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Induction of Dark-Adaptive Retinomotor Movement (Cell Elongation) in Teleost Retinal Cones by Cyclic Adenosine 3′,5′-Monophosphate

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ABSTRACT In the teleost retina, the photoreceptors and retinal pigment epithelium (RPE) undergo extensive movements (called retinomotor movements) in response to changes in light conditions and to an endogenous circadian rhythm. Photoreceptor movements serve to reposition the light-receptive outer segments and are effected by changes in inner segment length. Melanin granule movements within the RPE cells provide a movable melanin screen for rod outer segments. In the dark (night), cones elongate, rods contract, and pigment granules aggregate to the base of the RPE cell; in the light (day), these movements are reversed. We report here that treatments that elevate cytoplasmic cyclic adenosine 3′,5′-monophosphate (cAMP) provoke retinomotor movements characteristic of nighttime dark adapation, even in bright light at midday. To illustrate this response, we present a quantitative description of the effects of cyclic nucleotides on cone length in the green sunfish, *Lepomis cyanellus*. Cone elongation is induced when light-adapted retinas are exposed to exogenous cAMP analogues accompanied by phosphodiesterase (PDE) inhibitors (either by intraocular injection or in retinal organ culture). Cone movement is not affected by cyclic GMP analogues. Dose-response studies indicate that the extent, but not the rate, of cone elongation is proportional to the concentration of exogenous cAMP and analogue presented. As has been reported for other species, we find that levels of cAMP are significantly higher in dark- than in light-adapted green sunfish retinas. On the basis of these observations, we suggest that cAMP plays a role in the light and circadian regulation of teleost cone length.

INTRODUCTION

In the lower vertebrates, the retina adjusts to changing light conditions by movements of the photoreceptors and the melanin screening pigment of the

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retinal pigment epithelium (RPE) (cf. Walls, 1942; Ali, 1975). Usually found in animals with fixed pupils, these "retinomotor movements" serve to reposition photoreceptor outer segments and screening pigment so that rods are optimally exposed to incoming light in the dark (night) and optimally shielded in the bright light of day. In the day, cone myoids contract, rod myoids elongate, and pigment granules disperse into the long apical projections of the RPE, thus positioning the cone outer segments first in line for light reception and burying the rod outer segments in the shielding pigment of the RPE. In the night, these movements are reversed, thus exposing the rod outer segments and moving them into place for optimal light reception.

It is not yet clear how retinomotor movements are regulated. Over the last century, a vast literature has described effects of light on retinomotor movement in numerous species of fish, amphibians, and birds (cf. Walls, 1942); nonetheless, the mechanism by which light influences retinomotor movement remains unknown. More recently it has been shown that in several species of fish, retinomotor movements respond not only to light conditions but also to an endogenous circadian rhythm (cf. Welsh and Osborne, 1937; Levinson and Burnside, 1981). Even in constant darkness, cones elongate at subjective dusk and contract at subjective dawn, usually with reduced amplitude compared with the normal light/dark diurnal cycle.

A number of observations suggested to us that cyclic nucleotides might play a role in regulating retinomotor movements. Previous studies have shown that in all species so far examined, dark-adapted retinas exhibit higher levels of both cAMP and cGMP than light-adapted retinas (DeVries et al., 1978, 1979; Farber et al., 1981; Ferrendelli and Cohen, 1976). Thus light and dark retinomotor positions are correlated with different retinal cyclic nucleotide levels. In addition, we know that cAMP influences cell shape and cell movement in a large variety of cell types (cf. Dedman et al., 1979). For example, cAMP has been implicated in the regulation of pigment dispersion in the dermal melanophores of frogs and fishes (Bitensky and Burnstein, 1965; Novales and Fujii, 1970), in the induction of neurite elongation in cultured neurones and neuroblastoma (Roisen et al., 1972; Shapiro, 1973), and in the catecholamine-induced relaxation of smooth muscle cells (Mueller and van Breeman, 1979; Scheid et al., 1979). Moreover, cyclic AMP has a marked effect on the morphological characteristics of neuroepithelial PE cells in culture, including increased pigmentation, effects on cell and colony shape, and an increased number of microvilli (Redfern et al., 1976). Influenced by these observations, we set out in this study to ascertain whether cyclic nucleotides play a role in retinomotor movements.

In the green sunfish, *Lepomis cyanellus*, retinomotor movements exhibit extreme excursions and thus are particularly useful for quantitative study. The necklike "myoid" region of the cone elongates from 5–8 μm in the light to as much as 120 μm in the dark (Fig. 1), whereas pigment granules migrate over distances of >100 μm. For brevity, we present quantitative data in this report only for cones. Rod and RPE retinomotor movements (which also occur under the conditions described) will be reported in detail elsewhere. To

investigate possible roles of cyclic nucleotides in regulating sunfish retinomotor movements, we have exposed retinas to exogenous drugs by two experimental procedures: intraocular injections and retinal organ cultures.

Using both experimental procedures, we find that cAMP, when accompanied by phosphodiesterase (PDE) inhibitors, provokes retinomotor movements characteristic of dark adaptation (cone elongation), even in fully light-adapted retinas at midday. Cyclic GMP analogues have no effect on retinomotor movements. Dose-response and rate studies indicate that varying the concentration of exogenous cAMP analogue influences the extent, but not the rate, of cAMP-induced cone elongation. A preliminary report of this work has appeared in abstract form (Evans and Burnside, 1979).

MATERIALS AND METHODS

Green sunfish, *Lepomis cyanellus*, were obtained from local breeders and maintained in outdoor ponds or laboratory aquaria under ambient light cycle.

Intraocular injections were performed on fish of uniform size (75–85 mm length). Drugs were dissolved in Hanks' buffered salt solution (HBSS; 116 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 1 mM NaH_2PO_4, 4 mM $NaHCO_3$, 5.5 mM glucose). Isobutylmethylxanthine (IBMX) was dissolved (or suspended) by sonication in distilled water for several hours before adding buffer salts; under these conditions, no precipitate was visible, even at the higher concentrations. For each injection, 5 μl of solution was delivered to the center of the vitreous while observing the tip of the microsyringe needle through the lens. Calculated final concentrations were estimated by assuming dilution of the injected volume (always 5 μl) to the full eye volume. Eye volume was calculated from caliper-measured eye diameters, assuming a spherical shape. Because numerous factors contribute to the error of such an estimate, we should emphasize that we do not know the absolute concentrations bathing the retinas, or the time course of concentration changes. Nonetheless, since we use uniform injection volumes and eye sizes, we feel confident that the relative concentration relationships we report are informative. The reproducibility of our observations supports this confidence. Midday injections were carried out under fluorescent illumination (8 lx); midnight injections were done in dim red light from a clear red bulb with emission wavelengths >600 nm. Unless otherwise indicated, fish were killed 2 h after injection. The posterior eyecup was immersed in 3–6% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. An asymmetrically shaped block of retina was cut from the region just behind the optic nerve to ensure comparison of comparable regions from different fish. This block was either embedded in epon and sectioned at 2 μm, or chopped into 20-μm slices with a manual chopper modified from Werblin (1978) (Fig. 1). These sections or slices were used for cone length measurements.

Cone lengths were measured using Zeiss bright field optics (Carl Zeiss, Inc., New York) for 2-μm sections (Fig. 1*a–c*), or Zeiss Nomarski optics for 20-μm slices (Fig. 1*d–g*) with a 40× objective and ocular micrometer. For each retina, the myoid length, i.e., the distance from the outer limiting membrane to the cone ellipsoid, was ascertained for 20 representative cones (many more cones are visible in each section). In all tables and figures, *n* refers to numbers of retinas examined. 35-mm micrographs provide permanent records.

Organ cultures of light-adapted retinas with RPE attached were prepared from light-adapted fish. The RPE retina was gently removed from the choroid and the optic nerve was severed. Because the fish RPE retina is affixed to a gel-like vitreous,

it retained its hemispherical shape. The RPE retina was then bisected and the resulting half-retinas were immersed in 0.5 ml of HBSS containing the indicated drugs. Thus, one zero time (t_0) and three comparable experimental preparations were obtained from each fish. Half-retinas were positioned vitreous-down against the wall of the well of a Falcon multiwell plate (Falcon Labware, Oxnard, CA) and 0.3 ml of the culture medium was removed for optimal gassing. Cultures were gassed with 100% O_2 and rotated at 1 rev/s on a rotary shaker at 22°C. After 2 h, the half-retinas were removed to 6% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, and chopped into 20-μm slices for cone length measurements (cf. Figs. 1*d* and *e*).

To prepare organ cultures of light-adapted retinas without RPE, retinas were dissected in the dark-adapted state (where the RPE readily detaches) and then exposed to light culture before drug addition. Dark-adapted retinas were removed in dim red light (from fish kept in darkness for 2 h), bisected, and cultured in HBSS in fluorescent light (1.4 lx). After 30 min, one half-retina was fixed (t_0) and the other three were transferred to HBSS containing indicated drugs, for 2-h culture as described above for retinas with RPE attached (Fig. 1*f* and *g*). Cones shortened to the fully light-adapted position during the 30-min light preculture.

Cyclic nucleotide determinations were performed on light- and dark-adapted green sunfish retinas. Fish were killed by spinal cord section and pithing the brain; the fish remains alive with continued circulatory function for several minutes after this procedure. After removing the eye, retinas were dissected away from the eyecup in oxygenated HBSS and immediately placed in 10% perchloric acid solution (which stops all enzymatic reactions). The time lapse between opening the eye and placing the retina into the acid was 1–2 min. Dark-adapted retinas were dissected in dim red light. Removing the eye took ~30 s in either light condition. The relatively large size (8–10 mm Diam) of the eyes made it possible to dissect out retinas as easily in dim red as in white light; thus times of ischemia were similar for both light- and dark-adapted eyes. When dark-adapted, the neural retina separated from the RPE; therefore dark-adapted neural retina and RPE (with some attached choroid) were processed separately. When they were light-adapted, neural retina and RPE were firmly attached and thus were processed together.

Cyclic nucleotides were subsequently separated, purified, and succinylated (Frandsen and Krishman, 1976). Radioimmunoassay was performed by the procedure of Steiner et al. (1972) with minor modification (Krishman et al., 1976). Protein was determined by the method of Lowry et al. (1951) with bovine albumin as standard. Results are expressed as picomoles of cyclic nucleotide per milligram of protein.

RESULTS

To determine whether cyclic nucleotides influence retinomotor movements, we first used intraocular injections to expose retinas to a variety of cyclic nucleotide analogues and phosphodiesterase (PDE) inhibitors. When the cAMP analogue dibutyryl-cAMP (dbcAMP) was injected along with a PDE inhibitor into a light-adapted eye, the rods, cones, and RPE moved to assume retinomotor positions indistinguishable from normal dark adaptation (Figs. 1*a–c*) even in high intensity fluorescent light (8 lx) at noon. By 2 h after injection, cones had elongated, rods had contracted, and the screening pigment had aggregated to the base of the RPE cell. Retinomotor positions of rods, cones, and RPE were not affected by cyclic GMP analogues (Table I).

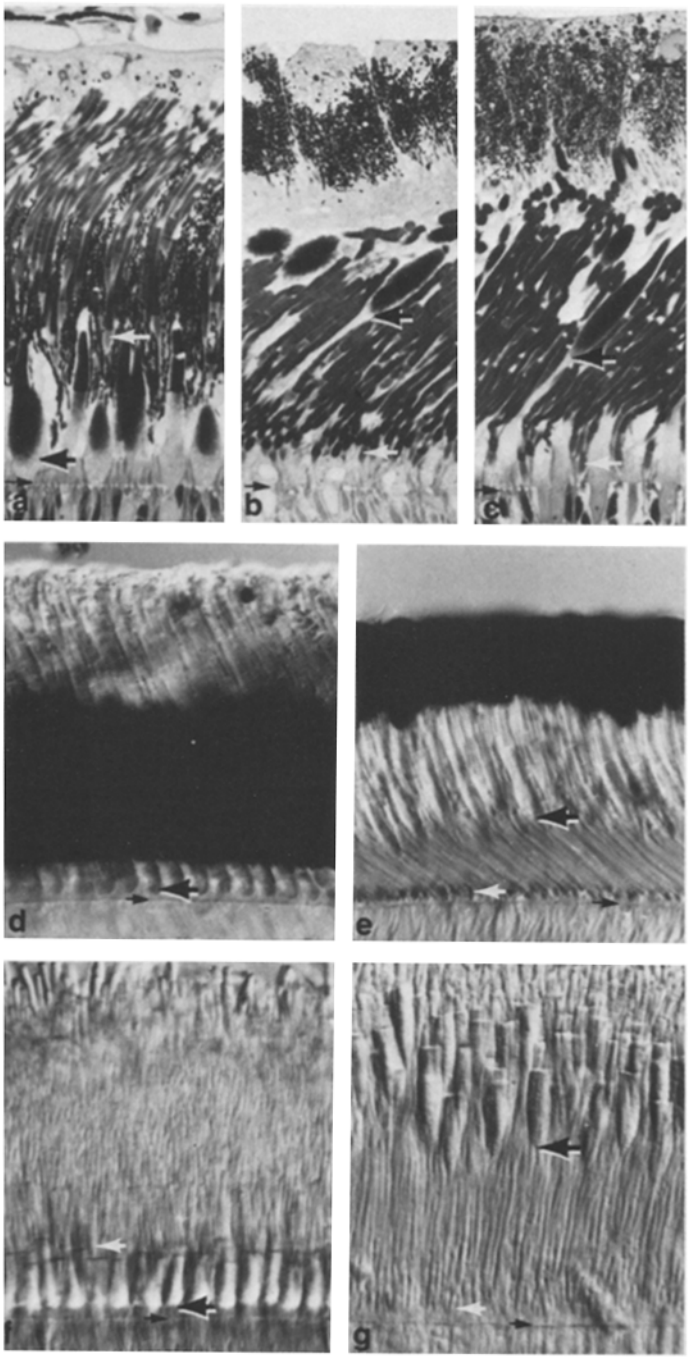
Table I provides a survey of the effects of several injected cyclic nucleotide

analogues and PDE inhibitors on cone retinomotor position. Cone myoid lengths were observed 2 h after intraocular injection. PDE inhibitors alone (isobutylmethylxanthine [IBMX], theophylline, papaverine) did not induce significant cone elongation. A moderate level of exogenous dbcAMP (2.5 mM calculated final concentration), in the absence of PDE inhibitor, also failed to produce cone elongation. However, this same moderate dbcAMP concentration, when accompanied by PDE inhibitor (0.6 mM IBMX), produced cone elongation equivalent to the maximal elongation observed during normal dark adaptation (20-fold). It was possible to induce elongation with dbcAMP in the absence of PDE inhibitor only if a very high level of dbcAMP was injected; 15 mM dbcAMP induced moderate (fourfold) elongation. Cone retinomotor position was not affected by cGMP analogues in the presence of IBMX. The long cones of fully dark-adapted retinas were not affected by either cAMP or cGMP analogues in the presence of PDE inhibitors.

To investigate further the effect of dbcAMP on cone elongation, we prepared dose-response curves for dbcAMP effects on cone myoid lengths measured 2 h after intraocular injection (Fig. 2A). The two curves in Fig. 2A illustrate dbcAMP effects at two IBMX concentrations (0.06 and 0.6 mM, calculated final concentration). In both curves the response was biphasic. In the ascending phase, cone myoid length was proportional to the concentration of dbcAMP injected. At high concentrations of dbcAMP, elongation appeared to be inhibited. In both curves the maximal cone length observed was comparable to the maximal normal dark-adapted cone length. Thus the full excursion of cone movement was induced by intraocular injection of exogenous nucleotide.

For Fig. 2A, we measured cone lengths achieved 2 h after injection. Such dose-response curves could have been produced by at least two different modes of dbcAMP action: (*a*) dbcAMP concentration might influence the rate of cone elongation, or (*b*) dbcAMP concentration might dictate the extent of cone elongation without influencing the rate. To distinguish between these possibilities, we investigated the time course of cone elongation observed during normal dark adaptation and at several dbcAMP concentrations (Fig. 2B and Table II). At each of the indicated dbcAMP concentrations, cones elongated at rates ranging from 1.0 to 1.2 μm/min. These rates did not differ significantly from the cone elongation rate during normal dark adaptation (1.1 ± 0.1 μm/min) (Table II). At all concentrations cone elongation was completed by 2 h. Thus the dose-response curve in Fig. 2A illustrates effects of dbcAMP concentration on extent rather than on rate of cone elongation. Exogenous dbcAMP concentrations clearly dictated different extents of elongation without altering the normal rate.

Intraocular injection procedures have the serious drawback that we can know neither the exact magnitude nor the time course of changes in dbcAMP concentrations bathing the retina. Therefore we extended our observations to retinal organ culture, where known concentrations of exogenous drugs can be maintained throughout the 2-h incubation period. Results with organ cultures were consistent with those observed with injections (Table III, Fig. 3). We saw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no effect of IBMX alone until the concentration was elevated to 1 mM, and we saw no effect of cyclic GMP analogues at any concentration examined (Table II). However, 0.5 mM IBMX plus 1 mM dbcAMP produced significant cone elongation (fourfold) in retinas with and without RPE.

Fig. 3 illustrates dose-response curves for effects of dbcAMP concentration on extent of cone elongation in retinas cultured with and without attached RPE (0.5 mM IBMX was included in all cultures; see Figs. 1*e–f*). Under these conditions, substantial cone elongation did occur, though the maximal elongation observed in culture (fivefold) was not as great as that observed in vivo (20-fold). Cone responses were similar in retinas cultured in the presence or absence of RPE. Thus cones appear to be capable of undergoing significant elongation (fivefold) and responding to dbcAMP without contribution from the RPE.

The results of our injection and culture surveys suggested that treatments that elevate cytoplasmic cAMP levels induced retinomotor movements characteristic of dark adaptation. To ascertain whether cyclic nucleotide levels were higher in dark-adapted than in light-adapted green sunfish retinas, we carried out determinations by radioimmunoassay (Table IV). Both cAMP and cGMP levels were significantly higher in dark-adapted retinas. Retinal cyclic AMP levels were approximately threefold higher in the dark.

FIGURE 1. (*opposite*) Bright field light micrographs of 2-μm Epon sections of green sunfish retinas. (*a*) Light-adapted retina fixed at noon; (*b*) dark-adapted retina fixed at midnight; (*c*) light-adapted retina fixed 2 h after an intravitreal injection calculated to produce a final concentration of 0.6 mM IBMX plus 2.0 mM dbcAMP in the eye (see Materials and Methods). Even in bright light at noon, IBMX plus dbcAMP injection produced retinomotor positions characteristic of the dark-adapted state: cones elongated, rods contracted, and pigment granules aggregated to the base of the RPE. Cone myoid lengths were measured between the outer limiting membrane (small black arrows) and the base of the cone ellipsoid (large black arrows). White arrows indicate the bottom tier of rod ellipsoids (× 500). Nomarski optics light micrographs of 20-μm slices chopped from light-adapted retinas with attached RPE: half of one retina was fixed before (*d*) and the other half was fixed after (*e*) 2 h culture in HBSS containing 1 mM IBMX plus 1 mM dbcAMP (*d* and *e* are from the same eye). Culture with IBMX plus dbcAMP provoked dark-adaptive retinomotor movements (cone elongation, large black arrows) though retinas were cultured in fluorescent light (4 lx). In these cultures the RPE remains attached to the retina and also displays pigment granule aggregation characteristic of dark adaptation. Rods also contract (white arrows) (× 500). Nomarski optics light micrographs of 20-μm slices chopped from retinas cultured without RPE: dark-adapted retinas are removed from the RPE, cultured in the light with HBSS for 30 min to permit cones to assume their light-adapted positions. At this point, one half of the retina was fixed (*f*) and the other half was placed in culture with 2.5 mM dbcAMP plus 0.5 mM IBMX, cultured in fluorescent light (4 lx) for 2 h (with 100% O_2) and then fixed (*g*). In the absence of the RPE, IBMX plus dbcAMP induced cone elongation (large black arrows) and rod contraction (white arrows) characteristic of dark adaptation (× 500).

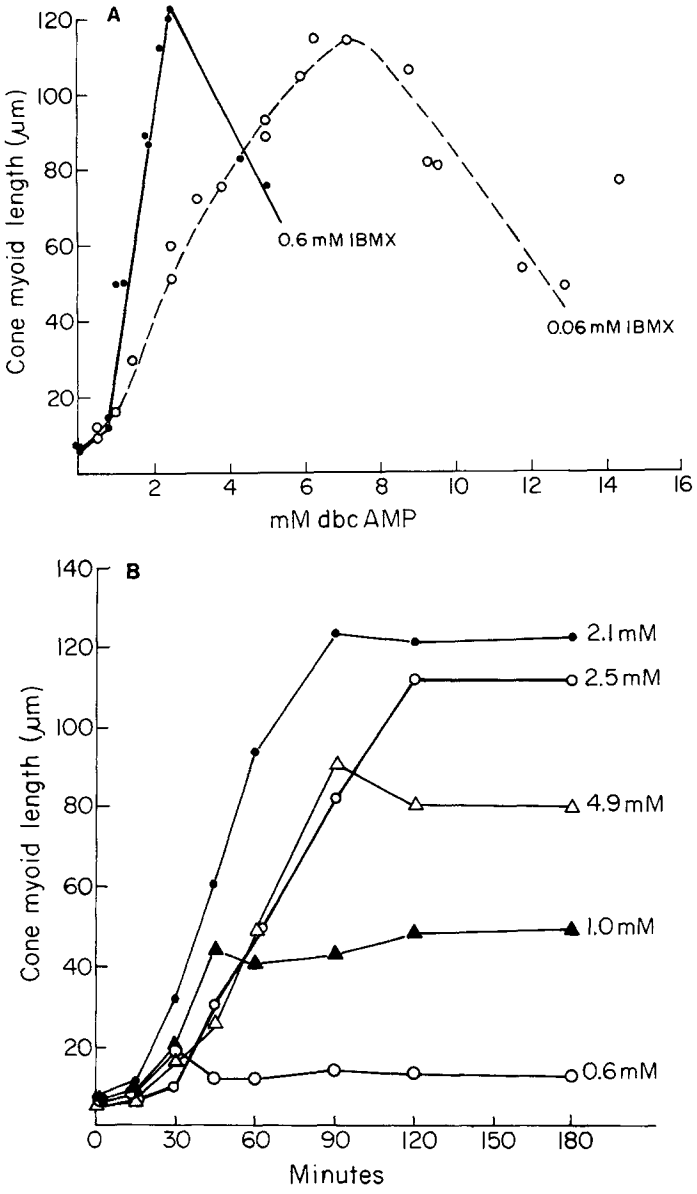

FIGURE 2. Effects of varying the concentration of intraocularly injected exogenous dbcAMP on cone myoid length. A. Dose-response curves for myoid lengths observed 2 h after intraocular injections with various concentrations of dbcAMP at either of two constant concentrations of IBMX (0.06 and 0.6 mM). Concentrations indicated were calculated as described in Materials and Methods. Each point indicates mean myoid length (from 20 measured cones) for one fish. Standard error of the mean is <10% mean for all points. B. Time courses of cone myoid elongation after injection of five indicated dbcAMP concentrations (calculated final concentrations from 0.6 to 4.9 mM), each accompanied by 0.6 mM IBMX (see curve in A). Time after injection is indicated. Each point

DISCUSSION

All the observations presented in this report are consistent with a specific role for cyclic AMP in regulation of cone retinomotor movements. Phosphodiesterase inhibitors alone have no effect on cone movement unless used in relatively high concentrations (e.g., 1 mM IBMX, in culture). However, dibutyryl cyclic AMP, when injected with a concentration of IBMX that causes no elongation when administered alone, can provoke the full extent of cone elongation (20-fold) observed under normal dark conditions. Cone

TABLE I

CONE MYOID LENGTHS: INTRAOCULAR INJECTIONS

	Cone myoid length	
	$\bar{x}$±SEM	n
	μm	
Light: midday		
No injection	6.4±0.1	(12)
HBSS	6.7±0.1	(12)
0.6 mM IBMX	7.0±0.3	(10)
2.5 mM dbcAMP	6.8±0.6	(8)
15.0 mM dbcAMP	28.6±7.9	(3)
0.6 mM IBMX + 2.5 mM dbcAMP	117.1±1.6	(7)
0.6 mM IBMX + 2.5 mM dbcGMP	5.4±0.2	(6)
0.6 mM IBMX + 4.5 mM dbcGMP	6.2±0.2	(4)
0.6 mM IBMX + 2.5 mM 8-bromo-cGMP	4.5±0.2	(6)
0.6 mM theophylline	9.9±1.8	(3)
0.6 mM papaverine	6.3±0.1	(2)
Dark: midnight		
HBSS	75.5±2.7	(6)
0.6 mM IBMX + 2.5 mM dbcAMP	74.7±4.8	(4)
0.6 mM IBMX + 2.5 mM dbcGMP	67.4±1.4	(4)

Cone myoid lengths were observed 2 h after intraocular injection with cyclic nucleotide analogues and phosphodiesterase inhibitors. Indicated concentrations are estimated by assuming dilution to full eye volume (see Materials and Methods). n = number of retinas examined; 20 cones were measured for each retina; $\bar{x}$ ± SEM = mean ± standard error of the mean (n − 1 method).

movement is not produced by dibutyryl cGMP or 8-bromo-cGMP, either alone or when combined with IBMX. This result underscores the specificity of the cAMP effect and obviates consideration of a butyrate effect. Exogenous dbcAMP induces cone elongation in a dose-dependent fashion, with optimal

represents combined mean for two fish (20 cones were measured for each fish); standard deviations were <10% mean for all points. Though there was some variability in the lag before onset of elongation, rates of elongation are similar for all concentrations (see Table II). Dark-induced elongation (not shown) superimposes on the 2.5 mM dbcAMP curve. At different dbcAMP concentrations, cones elongate at similar rates but plateau at different final lengths; 3 h after injection, plateau levels were still maintained.

dbcAMP concentrations producing the full possible excursion of cone movement in intraocular injection experiments.

Treatments that elevate cytoplasmic cAMP induce retinomotor movements characteristic of dark adaptation (cone elongation, rod contraction, RPE pigment aggregation). This effect is consistent with our finding that cAMP levels are approximately threefold higher in dark- than in light-adapted green sunfish retinas. Similar differences (approximately twofold) in dark vs. light retinal concentrations of cAMP have been reported for mice (DeVries et al., 1978; Ferrendelli and Cohen, 1976), ground squirrels (Farber et al., 1981), and rabbits (DeVries et al., 1979). The cAMP concentration we observe in light-adapted sunfish retinas (5 pmol cAMP/mg protein) is comparable to

TABLE II
RATES OF CONE ELONGATION

	Rate	
	$\bar{x}$±SEM	n
	μm/min	
Dark-induced (no injection)	1.1±0.1	9
1.0 mM dbcAMP + 0.6 mM IBMX	1.2±0	3
1.4 mM dbcAMP + 0.6 mM IBMX	1.1±0.2	4
2.5 mM dbcAMP + 0.6 mM IBMX	1.2±0.1	6
4.9 mM dbcAMP + 0.6 mM IBMX	1.0±0.2	6

Rates of cone length change were observed during normal dark adaptation and during dbcAMP-induced elongation after intraocular injection. To calculate rates, all time points during the linear phase of the curves in Fig. 2B were compared to the mean for the earliest point in the linear phase. $\bar{x}$ ± SEM = mean ± standard error of the mean; n = number of retinas (fish) examined. These rates do not differ significantly when tested by the one-way analysis of variance ($P > 0.4$).

those observed for rats, mice, and rabbits (6–10 pmol/mg protein; DeVries et al., 1978, 1979; Ferrendelli and Cohen, 1976; Farber et al., 1981), but much lower than that observed in the cone-dominant retina of the ground squirrel (40 pmol/mg protein; Farber et al., 1981).

It is not possible to ascertain from these observations of retinal cAMP levels whether light-dark cAMP changes are occurring in photoreceptors. Unlike cGMP, which appears to be preferentially localized in photoreceptors, cAMP is more evenly distributed over the whole retina (Orr et al., 1976; DeVries et al., 1979). Much of the retinal cAMP (and the light-dark change in cAMP) appears to be associated with dopaminergic interneurons (Dowling and Watling, 1981). Nonetheless, there are substantial levels of cAMP in the retinal layers occupied by photoreceptors (e.g., up to 22 pmol/mg protein in the rabbit inner segment; Orr et al., 1976; DeVries et al., 1979) and in isolated outer segments (Fletcher and Chader, 1976). Light is known to activate photoreceptor phosphodiesterases (cf. Lolley, 1980; Pober and Bitensky, 1979),

which could lower photoreceptor cAMP levels as well as cGMP levels in some species.

The role of cAMP in photoreceptor physiology is not yet clear. Cyclic GMP has received much more attention than cAMP over the last decade (cf. Pober and Bitensky, 1979; Lolley, 1980) because of its localization to photoreceptors (Orr et al., 1976) and its postulated role in rod phototransduction (cf. Hubbell and Bownds, 1979). Recently it has been suggested that cAMP might play a role in cone phototransduction analogous to that proposed of cGMP in rods (Farber et al., 1981). Given the broad range of physiological activities known to be regulated by cAMP in numerous cell types, including brain (cf. Daly, 1977; Rasmussen and Goodman, 1977; Dedman et al., 1979), it is not

TABLE III

CONE MYOID LENGTHS: RETINAL CULTURES

	Cone myoid length	
	$\bar{x}$±SEM	*n*
	μm	
With RPE		
0.1 mM IBMX	6.4±0.9	(8)
0.5 mM IBMX	6.8±0.3	(10)
1.0 mM IBMX	15.1±0.6	(12)
0.5 mM IBMX + 1 mM dbcAMP	34.0±1.1	(12)
0.5 mM IBMX + 1 mM dbcGMP	4.0±0.6	(3)
0.5 mM IBMX + 1 mM 8-bromo-cGMP	4.0±0.6	(3)
0.5 mM IBMX + 10 mM 8-bromo-cGMP	3.8±0.1	(2)
Without RPE		
0.5 mM IBMX + 1 mM dbcAMP	33.9±2.5	(3)

Cone myoid lengths were observed after 2 h culture with cyclic nucleotide analogues and phosphodiesterase inhibitors. $\bar{x}$ ± SEM = mean ± standard error of the mean. *n* = number of retinas examined; 20 cones were measured for each retina.

unreasonable to suspect that cAMP will turn out to play significant regulatory roles in photoreceptors. A cAMP-dependent protein kinase system has recently been described, for example, in isolated bovine outer segments (Farber et al., 1979). Among the numerous cellular activities that have been shown to be cAMP-regulated in other cell types are ion flux, enzyme activity, RNA and protein synthesis, protein secretion, cell-cell communication, and cell motility (cf. Daly, 1977; Dedman et al., 1979; Rasmussen and Goodman, 1977). Cell motility is clearly influenced by cAMP in teleost photoreceptors and RPE.

Because elevating cAMP induces three diverse movements in teleost retinas (elongation in cones, contraction in rods, pigment aggregation in RPE), and because these movements represent the characteristic responses of these cells to darkness, elevation of cAMP seems to serve as a general signal for "darkness" that is read differently in the different cell types. Most, if not all, cAMP effects in tissues appear to result from cAMP-induced phosphorylation of specific proteins, with consequent alteration of their biological activities (cf. Green-

gard, 1975; Corbin and Lincoln, 1978). Thus it seems likely that the different motile responses of retinal cells to cAMP result from different substrate specificities and different cascades of protein phosphorylations in the different cell types.

Our experiments with cultured retinas demonstrate clearly that cones can elongate in the absence of the RPE. Thus cone elongation is not produced by a contractile activity of the RPE cells, as has been suggested by Klyne and Ali (1980). Instead, the force-producing mechanism appears to reside in the cones themselves. We have also found that cytoplasmic cAMP concentration regulates the *extent* of cone elongation without modifying the *rate* of elongation. This observation suggests that cAMP might work as a switch to turn elonga-

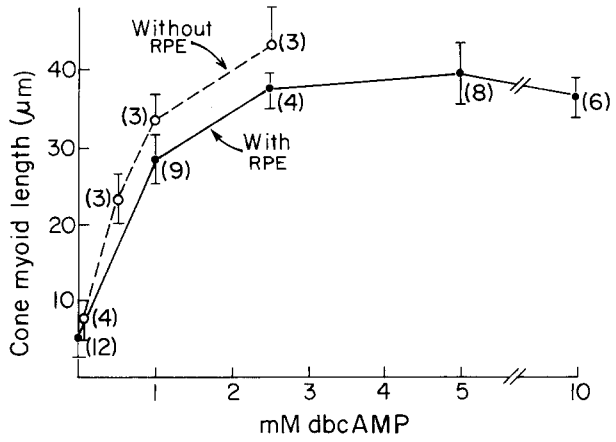

FIGURE 3. Effects of varying the concentration of exogenous dbcAMP on cone myoid length in cultured retinas: all cultures contained 0.5 mM IBMX plus the indicated concentrations of dbcAMP. Cone myoid length was measured from chopped slices of retinas fixed after 2 h in culture. Points represent mean ± standard error of the mean (error bars) for (*n*) retinas.

tion on and off rather than as a regulator of some rate-limiting component of the force-producing mechanism.

In constant darkness, teleost cones exhibit a circadian rhythm of length changes, contracting at subjective dawn and elongating at subjective dusk (cf. Levinson and Burnside, 1981). Although the amplitude of length change in constant darkness is less than that observed in the normal light/dark diurnal cycle, the circadian length changes are reproducible from day to day and from fish to fish. This reproducibility indicates that cones possess a mechanism for dictating specific intermediate lengths at specific times in the cycle. Because predictable intermediate cone lengths can be produced experimentally by exogenous dbcAMP, we suggest that the circadian regulation of cone length in vivo is mediated by changing cytoplasmic cAMP levels. This hypothesis is consistent with other proposed examples of cAMP participation in circadian regulation of neuronal activity. The pineal gland, for example, exhibits both

light-induced (cf. Weiss and Strada, 1972) and circadian (Mikuni et al., 1981) fluctuations in cAMP concentration, with higher cAMP levels associated with darkness and subjective night. It seems likely that pineal cAMP variation is in turn controlled by cycles of release of norepinephrine (Mikuni et al., 1981), which acts to stimulate cAMP production via a beta-adrenergic receptor: the adenylate cyclase system (Romero and Axelrod, 1975). In addition, pineal beta-adrenergic receptors regulate (apparently via cAMP) both induction and maintenance of the activity of *N*-acetyltransferase (NAT), and the enzyme involved in the biosynthesis of melatonin (Axelrod, 1974). It has recently been shown that retinas, like pineal glands, exhibit circadian and light-induced

TABLE IV
RETINAL CYCLIC NUCLEOTIDE LEVELS IN LIGHT- AND DARK-ADAPTED GREEN SUNFISH RETINAS

	cAMP			cGMP		
Tissue	$\bar{x}$±SEM	*n*		$\bar{x}$±SEM	*n*	
	pmol/mg protein					
Light-adapted						
Neural retina + RPE	4.7±0.3	(7)		23.6±2.1	(7)	
			p value (difference from LA)			*p* value (difference from LA)
Dark-adapted						
Neural retina	13.7±1.2	(6)	$P \ll 0.001$	36.4±2.8	(6)	$P \ll 0.001$
RPE (+ choroid)	4.8±1.2	(4)		2.1±0.6	(4)	

Values given are mean ± standard error of the mean ($\bar{x}$ ± SEM) for the indicated number of retinas (*n*). Duplicate radioimmuno-determinations at each of three different dilutions were averaged to get the final value for each retina. Statistical significance (*p* value) was determined using a *t* statistic evaluation (two-sample test) with a Texas Instruments (Dallas, TX) program.

variations in NAT activity and melatonin synthesis (Hamm and Menaker, 1980; Binkley et al., 1980) with maximal NAT activity in the dark or subjective night. Thus it is reasonable to suggest that these NAT fluctuations are associated with circadian changes in retinal cAMP level.

Circadian and light-induced fluctuations in photoreceptor cAMP levels might be expected to regulate other aspects of photoreceptor metabolism known to exhibit circadian variation, such as disc shedding (Besharse et al., 1977; LaVail, 1980). We have in fact found that dbcAMP inhibits disc shedding in vitro (Besharse et al., 1982).

In summary, we have shown in this report that treatments that elevate cytoplasmic cAMP induce dark-adaptive retinomotor movements in teleost retinas. We suggest, therefore, that alterations in photoreceptor cAMP levels might play a more general role in the light and circadian regulation of photoreceptor metabolism.

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