Prostaglandins E₁, E₂, and D₂ Induce Dark-Adaptive Retinomotor Movements in Teleost Retinal Cones and RPE
Brian Cavallaro and Beth Burnside

In teleosts, retinomotor movements of photoreceptors and retinal pigment epithelium are regulated both by light and by an endogenous circadian rhythm. Light induces cones to contract, rods to elongate and RPE cells to disperse their pigment granules into their long apical projections; darkness induces opposite movements. When fish are maintained in prolonged constant darkness, appropriate movements nonetheless occur at subjective dusk and dawn. To explore the mechanisms of this light and circadian regulation, we have been investigating effects of several extracellular messengers known to be present in retina on retinomotor movements in the green sunfish (Lepomis cyanellus). Here we report that prostaglandin E₁ (PGE₁) can induce movements characteristic of dark onset (or night) in both cones and RPE in isolated light-adapted retinas in the light; i.e., PGE₁ induces cone elongation and RPE pigment granule aggregation. The extent of PGE₁-induced cone and RPE movements were dose-dependent with maximal movement occurring at 250–500 nM; higher concentrations were not as effective. Incubations with PGE₂ and PGD₂ also induced dark-adaptive cone and RPE retinomotor movements, but PGF₂α did not. Further observations suggest that prostaglandins may play a role in mediating the induction of cone and RPE movements by dark onset: dark-induced movements were inhibited by pretreating light-adapted isolated retinas before dark culture with agents which inhibit endogenous prostaglandin synthesis. Both indomethacin (50 μM) and acetylsalicylic acid (50 μM), two inhibitors of the cyclooxygenase component of specific prostaglandin synthase, inhibited dark-induced cone elongation and pigment aggregation in cultured sunfish retinas. Another cyclooxygenase inhibitor, ibuprofen (50 μM) had no effect. Together the effectiveness of PGE₁ in inducing dark-adaptive movement and the inhibition of dark adaptive movement by cyclooxygenase inhibitors suggest that prostaglandins may play a role in vivo in mediating the induction of dark-adapted RPE and cone retinomotor movements by dark onset. Invest Ophthalmol Vis Sci 29:882–891, 1988.
light. Furthermore, inhibition of endogenous prostaglandin synthesis inhibits movements induced by dark onset.

Materials and Methods

Animals

Green sunfish, Lepomis cyanellus, were maintained in indoor aquaria, and entrained to a 24 hr light cycle (12 hr light/12 hr dark) for at least 1 month prior to use.

Drugs and Culture Media

PGE₁, PGE₂, PGF₂ alpha, 3-isobutyl-1-methylxanthine (IBMX), indomethacin, ibuprofen, acetylsalicylic acid, ketoprofen, dopamine, and forskolin were purchased from Sigma Chemical Company (St. Louis, MO). Eicosa-5,8,11,14,-tetranyoic acid was a generous gift from Hoffmann-La Roche (Nutley, NJ). Stock solutions were prepared by dissolving prostaglandins in absolute ethanol and stored at −20°C. Just prior to use the stock solutions were diluted to desired concentrations in modified Earle's Buffered Salt Solution (mEBSS: 116.4 mM NaCl; 5.4 mM KCl; 1.8 mM CaCl₂; 1.0 mM MgSO₄·7 H₂O; 25.6 mM glucose; 24.0 mM NaHCO₃; 3.0 mM N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES); 1.0 mM ascorbic acid; 5.0 mM taurine; pH 7.40). This medium was used for all dissections and incubations after gassing with 95% O₂/5% CO₂ unless otherwise noted. For Co++ studies bicarbonate, phosphate, and sulfate anions were replaced by chloride; HEPES was elevated to 6 mM; 100% O₂ was used for gassing.

Dissection and Retinal Culture

RPE-retina cultures were prepared as described previously. Intact RPE-retinas were more easily obtained during dissection if fish were exposed to intense light (700 footcandles or 2.66 × 10⁴ photons/sec/cm² at 546 nm) for 3 hr before dissection. Fish were killed by spinal section and brain pithing, eyes enucleated, and the anterior segment cut away to produce posterior eyecups. RPE-retinas were detached from the choroid by gently pipetting with mEBSS following optic nerve section. Four comparable retina samples were obtained per fish by bisecting the RPE-retina along the choroid fissure. One RPE-retina piece was immediately fixed for a time zero (T₀) sample, and the rest were placed vitreous down in wells of a Costar multiwell plate (Costar, Cambridge, MA) containing 0.5 ml culture media. All prostaglandin cultures contained 0.2 mM IBMX and 0.1% ethanol. An "experimental control" containing 0.2 mM IBMX and ethanol alone (without prostaglandin) was included for each fish. Ethanol (0.1%) had no effect on retinomotor movements and was present in all cultures unless otherwise indicated. To facilitate gas exchange, 0.3 ml of the culture media was removed immediately before culture. Retinal cultures were gassed with 95% O₂/5% CO₂ and rotated at 1 rps for 60 min. RPE and retina maintain normal association throughout the culture period. IBMX dose response experiments also included 0.1% ethanol in the medium.

In studies to determine whether retinas would undergo dark-induced retinomotor movements after preculture with cyclooxygenase inhibitors, isolated light-adapted RPE-retinas were preincubated in the light for 8 min with or without cyclooxygenase inhibitor and then incubated in the dark in the same media (within an opaque plexiglass chamber in a darkened room) for 60 min. For all cyclooxygenase inhibition studies the culture media contained ethanol (drug vehicle) adjusted to 0.5%. This ethanol concentration also had no effect on retinomotor movements.

The investigations reported in this manuscript conform to the ARVO resolution on the Use of Animals in research.

Isolated Cone Inner and Outer Segments (CIS/COS)

When fish are kept in darkness 90 min or more, the cones become so long that when the retina is removed during dissection the delicate long myoid breaks and the cone inner and outer segments (CIS/COS) remain attached to the RPE rather than the retina. To obtain CIS/COS, fish were dark-adapted in aerated tanks within a light-tight box for 90 min, sacrificed in the dark, and the eyes enucleated using infrared illumination. The retina was then gently pulled away from the RPE, removed by sectioning the optic nerve and discarded. The remaining RPE-eyecup was bisected to yield four samples per fish. Fragments of RPE with attached CIS/COS were dislodged from the choroid of each half eyecup by briskly pipeting culture medium (with or without drugs) into the eyecup, and collected in 0.9 ml medium in a 35 × 10 mm tissue culture dish (Falcon, Oxnard, CA).

A T₀ was fixed immediately after isolation of RPE-CIS/COS by the addition of 0.1 ml of a 10X stock glutaraldehyde/potassium ferrocyanide (K₃Fe(CN)₆) fix solution to the 0.9 ml mEBSS containing RPE-CIS/COS, for a final concentration of 1% glutaraldehyde and 0.016 g/ml K₃Fe(CN)₆. The remaining RPE-CIS/COS cultured 60 min in the light in 250 nM PGE₁ or 1.0 μM forskolin or mEBSS; each culture contained 0.2 mM IBMX and 0.1% ethanol.

Fixation and Measurement of Retinomotor Movements

All RPE-retinas were fixed with 6% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, chopped into
Fig. 1. Retinomotor positions of Rods, Cones and RPE in light- and dark-adapted green sunfish retinas. Cone myoids are short in the light and long in the dark. Pigment granules are dispersed in the light and aggregated in the dark. The extent of pigment aggregation is indicated by the Pigment Index, calculated as distance A divided by distance B, expressed as %.

25–50 μm slices and observed with Nomarksi optics as described elsewhere. Cone myoid length was measured as the distance from the outer limiting membrane (OLM) to the base of the ellipsoid (Fig. 1). Twenty measurements were made for each sample of RPE-retina. RPE retinomotor movements were measured as the percent of the distance from the OLM to Bruch's membrane occupied by pigment granules (the Pigment Index) (Fig. 1). The Pigment Index was measured in six locations per RPE-retina preparation. In all cases n refers to the number of retina preparations examined; data are presented as the mean ± the standard error of the mean (SEM).

For CIS/COS, cone myoid length was measured as the distance from the base of the ellipsoid to the tip of the myoid. Myoids were measured on the first 40 CIS/COS encountered per slide.

Results

Effects of Phosphodiesterase Inhibitors

Since in previous studies, we found that dbcAMP failed to induce cone elongation unless a phosphodiesterase (PDE) inhibitor was present, we reasoned that PDE inhibition might also be required to observe prostaglandin effects on retinomotor movements if prostaglandins functioned in retina by elevating cAMP. Therefore, for these studies we included the PDE inhibitor IBMX in all cultures at a concentration just above threshold for inducing cone elongation and pigment granule aggregation. Results of IBMX dose response experiments with RPE-retina tissue preparations are shown in Figure 2. At high concentrations (1 mM) IBMX alone induced maximal dark-adaptive cone and RPE movements. At 0.2 mM, IBMX induced 3–5% pigment granule aggregation (Fig. 2A) and 4–6 μm (<15%) cone myoid elongation (Fig. 2B). Similar effects of IBMX were reported previously for isolated retinas. Therefore in all subsequent prostaglandin experiments 0.2 mM IBMX was included in an attempt to insure that any cAMP produced in response to prostaglandins, whether directly or indirectly, might escape degradation and have the opportunity to activate movement.
Fig. 3. Nomarski photomicrographs of RPE-retinas fixed (A) immediately after dissection in the light (L0); (B) after 1 hr incubation in the dark in mEBSS; and (C) after 1 hr incubation in the light in the presence of 200 nM PGE1 plus 0.2 mM IBMX. Large arrows indicate the outer limiting membrane; small arrows indicate Bruch’s membrane. Cone myoid length is measured as the distance between large arrow and large arrowhead. The small arrowhead indicates distal-most extent of RPE pigment dispersion.

Prostaglandin Effects on RPE-Retina

Incubating light-adapted RPE-retinas with PGE1 alone (200 nM) did not induce pigment aggregation or cone elongation (cone myoid length after PGE1 culture = 5.1 ± 0.2 μm; n = 3; L0 = 7.8 ± 1.1 μm; n = 7). However, in the presence of 0.2 mM IBMX, PGE1 (200 nM) induced dark-adaptive retinomotor movements in both cones and RPE; ie, cones elongated and RPE pigment granules aggregated in response to PGE1 (Figs. 3, 4). The extents of PGE1-induced cone movement and RPE pigment aggregation (Fig. 3) were somewhat less than those observed in response to 1 hr culture in darkness (cone length = 40.9 ± 1.2 μm, n = 10; RPE pigment index = 43.5 ± 3.1%, n = 10). Cones were more responsive to PGE1, elongating to 66% of the maximal dark-induced length achieved in culture; RPE aggregation was only 23% of the maximal dark-induced extent achieved in culture.

In the presence of 0.2 mM IBMX, PGE1 effects on dark-adaptive retinomotor movements were dose-dependent in both cones and RPE, with maximal movement induced by 250–500 nM PGE1 (Fig. 4). Higher concentrations were less effective. PGE1 (2 μM) had no effect on cone and RPE position in dark-adapted RPE-retinas cultured in constant darkness (data not shown).

In order to investigate the specificity of prostaglandin effects on retinomotor movements we tested several other prostaglandins (Table 1). PGE2 and PGD2 also induced dark-adaptive retinomotor movements, while PGE2alpha did not. In fact, culture with PGA2alpha and IBMX produced more light-adapted retinomotor positions in cones and RPE than culture in IBMX alone (Table 1). However, PGE2alpha did not induce light-adaptive movements in previously dark-adapted retinas cultured in the dark (data not shown).
Table 1. Effects of prostaglandins on cone and RPE retinomotor movements

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Change in cone myoid length (μm) mean ± SEM (n)</th>
<th>Change in extent of pigment granule aggregation induced by prostaglandin (change in pigment index %) mean ± SEM (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>11.1 ± 3.5 (9)</td>
<td>11.2 ± 4.6 (9)</td>
</tr>
<tr>
<td>200 nM</td>
<td>16.5 ± 4.1 (6)</td>
<td>19.3 ± 3.2 (6)</td>
</tr>
<tr>
<td>500 nM</td>
<td>22.8 ± 2.5 (6)</td>
<td>22.7 ± 3.8 (6)</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>16.9 ± 9.7 (3)</td>
<td>24.7 ± 11.1 (3)</td>
</tr>
<tr>
<td>500 nM</td>
<td>17.6 ± 3.1 (4)</td>
<td>30.4 ± 3.3 (4)</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>-9.4 ± 1.6 (2)*</td>
<td>-11.0 ± 19.3 (2)*</td>
</tr>
</tbody>
</table>

Cone and RPE retinomotor positions were measured after 1 hr culture in the light with indicated concentration of drug plus 0.2 mM IBMX. Changes in cone myoid length and pigment granule aggregation were determined by subtracting the amount of movement observed in retina pieces cultured in IBMX alone from the extent of movement observed in retina pieces from the same fish cultured in 0.2 mM IBMX plus prostaglandin. Reported values are mean ± SEM, with n representing the number of fish examined. The mean values for cone length and pigment index in IBMX alone were 9.1 ± 1.2 μm and 83.7 ± 2.8% respectively (n = 14). Fully light-adapted cone and RPE pigment positions were 7.8 ± 1.1 μm and 80.2 ± 4.2% respectively (n = 7). *Negative values indicate that after culture with PGF<sub>2α</sub> plus IBMX cones and RPE had more light-adapted retinomotor positions than those cultured in IBMX alone.

Over the limited range of PGD<sub>2</sub> concentrations tested, the extents of PGD<sub>2</sub>-induced retinomotor movements appeared to be dose-dependent (Table 1).

Prostaglandin Effects on Isolated CIS/COS

Since previous studies had shown that dopamine can induce cone myoid shortening in isolated cone fragments containing inner and outer segments (CIS/COS), we also tested whether prostaglandins could directly influence myoid length in these cone fragments. CIS/COS were cultured with IBMX alone (0.2 mM) and with PGE<sub>1</sub> (250 nM) plus 0.2 mM IBMX in the light for 1 hr (Figs. 5, 6). CIS/COS cultured with PGE<sub>1</sub> plus IBMX did not have significantly longer myoids than CIS/COS cultured with IBMX alone (Fig. 6). In contrast, CIS/COS cultured with 1.0 μM forskolin plus 0.2 mM IBMX had significantly longer myoids (Figs. 5, 6). This result suggests that PGE<sub>1</sub> does not act directly on the adenylate cyclase present in cone inner-outer segments. Effects on more proximal parts of the cone cannot be ruled out.

Effects of Co<sup>2+</sup> Pretreatment on PGE<sub>1</sub> Culture

In order to examine whether PGE<sub>1</sub> effects on retinomotor movement require synaptic transmission, we attempted to inhibit synaptic transmission with Co<sup>2+</sup> and examine PGE<sub>1</sub> effects under these conditions (Table 2). Co<sup>2+</sup> has been reported by others to block synaptic transmission in isolated retinas. In the presence of 5 mM Co<sup>2+</sup>, culturing light adapted retinas with 250 nM PGE<sub>1</sub> plus 0.2 mM IBMX did not induce significant cone elongation beyond that induced by 0.2 mM IBMX alone (Table 2). However, debilitating effects of Co<sup>2+</sup> on retinal processes other than synaptic transmission appear likely since 5 mM Co<sup>2+</sup> also inhibited forskolin-induced cone elongation (Table 2). Since forskolin can act directly on CIS/COS to induce elongation, it clearly does not require synaptic transmission to be effective. Co<sup>2+</sup> inhibition of forskolin-induced elongation persisted when retinas were precultured in 5 mM Co<sup>2+</sup> for 10 min, rinsed in normal mEBSS and cultured for 1 hr with 1 mM forskolin and 0.2 mM IBMX. Therefore

![Fig. 5. Effects of PGE<sub>1</sub> and forskolin on myoid length in cone fragments composed of inner and outer segments (CIS/COS). Nomarski photomicrographs showing RPE-CIS/COS fixed (A) immediately after isolation, (B) after 1 hr light culture in 250 nM PGE<sub>1</sub> and 0.2 mM IBMX, or (C) after 1 hr light culture in 1.0 μM forskolin and 0.2 mM IBMX. Forskolin induced CIS/COS myoid elongation but PGE<sub>1</sub> did not. Arrows indicate cone myoids in (C). C = cone ellipsoid, bar = 10 μm.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933141/)
our Co⁺⁺ inhibition studies are not conclusive, since we cannot rule out the likelihood that cobalt treatment is destroying the ability of cones to elongate in some other way than by blocking synaptic transmission.

**Cyclooxygenase Inhibition**

To investigate whether prostaglandins might participate in the normal induction of retinomotor movement by dark onset in vivo, we tested the abilities of inhibitors of endogenous prostaglandin synthesis to interfere with the induction of retinomotor movements by dark onset in culture (Fig. 7). RPE-retinas were precultured with 50 μM indomethacin, acetylsalicylic acid, and ibuprofen for 8 min in the light followed by 60 min in the dark in the same medium. Indomethacin blocked and acetylsalicylic acid inhibited dark-induced cone and RPE pigment movement. Ibuprofen was ineffective. Inhibition of dark-induced retinomotor movements by indomethacin was not due to nonspecific toxicity since higher concentrations of indomethacin (100 μM) did not inhibit retinomotor movements in cultured RPE-retinas induced by light-onset (Table 3).

To investigate whether continuous prostaglandin release is required to maintain the dark-adapted retinomotor position in the dark, we tested effects of cyclooxygenase inhibitors on dark-adapted isolated retinas and RPE-retinas cultured in constant darkness (Table 4). Culturing RPE-retinas with indomethacin, ketoprofen or eicosatetraynoic acid (ETYA) (each at 100 μM) had no effect on cone or RPE retinomotor position. Thus inhibition of endogenous prostaglandin synthesis does not induce light-adaptive movements in retinas cultured in the dark.

Treatment with 100 μM indomethacin, ketoprofen, or ETYA in the dark also had no effect on induction of light-adaptive retinomotor movements either by light-onset or by 100 μM dopamine in the dark (Table 4). Thus inhibition of endogenous prostaglandin synthesis neither favors nor inhibits light-adap-

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**Table 2. Effects of Co⁺⁺ on PGE₁ and forskolin-induced cone myoid elongation**

<table>
<thead>
<tr>
<th>Co⁺⁺</th>
<th>Agents present</th>
<th>Mean ± SEM (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>1 μM FOR + 0.2 mM IBMX</td>
<td>43.6 ± 1.7 (3)</td>
</tr>
<tr>
<td>+</td>
<td>1 μM FOR + 0.2 mM IBMX</td>
<td>10.4 ± 1.3 (7)</td>
</tr>
<tr>
<td>+</td>
<td>5 mM Co⁺⁺ + 0.2 mM IBMX</td>
<td>8.9 ± 1.3 (4)</td>
</tr>
<tr>
<td>+</td>
<td>5 mM Co⁺⁺ + 0.2 mM IBMX + 250 mM PGE₁</td>
<td>9.8 ± 1.7 (4)</td>
</tr>
<tr>
<td>+</td>
<td>5 mM Co⁺⁺ + 0.2 mM IBMX + 1 μM FOR</td>
<td>11.4 ± 0.8 (4)</td>
</tr>
</tbody>
</table>

Experiments were performed on isolated retinas which had been fully light-adapted by 30 min culture in the light in mEBSS without taurine to allow full cone contraction; thus T₀ myoid length was 4.0 ± 0.4 μm (n = 4). FOR = forskolin.

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![Fig. 6. Effects of PGE₁ and forskolin on myoid elongation in CIS/COS. CIS/COS myoid lengths were not significantly longer in PGE₁ + IBMX than in IBMX alone. In contrast, CIS/COS myoid lengths were greater in forskolin plus IBMX. Culture conditions described in Figure 5.](image-url)

![Fig. 7. Effects of cyclooxygenase inhibitor on dark-induced retinomotor movements in cones and RPE. Light-adapted T₀ RPE-retinas were fixed immediately after dissection. Other RPE-retinas were precultured for 8 min in the light and then cultured in the dark for 1 hr with or without indomethacin (1 μM), acetylsalicylic acid (ASA), or ibuprofen (IBU), each at 50 μM. Indomethacin and acetylsalicylic acid inhibited dark-induced movement in both cones and RPE; ibuprofen was ineffective.](image-url)
Table 3. Indomethicin does not inhibit light-induced cone or RPE retinomotor movements

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Mean cone myoid length (µm) mean ± SEM</th>
<th>Pigment index (%) mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark → Light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t₀ (dark-adapted)</td>
<td>43.0 ± 1.2 (4)</td>
<td>42.7 ± 17.0 (4)</td>
</tr>
<tr>
<td>10⁻⁵ M Indomethicin</td>
<td>9.8 ± 3.7 (3)</td>
<td>73.3 ± 19.2 (3)</td>
</tr>
<tr>
<td>Ringer/ethanol control</td>
<td>6.4 ± 2.9 (4)</td>
<td>85.3 ± 2.7 (4)</td>
</tr>
</tbody>
</table>

These experiments were performed on RPE-retinas that had been previously dark-adapted by 1 hr dark culture in mEBSS. Immediately after dark-culture, the t₀ sample was fixed and the remaining RPE-retinas cultured in the light for 1 hr in mEBSS with or without indomethicin.

tive retinomotor movements; this treatment inhibits only dark-adaptive retinomotor movements triggered by dark onset.

Table 4. Cyclooxygenase inhibitors do not induce changes in cone or RPE retinomotor positions in dark-adapted retinas and RPE-retinas

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Mean cone myoid length (µm) mean ± SEM</th>
<th>Pigment index (%) mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark → Dark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t₀ (dark-adapted)</td>
<td>37.9 ± 2.3 (9)</td>
<td></td>
</tr>
<tr>
<td>Ringer/ethanol control</td>
<td>42.3 ± 1.7 (11)</td>
<td></td>
</tr>
<tr>
<td>10⁻⁵ M Indomethicin</td>
<td>44.5 ± 0.8 (4)</td>
<td></td>
</tr>
<tr>
<td>10⁻⁴ M Indomethicin plus 10⁻⁴ M dopamine</td>
<td>13.6 ± 3.1 (4)</td>
<td></td>
</tr>
<tr>
<td>10⁻³ M Ketoprofen plus 10⁻⁴ M dopamine</td>
<td>43.0 ± 1.2 (3)</td>
<td></td>
</tr>
<tr>
<td>10⁻¹ M M ETYA</td>
<td>7.3 ± 0.9 (4)</td>
<td></td>
</tr>
<tr>
<td>(B) RPE-retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark → Dark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t₀ (dark-adapted)</td>
<td>40.9 ± 1.2 (10)</td>
<td>43.5 ± 3.1 (10)</td>
</tr>
<tr>
<td>Ringer/ethanol control</td>
<td>52.4 ± 1.8 (11)</td>
<td>37.9 ± 1.5 (11)</td>
</tr>
<tr>
<td>10⁻⁴ M Indomethicin</td>
<td>35.6 ± 5.1 (9)</td>
<td>47.5 ± 5.2 (9)</td>
</tr>
<tr>
<td>10⁻⁴ M Dopamine</td>
<td>7.3 ± 2.3 (4)</td>
<td>91.6 ± 1.3 (4)</td>
</tr>
<tr>
<td>Dark → Light</td>
<td>7.8 ± 1.1 (7)</td>
<td>80.2 ± 4.2 (7)</td>
</tr>
</tbody>
</table>

(A) experiments were performed on dark-adapted retinas isolated from fish dark-adapted for 30 min prior to dissection (RPE detaches). A dark-adapted t₀ sample was fixed immediately after dissection and the remaining retinas cultured in the dark in the presence or absence of cyclooxygenase inhibitors for 60 min.

(B) experiments were performed on RPE-retinas isolated from light-adapted fish and then dark-adapted by 1 hr dark culture in mEBSS. At this time a t₀ sample was fixed and the other RPE-retina pieces cultured in the dark or light as indicated for 60 min.

Discussion

We have shown here that prostaglandins E₁, E₂, and D₂ induced dark-adaptive retinomotor movements in the teleost retina. Though the presence of prostaglandins in the retina has not been reported for teleosts, several other species have been shown to produce prostaglandins.¹⁸ Rabbit retinas spontaneously produced PGE₂ and PGF₂α from endogenous arachidonic acid sources, PGE₂ being the predominant eicosanoid formed.¹⁹ Both bovine and rat retinas can generate PGE₂, PGD₂, and PGF₂α from radiolabeled arachidonic acid.¹⁸,²⁰,²¹

The effect of prostaglandins E₁, E₂, and D₂ on teleost retinomotor movements reported here is similar to that previously reported for cAMP, i.e., they induce movements normally induced by the onset of darkness.¹⁻⁶ Maximal extents of dark-adaptive retinomotor movements can be induced by culturing light-adapted teleost retinas with dibutyl- cyclic AMP (dbcAMP), high concentrations of IBMX, or forskolin in the light.¹⁻⁶ In cultured chick embryo RPE, PGE₁, PGE₂, and dbcAMP were shown to induce similar changes in pigmentation and morphology²² and PGE₁ was found to increase intracellular cAMP levels.¹²

The effects of prostaglandins E₁, E₂, and D₂ on retinomotor movements appear to be selective since PGF₂α failed to elicit a response; however, since these treatments were carried out on intact retinas it is not possible to identify which cell types are the direct targets of prostaglandin action. To investigate whether prostaglandin E₁ could act directly on cones to induce elongation, we examined the effects of PGE₁ on cone cell fragments composed of inner and outer segments (CIS/COS) which retain their ability to elongate after detachment from the retina. These CIS/COS do elongate when cultured with forskolin, a diterpene which activates adenylate cyclase and thereby elevates intracellular cAMP levels through a direct interaction with a catalytic subunit of the enzyme.²³ This finding is consistent with previous studies in our laboratory using lysed cell models which showed that the microtubular machinery responsible for cone elongation requires elevated cAMP levels to produce cone elongation.⁷ The ability of forskolin to induce elongation in CIS/COS indicates that CIS/COS contain adenylate cyclase which can produce sufficient cAMP to induce cone elongation. When CIS/COS were cultured with PGE₁ and IBMX no significant myoid elongation beyond that obtained with IBMX alone was observed. This result indicates that under the conditions of our experiments, PGE₁ failed to activate the adenylate cyclase in CIS/COS. In contrast, previous studies have shown that the ade-
nlylate cyclase in CIS/COS can be regulated by dopamine: dopamine induced myoid contraction in CIS/COS via D2-receptor-mediated inhibition of adeny- late cyclase.14 Though our studies indicate that PGE1 does not stimulate the adenylate cyclase of CIS/COS, we cannot rule out the possibility that a PGE1-linked adenylate cyclase is present within the more proximal regions of the cone (the perinuclear, axonal, or synaptic terminal regions).

To further examine the question of direct effects of prostaglandins on cones, we tried to obtain functional isolation of the intact cone pharmacologically by blocking synaptic transmission with Co++ in intact retinas in culture.16,17 In the presence of 5mM Co++, PGE1 failed to induce cone elongation, thus suggesting a possible role for retinal interneurons in the prostaglandin effect. However, forskolin also failed to induce elongation in the presence of Co++, though it produced maximal cone elongation in the absence of Co++. This inhibition appeared to be irreversible for at least 1 hr, since a preincubation of just 10 min with Co++ completely blocked subsequent forskolin-induced elongation in an hour-long culture. Although earlier reports suggested that Co++ effects on electro-physiological responses of mudpuppy and turtle cones were reversible,15,17 our results indicate that Co++ appears to have irreversible effects on cone retinomotor movements. Even though the durations of the reported electrophysiological experiments were no greater than 10 min, our results suggest that within 10 min Co++ treatment has produced some irreversible effects on the photoreceptor's subsequent ability to undergo cone elongation that may not be immediately detectable in electrophysiological measurements. These technical limitations have, therefore, prevented us from further characterizing the retinal site(s) of action of prostaglandins E1, E2, and D2 responsible for inducing cone movement.

Other observations describing effects of prostaglandins E1 and E2 in the CNS and effects of neurotransmitters in the retina do suggest, however, that PGE1 might be influencing cone and RPE retinomotor movements indirectly by modulating dopamine release in the inner retina.5,14,23-30 We have previously shown that dopamine induces light-adaptive cone contraction and pigment granule dispersion in the green sunfish retina.5,14 The reported agonist and antagonist efficacies suggest that dopamine effects on cone and RPE movement are mediated by D2 receptors. In the rat brain PGE1 and PGE2 have been shown to inhibit dopamine release both in vivo and in vitro.27,29 Prostaglandins have also been shown to antagonize central dopamine actions postsynaptically.28 Similar effects of prostaglandins within the teleost retina may be the mechanism by which they influence retinomotor movements. Since blocking D2 receptors with the specific antagonist sulpiride is sufficient to induce cone elongation in retinas cultured in constant light,14 it seems plausible that prostaglandins could induce dark-adaptive retinomotor movements by decreasing dopamine release either by acting directly upon dopaminergic cells or indirectly through interneurons which comprise the circuit between photoreceptors and the dopaminergic cells.

Other investigators have reported that gamma-aminobutyric acid (GABA) modulates retinal dopamine release in the carp retina.31 In green sunfish, the GABA antagonists picrotoxin and bicuculline have been shown to induce light-adaptive cone contraction in retinas cultured in constant darkness.24 The light-adaptive retinomotor movements induced by those agents were blocked by the D2 antagonist sulpiride, thus suggesting that GABA influences retinomotor movements by inhibiting dopamine release. Since PGE1 has been shown to produce dose-dependent inhibition of the convulsant effects of the GABA antagonist picrotoxin and also to elevate whole brain levels of GABA in mice,25,26 a similar mechanism could be active in the retina such that PGE1 induces dark-adaptive retinomotor movements by increasing GABA release which subsequently inhibits dopamine release. Indeed, the reported ability of PGE1 and PGE2 to decrease the ERG b-wave amplitude in rabbits23 may reflect stimulatory effects on GABA activity, since GABA has also been shown to decrease b-wave amplitude both in vivo and in vitro32-35 and the effects of GABA were specifically blocked by the GABA antagonist picrotoxin.34,35 It would be interesting to determine whether picrotoxin could restore PGE1-depressed ERG b-wave amplitude.

Results of our studies with inhibitors of prostaglandin synthesis suggest that endogenous production of prostaglandins is necessary for normal induction of retinomotor movements by onset of darkness. Little is known about the mechanism by which dark onset initiates retinomotor movements. Lysed cell studies have shown that one of the ultimate steps in this process is the elevation of cAMP in the cone myoid cytoplasm,7 but how this increase in cAMP levels is stimulated is not clear. Dearry and Burnside14 have shown that culturing a light-adapted retina with the dopamine D2 antagonist sulpiride will induce dark-adaptive movements in the light. This result suggests that continual dopamine release is required to maintain the light-adapted cone position (presumably by inhibiting adenylate cyclase activity and thus keeping the cAMP levels low). If prostaglandins play a role in inhibiting dopamine release in the retina, this inhibition might be a relevant part of the dark-onset signal.

Our experiments with cyclooxygenase inhibitors
Further indicated that continued endogenous prostaglandin synthesis was not required for maintenance of the dark-adapted retinomotor positions in constant darkness. Thus the role of prostaglandins in regulating retinomotor movements appears to be restricted to events associated with the light-to-dark transition.

In summary, we have described here a new role for prostaglandins in the regulation of retinomotor movements in the teleost retina. Although we have not yet identified the specific cellular site of action of the prostaglandins in regulating these movements, we have been able to show that prostaglandins E₁, E₂, and D₂ can induce dark-adaptive retinomotor movements in cones and RPE cells in retinas cultured in constant light. Thus application of these prostaglandins to whole retinas in the presence of IBMX mimics the effect of darkness onset on retinomotor movements. In addition, we have shown that preventing endogenous prostaglandin synthesis in cultured retinas with cyclooxygenase inhibitors interferes with dark-induced cone elongation and RPE pigment granule aggregation, suggesting that prostaglandins play a role in the neurophysiological events associated with dark onset in the retina.

Previous reports of the retina's capacity to synthesize prostaglandins and prostaglandin effects on electrical properties of the retina strongly suggest that prostaglandins play important roles in retinal physiology in vivo. Our results suggest that prostaglandins may be particularly important to those events associated with onset of darkness.

Key words: prostaglandin E₁, retinomotor movement, dark-adaptation, cones, retinal pigment epithelium

Acknowledgments

The authors wish to thank Werner Loher, David Stanley-Samuelson, and Allen Dearry for critical review of the manuscript and Hoa Trinh and Camilla Joe for clerical help.

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