Elevation of Cyclic AMP Activates an Actin-dependent Contraction in Teleost Retinal Rods

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ABSTRACT Agents which elevate cyclic AMP (cAMP) cause teleost retinal rods to contract. We have characterized this cAMP effect and have evaluated the role of the cytoskeleton in cyclic nucleotide-induced contraction, using actin and microtubule inhibitors. The necklike myoid region of the rod contracts in the dark and elongates in the light. If long, light-adapted rods are cultured with cAMP analogs and IBMX, rods contract to their short dark-adapted position. Cyclic nucleotide-induced rod contraction occurs in constant light, requires a phosphodiesterase inhibitor, and is specific to cAMP (db cyclic GMP, 8-bromocyclic GMP, 5'AMP, and adenosine have no effect on rod myoid length). Cyclic AMP effects on rod length are consistent with observations from several species that cAMP levels are higher in dark-adapted than in light-adapted retinas. Since rod myoids contain paraxially aligned actin filaments and microtubules, we have used the motility inhibitors cytochalasin D and cold and nocodazole to investigate the roles of these cytoskeletal elements in rod contraction. Cyclic nucleotide-induced contraction is not inhibited when myoid microtubules are disrupted with cold and nocodazole treatments, but contraction is blocked if myoid actin filaments are disrupted with cytochalasin D. Thus, we conclude that actin filaments, but not microtubules, are required for rod contraction. We propose that rod contraction in vivo is triggered by a rise of cytoplasmic cAMP at onset of darkness and that this contraction is mediated by an actin-dependent mechanism.

Cyclic AMP (cAMP) and its analogs have been reported to influence cell motility in a variety of cell types (9, 35). In many cultured cell lines, exogenous cAMP analogs transform the cells from a flat epithelial-like morphology to a stellate morphology (15, 22, 30). Since mitotic inhibitors and cytochalasins prevent these cAMP-induced shape changes, it has been proposed that cAMP may be acting upon the microtubule and actin components of the cell's cytoskeleton (23). Cyclic AMP has also been reported to regulate other processes of motility including intracellular transport (19, 28), secretion (11, 16), and smooth muscle contractility (2). In this study, we report that cAMP induces contraction of teleost retinal rods, and we attempt to evaluate the role of the cytoskeleton in this cyclic nucleotide-induced cell shape change.

In teleost fish, adjustments to changes in light intensity are mediated by movements of the photoreceptors and the pigment granules within the processes of the retinal pigment epithelium (RPE) (1, 5). The net result of these so-called retinomotor movements is to rearrange the photoreceptors for optimal function in bright and dim light. In darkness, the cones elongate, the rods contract and the pigment granules aggregate to the base of the RPE, thereby exposing the rod outer segments. Light triggers the reverse of these movements: cones contract and rods elongate, burying their light-sensitive outer segments into the advancing pigment of the RPE.

Differences in retinomotor position can be correlated with differences in retinal cAMP levels. In retinas of most species examined to date, cAMP levels have been reported to be higher in dark-adapted than in light-adapted states (6, 10, 12, 13, 21). In addition, Van Buskirk and Dowling (32) have recently reported that there are substantial levels of cAMP in rod photoreceptors. These observations, along with the reports of cAMP effects on cellular motility, suggested to us that cAMP may act as a signal to induce dark-adaptive rod contraction.

Teleost retinal rods provide a favorable system in which to study nonmuscle contraction for several reasons. Rod contraction is uniaxial, slow (1.3 μm/min), and restricted to the necklike myoid region of the cell. Length changes occurring during contraction can therefore be easily and reliably quantitated. Rod myoids are small in diameter (2.9 μm) and contain

THE JOURNAL OF CELL BIOLOGY · VOLUME 95 NOVEMBER 1982 445-452

both longitudinally oriented microtubules and actin filaments. This simple paraxial alignment facilitates the analysis of cytoskeletal rearrangements.

We have investigated the role of cAMP in regulating rod contraction by culturing light-adapted retinas in the presence of cyclic nucleotide derivatives and the phosphodiesterase inhibitor, IBMX. We found that agents which elevate cAMP induce long, light-adapted teleost retinal rods to contract to their short dark-adapted position, even in constant light. To assess the role that microtubules and actin filaments play in cAMP-induced contraction, we treated cultured retinas with mitotic (nocodazole and cold) and actin (cytochalasin D) inhibitors, and then challenged the rods to contract with the appropriate cAMP concentrations. Contraction occurred in the absence of myoid microtubules, but was inhibited by cytochalasin disruption of the myoid actin filaments.

Since our morphological observations indicated that rod myoid microtubules disassemble during contraction in vivo, we investigated the effect of taxol, a drug which stabilizes microtubules (26, 27), on cyclic nucleotide-induced contraction. We found that taxol caused a partial inhibition of rod contraction.

MATERIALS AND METHODS

Animals

Retinas of the cichlid, *Sarotherodon mossambicus*, were used for all experiments. This fish was chosen because in its retina, the rods are arranged in a single tier, thereby facilitating measurement of rod cell length changes.

Fish were maintained in outdoor ponds (heated to 75–78°F) under ambient light conditions. Dark-adapted retinas were obtained from fish which had been placed in a dark aerated tank for 2–3 h before the start of an experiment.

Preparation of Light-adapted Retinas Isolated from the Retinal Pigment Epithelium (RPE)

Retinas to be used for culture were dissected from dark-adapted (DA) eyes. In this state, the neural retina and the RPE spontaneously separate. Under dim red light, DA eyes were enucleated, the lens removed and the retina detached by a gentle stream of oxygenated Hank's Balanced Salt Solution (HBSS). The retina was freed by cutting the optic nerve. Retinas were then bisected (yielding 4 half retinas/fish) and placed in a culture well (Falcon Multiwell #3008; Falcon Labware, Oxnard, CA) containing 0.5 ml of culture medium. These retinas were light-adapted by culturing them in oxygenated HBSS in fluorescent light (900 lux) for 30 min. Rods elongate to their maximum extent (23.6 ± 0.5 μm, N = 16) within the 30-min culture period. This length is somewhat shorter than the maximum light-adapted length in vivo (39.8 ± 1.0 μm, N = 4), but significantly longer than that observed in dark-adapted rods (11.6 ± 0.4 μm, N = 4).

Cyclic Nucleotide Studies

Cyclic nucleotides were dissolved in HBSS in the presence or absence of the PDE inhibitor, isobutylmethylxanthine (IBMX). To insure complete suspension, IBMX was sonicated in HBSS for 1 h before cyclic nucleotide addition. Effects of nucleotides were tested on light-adapted retinas prepared as described above. One half retina was fixed immediately after the 30-min light preculture to illustrate the starting point (t_0). The other three were transferred to HBSS containing the indicated cyclic nucleotide, cultured for 60 min and then fixed.

Microtubule Disruption Studies

To disrupt microtubules, retinas were cultured (in the dark) in the cold in nocodazole (20 μg/ml) dissolved in 0.4% dimethylsulfoxide (DMSO) in HBSS. For each experiment, four dark-adapted half retinas were cultured at 4°C for 30 min: two in the presence of nocodazole (experimental) and the other two in the absence of nocodazole (controls). After cold treatment, all half-retinas were then cultured at 22°C for 45 min in the light to allow rods to elongate (20). At this point one control and one experimental half-retina were fixed (t_0's) to show that rods had elongated. To challenge the rods to contract, the remaining two half-retinas were cultured in the presence of dbcAMP (1 mM) and IBMX (1 mM) for 60 min and then fixed. Experimental half-retinas were exposed to nocodazole throughout the time course of the experiment.

Cytochalasin Studies

To disrupt actin filaments, retinas were cultured in cytochalasin D (CD) (1 μg/ml) dissolved in 0.2% DMSO in HBSS. To allow time for the drug to act before challenging the rods to contract, two light-adapted half-retinas were cultured in the presence of CD (experimental) and two were cultured in the absence of CD (control) for 60 min. At that time, 1 control and 1 experimental half-retina were fixed, so that the state of rod actin could be assessed before the challenge to contract. The remaining two half-retinas were challenged to contract with dbcAMP as described above for nocodazole. DMSO controls were also carried out in parallel experiments.

Taxol

Taxol was a gift from the Natural Products Branch, Division of Cancer Treatment, NCI. A 50 mM (DMSO) stock of Taxol was diluted with HBSS to yield 50 μM taxol (0.1% DMSO). Light-adapted half retinas were cultured for 60 min with Taxol, HBSS, or, Hank's + DMSO and then challenged to contract with dbcAMP as described above for nocodazole.

Rod Length Measurements

Contraction was assayed by ocular micrometer measurement of rod inner segment length in 2 μm plastic sections cut parallel to the rod long axis. The rod inner segment length corresponds to the distance from the outer limiting membrane (OLM) to the base of the rod outer segment. Twenty rod inner segments were measured for each half retina. N refers to numbers of half-retinas examined. Values are expressed as mean ± standard error.

Electron Microscopy

Isolated retinas were fixed in 2% glutaraldehyde (TAAB EM grade; TAAB Laboratories, Reading, England), 0.1 M phosphate buffer (pH 7.0), 1 mM $MgCl_2$ and 0.2% tannic acid for 30–45 min. Postfixation was carried out in 1% OsO_4 for 45 min on ice. Dehydration and embedding procedures have been described elsewhere (20).

RESULTS

Teleost retinal rods contract when the fish is transferred from light to darkness. In the cichlid, *Saratherodon mossambicus*, rod inner segment length decreases from 39.8 ± 1.4 μm (N = 4) in the light to 11.6 ± 0.4 μm (N = 4) in the dark. These length changes take place in the myoid portion of the inner segment. The shape changes and ultrastructure of the rod myoid have been previously described (20) and are summarized in Fig. 1. Long, light-adapted rod myoids contain longitudinally oriented microtubules and bundles of 60-Å filaments. We have identified these filaments as actin by their ability to bind myosin subfragment-1 (20). With the onset of darkness, the long slender myoid of the light-adapted rod contracts to form a short, broad trunk. In these short, dark-adapted myoids, microtubules are visible, but 60-Å filaments are not detectable.

Effect of Nucleotides on Long Light-adapted Rods

Recently, we have defined conditions that support photoreceptor movement in cultured retinas. If isolated dark-adapted retinas are incubated in oxygenated HBSS for 30 min in the light, rods elongate from 10.9 ± 0.5 μm (N = 6) to 23.6 ± 1.5 μm (N = 16). The long, light-adapted rods of these retinas can then be induced to contract to their short, dark-adapted position by culturing the retinas with dbcAMP and the phosphodiesterase inhibitor, IBMX. This cyclic nucleotide-induced contraction occurs in constant light.

The concentration dependence of the rod response to dbcAMP is shown in Fig. 2. Higher concentrations of dbcAMP, in the presence of a constant IBMX concentration (0.25 mM), result in greater shortening of rod inner segment during the 1-

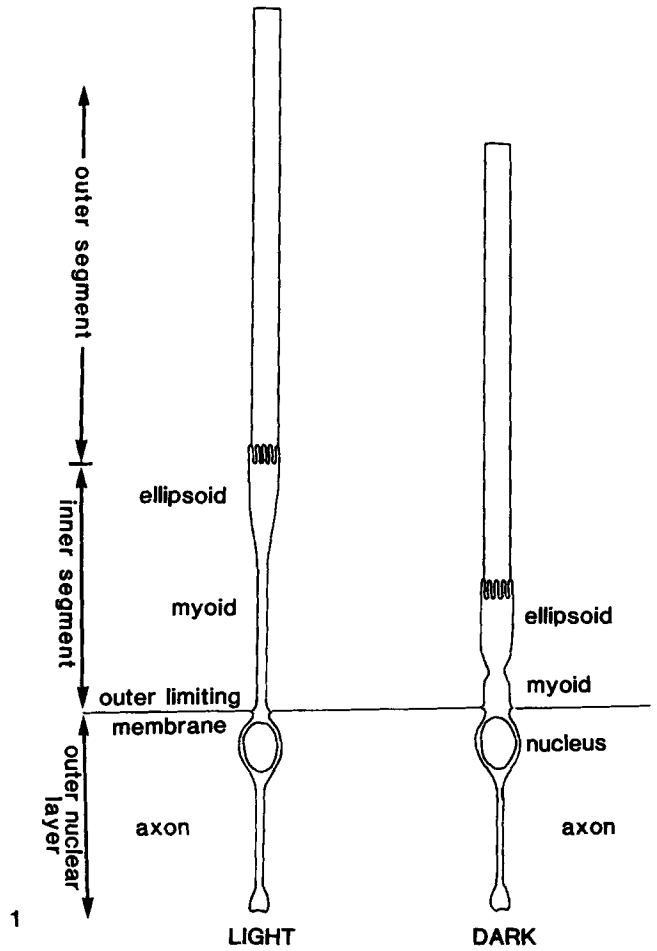

FIGURE 1 Schematic drawing of cell shape in light- and dark-adapted teleost retinal rods.

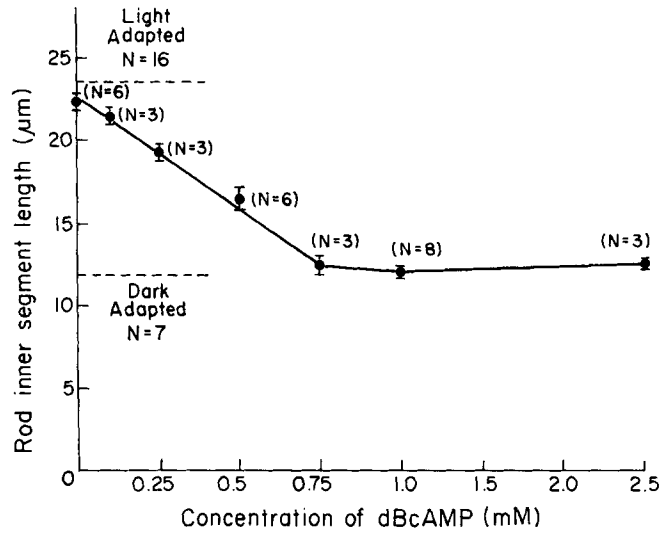

FIGURE 2 Effects of dbcAMP concentration on rod inner segment length achieved after 60 min in culture. Light-adapted retinas (without RPE) were cultured with indicated concentrations of dbcAMP in the presence of 0.25 mM IBMX. Values indicate mean ± SE (N, number of half-retinas examined).

h incubation period. Maximum contraction occurs at 1 mM dbcAMP. This final length achieved is equivalent to that of fully dark-adapted rods in vivo.

The presence of IBMX in the culture medium is required for dbcAMP-induced contraction. When used alone, neither 1 mM nor 10 mM dbcAMP has a significant effect on the length of light-adapted rods (Table I). Though 0.25 mM IBMX alone has no effect on rod length, IBMX alone at high concentrations (1 mM) can induce contraction in the absence of exogenous nucleotide (Table I).

Since we are required to use IBMX in the medium and since it is possible that IBMX could also influence cGMP levels, we tested derivatives of cGMP to see whether they could also induce shortening. Neither dbcGMP nor 8-bromo cGMP, in the presence of 0.25 mM IBMX, has any affect on the length of light-adapted rods (Table I). 5′ AMP and adenosine are also without effect (Table I). Hence induction of rod contraction appears specific to factors which elevate cAMP levels.

Effect of Cold and Nocodazole on Nucleotide-induced Contraction

Since long, light-adapted rods contain both microtubules and actin filaments, we were interested in what roles these cytoskeletal elements might play in cyclic nucleotide-induced contraction. We considered a possible role for microtubules because Klyne and Ali (17) previously reported that intraocular injections of colchicine block dark-induced rod contraction (though myoid microtubules were not completely disrupted in their study). We also have observed that intraocular injections of colchicine block rod contraction of *Sarotherodon* in vivo. Due to technical difficulties in fixing retinas treated with colchicine in vivo, we were unable to determine whether rod myoid microtubules were disrupted by the drug in our studies. Because Klyne and Ali's (17) studies suggested the possibility that the observed colchicine block was not a specific effect on myoid microtubules, we tested the effects of other microtubule disruption treatments (cold and nocodazole) on nucleotide-induced contraction in vitro. We found that complete disruption of myoid microtubules with cold and nocodazole had no effect on cyclic nucleotide-induced rod contraction (Table II, Fig. 3, 4).

Isolated dark-adapted retinas were cultured in the presence or absence of 20 μg/ml nocodazole at 4°C for 30 min. Cold treated retinas were subsequently cultured at 22°C for 45

TABLE I

Effect of Cyclic Nucleotides on Rod Inner Segment Length in Cultured Light-adapted Retinas

	Rod inner segment length	No. of fish
	*μm**	
Light-adapted position (control)	23.6 ± 0.5	16
After 60 min in culture in light with:		
1 mM dbcAMP + 0.25 mM IBMX	12.2 ± 0.5	8
1 mM dbcAMP	21.6 ± 1.0	3
10 mM dbcAMP	23.6 ± 0.7	3
0.25 mM IBMX	22.4 ± 0.8	3
1 mM IBMX	12.0 ± 2.2	2
1 mM 5′ AMP + 0.25 mM IBMX	28.1 ± 2.5	3‡
1 mM adenosine + 0.25 mM IBMX	27.7 ± 1.4	3‡
1 mM dbcGMP + 0.25 mM IBMX	23.4 ± 1.7	3
5 mM dbcGMP + 0.25 mM IBMX	22.5 ± 0.7	3
1 mM 8-bromo-cGMP + 0.25 mM IBMX	26.7 ± 2.9	6

* Mean ± SE.

‡ These values were not significantly different from the T_0 values (27.7 ± 2.5, N = 3) in the experiment.

TABLE II

Effect of Cold and Nocodazole on cAMP-induced Rod Contraction and Rod Myoid Microtubules in Cultured Light-adapted Retinas

	Rod inner segment length	No. of fish	No. of microtubules per rod myoid*	No. of rods
	μm*			
After 30 min at 4°C and 45 min at 22°C:				
0 μg/ml nocodazole (control)	23.7 ± 0.3	6	5.0 ± 0.7	34
20 μg/ml nocodazole	22.3 ± 0.5	6	0.0	20
Same as above, followed by 60 min at 22°C in 1 mM dbcAMP + 1 mM IBMX:				
0 μg/ml nocodazole (control)	11.9 ± 0.7	6	4.6 ± 0.9	21
20 μg/ml nocodazole	13.2 ± 0.9	6	0.2 ± 0.1	17

* Mean ± SE.

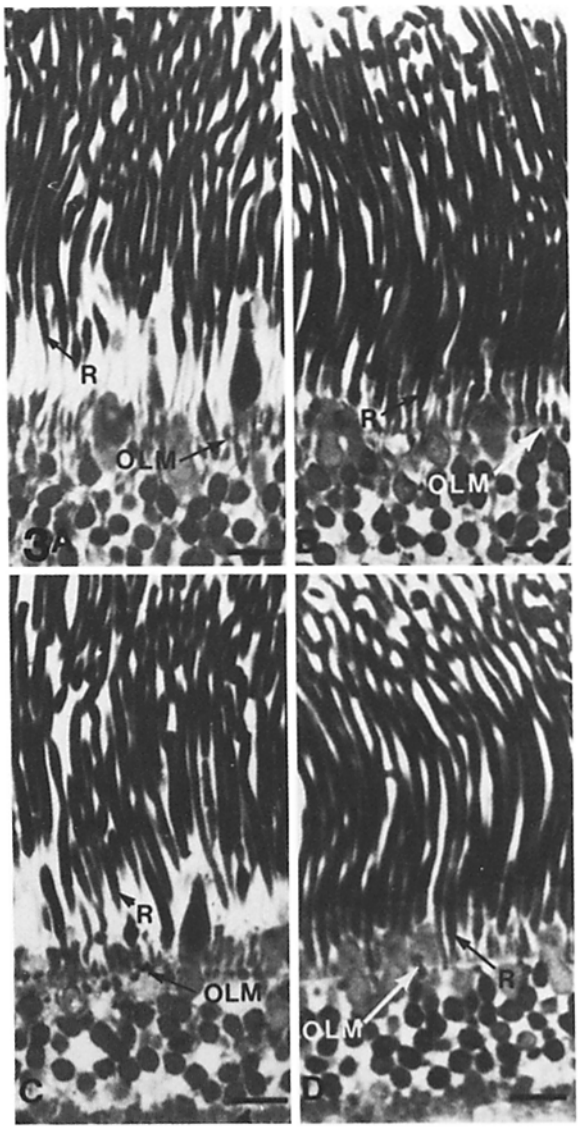

FIGURE 3 Effects of cold and nocodazole on cyclic nucleotide-induced rod contraction: light micrographs of cichlid retinas cultured in the presence (*A, B*) or absence (*C, D*) of nocodazole. All retinas (*A-D*) were cultured at 4°C for 30 min and then at 22°C for 45 min. At this point, control (*A*) and nocodazole (*C*) were fixed. *B* and *D* were then cultured at 22°C in the presence of 1 mM dbcAMP and 1 mM IBMX for 60 min and fixed. Cyclic nucleotide treatment induced rod contraction in both control (*B*) and nocodazole (*D*) cultures. *R*, rod ellipsoid; *OLM*, outer limiting membrane. Bar, 10 μm. × 725.

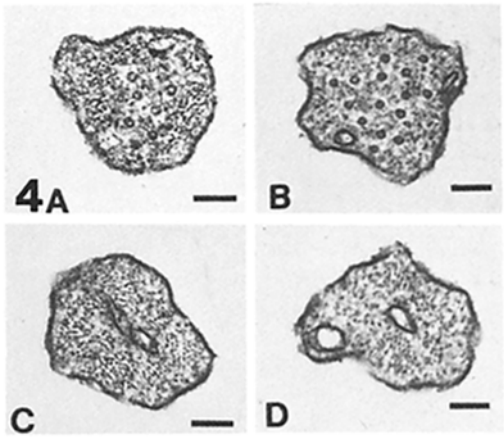

FIGURE 4 Electron micrographs of cross sections through long light-adapted rod myoids from cultured retinas shown in *A* (control) and *C* (nocodazole). Microtubules are present in rod myoids of control retinas (*A, B*) but absent in rod myoids of nocodazole-treated retinas (*C, D*). Bar. 0.2 μm. × 52000.

minutes in the light. Rods elongated to similar lengths in nocodazole and nocodazole-free cultures. Microtubules, however, were present only in rods of the nocodazole-free cultures (Table II, Fig. 3). The microtubule independence of light-induced rod elongation has been previously reported (20). Nocodazole and nocodazole-free cultures were then incubated in the presence of 1 mM dbcAMP and 1 mM IBMX for 60 min at 22°C. Rods contracted to dark-adapted lengths in both nocodazole and nocodazole-free cultures (Table II, Fig. 3). We are certain that microtubules did not repolymerize during contraction in the rods of nocodazole-treated retinas since microtubules were not present in these cells but were present in the contracted rod of myoids of nocodazole free retinas (Table II; Figure 5). From these observations, we conclude that microtubules are not required for force generation in retinal rod contraction.

Effect of Taxol on Cyclic Nucleotide-induced Contraction

Although disruption of microtubules had no effect on cyclic nucleotide-induced contraction, rod contraction was partially inhibited by taxol (Table III), a drug which stabilizes microtubules (26, 27). If light-adapted retinas were precultured for 60 min in 50 μM taxol and subsequently cultured for 60 min in the presence of 1 mM dbcAMP and 1 mM IBMX, rod contraction was inhibited by ~50%. Rods of control (HBSS and

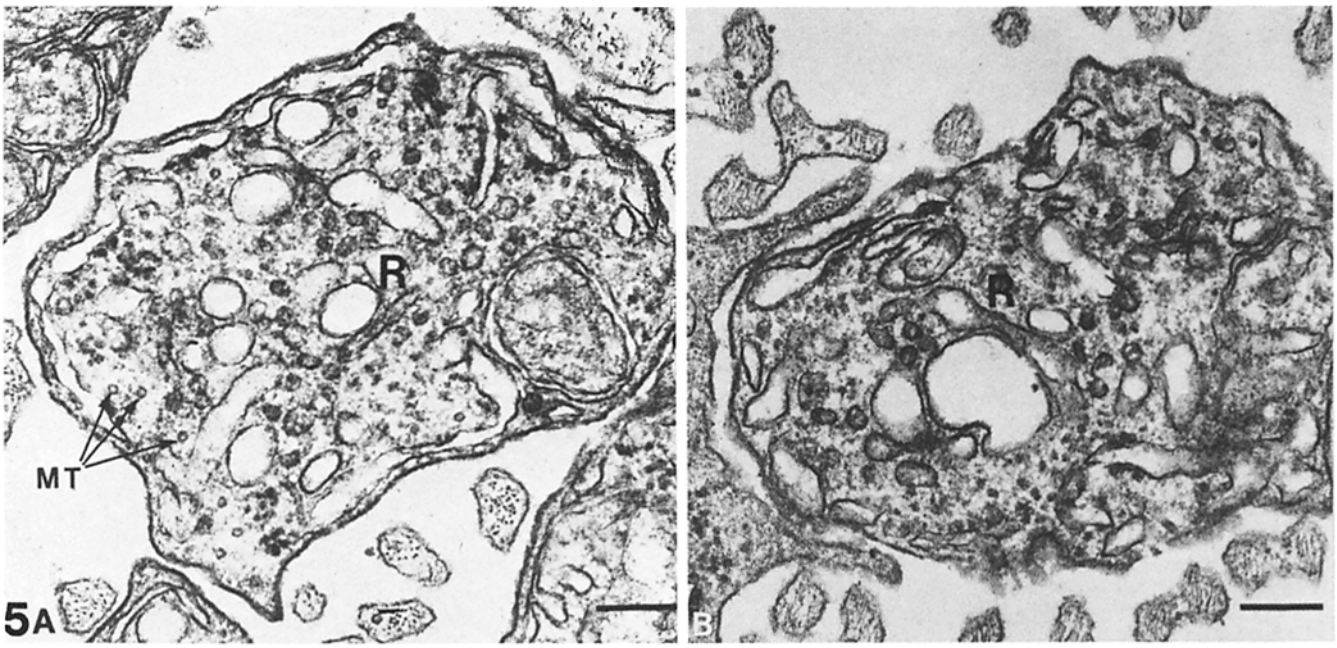

FIGURE 5 Electron micrographs of cross sections through short dark-adapted rod myoids from cultured retinas shown in Fig. 4 *B* (control) and 4 *D* (nocodazole). Microtubules are present in rod myoid of control retina (*A*) but absent in rod myoid of nocodazole-treated retina (*B*). Hence, microtubules did not repolymerize in nocodazole-treated retinas during contraction. Bar, 0.2 μm. × 52,000.

HBSS + DMSO) retinas contracted normally. This inhibition by taxol suggests that microtubule disassembly is required for normal contraction.

Morphological analysis of total microtubule length supports the hypothesis that microtubules disassemble as rods shorten. Long light-adapted rod myoids contain 5.0 ± 0.7 (N = 34) microtubules per myoid cross section and short dark-adapted rod myoids contain 5.4 ± 0.5 (N = 26) microtubules per myoid cross section. The total microtubule length,

$$\frac{\text{no. of microtubules}}{\text{myoid cross section}} \times \text{myoid length}$$

is significantly greater in long rods (118.0 μm) than in short rods (58.9 μm). Thus, these observations indicate that microtubules disassemble during contraction in vivo and also suggest that interference with disassembly can impair normal contraction.

Effect of Cytochalasin D on Nucleotide-induced Contraction

Cytochalasin D (1 μg/ml) strongly inhibits nucleotide induced rod contraction in cultured retinas. Light-adapted retinas were prepared in culture as described above and then preincubated in the light for 60 min at 22°C in the presence or absence of cytochalasin D. Cytochalasin and cytochalasin-free retina cultures were then challenged to contract by incubating them for 60 min in the presence of 1 mM dbcAMP and 1 mM IBMX. The rods of the cytochalasin-free (control) cultures contracted normally, whereas the rods of the cytochalasin-treated retinas failed to contract (Fig. 6, Table IV).

To investigate the site of action of cytochalasin D, we examined the myoids of long light-adapted rods of cytochalasin and control retinas at the time in culture just before rods were challenged to contract by addition of cyclic nucleotide. In rods examined from control retinas, myoid thin filaments were easily discernible (Fig. 7). On the other hand, in rods examined from cytochalasin-treated retinas, no thin filaments were visible in the rod myoids (Fig. 7). Thus, we are confident that cytochalasin D disrupted rod myoid thin filaments before we gave the signal to induce contraction. These observations are consistent with the suggestion that the failure of cytochalasin D-treated rods to contract results from disruption of myoid thin filaments.

DISCUSSION

We have shown that treatments which elevate intracellular concentrations of cAMP promote dark-adaptive retinomotor movements in teleost retinal rods. If light-adapted retinas are cultured with dbcAMP and the PDE inhibitor, IBMX, rods contract to their short dark-adapted positions. The effect occurs in constant light, requires the presence of IBMX in the culture medium, and is specific to cAMP. dbcGMP, 5′AMP, and adenosine have no effect on rod retinomotor position.

Our findings are also consistent with observed light-dark variations of cAMP level in neural retinas. In most species examined (10, 12, 13, 21), including teleost fish (6), cAMP levels have been reported to be two- to threefold higher in dark-adapted retinas than in light-adapted retinas. Though highest levels of cAMP are found in the outer plexiform layer of the retina, a significant level of cAMP has been reported in the photoreceptor layer (21), and in isolated rods (32). Most of the light-dark cAMP changes are attributed to the outer plexiform layer (21). However, a twofold dark-induced increase does occur in certain photoreceptor regions (21). Hence, the distributions of these light-dark changes in cAMP level can be correlated with our observed effects of cAMP on rod retinomotor movements. These observations strongly suggest that cAMP functions in the in vivo regulation of retinomotor motility.

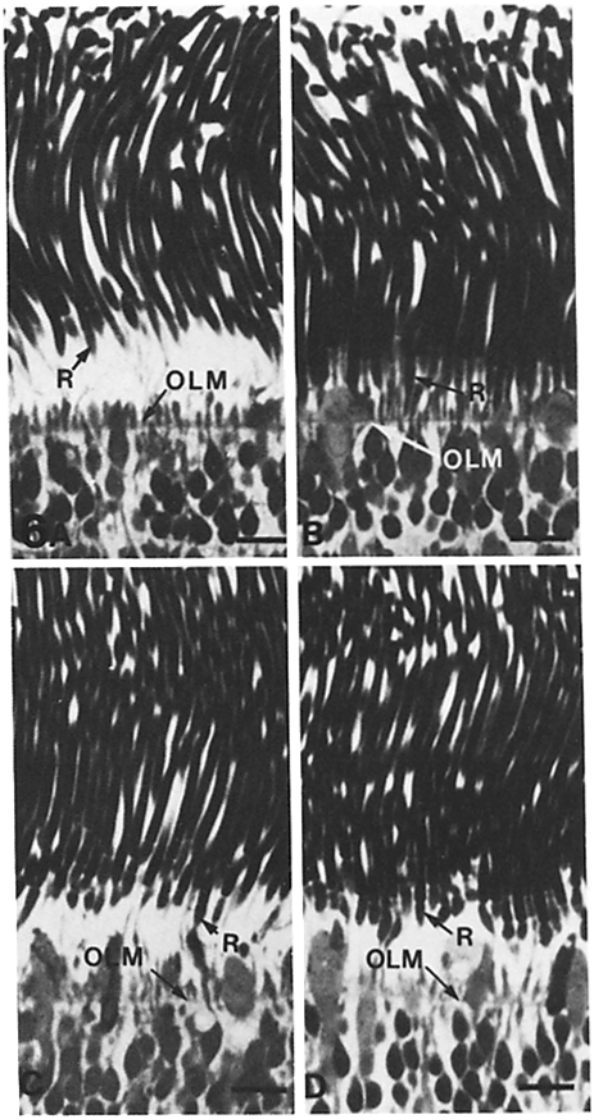

FIGURE 6 Effects of cytochalasin D on cyclic nucleotide-induced rod contraction: light micrographs of cultured light-adapted cichlid retinas. Retinas were cultured without (*A*, *B*) or with (*C*, *D*) cytochalasin D for 60 min. At this point, control (*A*) and cytochalasin D (*C*) retinas were fixed. Then *B* and *D* were cultured with 1 mM dbcAMP and 1 mM IBMX for 60 min. Rods contracted in control (*B*) cultures but failed to contract in cytochalasin D (*D*) cultures. *R*, rod ellipsoid; *OLM* outer limiting membrane. Bar, 10 μm. × 725.

TABLE III

Effect of Taxol on cAMP-induced Rod Contraction in Cultured Light-adapted Retinas

	Rod inner segment length	No. of fish
	μm*	
After 60 min in culture:		
0 μM Taxol (control)	27.9 ± 1.0	5
Same as above, followed by 60 min in 1 mM dbcAMP + 1 mM IBMX:		
0 μM Taxol	14.7 ± 0.8	6
50 μM Taxol	21.3 ± 1.3	6
DMSO	13.9 ± 0.6	5

* Mean ± SE.

TABLE IV

Effect of Cytochalasin D on cAMP-induced Rod Contraction in Cultured Light-adapted Retinas

	Rod inner segment length	No. of fish
	μm*	
After 60 min in culture:		
0 μg/ml cytochalasin D (control)	23.0 ± 0.2	3
1 μg/ml cytochalasin D	21.3 ± 0.6	3
Same as above, followed by 60 min in 1 mM dbcAMP + 1 mM IBMX:		
0 μg/ml cytochalasin D (control)	11.1 ± 0.6	3
1 μg/ml cytochalasin D	21.1 ± 0.4	3
DMSO	13.9 ± 0.6	5

* Mean ± SE.

Treatments which elevate cAMP also induce cones (5) and retinal pigment epithelium (Burnside and Basinger, 1982, *Invest. Ophthalmol. Visual Sci.*, In press.) to assume their dark-adapted retinomotor positions. In addition, it has been recently demonstrated that rod outer segment shedding, an event regulated by light (3) and an endogenous circadian rhythm (18), can be inhibited in vivo (Eckmiller and Burnside. Manuscript submitted for publication.) and in vitro (4) by cAMP analogs. Thus, cAMP not only effects rod photomechanical movements, but may act as a more general signal to mediate light regulated processes within photoreceptors and RPE.

Finally, our cAMP results demonstrate unequivocally that rods can elongate and contract in retinas which do not have attached RPE. This observation rules out the suggestion of Couillard (7) that RPE retinomotor movements provide the motive force for photoreceptor movements.

Role of the Cytoskeleton

As an initial step in investigating how cAMP could regulate photoreceptor motility, we examined the role of the myoid cytoskeletal elements in cyclic-nucleotide induced rod contraction. We found that if myoid microtubules were disrupted with cold and nocodazole treatments, rods contracted normally in response to cAMP stimulation. If, however, the myoid actin filaments were disrupted with cytochalasin D, rods failed to contract when challenged with appropriate cyclic nucleotide treatments. Therefore, we conclude that force generation during rod contraction is independent of myoid microtubules but does require myoid actin filaments. Although microtubules do not play an active role in contraction, their disassembly appears to be necessary for contraction to occur. Taxol, an agent which stabilizes microtubules in vitro (26) and in vivo (27), partially inhibits rod contraction. Though it is not possible from our data to ascertain whether taxol is affecting the extent of contraction or the rate of contraction, our taxol results do suggest that concommitant microtubule disassembly plays a part in normal rod contraction in vivo.

Our results help to clarify previous observations that colchicine inhibits rod contraction in vivo. In studies with trout, Klyne and Ali (17) reported that intraocular injections of colchicine blocked rod contraction but failed to completely disrupt myoid microtubules. We also have observed similar effects of colchicine on rod movements in *S. mossambicus*. These observations could be interpreted to mean that partial disruption of myoid mucrotubules is sufficient to block con-

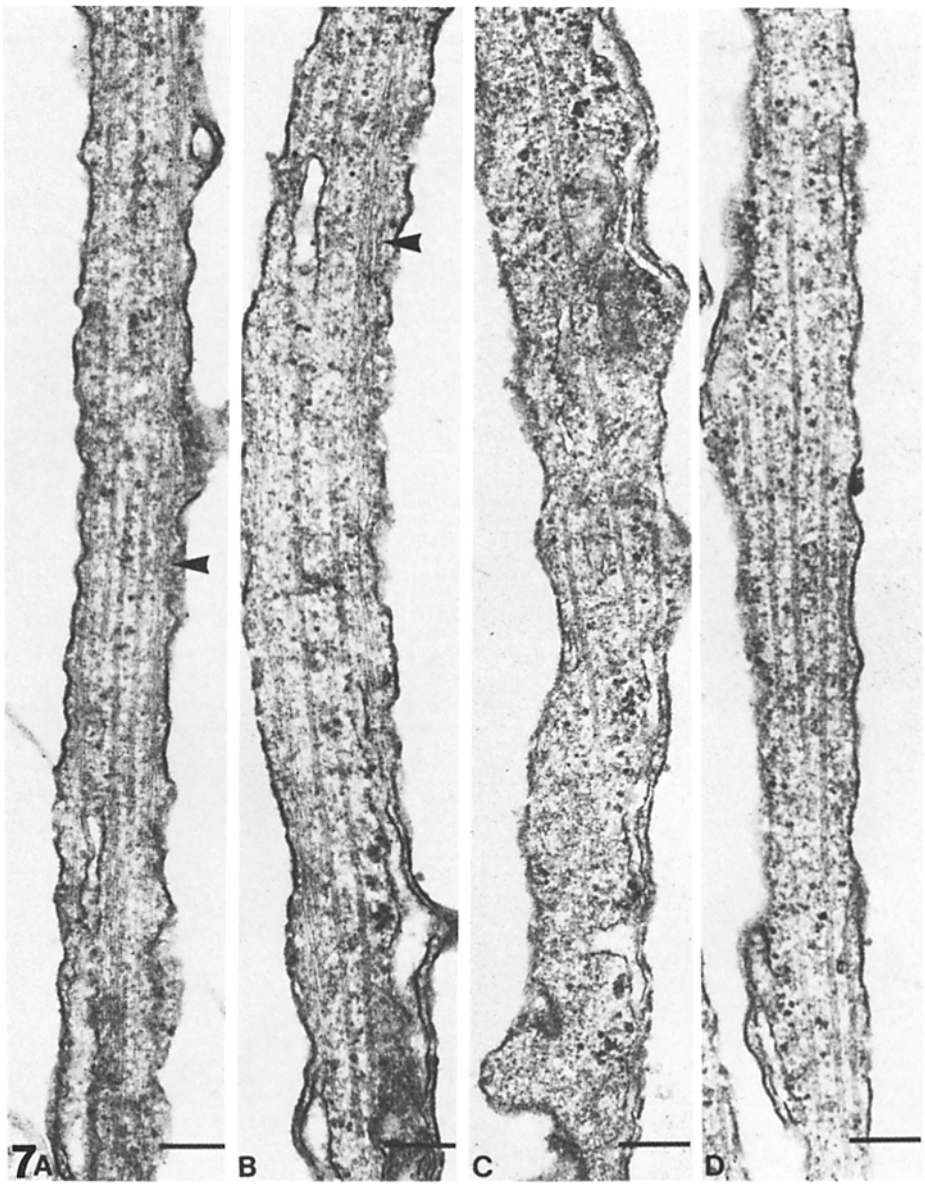

FIGURE 7 Electron micrographs of longitudinal sections through long light-adapted rod myoids of retinas cultured for 60 min in the presence or absence of cytochalasin D (1 μg/ml). Thin filaments (arrow) are present in rod myoids of control (*A*, *B*) retinas but absent in rod myoids of cytochalasin-treated (*C*, *D*) retinas. Bar, 0.2 μm. × 39,000.

traction or that colchicine inhibition is unrelated to its effect on myoid microtubules. Since we find that microtubules are not required for contraction, we favor the latter interpretation. Further investigation into the mechanism by which colchicine inhibits rod contraction are in progress in our lab.

We concluded that rod contraction is actin-dependent because rod contraction is cytochalasin-sensitive. We used cytochalasin D in our experiments because it is a more potent and specific actin inhibitor than other cytochalasin derivatives (31). 1 μg/ml cytochalasin D disrupts myoid actin filaments and significantly inhibits rod contraction. Myoid microtubules however were unaffected by cytochalasin D treatment (Fig. 7). This observation further corroborates the suggestion that myoid microtubules are not responsible for the motive force during contraction.

Because these studies were done with short term primary cultures of retinas, we have not done cytochalasin recovery experiments with this system. However, in a previous study (20), using intraocular injections we have shown that cytochalasin D (estimated at 4 μg/ml final concentration) also blocks rod elongation and that this inhibition was reversed when tested 48 h later. Therefore, we feel confident that the dosages used in this experiment were not toxic to be retinal photoreceptors.

Although we know that actin filaments play a role in cAMP-induced rod contraction, we do not yet understand how the rise in cAMP activates the contractile apparatus. It is generally thought that cAMP effects are mediated through activation of protein kinases, which catalyze phosphorylation of target proteins, thereby altering their activities (25). Cyclic AMP might activate rod contraction by phosphorylation of some component of the contractile machinery. Several cytoskeletal proteins have been found to be phosphorylated by cAMP-dependent kinases (29, 34). One such protein is filamin, an actin binding protein which cross-links actin filaments and also interferes with actin activation of myosin ATPase (8, 33). Clearly, if in rods cAMP-dependent phosphorylation decreased the affinity of some filamin-like protein for actin, activation of contraction might result.

Alternatively, cAMP might regulate the cytoskeleton indirectly by altering ion fluxes across the plasma membrane and/or internal membranes and thereby altering the ionic environ-

ment of the contractile apparatus (2, 14, 24). Evidence for interrelated metabolism of cytoplasmic Ca^{++} and cAMP (24), and the regulation of acto-myosin systems by Ca^{++} (9, 14) is well established. Thus, there is precedent for suggesting that cAMP might act directly and/or indirectly on the contractile apparatus of retinal rods.

In summary, we have shown that agents which elevate cytoplasmic cAMP induce teleost retinal rods to contract. We have demonstrated that this cyclic nucleotide-induced contraction is dependent on an actin-based mechanism. In addition, our taxol studies suggest that concurrent microtubule disassembly is required for normal contraction.

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