

Circadian Regulation of Retinomotor Movements

I. Interaction of Melatonin and Dopamine in the Control of Cone Length

MARY E. PIERCE and JOSEPH C. BESHARSE

From the Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322

ABSTRACT In lower vertebrates, cone retinomotor movements occur in response to changes in lighting conditions and to an endogenous circadian clock. In the light, cone myoids contract, while in the dark, they elongate. In order to test the hypothesis that melatonin and dopamine may be involved in the regulation of cone movement, we have used an *in vitro* eyecup preparation from *Xenopus laevis* that sustains light- and dark-adaptive cone retinomotor movement. Melatonin mimics darkness by causing cone elongation. Dark- and melatonin-induced cone elongation are blocked by dopamine. Dopamine also stimulates cone contraction in dark-adapted eyecups. The effect of dopamine appears to be mediated specifically by a dopamine receptor, possibly of the D₂ type. The dopamine agonist apomorphine and the putative D₂ agonist LY171555 induced cone contraction. In contrast, the putative D₁ agonist SKF38393-A and specific α_1 -, α_2 -, and β -adrenergic receptor agonists were without effect. Furthermore, the dopamine antagonist spiroperidol not only blocked light-induced cone contraction, but also stimulated cone elongation in the light. These results suggest that dopamine is part of the light signal for cone contraction, and that its suppression is part of the dark signal for cone elongation. Melatonin may affect cone movement indirectly through its influence on the dopaminergic system.

INTRODUCTION

It is now apparent that several aspects of photoreceptor metabolism are regulated in relation to the daily light-dark cycle (Besharse, 1982). Prominent among these are photoreceptor membrane turnover (LaVail, 1976) and photoreceptor movement (Welsh and Osborne, 1937; Levinson and Burnside, 1981). Retinomotor movements occur in some lower vertebrates, presumably to position photoreceptor outer segments for optimal exposure to incoming light (see Burnside and Nagle, 1983). In the light, cone myoids contract, rod myoids elongate, and, in some species, the pigment granules in the retinal pigment epithelium (RPE)

Address reprint requests to Dr. Mary E. Pierce, Dept. of Anatomy, Emory University School of Medicine, Atlanta, GA 30322.

move vitreally. In the dark, the movements are reversed: cones elongate, rods contract, and pigment granules move sclerally.

An analysis of reactivated movements in detergent-lysed photoreceptor cells has increased our understanding of the mechanisms of elongation and contraction. Elongation appears to be a microtubule-based process in cones (Warren and Burnside, 1978; reviewed by Burnside and Nagle, 1983), whereas contraction is actin-based (Burnside, 1976, 1978; reviewed by Burnside and Nagle, 1983). Furthermore, cAMP and Ca^{2+} are probably directly involved in regulating the motile machinery. In particular, cAMP blocks reactivated cone contraction and is required for reactivated elongation (Burnside et al., 1982*b*; Porrello and Burnside, 1984). This is consistent with earlier data showing that increases in cAMP in intact cells also cause dark-adaptive retinomotor movements (Burnside et al., 1982*a*; Besharse et al., 1982; Burnside and Basinger, 1983). These observations have led to the general view that nighttime increases in cAMP in the photoreceptor-pigment epithelial complex lead to the dark-adaptive movements (Burnside and Nagle, 1983).

Although numerous investigators have described the effects of light and darkness on retinomotor movements and other aspects of rhythmic photoreceptor metabolism (reviewed by Besharse, 1982; Burnside and Nagle, 1983), little is known about the mechanism of their effects. To further complicate the issue, rhythmic photoreceptor metabolism is influenced by an endogenous circadian clock (LaVail, 1976; Besharse et al., 1977; Welsh and Osborne, 1937; Levinson and Burnside, 1981). The latter observation has led to the suggestion that melatonin may be involved in the circadian regulation of photoreceptor metabolism (Besharse, 1982). This view is consistent with reports that melatonin activates photoreceptor disk shedding (Besharse and Dunis, 1983*b*) and promotes dark-adaptive pigment aggregation in the RPE (Kraus-Ruppert and Lembeck, 1965; Chéze and Ali, 1976; Pang and Yew, 1979). Furthermore, an indoleamine-synthesizing pathway has been identified in the retina (Baker et al., 1965; Gern and Ralph, 1979), where the activity of a key enzyme in the synthesis of melatonin, serotonin *N*-acetyltransferase (NAT), exhibits a circadian rhythm with peak activity at night (Hamm and Menaker, 1980; Binkley et al., 1980; Iuvone and Besharse, 1983).

Recent evidence suggests that melatonin modulates the release of dopamine (Dubocovich, 1983), a major catecholamine in retina (reviewed in Iuvone, 1985*a*), and that dopamine regulates melatonin biosynthesis (Besharse et al., 1984; Iuvone and Besharse, 1985*a, b*). The activity of tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, occurs rhythmically in retina, but the peak activity of tyrosine hydroxylase and dopamine levels occurs in the daytime (Iuvone et al., 1978; Remé et al., 1983; Wirz-Justice et al., 1984). Dubocovich (1983) has demonstrated that melatonin inhibits the Ca^{2+} -dependent release of [^3H]dopamine in rabbit retinal slices. In contrast, dopamine receptor agonists inhibit the nighttime rise in NAT activity (Besharse et al., 1984; Iuvone, 1985*a*). These data have led to the suggestion that melatonin and dopamine are components of a retinal regulatory pathway for the control of rhythmic photoreceptor metabolism (Iuvone and Besharse, 1985*a, b*).

In order to investigate the mechanisms involved in the temporal regulation of cone movement, we have used an in vitro preparation, eyecups from *Xenopus laevis*, that sustains the processes of photoperiod-related disk shedding (Besharse et al., 1980), cone retinomotor movement (Besharse et al., 1982), and melatonin biosynthesis (Besharse and Iuvone, 1983). In the present study, we have taken advantage of the fact that cone movements occur in vitro to investigate the possible role of melatonin and dopamine in the control of those movements. The principal finding is that melatonin mimics darkness by causing cone elongation, while dopamine mimics light by causing cone contraction. These observations suggest that a retinal dopaminergic system is important for the control of cone position, and that melatonin may influence cone position indirectly through its effects on dopaminergic neurons.

MATERIALS AND METHODS

Experiments were performed using eyecups prepared from postmetamorphic *Xenopus laevis* that were 3.5–5.0 cm in length (Nasco Biologicals, Inc., Fort Atkinson, WI). Animals were maintained at 24–26°C on a cyclic light schedule (12 h light:12 h dark) for at least 1 mo before use. For constant-light experiments, eyecups were prepared in room light from animals that had been maintained for 4 d in constant light (3×10^{-4} W/cm², 25°C). In cyclic-light experiments, eyecups were also prepared in room light just before light offset. Eyecups were obtained by surgical removal of the cornea, iris, and lens (Besharse et al., 1980). For all experiments, a defined culture medium containing 35 mM NaHCO₃ (Besharse and Dunis, 1983a) was supplemented with 100 μM ascorbic acid (Fisher Scientific Co., NJ). This ascorbate medium, gassed with 5% CO₂/95% O₂ (pH 7.4), was used during dissection and incubation. All drugs were added to this medium just before use. Melatonin, dopamine, and isoproterenol were obtained from Sigma Chemical Co., St. Louis, MO; apomorphine from Merck, Sharp & Dohme, Rahway, NJ; clonidine from Boehringer-Ingelheim, Ridgefield, CT; spiroperidol from Janssen, Piscataway, NJ; trans-(–)-4aR-4, 4a,5,7,8,8a,9-octahydro-5 propyl-1H (or 2H) 4-pyrazolo [3,4-g]-quinoline monohydrochloride (LY171555) from Lilly Research Laboratories, Indianapolis, IN; and 2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol, hydrochloride (SKF38393-A) from Smith, Kline and French Laboratories, Philadelphia, PA. During incubation, eyecups were maintained in plastic culture dishes containing 4 ml of medium. The dishes were placed in a gassed (5% CO₂/95% O₂) incubation chamber on a rotary shaker (60 rpm) for 3–6 h in the light (1.85×10^{-3} W/cm² incident at level of culture dishes) or in the dark.

After incubation, eyecups were fixed in 1% OsO₄, 1.65% glutaraldehyde, and 0.075 M cacodylate buffer on ice for 1.5 h. They were then dehydrated in ethanol and embedded in Polybed 812-araldite (Polysciences, Inc., Warrington, PA). Eyecups were oriented in the blocks along their dorso-ventral axis, and sections were taken at the level of the optic nerve. Data were obtained from the light-microscopic viewing of 1-μm-thick sections stained with Azure II (Sigma Chemical Co.). As previously described (Besharse et al., 1982), the cone length was operationally defined as the distance from the external limiting membrane to the proximal side of the oil droplet, as measured in a plane parallel to the long axis of the adjacent rod outer segments (see Fig. 1). Because the cone length is generally 5–10 μm shorter in the dorsal retina for both elongated and contracted states, measurements were made only in the ventral field. 20 cones were measured beginning 100 μm from the optic nerve in each eyecup, and an average value was obtained for each eyecup. Statistical evaluation involved an analysis of variance followed by the Student Neumann-Kuels test (Scheffler, 1979). In this paper, data are expressed as the percent

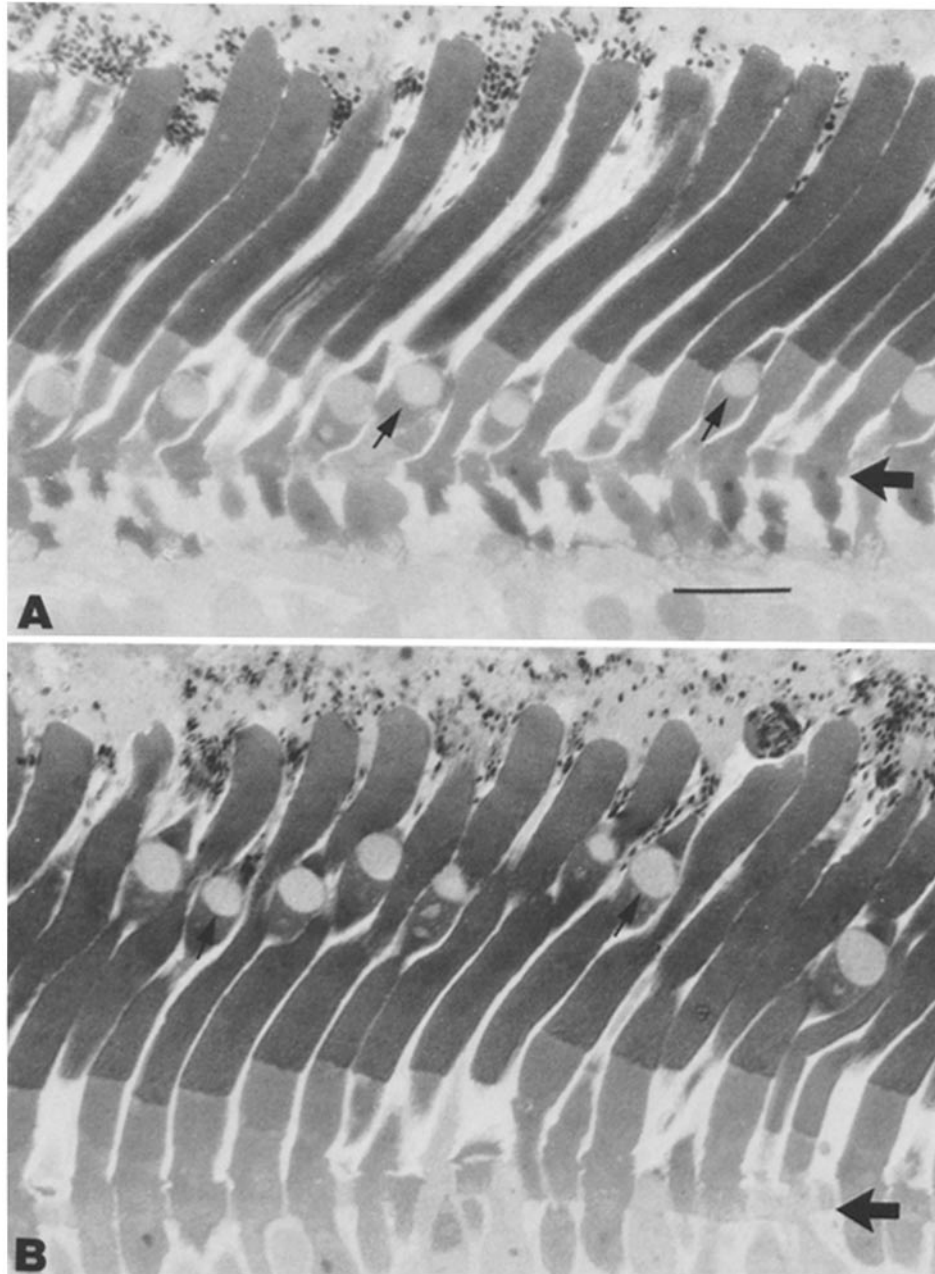


FIGURE 1. Light micrographs of light- and melatonin-treated retinas from 4-d constant-light-treated *X. laevis*. (A) Azure II-stained section ($1 \mu\text{m}$) of a retina maintained in vitro for 3 h in the light. The small arrows indicate the proximal edge of the oil droplet. Note the close proximity of the cone oil droplet to the external limiting membrane, indicated by the large arrow. (B) A similar section from an eyecup maintained in the light in the presence of $0.5 \mu\text{M}$ melatonin. Note that the oil droplet is displaced sclerally. The calibration bar equals $20 \mu\text{m}$.

change in cone length compared with the light control $[(\bar{X} \text{ experimental} - \bar{X} \text{ light control}) / \bar{X} \text{ light control}] \times 100$. A 100% change generally involved a total movement of 25–35 μM .

RESULTS

Effects of Melatonin

As previously reported (Besharse et al., 1982; Besharse, 1982), dark incubation of eyecups obtained from 4-d constant-light-treated animals results in cone elongation. The dark-induced increase approaches a maximum by 2 h (Besharse,

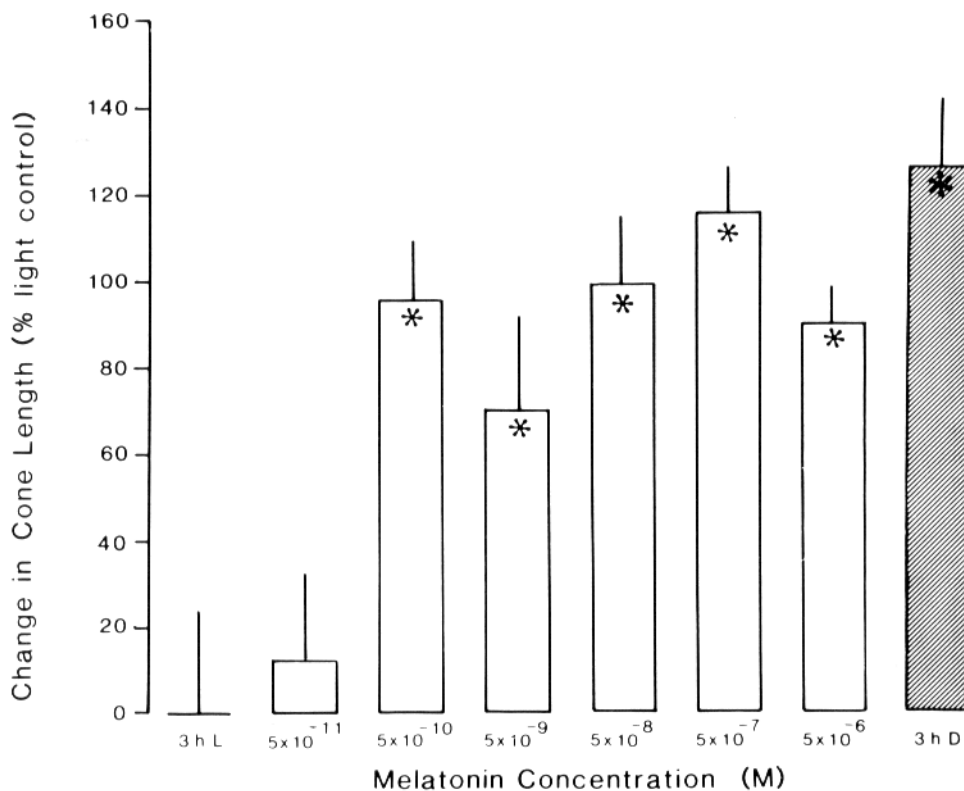


FIGURE 2. Melatonin stimulates cone elongation in eyecups prepared in the light from 4-d constant-light-treated animals. All groups were treated for 3 h in the light (L) (open bars) or in darkness (D) (shaded bar). As for all experiments, the drug concentration indicated is that of the culture medium. The histogram bar height indicates the mean ($n = 4-5$ per group) percent change in cone length as compared with light control. The percent standard error is indicated by the line above each bar. Asterisks signify a statistically significant difference compared with the light control: $P < 0.01$.

1982) and typically exceeds a 100% increase over the cone length observed in the light (see Fig. 2).

Melatonin stimulates cone elongation in eyecups from constant-light-treated animals to an extent comparable to dark treatment (Figs. 1 and 2). When eyecups

prepared in the light were maintained in the light, the cones remained contracted. However, when they were incubated in the dark or in the light in the presence of melatonin, the cone length increased by >100%. Melatonin was highly potent, yielding the same effect over a concentration range of 500 pM to 5 μ M. Although melatonin was reproducibly effective in eyecups from constant-light-treated

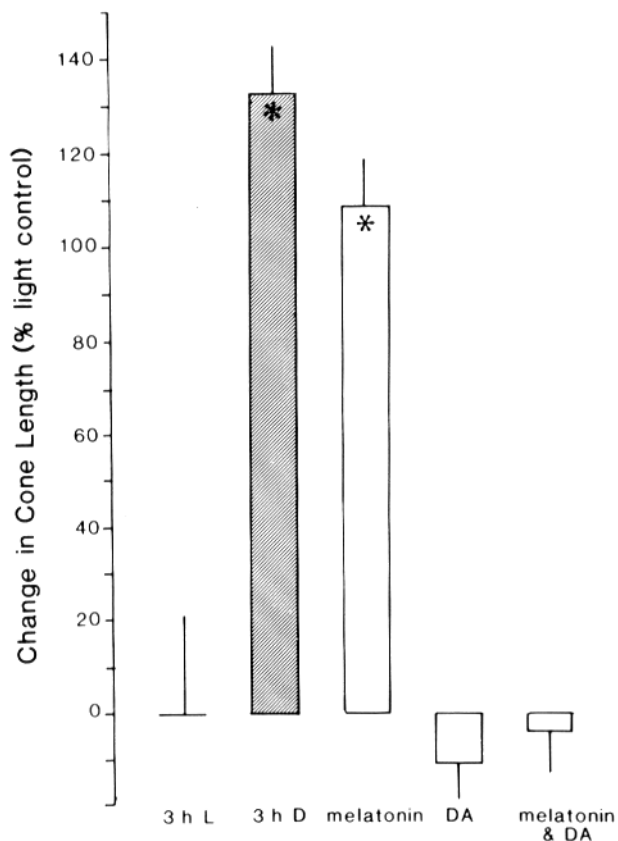


FIGURE 3. Dopamine (DA) blocks melatonin-induced cone elongation. Eyecups from constant-light-treated animals were cultured in darkness (D) (shaded bar), in the light (L) (open bar) without drugs, or in the light in the presence of melatonin (0.5 μ M), dopamine (50 μ M), or both drugs for 3 h. Data are expressed as in Fig. 2. $n = 8-9$ per group. * $P < 0.01$ compared with light control.

animals, a significant effect of melatonin was not detected in eyecups prepared at the time of normal light offset from animals that had been maintained on a 12 h light:12 h dark lighting schedule (data not shown). In some individual cases, cones appeared to elongate, but never to an extent comparable to that occurring at night. The lack of a reproducible effect of melatonin under this condition was confirmed in several experiments using concentrations of melatonin from 50 nM to 200 μ M.

Effects of Dopamine

Since it has been demonstrated that melatonin inhibits the Ca^{2+} -dependent release of [^3H]dopamine in rabbit retinal slices (Dubocovich, 1983), we asked what effect dopamine would have on melatonin-induced cone movement. Dopamine ($50\ \mu\text{M}$) inhibited melatonin-induced cone elongation (Fig. 3). Both dark treatment and $0.5\ \mu\text{M}$ melatonin caused cones to double in length compared

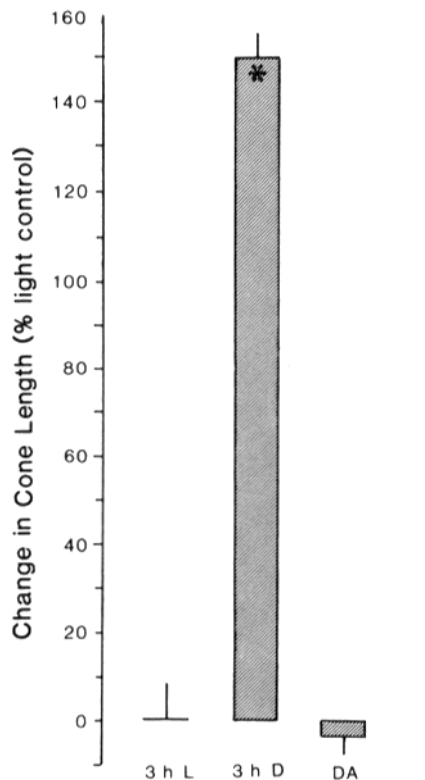


FIGURE 4. Dopamine blocks dark-induced cone elongation. Eyecups from constant-light-treated animals were prepared in the light and dopamine ($50\ \mu\text{M}$) was added to the culture medium at the beginning of the 3-h dark incubation. Data are expressed as in Fig. 2. $n = 4$ per group. $*P < 0.01$ compared with light control.

with the light control. However, when both melatonin and dopamine were added to the culture medium in the light, dark-adaptive movements no longer occurred. The addition of dopamine alone in the light did not alter the cone length significantly; the mean lengths declined slightly (Fig. 3).

The above results suggest that dopamine might be important in the control of cone contraction. To test this hypothesis, we investigated the ability of dopamine to block dark-induced cone elongation and to induce cone contraction in constant-light-treated animals. Burnside and colleagues (reviewed by Burnside and Nagle, 1983) have shown that the actual motile mechanisms for these processes

differ. For both processes, the addition of dopamine to the culture medium mimicked the effects of light. When eyecups prepared in the light were cultured in the dark with 50 μM dopamine, dark-induced cone elongation was blocked (Fig. 4). When cones were allowed to elongate by incubation of eyecups in normal medium for 2 h in the dark followed by addition of dopamine, cones contracted

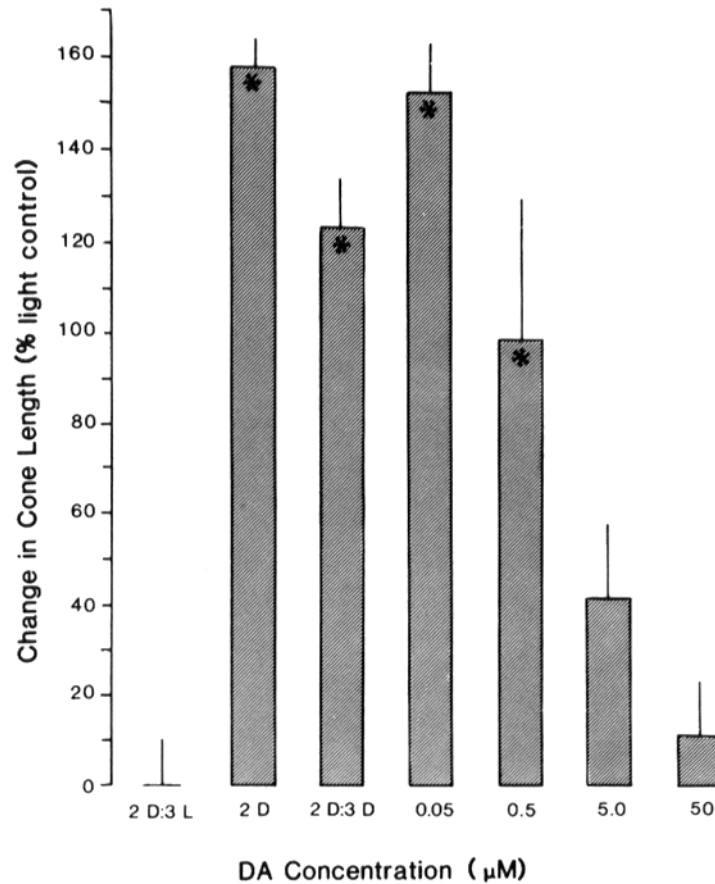


FIGURE 5. Dopamine stimulates cone contraction in the dark in eyecups prepared from constant-light-treated animals. Eyecups were preincubated in the dark for 2 h to elongate cones. Dopamine was added during the subsequent 3-h incubation. Dopamine and light caused cone contraction, but cones remained elongated in eyecups kept in the dark. Data are expressed as in Fig. 2. $n = 4$ per group. $*P < 0.01$ compared with light control.

to their light-adapted positions (Fig. 5). Significant contraction occurred with 50 and 5 μM dopamine; 0.5–0.05 μM dopamine was not effective.

Dopamine also effects cone contraction in cyclic-light-treated animals (Fig. 6). This experiment was performed exactly as that for the constant-light-treated eyecups, except that eyecups were prepared at the time of normal light offset and were preincubated for 3 h rather than for 2 h. A 2-h preincubation in

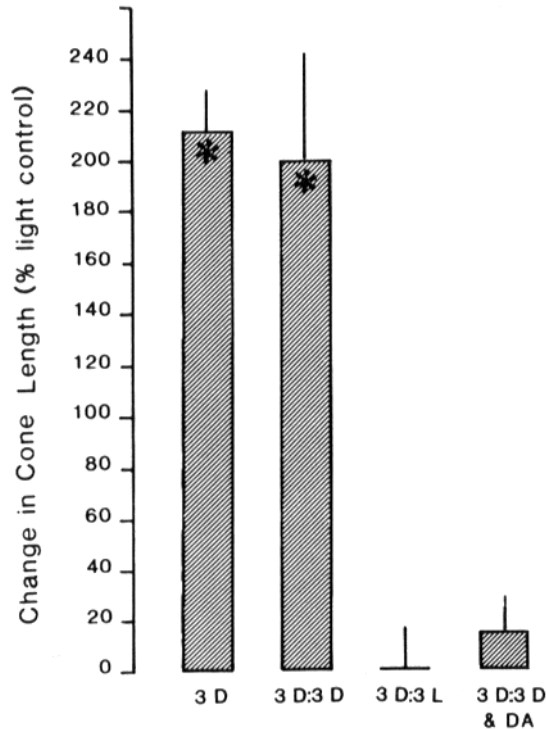


FIGURE 6. Dopamine induces cone contraction in eyecups prepared in the light from animals maintained on a 12 h light:12 h dark lighting schedule. A 3-h dark incubation was necessary to reproducibly elongate cones (bar on right). Dopamine (50 μ M) was added during the subsequent 3-h incubation. Data are expressed as in Fig. 2. $n = 4$ per group. * $P < 0.01$ compared with light control.

TABLE I
*Effects of Catecholamine Agonists on Cone Contraction
in Eyecups from 4-d Constant-Light-treated Animals*

Condition	n	Percent change in cone length
2 h dark preincubation	5	91 \pm 10*
2 h dark:3 h light	5	0 \pm 14
2 h dark:3 h dark	4	99 \pm 13*
2 h dark:3 h dark + apomorphine [‡]	5	-9 \pm 11
2 h dark:3 h dark + isoproterenol [‡]	5	86 \pm 9*
2 h dark:3 h dark + clonidine [‡]	5	88 \pm 6*
2 h dark:3 h dark + phenylephrine [‡]	5	90 \pm 7*
2 h dark:3 h dark + LY171555 [‡]	4	-14 \pm 3
2 h dark:3 h dark + SKF38393 [‡]	4	80 \pm 10*

Values are expressed as percent change in cone length compared with light control \pm percent SEM based on the number of eyecups (n) indicated.

* $P < 0.01$ compared with light control.

[‡] All drugs were added at a concentration of 1 μ M.

darkness did not completely elongate cones in cyclic-light animals. Both light and dopamine ($50 \mu\text{M}$) caused complete cone contraction in these eyecups.

Dopamine Agonists and Antagonists

An analysis of the effects of catecholamine agonists on cone length suggests that the effect reported here is specifically mediated by a dopamine receptor. When eyecups were preincubated in the dark, the cones elongated (Table I). If, during the subsequent incubation, eyecups were exposed to light or to darkness plus $1 \mu\text{M}$ apomorphine (a dopamine receptor agonist), cones contracted. The same

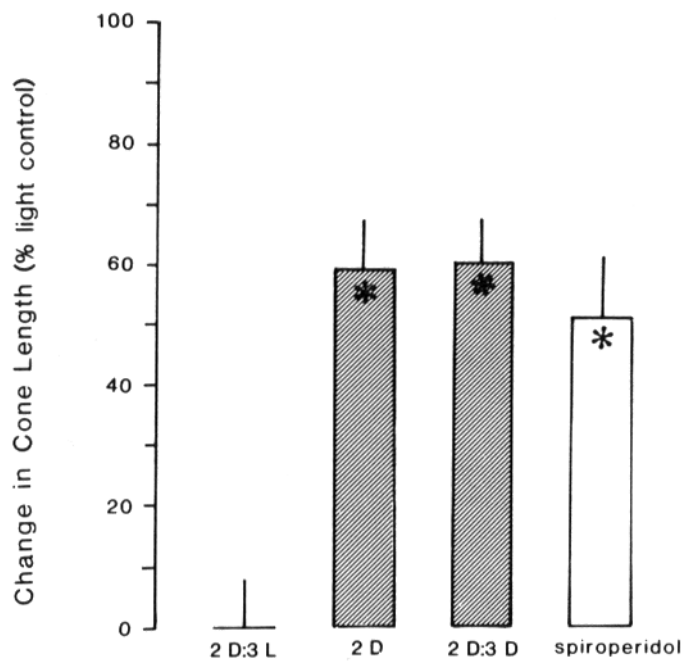


FIGURE 7. Spiroperidol, a dopamine antagonist, blocks light-induced cone contraction. After a 2-h dark preincubation to elongate cones, spiroperidol ($10 \mu\text{M}$) was added just before transferring eyecups to the light. See Fig. 2 for details. $n = 4$ per group. * $P < 0.01$ compared with light control.

concentrations of isoproterenol (β -adrenergic agonist), phenylephrine (α_1 -adrenergic agonist), or clonidine (α_2 -adrenergic agonist) did not cause cone contraction.

We attempted to further characterize the dopamine receptor using a specific D_2 receptor agonist, LY171555, the active isomer of the racemic mixture LY141865 (Bach et al., 1980; Stoof and Keabian, 1981; Tsuruta et al., 1981), and a specific D_1 receptor agonist, SKF38393-A (Setler et al., 1978; Roberts and Messent, 1980; Sibley et al., 1982). The D_1 receptor subtype is thought to stimulate adenylate cyclase and hence increase levels of cAMP, while the D_2 receptor is believed to block or to reduce the cyclase activity and thus decrease cAMP levels (Keabian and Calne, 1979). Light and $1 \mu\text{M}$ LY171555 induced

cone contraction (Table I), but the same concentration of the D₁ agonist had no effect.

The dopamine receptor antagonist spiroperidol, which is relatively specific for D₂ receptors (Creese et al., 1983), blocked light-induced cone contraction (Fig. 7). In this experiment, the eyecups were preincubated for 2 h in the dark. 15 min before transferring the eyecups to the light, 10 μ M spiroperidol was added

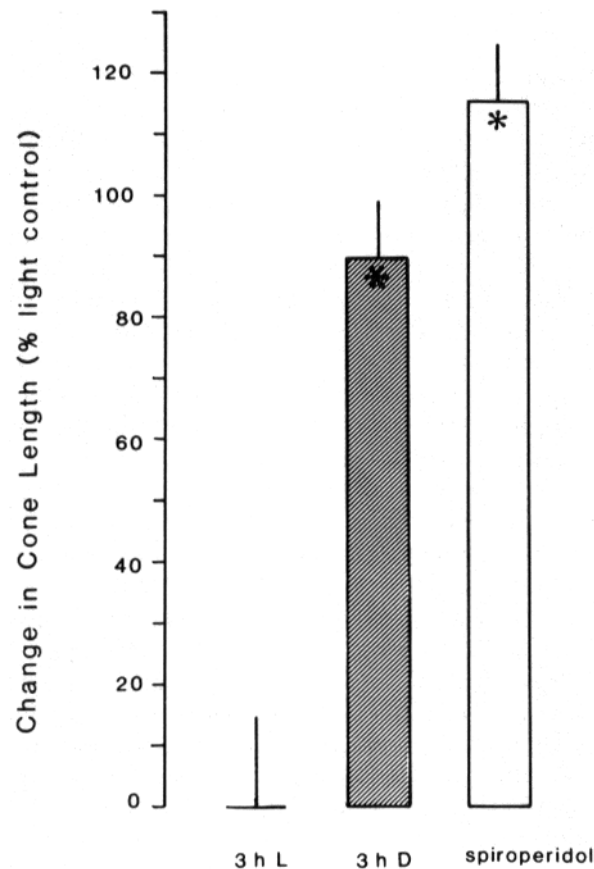


FIGURE 8. Addition of spiroperidol (10 μ M) to eyecups from 4-d constant-light-treated animals causes cone elongation. The antagonist was added at the beginning of a 3-h light incubation. Data are expressed as in Fig. 2. $n = 4$ per group. * $P < 0.01$ compared with light control.

to the culture medium. When the cones were brought back into the light, they no longer contracted, but rather maintained their elongated state. Furthermore, the addition of 10 μ M spiroperidol in the light induced cone elongation to an extent comparable to the dark control (Fig. 8). Blocking the dopamine receptors maintained or induced the dark-adaptive state, which further supports the hypothesis that dopamine is at least part of the light signal for cone contraction.

DISCUSSION

Melatonin and Dopamine in the Retina

There is substantial evidence that the retina contains the neuromodulator melatonin, and that this or a related indoleamine may play an important role in the control of rhythmic photoreceptor metabolism. The enzyme system for melatonin synthesis has been identified in the retina of several species (Baker et al., 1965; Cardinali and Rosner, 1971; Gern and Ralph, 1979; Binkley et al., 1980). At present, the melatonin-synthesizing cell has not been unequivocally identified, although the photoreceptor is a candidate (Bubenik et al., 1974; Vivien-Roels et al., 1981; Wiechmann et al., 1985). As in the pineal gland (reviewed by Reiter, 1981), melatonin appears to be synthesized and released in a circadian fashion in the retina. Hamm and Menaker (1980) have detected melatonin-like immunoreactivity in the chicken retina, and have demonstrated that peak levels occur at night. Many recent studies demonstrate that the enzyme NAT, which is believed to be responsible for the rhythmic production of melatonin in the pineal (Klein and Weller, 1970), also expresses a circadian rhythm in retinal tissue (Binkley et al., 1980; Hamm and Menaker, 1980; Iuvone and Besharse, 1983; Besharse and Iuvone, 1983; Besharse et al., 1984). The proposed functions for locally synthesized melatonin include the regulation of retinomotor movements (Kraus-Ruppert and Lembeck, 1965; Chêze and Ali, 1976; Pang and Yew, 1979), circadian photoreceptor membrane turnover (Besharse and Dunis, 1983*b*; Besharse et al., 1984), and retinal dopamine release (Dubocovich, 1983).

The retina also contains enzyme systems for the synthesis of dopamine (reviewed by Iuvone, 1985*a*). Dopamine appears to be localized to a subclass of amacrine cells in most species examined (reviewed by Iuvone, 1985*a*), and it may also be the neurotransmitter of some interplexiform cells in teleosts and in New World monkeys (Laties and Jacobowitz, 1966; Dowling and Ehinger, 1975). The effects of dopamine mediated by D₁ receptors that increase adenylate cyclase activity in fish horizontal cells have been well characterized (reviewed by Iuvone, 1985*a*). In *X. laevis*, a subpopulation of dopaminergic amacrine cells has been identified, but there is no evidence for a dopamine-containing interplexiform cell (Sarthy et al., 1981). Evidence indicates that retinal dopamine not only functions as a neurotransmitter (reviewed by Iuvone, 1985*a*), but it also appears to have a neuromodulatory role and may influence the response of cells to other neurotransmitters (Yeh et al., 1984). Whether dopamine's effect on cone contraction is indirect via another cell synapse, or whether dopamine actually diffuses through the retina to affect the cone directly awaits further experimentation. In either case, the effects of dopamine on photoreceptors imply the existence of a retinal feedback pathway.

Melatonin and Dopamine Effects on Cone Movement

We have previously demonstrated that both light-induced cone contraction and dark-induced cone elongation are sustained in eyecups from 4-d constant-light-treated *X. laevis* (Besharse et al., 1982). Constant-light treatment blocks rod photoreceptor disk shedding (Besharse et al., 1977), retinomotor movements (Besharse et al., 1982), and NAT rhythmicity (Iuvone and Besharse, 1983). The

subsequent exposure of either intact animals or eyecups to darkness results in an increase in each activity. In the present study, we have found that melatonin mimics the effects of darkness (cone elongation), while dopamine mimics the effects of light (cone contraction). We have also found that dopamine's effect can be demonstrated in eyecups from animals maintained in cyclic light. In contrast, the addition of melatonin is not sufficient to cause cone elongation at subjective dusk in eyecups from cyclic-light-treated animals. It was previously reported in a frequently quoted abstract that melatonin induced cone contraction in *X. laevis* (Quay and McLeod, 1968). In our experiments, however, melatonin induced movements characteristic of darkness.

Dark-adaptive cone movements can also be stimulated by conditions expected to increase intracellular cAMP (Besharse et al., 1982; Burnside et al., 1982*b*). It has been suggested that the nighttime rise in cAMP may act as a general signal for darkness (Burnside et al., 1982*b*), which is interpreted differently by the different cells exhibiting retinomotor movement. For example, by stimulating a force-producing microtubule-dependent process, cAMP induces cone elongation (Warren and Burnside, 1978; Dedman et al., 1979). However, in intact cells, the effects of cAMP are probably more complicated (Besharse, 1982). Retinal NAT activity, which is thought to be responsible for generating a rhythm of melatonin biosynthesis, peaks during the dark phase of the light-dark cycle. The effects of darkness on NAT activity are mimicked by cAMP analogues (Iuvone and Besharse, 1983, 1985*a, b*). Hence, increasing levels of cAMP probably elevate levels of melatonin, which we have shown to induce cone elongation. Furthermore, RPE pigment migration is similarly affected by both cAMP and melatonin. In the dark, melanin granules migrate to the base of the RPE cell (Ali, 1975; Burnside and Laties, 1979). This effect can be mimicked by melatonin (Kraus-Ruppert and Lembeck, 1965; Chéze and Ali, 1976; Pang and Yew, 1979) and by cAMP (Burnside et al., 1982*b*; Burnside and Basinger, 1983). The addition of exogenous cAMP to the retina probably affects both the motile machinery as well as the melatonin synthetic pathway.

The mechanism by which melatonin affects cone position is unclear. Some reports suggest that at high (millimolar) concentrations, melatonin may affect microtubule-mediated events directly (reviewed by Cardinali, 1980). Since cone elongation is a microtubule-dependent process (Warren and Burnside, 1978), it is possible that melatonin stimulates cone elongation by directly affecting the motile mechanism. Although we cannot rule out a direct effect on the motile mechanism, the low concentration of melatonin used in these experiments makes this unlikely. Melatonin could also interact with an adenylate cyclase-linked receptor and stimulate a rise in cAMP that could activate motile events in the photoreceptors or RPE. Although no evidence supports such a model, it is a testable hypothesis that cannot be ruled out at present.

Another possibility consistent with the results of our experiments is that melatonin may influence cone movement by modulating dopamine release in the retina. Dubocovich (1983) has recently demonstrated that picomolar concentrations of melatonin inhibit the Ca^{2+} -dependent release of [^3H]dopamine in rabbit retinal slices. Similarly, in the hypothalamus, melatonin inhibits the uptake and release of catecholamines (Cardinali et al., 1975; Zisapel and Laudon, 1982). We

have shown that melatonin-induced cone elongation is inhibited by dopamine, which suggests that melatonin may regulate cone movement by modulating dopamine release. This hypothesis is further supported by the observation that in *X. laevis* eyecups, melatonin decreased the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid in light-exposed retinas, which suggests that, in this system, melatonin also inhibits dopamine release (Pierce et al., 1984).

Dopamine also influences melatonin biosynthesis in *X. laevis* eyecups. As with cone elongation in the eyecup preparation, catecholamines mimic the effects of light and block the nighttime rise in NAT activity through a process mediated by a specific dopamine receptor (Iuvone and Besharse, 1983, 1985*a, b*; Besharse et al., 1984; Iuvone, 1985*a*). Iuvone (1985*b*) has suggested that this effect may be mediated by a D₂ receptor that is believed to inhibit adenylate cyclase activity (Kebabian and Calne, 1979). He has demonstrated that the D₂ agonist LY171555 not only inhibits the dark-induced rise in NAT activity, but also decreases cAMP accumulation in dark-adapted retinas (Iuvone, 1985*b*). Thus, in the light, tyrosine hydroxylase activity peaks (Iuvone et al., 1978), while NAT activity is low (Iuvone and Besharse, 1983). In darkness, the enzyme activity levels are reversed: NAT activity peaks and tyrosine hydroxylase activity is suppressed. The reciprocal nature of the melatonin and dopamine synthetic pathways suggests that an effective feedback mechanism exists for these systems in the retina. Whether the feedback is direct or is modulated trans-synaptically is not currently known.

It seems unlikely that this melatonin/dopamine relationship is solely responsible for the dark-adaptive position of cones, because in eyecups from cyclic-light animals, melatonin did not induce elongation in the light. Although melatonin may be a part of the dark signal for elongation, other factors may also be needed. For example, some as yet unidentified positive effector or an additional mechanism for the suppression of dopamine may be required. There is considerable evidence that the neurotransmitter γ -aminobutyric acid (GABA) has an inhibitory influence on dopamine-containing amacrine cells in rat retina (reviewed by Iuvone, 1985*a*). We have evidence that a GABA agonist, muscimol, stimulates cone elongation in our constant-light preparation and that, in concert with melatonin, it causes elongation in the cyclic-light preparation (Pierce, M. E., and J. C. Besharse, manuscript in preparation). This suggests that in the cyclic-light preparation, melatonin, dopamine, and GABA interact to determine cone position.

The difference in melatonin sensitivity between cyclic-light- and constant-light-treated eyecups may be related to decreased melatonin biosynthesis and to down-regulation of dopamine sensitivity, which is expected in constant light. It has been reported that constant-light treatment not only decreases the level of immunoreactive melatonin, but also decreases the dopamine receptor number in rabbit retina (Lucas et al., 1984; Dubocovich et al., 1985) and in chick retina (de Mello et al., 1982). If our constant-light system is already down-regulated with respect to dopamine sensitivity, any treatment that further lowers dopamine levels may be sufficient to induce elongation.

Our evidence suggests that dopamine is an important part of the light signal for cone contraction. Retinal dopamine biosynthesis occurs in a rhythmic fashion

with peak activity in the light (Iuvone et al., 1978). We have demonstrated that exogenous dopamine mimics the effects of light. It not only blocks dark- and melatonin-induced cone elongation, but it also stimulates cone contraction in dark-adapted eyecups. Its effects are the same in eyecups from both cyclic- and constant-light-treated animals. The dopamine agonist apomorphine stimulates cone contraction, while the β -, α_1 -, and α_2 -adrenergic agonists do not, which implies that the catecholamine effect is probably mediated by a specific dopamine receptor. Furthermore, the effects of both dopamine and light are blocked by the dopamine antagonist spiroperidol, which implies that it is endogenous dopamine that affects cone contraction. Dearry and Burnside (1985) have also shown in the sunfish that dopamine induces cone contraction that is blocked by dopamine antagonists. Simply blocking the dopamine receptors in our system with spiroperidol stimulated cone elongation, which supports the hypothesis that dopamine suppression is part of the dark signal.

Our data also support a role for a D_2 receptor in the retina and suggest that dopamine's effect on cone contraction is mediated by such a receptor. The D_1 receptor agonist SKF38393-A did not cause light-adaptive responses, but the D_2 receptor agonist LY171555 did. The relative potency of dopamine can be used as a criterion for the categorization of dopamine receptors (Kebabian and Calne, 1979). Nanomolar concentrations stimulate D_2 receptors (Kebabian and Calne, 1979). Our dose-response data indicate that cone contraction is affected by micromolar concentrations of dopamine. However, since the concentration of dopamine reported is for the bathing medium, the actual effective concentration may be significantly lower since dopamine must diffuse through the retina, and a high-affinity uptake system exists in *X. laevis* retina for this compound (Sarthy et al., 1981). Interestingly, a low level of cAMP is one of the factors necessary for reactivated cone contraction (Porrello and Burnside, 1984), but cAMP is required for reactivated cone elongation (Burnside et al., 1982a). Recently, Dearry and Burnside (1985) have demonstrated that the adenylate cyclase activator forskolin and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine each induces dark-adaptive retinomotor movements that are blocked by dopamine, which further supports a role for a D_2 receptor in the modulation of cone position through effects on cAMP.

In summary, our data suggest that dopamine is an important effector of cone movement and that retinal melatonin may affect cone movement via modulation of dopamine release. Dopamine appears to be an integral part of the light signal, and its suppression may be a necessary part of the dark signal for cone elongation. These results emphasize the importance of investigating the role of post-receptor retinal neurons in the regulation of circadian phenomena in the photoreceptor-pigment epithelial complex.

We thank Mike Iuvone for his critical comments and discussions both on work in progress and on the manuscript, Gwendolyn Spratt and Donna Forestner for technical assistance, and Janet Vogt for preparing the manuscript.

This work was supported by National Institutes of Health grants EY02414 and EY04864 and Research Career Development Award EY0169.

Original version received 23 April 1985 and accepted version received 1 July 1985.

REFERENCES

- Ali, M. A. 1975. Retinomotor responses. *In* Vision in Fishes: New Approaches in Research. M. A. Ali, editor. Plenum Press, New York. 313–355.
- Bach, N. J., E. C. Kornfeld, N. D. Jones, D. E. Dorman, J. W. Paschal, J. A. Clemens, and E. B. Smalstig. 1980. Bicyclic and tricyclic ergoline partial structures. Rigid 3-(2-aminoethyl)pyrazoles and 3- and 4-(2-aminoethyl)pyrazoles as dopamine agonists. *J. Med. Chem.* 23:481–491.
- Baker, P. C., W. B. Quay, and J. Axelrod. 1965. Development of hydroxyindole-O-methyl transferase activity in eye and brain of the amphibian, *Xenopus laevis*. *Life Sci.* 4:1981–1987.
- Besharse, J. C. 1982. The daily light-dark cycle and rhythmic metabolism in the photoreceptor-pigment epithelial complex. *In* Progress in Retinal Research. N. Osborne and G. Chader, editors. Pergamon Press, New York. 81–124.
- Besharse, J. C., and D. A. Dunis. 1983a. Photoreceptor disc shedding in eyecups: relationship to bicarbonate and amino acids. *Exp. Eye Res.* 36:567–580.
- Besharse, J. C., and D. A. Dunis. 1983b. Methoxyindoles and photoreceptor metabolism: activation of rod shedding. *Science (Wash. DC)*. 219:1341–1343.
- Besharse, J. C., D. A. Dunis, and B. Burnside. 1982. Effects of cyclic adenosine 3',5'-monophosphate on photoreceptor disc shedding and retinomotor movement. Inhibition of rod shedding and stimulation of cone elongation. *J. Gen. Physiol.* 79:775–790.
- Besharse, J. C., D. A. Dunis, and P. M. Iuvone. 1984. Regulation and possible role of serotonin *N*-acetyltransferase in the retina. *Fed. Proc.* 43:2704–2708.
- Besharse, J. C., J. G. Hollyfield, and M. E. Rayborn. 1977. Photoreceptor outer segments: accelerated membrane renewal in rods after exposure to light. *Science (Wash. DC)*. 196:536–538.
- Besharse, J. C., and P. M. Iuvone. 1983. Circadian clock in the *Xenopus* eye controlling retinal serotonin *N*-acetyltransferase. *Nature (Lond.)*. 305:133–135.
- Besharse, J. C., R. O. Terrill, and D. A. Dunis. 1980. Light-evoked disc shedding by rod photoreceptors *in vitro*: relationship to medium bicarbonate concentration. *Invest. Ophthalmol. Visual Sci.* 19:1512–1517.
- Binkley, S., K. B. Reilly, and M. Hryshchshyn. 1980. *N*-acetyltransferase in the chick retina. I. Circadian rhythms controlled by environmental lighting are similar to those in the pineal gland. *J. Comp. Physiol. B Biochem. System. Environ. Physiol.* 139:103–108.
- Bubenik, G. A., G. M. Brown, I. Uhler, and L. J. Grotta. 1974. Immunohistological localization of *N*-acetylindolealkylamines in pineal gland, retina, and cerebellum. *Brain Res.* 81:233–242.
- Burnside, B. 1976. Microtubules and actin filaments in teleost visual cone elongation and contraction. *J. Supramol. Struct.* 5:257–275.
- Burnside, B. 1978. Thin (actin) and thick (myosin-like) filaments in cone contraction in the teleost retina. *J. Cell Biol.* 78:227–246.
- Burnside, B., and S. Basinger. 1983. Pigment migration in the teleost retinal pigment epithelium. II. Cyclic 3',5'-adenosine monophosphate induction of dark-adaptive retinomotor movement *in vitro*. *Invest. Ophthalmol. Visual Sci.* 24:16–23.
- Burnside, B., M. Evans, R. T. Fletcher, and G. J. Chader. 1982a. Induction of dark-adaptive retinomotor movement (cone elongation) in teleost retinal cones by cyclic adenosine 3',5'-monophosphate. *J. Gen. Physiol.* 79:759–774.
- Burnside, B., B. Smith, M. Nagata, and K. Porrello. 1982b. Reactivation of contraction in detergent-lysed teleost retinal cones. *J. Cell Biol.* 92:199–206.
- Burnside, B., and A. Laties. 1979. Pigment movement and cellular contractility in the retinal

- pigment epithelium. *In* The Retinal Pigment Epithelium. K. M. Zinnand and M. F. Marmor, editors. Harvard University Press, Cambridge, MA. 175–191.
- Burnside, B., and B. Nagle. 1983. Retinomotor movements of the photoreceptors and retinal pigment epithelium: mechanisms and regulation. *In* Progress in Retinal Research. N. Osborne and G. Chader, editors. Pergamon Press, New York. 67–109.
- Cardinali, D. P. 1980. Molecular biology of melatonin: assessment of the “microtubule hypothesis of melatonin action.” *In* Melatonin: Current Status and Perspectives. N. Birau and W. Schloot, editors. Pergamon Press, New York. 247–256.
- Cardinali, D. P., C. P. Nagle, F. Freire, and J. M. Rosner. 1975. Effects of melatonin on neurotransmitter uptake and release by synaptosome-rich homogenates of the rat hypothalamus. *Neuroendocrinology*. 18:72–85.
- Cardinali, D. P., and J. M. Rosner. 1971. Retinal localization of the hydroxyindole-O-methyl transferase (HIOMT) in the rat. *Endocrinology*. 89:301–303.
- Chéze, G., and M. A. Ali. 1976. Role de l'épiphyse dans la migration du pigment epithelial retinien chez quelques Teleosteens. *Can. J. Zool.* 54:475–481.
- Creese, I., D. R. Sibley, M. W. Hamblin, and S. E. Leff. 1983. The classification of dopamine receptors: relationship to radioligand binding. *Annu. Rev. Neurosci.* 6:43–71.
- Dearry, A., and B. Burnside. 1985. Dopamine inhibits forskolin- and 3-isobutyl-methylxanthine-induced dark-adaptive retinomotor movements in isolated teleost retinas. *J. Neurochem.* 44:1753–1763.
- Dedman, J. R., B. R. Brinkley, and A. R. Means. 1979. Regulation of microfilaments and microtubules by calcium and cyclic AMP. *Adv. Cyclic Nucleotide Res.* 11:131–174.
- de Mello, M. C. F., A. L. M. Ventura, R. Paes de Carvalho, W. L. Klein, and F. G. de Mello. 1982. Regulation of dopamine- and adenosine-dependent adenylate cyclase systems of chicken embryo retina cells in culture. *Proc. Natl. Acad. Sci. USA.* 79:5708–5712.
- Dowling, J. E., and B. Ehinger. 1975. Synaptic organization of the amine-containing interplexiform cells of the goldfish and Cehus monkey retinas. *Science (Wash. DC)*. 188:270–273.
- Dubocovich, M. L. 1983. Melatonin is a potent modulator of dopamine release in the retina. *Nature (Lond.)*. 204:183–184.
- Dubocovich, M. L., R. C. Lucas, and J. S. Takahashi. 1985. Light-dependent regulation of dopamine receptors in mammalian retina. *Brain Res.* 335:321–325.
- Gern, W. A., and C. L. Ralph. 1979. Melatonin synthesis by the retina. *Science (Wash. DC)*. 204:183–184.
- Hamm, H. E., and M. Menaker. 1980. Retinal rhythms in chicks—circadian variation in melatonin and serotonin *N*-acetyltransferase activity. *Proc. Natl. Acad. Sci. USA.* 77:4998–5002.
- Iuvone, P. M. 1985a. Neurotransmitters and neuromodulators in the retina: regulation, interaction and cellular effects. *Adv. Cell. Neurobiol.* In press.
- Iuvone, P. M. 1985b. Evidence for a dopamine receptor in retina that decreases cyclic AMP accumulation and serotonin *N*-acetyltransferase activity. *Invest. Ophthalmol. Visual Sci. Abstr. Suppl.* 26:279.
- Iuvone, P. M., and J. C. Besharse. 1983. Regulation of indoleamine *N*-acetyltransferase activity in the retina: effects of light and dark, protein synthesis inhibitors, and cyclic nucleotide analogues. *Brain Res.* 273:111–119.
- Iuvone, P. M., and J. C. Besharse. 1985a. Dopamine receptor-mediated inhibition of serotonin *N*-acetyltransferase in retina. *Brain Res.* In press.
- Iuvone, P. M., and J. C. Besharse. 1985b. Cyclic AMP stimulates serotonin *N*-acetyltransferase activity in *Xenopus* retina in vitro. *J. Neurochem.* In press.

- Iuvone, P. M., C. L. Galli, C. K. Garrison-Gund, and N. H. Neff. 1978. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. *Science (Wash. DC)*. 202:901–902.
- Kebabian, J. W., and D. B. Calne. 1979. Multiple receptors for dopamine. *Nature (Lond.)*. 277:93–96.
- Klein, D. C., and J. L. Weller. 1970. Indole metabolism in the pineal gland: a circadian rhythm in *N*-acetyltransferase. *Science (Wash. DC)*. 169:1093–1095.
- Kraus-Ruppert, R., and F. Lembeck. 1965. Die wirkung von melatonin auf die pigmentzellen der retina von fischen. *Pflügers Arch. Eur. J. Physiol.* 284:160–168.
- Laties, A., and D. Jacobowitz. 1966. A comparative study of the autonomic innervation of the eye in monkey, cat, and rabbit. *Anat. Rec.* 156:383–396.
- LaVail, M. M. 1976. Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science (Wash. DC)*. 194:1071–1074.
- Levinson, G., and B. Burnside. 1981. Circadian rhythms in teleost retinomotor movements. *Invest. Ophthalmol. Visual Sci.* 20:294–303.
- Lucas, R. C., J. S. Takahashi, and M. L. Dubocovich. 1984. Effect of light and dark on retinal melatonin levels and dopamine (DA) receptor number. *Soc. Neurosci. Abstr.* 10:328.11.
- Pang, S. G., and D. T. Yew. 1979. Pigment aggregation by melatonin in the retinal pigment epithelium and choroid of guinea pigs, *Cavia procellus*. *Experientia*. 35:231–233.
- Pierce, M. E., P. M. Iuvone, and J. C. Besharse. 1984. Melatonin and photoreceptor metabolism: regulation of cone retinomotor movement by melatonin and dopamine. *Soc. Neurosci. Abstr.* 10:10.1.
- Porrello, K., and B. Burnside. 1984. Regulation of reactivated contraction in teleost retinal cone models by calcium and cyclic adenosine monophosphate. *J. Cell Biol.* 98:2230–2238.
- Quay, W. B., and R. W. McLeod. 1968. Melatonin and photic stimulation of contraction in the retina of larval *Xenopus laevis*. *Anat. Rec.* 160:491a. (Abstr.)
- Reiter, R. J. 1981. The mammalian pineal gland: structure and function. *Am. J. Anat.* 162:287–313.
- Remé, C. E., A. Wirtz-Justice, and M. DaPrada. 1983. Retinal rhythms in photoreceptors and dopamine synthetic rate: the effect of a monoamineoxidase inhibitor. *Trans. Ophthalmol. Soc. UK*. 103:405–410.
- Roberts, D. M., and D. O. H. Messent. 1980. Competition by sk&f 38393 for ³H-apomorphine and ³H-spiroperidol binding sites in the guinea pig corpus striatum. *Fed. Proc.* 39:3897. (Abstr.)
- Sarthy, P. V., M. E. Rayborn, J. G. Hollyfield, and D. M. K. Lam. 1981. The emergence, localization and maturation of neurotransmitter systems during development of the retina. III. Dopamine. *J. Comp. Neurol.* 195:595–602.
- Scheffler, W. C. 1979. Analysis of variance. In *Statistics for the Biological Sciences*. Addison-Wesley Publishing Co., The Phillipines. 121–142.
- Setler, P. E., H. M. Sarau, C. L. Zirkle, and H. L. Saunders. 1978. Central effects of a novel dopamine agonist. *Eur. J. Pharmacol.* 50:419–430.
- Sibley, D. R., S. E. Leff, and I. Cress. 1982. Interactions of novel dopaminergic ligands with D-1 and D-2 dopamine receptors. *Life Sci.* 31:637–645.
- Stoof, J. C., and J. W. Kebabian. 1981. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature (Lond.)*. 294:366–368.
- Tsuruta, K., A. Frey, C. W. Grewe, R. L. Eskay, and J. W. Kebabian. 1981. Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor. *Nature (Lond.)*. 292:463–465.

- Vivien-Roels, B., P. Pevet, M. P. Dubois, J. Arendt, and G. M. Brown. 1981. Immunohistochemical evidence for the presence of melatonin in the pineal gland, the retina, and the Harderian gland. *Cell Tissue Res.* 217:105–115.
- Warren, R. H., and B. Burnside. 1978. Microtubules in cone myoid elongation in the teleost retina. *J. Cell Biol.* 78:247–259.
- Welsh, J. H., and C. M. Osborne. 1937. Diurnal changes in the retina of the catfish, *Ameiurus nebulosis*. *J. Comp. Neurol.* 66:349–359.
- Wiechmann, A. F., D. Bok, and J. Horwitz. 1985. Localization of hydroxyindole-O-methyltransferase in the mammalian pineal gland and retina. *Invest. Ophthalmol. Visual Sci.* 26:253–265.
- Wirz-Justice, A., M. DaPrada, and C. Remé. 1984. Circadian rhythm in rat retinal dopamine. *Neurosci. Lett.* 45:21–25.
- Yeh, H. H., B. A. Battelle, and D. G. Puro. 1984. Dopamine regulates synaptic transmission mediated by cholinergic neurons of the rat retina. *Neuroscience.* 13:901–909.
- Zisapel, N., and M. Laudon. 1982. Dopamine release induced by electrical field stimulation of rat hypothalamus: inhibition by melatonin. *Biochem. Biophys. Res. Commun.* 104:1610–1616.