illustrate the effects of free calcium concentration on reactivated cone elongation. Maximum reactivated cone elongation occurred if free calcium concentration was ≤10^{-8} M. Complete inhibition was observed at 10^{-5} M free Ca^{++} (cones elongate 4–5 μm under all reactivation conditions observed so far). Comparison of the extents of cone elongation after an 18- or a 33-min incubation in media containing 10^{-7} M free Ca^{++} indicated that increased free calcium concentration limited the rate, not the extent, of reactivated cone elongation. Cones elongated 13.4 ± 1.4 μm (n = 3) in 33 min. This extent was greater than that observed in 18 min (8.4 ± 2.1 μm; n = 3) but did not represent the full elongation

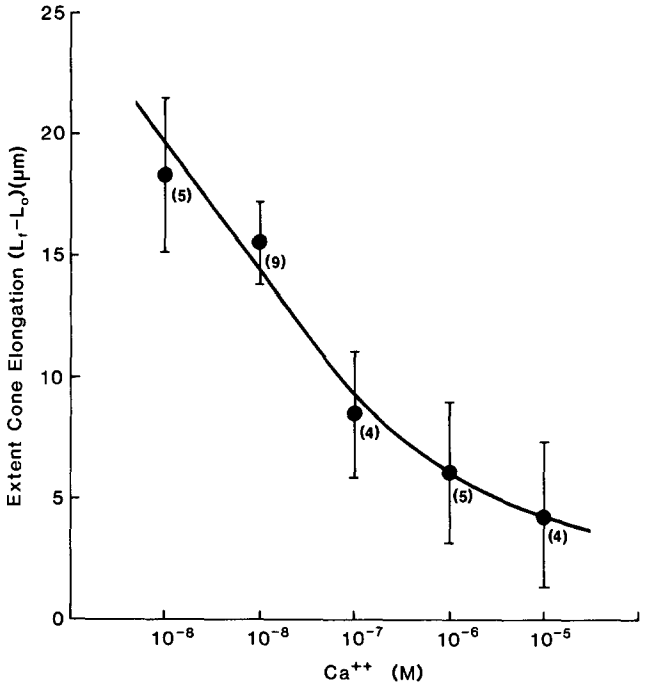

Figure 10. The effects of free calcium concentration on the extent of inhibition of reactivated cone elongation in 18 min. Reactivation media contained 1 mM cAMP and 1 mM Mg-ATP. Free Ca^{++} concentrations were based on a formula for Ca^{++}/EGTA buffers from Steinhardt et al. (61) (Table I).

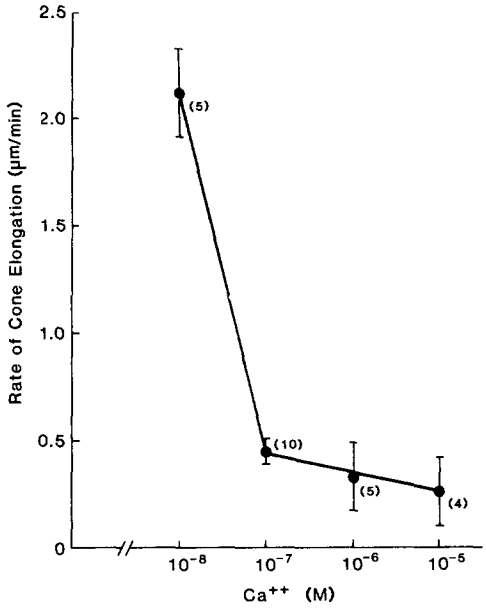

Figure 11. The effects of free calcium concentration on the rate of reactivated cone elongation. The rate of reactivated cone elongation was decreased 78–87% when the free Ca^{++} concentration was $\geq 10^{-7}$ M. The rates observed in 10^{-7} and 10^{-5} M free Ca^{++} were not significantly different ($P = 0.05$).

response observed in media containing 10^{-8} M free Ca^{++} (16.6 ± 0.7 μm; $n = 3$). The rates of cone elongation calculated for the 18- and 33-min samples in media containing 10^{-7} M free Ca^{++} were not significantly different from each other (0.46 ± 0.14 μm/min and 0.41 ± 0.05 μm/min, respectively; $P = 0.8$), but represented a considerable decrease from the maximum rate possible (2.0 μm/min).

Cytochalasin D Studies

Inhibition of cone elongation occurs at the same free calcium concentrations which have been shown elsewhere to produce reactivated cone contraction (50). To investigate the possibility that calcium inhibition of cone elongation could be mediated by initiation of a competing calcium-induced cone contraction, cytochalasin D was used to eliminate the capability of the models to contract. When dark-adapted retinas containing long cones were subjected to the 3-min lysis/15-min reactivation protocol in contraction media, cones contracted 18.2 ± 2.2 μm ($n = 5$). However, when 5 μg/ml cytochalasin D (with 0.5% dimethyl sulfoxide) was added to the contraction media, contraction was blocked (2.3 ± 2.4 μm; $n = 5$). Adding 0.5% dimethyl sulfoxide alone had no effect (19.1 ± 0.7 μm; $n = 4$). Thus, cytochalasin D blocks reactivated cone contraction. When light-adapted retinas containing short cones were subjected to the 3-min lysis/15-min reactivation protocol in elongation media, cones elongated 15.5 ± 1.6 μm ($n = 5$). When 5 μg/ml cytochalasin D (with 0.5% dimethyl sulfoxide) or 0.5% dimethyl sulfoxide alone were added to the elongation media, maximal elongation was still observed (18.0 ± 2.2 μm, $n = 3$; and 18.7 ± 0.6 μm, $n = 2$, respectively). Thus cytochalasin D has no effect on reactivated cone elongation.

To investigate whether calcium inhibition of cone elongation was retained after disruption of the contractile machinery by cytochalasin D, we prepared models in elongation media containing 10^{-5} M free Ca^{++} plus 5 μg/ml of cytochalasin D. The extent of cone elongation was compared to that of control samples in which elongation media contained 10^{-5} M free Ca^{++} or no additions (10^{-8} M free Ca^{++}). When 10^{-5} M free Ca^{++} was added to the elongation media, reactivated elongation was blocked (4.9 ± 3.0 μm; $n = 3$). Addition of 5 μg/ml cytochalasin D to the calcium media did not relieve calcium inhibition of elongation (3.7 ± 3.4 μm; $n = 3$). Thus, calcium inhibition of reactivated cone elongation does not appear to be mediated by an onset of cone contraction.

Effects of Delayed Exposure to Ca^{++}

Calcium inhibition of reactivated cone elongation occurs only if calcium is added to the medium at the time of lysis. Fig. 12 illustrates the results of adding calcium subsequent to the lysis step. If 10^{-5} M free Ca^{++} was added to both the 3-min lysis and the 15-min reactivation steps, full inhibition of elongation was observed (Fig. 12*A*). However, if 10^{-5} M free Ca^{++} was added only to the 15-min reactivation step, no inhibition was observed (Fig. 12*B*). We have previously shown that the full elongation response can be induced if cAMP is present during only the initial 3-min lysis step; thus cAMP had exerted its full effect before the addition of calcium when calcium was added to the 15-min reactivation step only. This suggests that the inability of calcium to inhibit elongation in this case could be due to the inability of calcium to reverse the effects of cAMP. To examine this possibility, cone models were lysed in the absence of cAMP. When no cAMP was present during the 3-min lysis step followed by 15 min in media containing 1 mM cAMP plus either 10^{-8} or 10^{-5} M free Ca^{++}, the full elongation response was observed (Fig. 12, *C* and *D*, respectively). Thus, calcium still failed to inhibit reactivated cone elongation even when added to the medium simultaneously with cAMP, following the 3-min cell lysis step.

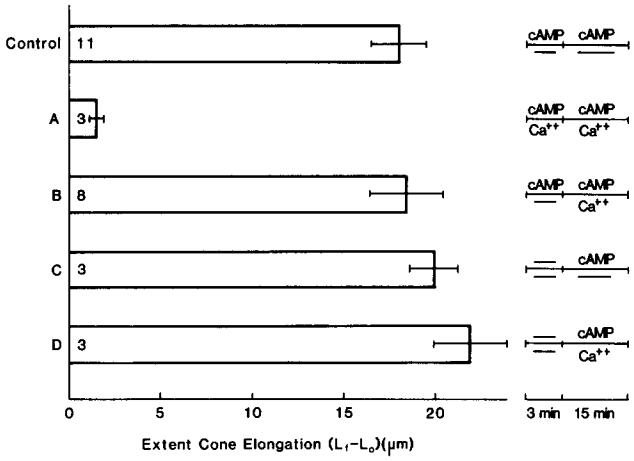

Figure 12. Calcium inhibition of reactivated cone elongation occurred only if calcium was added to the medium at the time of lysis. (*A*) Cones were lysed for 3 min in media containing cAMP plus high Ca^{++}, followed by 15 min in similar medium. (*B*) Cones were lysed for 3 min in media containing cAMP only, followed by 15 min in media containing cAMP plus high Ca^{++}. (*C*) Cones were lysed for 3 min in media containing neither cAMP nor high Ca^{++}, followed by 15 min in media containing cAMP only. (*D*) Cones were lysed in media containing neither cAMP nor high Ca^{++}, followed by 15 min in media containing both cAMP plus high Ca^{++}. In each case, reactivation media contained 1 mM Mg-ATP and 10^{-8} M free Ca^{++}. cAMP (1 mM) and Ca^{++} (10^{-5} M free Ca^{++}) were added to the media as noted.

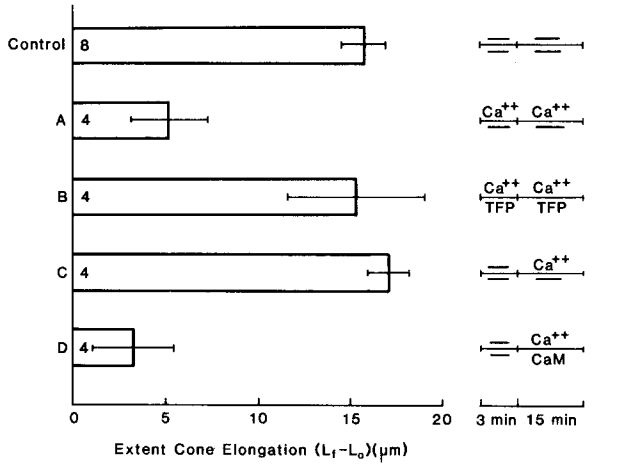

Figure 13. The effect of TFP and exogenous calmodulin on calcium inhibition of reactivated elongation. Reactivation media contained 1 mM cAMP, 1 mM Mg-ATP, and 10^{-8} or 10^{-5} M free Ca^{++} as noted. When 10^{-5} M free Ca^{++} was added to the media for both the 3-min lysis and the 15-min reactivation steps, reactivated elongation was inhibited (*A*). If 10 μM TFP plus 10^{-5} M free Ca^{++} were added to these media, calcium was unable to inhibit cone elongation (*B*). The addition of exogenous calmodulin returned the ability of Ca^{++} to inhibit reactivated elongation when Ca^{++} was added subsequent to cone lysis. If elongation medium contained 10^{-8} M free Ca^{++} for the 3-min lysis step followed by 15 min in medium containing 10^{-5} M free Ca^{++}, the full elongation response was observed (*C*). However, if 10^{-5} M free Ca^{++} plus 10^{-6} M calmodulin were added to the medium for the 15-min reactivation step, elongation was blocked (*D*).

Calmodulin Inhibitor Studies

To investigate the possible role of calmodulin in the calcium inhibition of reactivated cone elongation, we determined the effect of the calmodulin inhibitor, TFP, on calcium inhibition. Results are illustrated in Fig. 13. The extent of cone elongation in media containing 10 μM TFP plus 10^{-5} M free Ca^{++} was compared to that of control samples in which elongation media contained 10^{-5} M free Ca^{++} or no additions (10^{-8} M free Ca^{++}). When 10^{-5} M free Ca^{++} was added to the media for both the 3-min lysis and the 15-min reactivation steps, reactivated elongation was blocked. Addition of 10 μM TFP to the calcium media relieved this inhibition, suggesting that calcium inhibition of reactivated cone elongation may be mediated by a Ca^{++}–calmodulin interaction.

Calmodulin Studies

To determine whether calcium lost its inhibitory capacity when added subsequent to cell lysis due to the loss of soluble calmodulin, calcium plus calmodulin were added to the medium during the 15-min reactivation step. Results are illustrated in Fig. 13. When medium contained 10^{-8} M free Ca^{++} for the 3-min lysis step, followed by 15 min in medium containing 10^{-5} M free Ca^{++}, no inhibition of elongation was observed. When the medium contained 10^{-8} M free Ca^{++} for the 3-min lysis step followed by 15 min in medium containing both 10^{-5} M free Ca^{++} plus 10^{-6} M calmodulin the full inhibitory effect of calcium was restored.

Discussion

Reactivation of Elongation in Lysed Cone Models

Detergent-treated teleost retinal cones undergo cAMP- and ATP-dependent elongation with a rate and morphology comparable to that observed in vivo. Several observations indicate that this movement represents true reactivated elongation in a motile cell model. Although a slight elongation (≤5 μm) is observed under all incubation conditions, an additional 10–15-μm reactivated elongation is observed only if cells are lysed and if cells are incubated in media containing both cAMP and Mg-ATP. Furthermore, membranes are still fully permeabilized even after 18 min. Transfer to fresh medium can induce continued elongation after a 3-min lysis/15-min incubation in media containing 10 μM Mg-ATP during which cone elongation has ceased.

Ultrastructural examination reveals that the cytoplasmic matrix has been extensively extracted. Our lab has previously shown that plasma membranes are sufficiently permeabilized by this lysis protocol to allow entry of myosin subfragment-1 (molecular weight 100,000) (51). The cytoskeleton, however, remains intact. Microtubules and actin filaments are still present in similar number and distribution to those seen in unlysed preparations. Warren and Burnside (71) have reported that myoid microtubules are required for cone elongation in blue striped grunts. Intraocular colchicine injections disrupted myoid microtubules of light-adapted cones and prevented subsequent dark-induced cone elongation. Since morphometric analysis of myoid microtubule number and distribution during cone elongation showed no net increase in total microtubule length, Warren and Burnside (71) suggested that cone elongation might result from a sliding microtubule mechanism.

The maximum rates of elongation observed in cone models and in cones in vivo are identical (2 μm/min). This suggests that the motile machinery for cone elongation is not compromised by our lysis protocol. Although the maximum final

cone myoid length observed in cone models was less than that observed in vivo, this limitation is not exerted by the lysis protocol itself. This reduced maximum myoid length has been observed under all conditions that have been used to induce cone elongation in retinas in vitro. Thus we conclude that it represents a limitation, perhaps mechanical, resulting from retinal isolation and incubation, rather than from cone lysis.

Elongation starts immediately upon lysis in cone models and proceeds at a constant rate. In unlysed retinas in vitro and in retinas in vivo, the onset of elongation follows a delay of ~10 min. These 10 min may represent time taken to accumulate sufficient intracellular cAMP concentration to initiate elongation. Since the cAMP concentration is increased immediately upon lysis in cone models, this delay period would not be observed. The 10-min delay may also represent time taken to reach a threshold level of protein phosphorylation or to undergo an unknown initiation process required for the onset of cone elongation. While producing cone models, we induced a slight cone elongation (15–20 μm) before lysis (see Materials and Methods); thus any required initiation process had already taken place. In fact, in the few cases where we did obtain lysis of fully light-adapted, short cones, those cone models did not elongate. These observations suggest that an initiation process, which does not occur under our lysis conditions, may be required for cone elongation. Our reactivation conditions clearly support continued elongation; however, they do not appear to be able to initiate elongation in a fully light-adapted, maximally shortened cone.

The induction of partial cone elongation prior to lysis may also explain the 4–5-μm elongation observed under all reactivation conditions. Since cones are in the process of elongation before lysis, they may move an additional few micrometers before they have completed their current cycle of motility or have been depleted of endogenous cAMP and/or ATP.

cAMP Requirement for Reactivated Cone Elongation

The requirement for cAMP suggests a role for protein phosphorylation in the regulation of cone elongation (18, 36). cAMP concentrations $\geq$10 μM supported the full elongation response, although the maximum rate of elongation (2 μm/min) was observed only at $\geq$0.5 mM cAMP. No reactivated elongation was observed if cAMP concentration was $\leq$5 μm. cAMP-dependent protein kinases exhibit K_m values for cAMP in the range of 0.01 to 0.3 μM, depending on the substrate and the ATP concentration (1, 37, 43, 54, 73). Thus the concentrations of cAMP that support reactivated cone elongation are sufficient to activate these enzymes.

The finding that a transient (3-min) exposure to 1 mM cAMP is sufficient to support the full subsequent elongation response is consistent with a role for cAMP in the phosphorylation of a regulatory protein which, once phosphorylated, retains its activity even in the absence of cAMP. The finding that the rate of cone elongation increases as the cAMP concentration increases suggests that the number of force-producing elements that are activated increases with the cAMP concentration.

The effect of cAMP concentration on the rate of cone elongation may explain the variability in elongation rate observed in vivo. The maximum rates of elongation observed in vivo and in cone models were identical (2 μm/min) but rates as low as 1 μm/min have been observed in vivo. This variability may be due to seasonal variations in endogenous adenylate cyclase or phosphodiesterase activities, with consequent variations in cAMP levels.

The cAMP dependence of cone elongation is consistent with several similar observations in cilia and flagella, thus favoring the suggestion that cone elongation involves a sliding microtubule mechanism. In cilia and flagella of mammals, fish, and invertebrates, motility is increased by cAMP (5, 6, 30, 41, 44–46, 64). In addition to increasing the per cent activation of flagella, cAMP increases flagellar beat frequency (41, 44, 46, 64). This finding is consistent with our finding that cAMP increases the rate of cone elongation. Just as in cone models, transient exposure to cAMP is sufficient to induce sustained increased motility in demembranated sperm flagella (6, 45).

There are several possible targets for protein phosphorylation in a microtubule sliding system. The most obvious are microtubule-associated proteins, which are phosphorylated by cAMP-dependent protein kinases (52, 58, 59, 69). Another likely primary target for cAMP activation is a soluble protein isolated from demembranated sperm flagella from several species, both mammalian and invertebrate (5, 30, 64). This protein has a molecular mass of 55,000–56,000 daltons and is neither tubulin nor the regulatory subunit of cAMP-dependent protein kinase (5, 64). The protein acts as a substrate for cAMP-dependent phosphorylation, and in its phosphorylated form will substitute for cAMP in the activation of motility in demembranated sperm. Furthermore, removal of this soluble protein from the supernatant of demembranated sperm renders them insensitive to cAMP. Tash et al. have shown that the detergent extracts from sperm flagella of several species (mammalian and invertebrate) are all capable of reactivating demembranated dog sperm flagella, and all contain the 56,000-dalton protein (64).

Cone models incubated without cAMP for 33 min lose their ability to elongate maximally when cAMP is subsequently added. Another observation suggests that this loss of elongation capacity results from the loss of a soluble factor rather than from degradation of the motile machinery. Cone models incubated for 18 min in 10 μM Mg-ATP plus 1 mM cAMP can only partially elongate, but if Mg-ATP is subsequently replenished they will continue to elongate to maximal lengths. Thus, if cAMP is supplied earlier the elongation machinery is still functional 30 min after lysis. Consequently, the inability of cones to elongate after the 33-min incubation without cAMP could result from the gradual loss of a soluble factor like the 56,000-dalton protein described above. Tash et al. have identified a 56,000-dalton protein in dog retina which, when phosphorylated by cAMP-dependent protein kinase, will activate motility in demembranated dog sperm (64).

The concentrations of cAMP which induce reactivated cone elongation also inhibit reactivated cone contraction. Since the cAMP concentration required to initiate cone elongation is 100-fold higher than that needed to inhibit cone contraction (10 μM vs. 0.1 μM), it seems unlikely that cAMP is stimulating cone elongation and inhibiting cone contraction by the same mechanism.

ATP Requirement for Reactivated Cone Elongation

Mg-ATP is required for reactivated cone elongation for both a cAMP-dependent and a cAMP-independent process. These

requirements suggest a role for ATP in a cAMP-dependent phosphorylation regulatory step, and as the energy source for force production. Mg-ATP concentrations ≥ 10 μM can induce the full elongation response, although in 10 μM Mg-ATP, the ATP is depleted and must be replenished before the completion of elongation. No reactivated elongation was observed if the Mg-ATP concentration was ≤ 5 μM. Since cAMP-dependent protein kinases exhibit a K_m of 5–16 μM for ATP (37, 43, 54, 73), the concentrations of Mg-ATP that support reactivated cone elongation are sufficient to serve as a phosphate source for these enzymes.

Dynein ATPases use Mg-ATP as the energy source for force production in microtubule sliding in cilia and flagella (20, 56, 62). Dynein ATPases exhibit K_m values in the range of 1 to 110 μM for ATP depending on the species and the assay conditions (7, 22, 24, 63, 66). Thus the Mg-ATP concentrations that support cone elongation are sufficient to activate these enzymes. Furthermore, we have preliminary data showing an inhibition of reactivated cone elongation by Vanadate (100 μM) and EHNA (0.5 mM), two agents known to inhibit dynein ATPase (4, 35). Thus our results are consistent with a role for a dynein-like ATPase in force production for cone elongation, possibly by a sliding microtubule mechanism.

In reactivated cilia and flagella, beat frequency is dependent on ATP concentration (19, 23). However, we found that the rate of reactivated cone elongation was not affected by Mg-ATP concentration. The inability of Mg-ATP to affect cone elongation rate may be a result of the relatively slow maximum rate of cone elongation (2 μm/min). This low rate may be caused by factors other than ATP. In trypsin-treated axonemes, the rate of sliding disintegration depends on the ATP concentration and is in the range of 1–2 μm/s in 10 μM ATP (23, 27, 62). This rate is 30–60 times greater than the maximum elongation rate observed in cones. Thus, if a similar sliding mechanism exists in cones, the minimum ATP concentration required to induce cone elongation (10 μM) may be sufficient to induce a rate of microtubule sliding far in excess of the maximum cone elongation rate observed.

Calcium Inhibition of Reactivated Cone Elongation

Calcium inhibits reactivated cone elongation by decreasing the elongation rate. Calcium concentrations $\geq 10^{-7}$ M cause a decrease in the elongation rate of 78% or more. In medium containing 10^{-5} M free Ca^{++}, no reactivated elongation is observed in 18 min.

Although microtubules are disrupted in several cell types by calcium concentrations of 10^{-6} M (31, 33, 39), the microtubules of green sunfish cone models are stable in the presence of calcium. They are not disrupted by an 18- or 30-min incubation in medium containing 10^{-5} M free Ca^{++} (11; Porrello, K., and B. Burnside, unpublished observations). Thus calcium does not appear to be exerting its inhibitory effects by the disruption of cytoplasmic microtubules.

Calcium concentrations that inhibit reactivated cone elongation have previously been shown to activate reactivated cone contraction (50). Thus the possibility had to be considered that calcium inhibits elongation by initiating an antagonistic contractile response. We have shown that disrupting the contractile machinery with cytochalasin D did not abolish the calcium inhibition of reactivated cone elongation. This observation suggests that calcium inhibits cone elongation by a direct action on the cones' elongation mechanism, not by activation of an antagonistic cone contraction.

Calcium had to be present during the initial 3-min lysis step to exert its inhibitory effect; adding calcium after 3 min had no effect. This loss of calcium sensitivity did not result because calcium was unable to reverse previous effects of cAMP which had occurred in the first 3 min. Deletion of cAMP from the 3-min lysis step did not return the ability of calcium to block elongation when calcium plus cAMP were added together subsequent to the 3-min cell lysis step. These results suggest that the ability of calcium to inhibit cone elongation depends on the presence of some soluble factor which is lost during the initial 3-min lysis step.

Two lines of evidence suggest that this soluble factor is calmodulin. First, calmodulin is required for calcium inhibition of cone elongation: TFP, a calmodulin inhibitor (39, 53), prevents calcium inhibition of cone elongation. Second, the addition of 10^{-6} M calmodulin to the reactivation medium restores the ability of calcium to inhibit cone elongation when calcium is added subsequent to the 3-min lysis step. These observations imply that the calmodulin which mediates calcium inhibition of cone elongation is soluble under our lysis conditions and is lost during the 3-min lysis step in medium containing low calcium. This calmodulin loss is consistent with the finding that calmodulin binds to both microtubule-associated proteins and τ protein in a calcium-dependent manner (38). We suggest that this loss of soluble calmodulin prevents subsequent inhibition of cone elongation by added calcium. This observation contrasts with one we reported previously for contracting cone models, which also require calmodulin (50). In contraction models, the entire machinery is stable for at least 90 min in $<10^{-8}$ M free Ca^{++}; adding back calcium at that time induced maximal contraction. No exogenous calmodulin was required. Thus the elongation and contraction mechanisms appear to depend on different pools of calmodulin.

The inhibition of cone elongation by calcium resembles the effects of calcium on ciliary and flagellar motility in several species. Micromolar calcium concentrations have been shown in some species to cause complete arrest of motile ciliary and flagella axonemes (21, 28, 47, 53, 65, 70) in a calmodulin-dependent manner (53, 55). Thus both in cilia and flagella and in cones, calcium can act directly on the motile machinery.

Although the mechanism by which calcium inhibits cone elongation is not at all clear, possible mechanisms through which calcium/calmodulin may be exerting its effect are suggested by observations in other systems. For example, calcium/calmodulin could be acting through the regulation of protein phosphorylation. The activities of several cytoplasmic protein kinases and phosphoprotein phosphatases are altered by calcium/calmodulin (14, 15, 26, 32, 34, 48, 49, 57, 74). Microtubule-associated proteins have been reported to be phosphorylated in a calcium-dependent manner (25, 72). Tash and Means (65) have shown that calcium inhibition of motility in dog sperm flagella is accompanied by a decrease in the phosphorylation of a 98,000-mol-wt protein which is phosphorylated in a cAMP-independent manner. Calcium/calmodulin might also be directly modulating dynein ATPase, and thus influencing microtubule sliding. Calcium has been shown to influence dynein ATPase activity in cilia and flagella

(2, 3); and calcium has been shown to inhibit dynein arm detachment during microtubule sliding (75).

Conclusion

We have succeeded in reactivating a microtubule-dependent cone elongation in a lysed cell model. This elongation is both Mg-ATP- and cAMP-dependent, and is inhibited by calcium. The rate of reactivated cone elongation is proportional to the cAMP concentration, with the maximum rate (2.0 μm/min) being the same as the maximum cone elongation rate observed in vivo. This cAMP requirement suggests a role for protein phosphorylation in the regulation of cone elongation. Mg-ATP is required for reactivated elongation for both a cAMP-dependent, and a cAMP-independent process. The cAMP-independent Mg-ATP requirement may represent the energy source for force production.

Free calcium concentrations $\geq 10^{-7}$ M inhibit reactivated cone elongation by decreasing the elongation rate by 78% or more. Our results indicate that this calcium inhibition is mediated by a soluble calmodulin, and that calcium/calmodulin does not inhibit elongation by activating the antagonistic cone contraction, but by directly affecting the elongation mechanism. All these observations resemble previously reported findings in cilia and flagella and are consistent with a role for microtubule sliding in force production for cone elongation.

The requirements of reactivated cone elongation contrast with those of reactivated cone contraction. Cone elongation is activated by cAMP and inhibited by calcium, while cone contraction is activated by calcium and inhibited by cAMP. These findings imply that in vivo, the cytoplasmic cAMP concentration in the cone myoid is high at dark onset and low in the light, while conversely the calcium concentration is high at light onset and low in the dark.

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