Circadian Regulation of Teleost Retinal Cone Movements In Vitro

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ABSTRACT In the retinas of many species of lower vertebrates, retinal photoreceptors and pigment epithelium pigment granules undergo daily movements in response to both diurnal, and in the case of teleost cone photoreceptors, endogenous circadian signals. Typically, these cone movements take place at dawn and at dusk when teleosts are maintained on a cyclic light (LD) regime, and at expected dawn and expected dusk when animals are maintained in continuous darkness (DD). Because these movements are so strictly controlled, they provide an overt indicator of the stage of the underlying clock mechanism. In this study we report that both light-induced and circadian-driven cone myoid movements in the Midas cichlid (*Cichlasoma citrinellum*), occur normally in vitro. Many of the features of retinomotor movements found in vivo also occur in our culture conditions, including responses to light and circadian stimuli and dopamine. Circadian induced predawn contraction and maintenance of expected day position in response to circadian modulation, are also normal. Our studies suggest that circadian regulation of cone myoid movement in vitro is mediated locally by dopamine, acting via a D_2 receptor. Cone myoid contraction can be induced at midnight and expected mid-day by dark culture with dopamine or the D_2 receptor agonist LY171555. Further, circadian induced predawn contraction can be increased with either dopamine or LY171555, or may be reversed with the dopamine D_2 antagonist, sulpiride. Sulpiride will also induce cone myoid elongation in retinal cultures at expected mid-day, but will not induce cone myoid elongation at dusk. In contrast, circadian cone myoid movements in vitro were unaffected by the D_1 receptor agonist SCH23390, or the D_1 receptor antagonist SKF38393.

Our short-term culture experiments indicate that circadian regulation of immediate cone myoid movement does not require humoral control but is regulated locally within the retina. The inclusion of dopamine, or dopamine receptor agonists and antagonists in our cultures, has indicated that retinal circadian regulation may be mediated by endogenously produced dopamine, which acts via a D_2 mechanism.

INTRODUCTION

In species of lower vertebrates, some teleosts, anurans, urodeles and birds, retinal photoreceptors and pigment granules exhibit retinomotor movements in response to

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both light and endogenous circadian signals (cf Burnside and Nagle, 1983; Burnside and Dearry, 1986). Early reviews on the discovery and phylogenetic distribution of movements including, Garten (1907), Arey (1915), Parker (1932), Walls (1942), Detwiler (1943), and von Studnitz (1952) are followed by more recent reviews concerned with the regulation of movements, Burnside (1976), Burnside and Laties (1979), Besharse (1982), and Burnside and Nagle (1983). Typically, for most animals maintained on a cyclic light/dark (LD) routine, in darkness at night, cone myoids elongate, rod myoids contract, and the screening melanin pigment granules of the retinal pigment epithelium (RPE) aggregate into the RPE cell body. In the light during the day, movements are reversed: cone myoids contract, rod myoids elongate and the retinal pigment epithelial cell pigment granules disperse into the apical processes of the RPE cells (Burnside and Nagle, 1983). Of these movements all three, rod, cone, and pigment movements are seen to be light regulated, whereas only cone movements are seen to take place in response to circadian regulation. We are studying control of circadian retinomotor movements in teleost fish retinae. Within the retinae of our current experimental species, the Midas cichlid (*Cichlasoma citrinellum*), circadian control of retinomotor movements is highly entrained. The change from night to day at dawn is anticipated within the retina, where, in response to an endogenous circadian signal mechanism, cones begin to contract by up to 50–60%, in the 2 h before expected dawn. When fish are maintained in continuous darkness (DD), they display both the normal dark adaptive cone elongations at night and the predawn cone contractions. In the absence of ambient illumination in expected day, cones maintain a partially contracted position, which is again mediated by the endogenous circadian signal mechanism. This predawn cone myoid contraction and the expected day cone position, makes these animals retinas an ideal model system for the study of circadian retinal cell biology.

Previous studies have shown that retinomotor movements take place in response to both light and endogenous circadian signals (cf Besharse, 1982; Mc Cormack and Burnside, 1991). In a previous study we have shown that circadian control of retinomotor movement is not only particularly well entrained in the Midas cichlid (*Cichlasoma citrinellum*), but does not require efferent input, and may be modulated by endogenously produced dopamine, which acts via a D_2 mechanism to mimic light (Mc Cormack and Burnside, 1992). In the present study we wished to assess the ability of cones to respond to both light and circadian signals in vitro, and further to assess the role of dopamine in those responses.

There is significant evidence to suggest that dopamine may be involved in both the light and circadian signal mechanisms in the retina. Dopamine is known to be synthesised, (cf Negishi, Kato, and Teranishi, 1981) and released, in response to light stimulation, in the retina of several species; rabbit (Nowak and Zurawska, 1989), rat (Brainard and Morgan, 1987) and fish retina (Dearry, 1991; Kirsch and Wagner, 1989; Wagner and Wulle, 1990; Mc Cormack and Burnside, 1993). Such endogenously produced dopamine is thought to act in a paracrine fashion via D2 dopaminergic receptors, to mimic the effect of light onset (Besharse and Iuvone, 1992). Dopamine has been shown to induce light-adaptive cone retinomotor movements in both teleost fish (Dearry and Burnside, 1986*a*,*b*) and frog retinas (Pierce and Besharse, 1985), and to mediate circadian regulation of cone position in the cichlid

retina, apparently via a D_2 mechanism (McCormack and Burnside, 1992). Furthermore, experiments with isolated cone cell fragments have demonstrated that D_2 receptors are located on the inner/outer segments of cone cells prepared from teleost fish retina (Dearry and Burnside, 1986*a*).

We report here that both light- and circadian-driven cone myoid movements persist in vitro. Furthermore, these studies demonstrate a role for endogenous dopamine in this circadian control mechanism.

MATERIALS AND METHODS

Experimental Animals

Adult farm raised Midas cichlids, (*Cichlasoma citrinellum*), were purchased from a local supplier (New World Aquatics, Taffs Well, Mid-Glamorgan Wales UK) and maintained in 225 liter indoor aquaria with filtered aerated tap water at 24°C and fed daily (0.5–3.5% of body weight) on Trout Fingerling 1 (BP Nutrition Ltd, Preston, UK). Animal density was maintained at 50 per aquarium. Fish were entrained for at least 28 d (usually >50 d) before experimentation to a 12 h light/dark cycle with light onset (Ct 00.00) at 0800 h and offset (Ct 12.00) at 2,000 h. Aquaria were illuminated with full spectrum lights (1,000 lux ~92 foot candles) at the water surface (General Electric, cool white fluorescent; PANLUX electronic 2 light meter, GOSSEN West Germany).

Normal Circadian- and Light-regulated Cone Position

Circadian- and light-regulated cone position was verified in control animals at experimental time points Ct 00.00, Ct 06.00ED (expected day), Ct 12.00, Ct 18.00, Ct 22.00 and Ct 23.00, in the normal light/dark (LD) cycle and in continuous darkness (DD), (noted hereafter as "To control" time points), as in Mc Cormack and Burnside (1991). Once verified, these circadian- and light-regulated cone positions were used throughout the remainder of the study as "To time" points, where "To" was the time at which experimental retinal cultures were initiated. As before, cone position was analyzed from pairs of eyes taken from a number of fish at selected time points in both the normal light/dark cycle and in continuous darkness. At all sample points, fish were killed by spinal cord section with pithing before both eyes were enucleated and hemisected along the ora serrata to prepare eye cups. When in darkness, animals were killed and eye cups were prepared under infrared illumination, (>880 nm) (Find-R-Scope Infrared Viewer, FJW Optical Systems, Inc; modified Keeler bar type near telescope, Keeler Windsor, UK; Kodak Safelight No. 11, Kodak, UK; Farnell 950 nm IR-LED, Farnell Electronic Components Ltd., UK).

Modulation of Circadian Cone Myoid Movements In Vitro

Eye cups were prepared as above from adult fish of uniform size (10 cm nose to caudal fin peduncle). Whole retinas were removed from freshly prepared eyecups to culture after the protocol of Dearry and Burnside (1986*a*). Animals were sacrificed at 6 time points (corresponding to To time points), throughout both the normal light/dark cycle and in continuous darkness. At midnight (Ct 18.00), at expected dawn (Ct 22.00 and Ct 23.00) and at expected mid-day (Ct 06.00ED), animals were killed, retinas prepared in darkness and all retinal cultures, with the exception of those noted as in the light, were carried out in darkness. At dawn (Ct 00.00), animals were killed and retinas prepared in darkness, before lights on; retinas were then cultured in the light after normal lights on. At dusk (Ct 12.00), animals were killed and retinas were prepared under dim red light, before lights off. With the exception of experiments

where cultures were conducted in the light, retinae were then cultured in the dark. At all times (with the exception of To control animals as noted in the previous section), for each animal killed its left retina was cultured in medium alone, and thus served as a culture control, whereas its right retinas was used for experimental treatment. Such control retinas were always cultured under the lighting conditions prevailing at the time of dissection. Thus, for example at Ct 00.00, where and dissection were conducted in the dark, control retinas were cultured for 30' in the dark. In comparison, at Ct 12.00 where dissection was conducted under dim red light, control retinas were cultured for 30' under the same dim red light conditions. This added experimental precaution was taken in order to eliminate the possible confounding effects of dissection induced stress in the culture preparation.

The experimental treatments used were; 30' light culture, where retinae were cultured in the light (Ct 18.00, Ct 22.00, Ct 23.00, Ct 06.00ED, and Ct 12.00), or 30 min dark culture, where retinae were cultured in the dark (Ct 00.00), at which times (as noted above) the converse lighting condition was used for the left eye control; and 30 min culture in the presence of drug. At each time point an additional experimental control grouping was included in which retinas were cultured for 30 min in the dark, and then exposed to 10 min ambient illumination; in an attempt to assess retinal viability in culture. These time points were chosen since our previous studies (McCormack and Burnside, 1991, and 1992) have indicated that at midnight, cone myoids are fully elongate in the absence of either a circadian or a light signal; before expected dawn circadian-driven cone myoid contraction takes place; at dawn, light-induced cone myoid contraction is initiated; at expected midday cone myoids are normally partially contracted and are seen to be held in this position by a circadian signal in the absence of a light signal, and finally at dusk, cone myoids elongate in the absence of either a circadian or a light signal. We chose to assess circadian cone contraction in vitro at two time points before expected dawn, Ct 22.00 and Ct 23.00. Our rational was that at Ct 22.00, circadian induction of cone contraction should just be beginning, whereas at Ct 23.00 circadian induction should be well established. We reasoned that the result would therefore be two "To positions," Ct 22.00 and Ct 23.00, reflecting an increase in the strength of the underlying circadian signal. The "To position" at Ct 22.00 should be close to the fully dark adapted position, whereas the "To position" at Ct 23.00 should be somewhat shorter. The experimental conditions chosen allowed us to remove retinas to culture at times during which their cones were under the influence of a variety of combinations of either ± light and/or ± circadian influences. In this way we hoped to ascertain whether there was a humoral component to the short term circadian signaling mechanism. Dopamine and sulpiride were purchased from Sigma Chemical (Dorset UK) and LY171555, SKF39393, and SCH23390, from RBI Research Biochemicals Inc. (Semat Technical, Herts, UK). Drugs were added to culture media at final concentrations as noted. Each retina was cultured in 500 μl of freshly oxygenated modified Earle's buffered salt solution (± drug) containing; 120 mM NaCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.4 mM KCl, 1.0 mM Na_2HPO_4, 24 mM $NaHCO_3$, 20 mM glucose, 3 mM HEPES, 1 mM EGTA, 0.1 mg/ml BSA, 1 mM ascorbic acid, 0.01% DMSO, pH 7.4 at 24°C. No attempt was made to oxygenate or circulate media during culture. Cultures were terminated by the addition of an equal volume of fixative to the culture medium.

Histological Analysis of Cone Myoid Length

Eye cups prepared from "To control" animals were immediately fixed in either light or dark as appropriate with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 for ≥ 12 h. To enhance retinal-RPE adhesion cetylpyridium chloride (0.5%) (Sigma Chemical) was added to this fixative. Retinas which had been cultured were fixed under their culture conditions for 30 min with 4% glutaraldehyde in 0.2 M phosphate buffer, pH 7.0, and then for a further ≥ 12 h, with a fresh solution of 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. For histological

analysis, a central region was cut from the fundus of each retina and chopped into approximately 20 μm thick radial slices. These slices were then examined unstained with a light microscope (Nikon) under Nomarski interference contrast optics. From each retina, 30 representative cone myoid lengths were measured manually using a graduated eye piece (Ealing, UK), as the distance from the outer limiting membrane to the base of the cone ellipsoid. Data are presented as mean ± standard error as noted. At all time points and for all treatments, *n* refers to the number of fish examined.

RESULTS

Normal Circadian- and Light-regulated Cone Position and Movements are Maintained In Vitro

We have examined the position occupied by cones in retinas immediately before their removal to culture. These results are presented in Fig. 1 and are comparable to and representative of cone positions in this species in vivo under continuous dark (DD) and cyclic light (LD), previously reported (McCormack and Burnside, 1991, 1992).

Modulation of Circadian Cone Movements In Vitro

To ascertain whether either light- or circadian-induced cone myoid movements could take place without humoral input, we chose to examine cone movements in short term culture. We further examined the effects of dopamine, and D_1 (SCH23390 and SKF 38393) and D_2 (LY171555 and Sulpiride) type dopamine receptor agonists and antagonists, at various time points throughout the light/dark cycle and in continuous darkness.

At midnight. At midnight ("To cone" myoid length, 55.6 ± 1.9 μm), 30 min culture in darkness had no effect on cone myoid length (54.5 ± 2.1 μm), whereas both 30 min light culture and 10 min light culture after 30 min dark culture, induced complete cone myoid contraction to 5.6 ± 1.8 μm and 5.8 ± 1.7 μm, respectively (Fig. 1). When added to the culture medium dopamine or the D_2 agonist LY171555 induced cone myoids to contract in dark culture to 6.1 ± 1.6 μm and 5.9 ± 1.5 μm, respectively. The D_2 antagonist sulpiride and both of the D_1 compounds, had no effect on cone position at this time, as compared to cone myoid length before experimentation (To) ($P < 0.05$) (Fig. 2). Final concentrations of agents in culture medium were calculated to be 10^{-6} M for dopamine and the D_2 compounds, and 10^{-4} M for both the D_1 compounds. These concentrations were those used throughout this study, unless otherwise noted.

At expected dawn. At Ct 22.00, the "To cone" myoid length was 57.3 ± 3.2 μm. After 30 min dark culture cones had begun to contract as predicted to reach 45.6 ± 3.6 μm, a contraction rate of 0.39 μm/min. In comparison, both 30 min light culture and 10 min light culture after 30 min dark culture induced complete cone myoid contraction at this time (Fig. 1). When added to the culture medium dopamine or the D_2 agonist LY171555 again induced cone myoids to contract in dark culture to 6.1 ± 2.7 μm and 6.3 ± 1.9 μm respectively. The D_2 antagonist, sulpiride, prevented the circadian induced cone contraction seen in the dark cultures, and induced cone myoid elongation to 55.3 ± 4.3 μm. Neither of the D_1 compounds (agonist 46.1 ± 3.5 μm; antagonist 44.3 ± 5.3 μm), effected cone position at this time, as

compared to the degree of circadian induced cone myoid contraction seen in dark culture ($P < 0.05$) (Fig. 2).

At Ct 23.00 (Fig. 2), the "To cone" myoid length was 41.3 ± 4.4 μm, so circadian induced contraction in the hour from Ct 22.00 to Ct 23.00 had reduced cone myoid length by ~16 μm, a contraction rate of 0.26 μm/min. After 30 min dark culture cones had further contracted to 36.9 ± 5.3 μm. Again, as at Ct 22.00, both 30 min light culture and 10 min light culture after 30 min dark culture induced complete

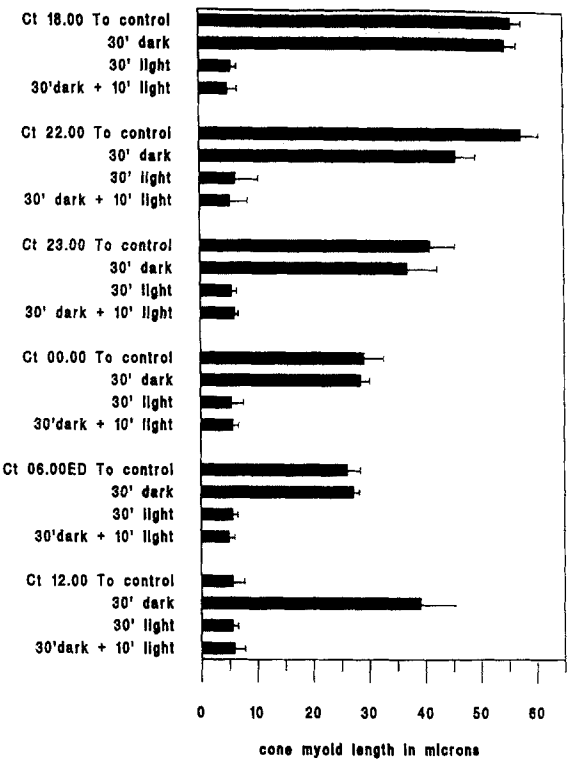

FIGURE 1. Existing cone positions were maintained and/or ongoing cone movements continued when retinas were removed to culture at any stage of either the LD or the DD cycle. For example, at midnight and at expected midday, the cone position seen in vivo is maintained in vitro. In comparison, at expected dawn, ongoing circadian induced contractions are continued in vitro. The continued viability of retinas in vitro is indicated by their ability to respond to light in both 30 min light culture and more especially in 10 min light culture after 30 min dark culture, and in their ability to elongate in vitro at dusk. In all cases, "To control" represents cone myoid length in the retina at the time of killing and dissection. (Ct 18.00 = midnight, Ct 22.00 and Ct 23.00 = expected dawn. [n = 12 fish {24 eyes} at all "To controls," n = 12 left retinas at 30 min dark and 30 min light points]. Data are presented as mean ± standard error).

cone myoid contraction (Fig. 1). When added to the culture medium, dopamine or the D_2 agonist LY171555 again induced cone myoids to contract in dark culture to 6.3 ± 1.1 μm and 6.5 ± 1.5 μm, respectively. The D_2 antagonist, sulpiride again prevented circadian induced cone contraction as seen in the dark cultures, and induced cone myoid elongation to 48.1 ± 4.6 μm. Interestingly, the sulpiride induced elongation in 30 min culture at Ct 23.00, was somewhat less than the sulpiride induced elongation in 30 min culture seen at Ct 22.00, where cones elongated by a further 7.2 μm, approximately. Both of the D_1 compounds (agonist

35.7 ± 4.6 μm; antagonist 38.1 ± 5.1 μm), failed to effect cone position at this time, as compared to the degree of circadian induced cone myoid contraction seen in dark culture ($P < 0.05$) (Fig. 2).

At dawn. Having thus ascertained that circadian induced pre-dawn cone myoid contraction continued in vitro, and could be accelerated by dopamine and reversed by sulpiride, we wished to assess whether light-induced cone myoid contraction at normal dawn occurred in vitro in similar fashion. At Ct 00.00, the "To cone" myoid length for animals which were sacrificed in the dark was 29.1 ± 3.6 μm, reflecting the degree to which circadian induced contraction had taken place in the 2 h before

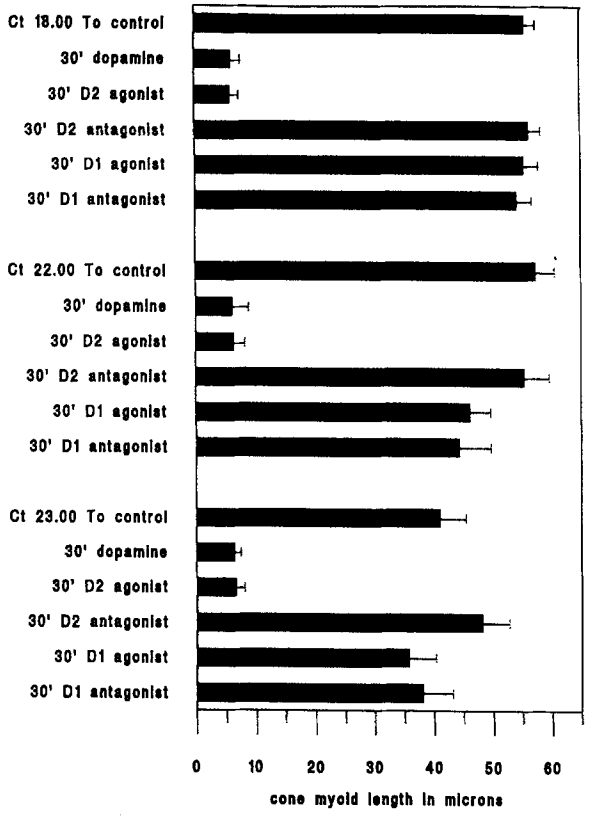

FIGURE 2. Dopamine or the D_2 agonist LY171555 induced cone contraction in cultured retinas at midnight (Ct 18.00) and at expected dawn (Ct 22.00 and Ct 23.00). The D_2 antagonist sulpiride had no effect on cone position at Ct 18.00 but blocked circadian induced pre-dawn cone myoid contraction at Ct 22.00 and Ct 23.00. Both the D_1 receptor compounds failed to effect cone position at midnight and at expected dawn. In all cases, "To control" represents cone myoid length in the retina at the time of killing and dissection. (Ct 18.00 = midnight; Ct 22.00 and Ct 23.00 = expected dawn; Ct 00.00 = dawn; Ct 06.00ED = expected mid-day; and Ct 12.00 = dusk. [n = 12 fish {24 eyes both left and right] at all "to controls," n = 6 right retinas for all other treatments]. Data are presented as mean ± standard error).

lights on Ct 22.00 to Ct 00.00. When animals were killed in the dark at this time and their retinas cultured in the dark for 30 min cone myoids failed to contract further, resulting in myoid lengths of 28.5 ± 1.6 μm. This cone position agrees closely with the results of an earlier study, in which we demonstrated that the expected day cone position was reliably fixed at ~25 μm (McCormack and Burnside, 1992). In comparison, both 30 min light culture and 10 min light culture after 30 min dark culture induced complete cone myoid contraction to 5.5 ± 2.1 μm and 5.7 ± 1.1 μm, respectively (Fig. 1). At this time the addition of dopamine, or of either of the D_2 or the D_1 compounds, to the media of retinas cultured in the light (1000 lux) did

nothing to interfere with the attainment of fully light adapted, shortened cone myoids (5.8 ± 1.1 μ, n = 42 right retinae) (Fig. 3).

At expected midday. Because we had previously shown that maintenance of expected day cone position was mediated in vivo by dopamine via a D_2 mechanism (McCormack and Burnside, 1992), we wished to ascertain whether a similar mechanism existed in vitro. The "To cone" myoid length for animals which were killed in the dark at Ct 06.00ED was 26.1 ± 2.2 μm, reflecting the partially contracted position occupied by cones in the Midas retina in expected day. Removal of retinae to

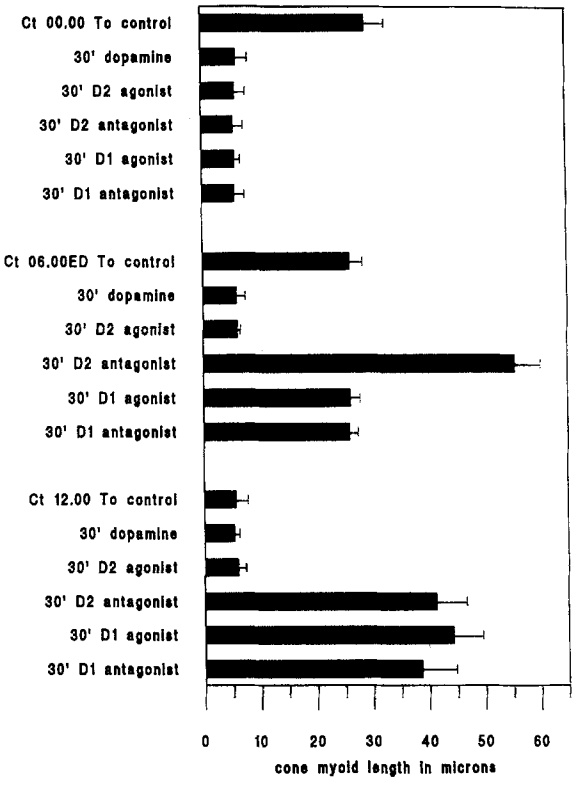

FIGURE 3. Circadian regulated cone movement. Dopamine or the D_2 agonist LY171555 did not effect cone position at dawn Ct 00.00, but induced cone contraction in cultured retinas at expected midday (Ct 06.00ED) and inhibited cone elongation at dusk (Ct 12.00). The D_2 antagonist sulpiride induced cone myoid elongation at Ct 06.00ED, but did not affect cone position at dawn Ct 00.00 or at dusk Ct 12.00. Neither of the D_1 compounds had an effect on cone position at dawn, at midnight and at expected dusk. In all cases, "To control" represents cone myoid length in the retina at the time of killing and dissection. (Ct 00.00 = dawn; Ct 06.00ED = expected mid-day; and Ct 12.00 = dusk. [n = 12 fish {24 eyes both left and right} at all "to controls," n = 6 right retinas for all other treatments]. Data are presented as mean ± standard error).

30 min dark culture at this time did not significantly alter cone myoid lengths (27.2 ± 1.8 μm), whereas both 30 min light culture and 10 min light culture after 30 min dark culture induced complete cone myoid contraction to 5.6 ± 0.9 μm and 5.8 ± 1.8 μm, respectively (Fig. 1). Addition of either dopamine or the D_2 agonist to the culture medium in the dark, mimicked the effects of light and induced cone myoid contraction, whereas sulpiride, the D_2 antagonist, induced significant cone myoid elongation to 55.4 ± 4.7 μm. In comparison neither of the D_1 compounds had any significant effect upon cone myoid length in 30 min dark culture (Fig. 3).

At dusk. To conclude this series of experiments, we assessed the ability of cones to elongate at normal dusk, Ct 12.00, in vitro. At Ct 12.00 animals were sacrificed in dim red light, before lights off. "To" cone myoid length was 5.6 ± 2.1 μm, reflecting the fully contracted position occupied by cones in the Midas retina in normal day. Removal of retinas to 30 min dark culture at this time resulted in cone myoid elongation to 39.1 ± 6.3 μm, representing an average elongation rate of ~1 μm/min. 30 min light culture, under dim red light, maintained cones in their fully contracted, light adapted position, whereas 10 min light culture after 30 min dark culture, induced cone myoids to recontract to 5.9 ± 1.9 μm (Fig. 1). Addition of both dopamine and the D_2 agonist to the culture medium in the dark, prevented cone myoid elongation, cones remained contracted at 5.3 ± 0.9 μm, and 5.9 ± 1.4 μm, respectively. The addition of sulpiride, the D_2 antagonist to culture medium did not increment nor interfere with normal dark induced cone myoid elongation at this time. After 30 min culture in the dark with sulpiride, cone myoids had elongated to 41.2 ± 5.5 μm. Again both of the D_1 compounds, had no effect upon cone myoid length in 30 min dark culture; averaged cone myoid lengths after these treatments was 40.2 ± 1.8 μm (n = 36 retinas) (Fig. 3).

DISCUSSION

Cone Movements In Vitro

The results of this study demonstrate that both light-induced and circadian-driven cone myoid movements can occur normally in the Midas cichlid retina in vitro. The catecholamine dopamine acting via a D_2 type receptor mechanism, mimics the effects of ambient illumination inducing cone myoid contraction and preventing cone myoid elongation. Further, the effects of the circadian signal mechanism, which favors the light-adapted state promoting pre-dawn cone contraction and maintaining partially contracted cone myoids in expected day, can be overridden by the D_2 antagonist sulpiride. Removing retinas to culture did not effect their ability to maintain cones fully elongate, and dark adapted at night, nor their ability to respond to normal ambient illumination at dawn, and so contract, or to maintain fully contracted cones in normal daylight. Further, cones remained fully responsive to ambient illumination after 30 min dark culture at any of the experimental time points, indicating the continued viability of these retinas in vitro.

The findings of this study are consistent with the results of several in vitro studies performed on green sunfish retinas (Dearry and Burnside, 1986*a*,*b*). The circadian characteristics of cone movements seen in the cichlid retina in vivo (McCormack and Burnside, 1991) are also retained in vitro. Cone myoids exhibit circadian induced contraction in the two hours before dawn, with approximately the same extent as that seen in vivo. Further, circadian regulation of the partially contracted expected mid-day cone position, previously reported in intact eyes is also retained in culture. These observations suggest that the circadian signal mechanism required for both successful continuation of predawn cone myoid contraction and maintenance of partially contracted cones in expected day requires no immediate humoral input. Finally, the ability of cones to elongate normally in response to the absence of

ambient illumination at dusk, is further evidence that these retinas are behaving normally in culture.

Dopaminergic Regulation of Cone Movements In Vitro

Results of studies with retinal cultures in the presence of dopamine, or D_1 or D_2 agonists/antagonists, indicate that dopamine acting via D_2 receptors, may mimic the effect of light and circadian signals, to induce cone myoid contractions. This result is consistent with the results of previous intraocular injection studies performed on this species (McCormack and Burnside, 1992), and with reports of dopamine acting as a light signal in the green sunfish retina (Dearry and Burnside, 1986*a*,*b*). In 30 min dark culture, at midnight, expected dawn, expected mid-day or dusk, dopamine mimics the effect of light culture, to either induce cone myoid contraction or prevent cone myoid elongation. The D_2 agonist LY171555 has a similar effect to dopamine at these time points. The D_2 antagonist, sulpiride, (*a*) does not alter cone myoid length at midnight; (*b*) reverses circadian induced contraction at expected dawn in a time dependent fashion, it is less effective at Ct 23.00 than at Ct 22.00, implying an underlying increase in retinal dopamine release; (*c*) induces almost complete cone myoid elongation at expected mid-day; and (*d*) does not interfere with normal cone elongation at dusk. These findings are consistent with our previous findings from intraocular injection studies, and suggest that it is the titer or concentration of available endogenous dopamine, and the availability of D_2 receptors, which control circadian cone myoid movements. Finally, D_1 receptor compounds had no measurable effect upon either light or circadian regulated cone movements or positions, at the time points tested.

Dopamine as a Retinal Signal

Our findings, that intraocularly produced dopamine is involved in mediating light and circadian regulation of cone myoid position in vitro concur with the reported effects of dopamine on other retinal processes. Retinal dopamine production is known to be regulated in diurnal and circadian fashion in parallel with retinomotor movements in the cichlid retina. The activity of tyrosine hydroxylase, the rate limiting enzyme in the pathway by which dopamine is produced from precursor tyrosine, exhibits fluctuations of sixfold in response to circadian signals, and of twofold in response to light (McCormack and Burnside, 1993). Dopaminergic activity and dopamine release are known to increase in response to light stimulation in rabbit, rat, *Xenopus,* and fish retinas, (Godley and Wurtman, 1988; Nowak and Zurawska, 1989; Brainard and Morgan, 1987; Boatright, Hoel, and Iuvone, 1989; Witkovsky and Xiao-Ping Shi, 1990; Kirsch and Wagner, 1989). Of the many rhythmic activities known to occur in vertebrate retinae, eg, retinomotor movements (Besharse, 1982), horizontal cell spinule formation (Weiler and Wagner, 1984), opsin synthesis (Korenbrot and Fernald, 1989), and outer segment disc assembly and shedding (LaVail, 1976); dopamine is now thought to regulate most in a light-adaptive fashion. Those cyclic activities now known to be regulated at least in part by dopamine include, cone myoid movements in other teleost fish species (Dearry and Burnside, 1986*a;* Kohler, Kolbinger, Kurz-Isler, and Weiler, 1990); retinal pigment epithelium pigment granule migration in teleost fish and bull frogs (Dearry and Burnside, 1989); cone myoid

movements in bullfrog (Dearry, Edelman, Miller, and Burnside, 1990) and in *Xenopus* retinas (Pierce and Besharse, 1985); horizontal cell spinule number and gap junction connexon density in fish (Weiler, Kohler, Kolbinger, Wolburg, Kurz-Isler, and Wagner, 1988; Kohler and Weiler, 1990; Kohler et al., 1990); activity at horizontal cell synapses in *Xenopus* (Witkovsky, Stone, and Besharse, 1988; Witkovsky and Xiao-Ping Shi, 1990); cAMP concentration in mouse photoreceptors (Cohen and Blazynski, 1990) and serotonin *N*-acetyltransferase (NAT) activity and melatonin synthesis in *Xenopus* retinas (Nowak, Kazula, and Golembiowska, 1992; Zawilska and Iuvone, 1992; Cahill and Besharse, 1992).

The results of this study indicate that both light- and circadian-driven cone myoid movements occur normally in vitro. Further they suggest that immediate circadian regulated cone movements do not require humoral input. Our whole retinal culture studies suggest that endogenous dopamine, acting via D_2 receptors, plays an important role in the circadian regulatory mechanism. Blocking D_2 receptors prevents the circadian-induced cone contraction that occurs at expected dawn and reverses the maintainence of partially contracted cone position in expected day. This result suggests that both the titre of endogenously produced dopamine and the availability of D_2 receptors is critical to the circadian response.

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REFERENCES

Arey, L. B. 1915. The occurance and significance of photomechanical changes in the vertebrate retina—an historical survey. *Journal of Comparative Neurology.* 25:535–554.

Besharse, J. C. 1982. The daily light-dark cycle and rhythmic metabolism in the photoreceptor-pigment epithelium complex. *In* Progress in Retinal Research. N. N. Osbourne and G. J. Chader, editors. Pergammon Press, Oxford. 81–124.

Besharse, J. C., and P. M. Iuvone. 1992. Is dopamine a light-adaptive or a dark-adaptive modulator in retina? *Neurochemistry International.* 20:192–199.

Boatright, J. H., M. J. Hoel, and P. M. Iuvone. 1989. Stimulation of endogenous dopamine release and metabolism in amphibian retina by light- and K^+-evoked depolarization. *Brain Research.* 482:164–168.

Brainard, G. C., and W. W. Morgan. 1987. Light-induced stimulation of retinal dopamine: a dose response relationship. *Brain Research.* 424:199–203.

Burnside, B., and B. Nagle. 1983. Retinomotor movements of photoreceptors and retinal pigmented epithelium: mechanisms and regulations. *In* Progress in Retinal Research. Vol. 2. N. N. Osbourne and G. J. Chader, editors. Pergamon Press, Oxford, UK. 67–110.

Burnside, B., and A. Dearry. 1986. Cell motility in the retina. *In* The Retina: A Model for Cell Biology Studies R. Adler, and D. Farber, editors. Academic Press, New York. 151–206.

Burnside, B., and A. M. Laties. 1979. Pigment movement and cellular contractility in the retinal pigment epithelium. *In* The Retinal Pigment Epithelium. K. M. Zinn and M. F. Marmor, editors. Harvard University Press, Cambridge, MA. 175–191.

Cahill, G. M., and J. C. Besharse. 1992. Light-sensitive melatonin synthesis by *Xenopus* photoreceptors after destruction of the inner retina. *Visual Neuroscience.* 8:487–490.

Cohen, A. I., and C. Blazynski. 1990. Dopamine and its agonists reduce a light-sensitive pool of cyclic AMP in mouse photoreceptors. *Visual Neuroscience.* 4:43–52.

Dearry, A. 1991. Light onset stimulates tyrosine hydroxylase activity in isolated teleost retinas. *Vision Research.* 31:395–399.

Dearry, A., and B. Burnside. 1986*a*. Dopaminergic regulation of cone retinomotor movement in isolated teleost retinas. I. Induction of cone contraction is mediated by D2 receptors. *Journal of Neurochemistry.* 46:1006–1021.

Dearry, A., and B. Burnside. 1986*b*. Dopaminergic regulation of cone retinomotor movement in isolated teleost retinas. II. Modulation by γ-aminobutryic acid and serotonin. *Journal of Neurochemistry.* 46:1022–1031.

Dearry, A., and B. Burnside. 1989. Light induced dopamine release from teleost retinas acts as a light-adaptive signal to the retinal pigment epithelium. *Journal of Neurochemistry.* 53:870–878.

Dearry, A., J. L. Edelman, S. Miller, and B. Burnside. 1990. Dopamine induces light-adaptive retinomotor movements in Bullfrog cones via D2 receptors and in retinal pigment epithelium via D1 receptors. *Journal of Neurochemistry.* 54:1367–1378.

Detwiler, S. R. 1943. Vertebrate Photoreceptors. MacMillan Publishing Co., New York. 184 pp.

Garten, S. 1907. Die Veranderungen derNetzhaut Durch Licht. Graefe-Saemisch Handbuch der Gesamten. *Augenheilkunde.* Leipzig. 2:12:250–280.

Godley, B. F., and R. J. Wurtman. 1988. Release of endogenous dopamine from the superfused rabbit retina in vitro: effect of light stimulation. *Brain Research.* 452:393–395.

Kirsch, M., and H. J. Wagner. 1989. Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation. *Vision Research.* 29:147–154.

Kohler, K., and R. Weiler. 1990. Dopaminergic modulation of transient neurite outgrowth from horizontal cells of the fish retina is not mediated by cAMP. *European Journal of Neuroscience.* 2:788–794.

Kohler, K., W. Kolbinger, G. Kurz-Isler, and R. Weiler. 1990. Endogenous dopamine and cyclic events in the fish retina. II. Correlation of retinomotor movement, spinule formation, and density of gap junctions with dopamine activity during light/dark cycles. *Visual Neuroscience.* 5:415–428.

Korenbrot, J. I., and R. D. Fernald. 1989. Circadian rhythm and light regulate opsin mRNA in rod photoreceptors. *Nature.* 3337:454–457.

LaVail, M. M. 1976. Rod outer segment disc shedding in relation to cyclic lighting. *Eye Research.* 23:277–280.

Mc Cormack, C. A., and B. Burnside. 1991. Effects of circadian phase on cone retinomotor movements in the Midas cichlid. *Experimental Eye Research.* 52:431–438.

Mc Cormack, C. A., and B. Burnside. 1992. A role for endogenous dopamine in circadian regulation of retinal cone movement. *Experimental Eye Research.* 55:511–520.

Mc Cormack, C. A., and B. Burnside. 1993. Light and circadian modulation of teleost retinal tyrosine hydroxylase activity. *Investigative Ophthalmology and Visual Science.* 34:1853–1860.

Negishi, K., S. Kato, and T. Teranishi. 1981. Density ratio of dopaminergic cells in the amacrine cell layer of the fish retina. *Acta Histochemica et Cytochemica* 14:317–324.

Nowak, J. Z., and E. Zurawska. 1989. Dopamine in the rabbit retina and striatum: diurnal rhythm and effect of light stimulation. *Journal Neural Transmission.* 75:201–212.

Nowak, J. Z., A. Kazula, and K. Golembiowska. 1992. Melatonin increases serotonin *N*-acetyltransferase activity and decreases dopamine synthesis in light-exposed chick retina: in vivo evidence supporting melatonin dopamine interaction in retina. *Journal of Neurochemistry.* 59:1499–1505.

Parker, G. H. 1932. The movements of the retinal pigment. *Ergebnisse Biologie.* 9:239–292.

Pierce, M. E., and J. C. Besharse. 1985. Circadian regulation of retinomotor movements. I. Interaction of melatonin and dopamine in the control of cone length. *Journal of General Physiology.* 86:671–689.

von Studnitz, G. 1952. Physiologie des Schens. Akademische Verlagagesellachaft, Leipzig. 1–256.

Wagner, H. J., and I. Wulle. 1990. Dopaminergic interplexiform cells contact photoreceptor terminals in catfish retina. *Cell Tissue Research.* 359–365.

Walls, G. L. 1942. The Vertebrate Eye. Hafner Publishing Co., New York. 780 pp.

Weiler, R., and H. J. Wagner. 1984. Light-dependent change of cone-horizontal cell interactions in carp retina. *Brain Research.* 298:1–9.

Weiler, R., K. Kohler, W. Kolbinger, H. Wolburg, G. Kurz-Isler, and H. J. Wagner. 1988. Dopaminergic neuromodulation in the retinas of lower vertebrates. *Neuroscience Research Supplement.* 8:S183–S196.

Witkovsky, P., and Shi Xiao-Ping. 1990. Slow light and dark adaptation of horizontal cells in *Xenopus* retina: a role for endogenous dopamine. *Visual Neuroscience.* 5:405–413.

Witkovsky, P., S. Stone, and J. C. Besharse. 1988. Dopamine modifies the balance of rod and cone inputs to horizontal cells of *Xenopus* retina. *Brain Research.* 449:332–336.

Zawilska, J. B., and P. M. Iuvone. 1992. Melatonin synthesis in chicken retina: effect of kainic acid-induced lesions on the diurnal rhythm and D_2-dopamine receptor-mediated regulation of serotonin *N*-acetyltransferase activity. *Neuroscience Letters.* 135:71–74.