Enhanced Prostaglandin Synthesis After Ultraviolet-B Exposure Modulates DNA Synthesis of Lens Epithelial Cells and Lowers Intraocular Pressure In Vivo

Usha P. Andley,* Bernard Becker,* Jason S. Hebert,* John R. Reddan,† Aubrey R. Morrison,‡ and Alice P. Pentland§

Purpose. To study the functional significance of prostaglandin synthesis after ultraviolet-B (UVB) exposure of cultured human lens epithelial cells and rabbit eyes in vivo.

Methods. Prostaglandin E₂ (PGE₂) was assayed using a radioimmunoassay (RIA) and mass spectroscopy. An immortalized human lens epithelial cell line (HLE-B3) was exposed to UV irradiation, and the synthesis of PGE₂ was compared with the rabbit lens epithelial cell line N/N1003A. Intact human lenses were exposed to UVB in organ culture. [³H]Thymidine incorporation was measured in cultured lens epithelial cells by incubation with the radiolabel. The effects of isobutyl methyl xanthine (IBMX), an inhibitor of phosphodiesterase and of dibutyl cyclic adenosine monophosphate (cAMP), an analog of cAMP, on PGE₂ synthesis and DNA synthesis, were determined. Rabbit eyes were exposed to UVB radiation in vivo. Intraocular pressure was measured at specific times after exposure. Aqueous humor was removed from rabbit eyes, and its PGE₂ content was measured by RIA.

Results. Cultured human lens epithelial cells (HLE), like rabbit lens epithelial cells (RLE), showed a dose-dependent increase in basal PGE₂ synthesis 24 hours after UVB exposure. However, the amount of PGE₂ synthesis was 2000-fold higher in the rabbit cells. Ultraviolet-B radiation enhanced the incorporation of [³H]thymidine in lens epithelial cells. Pretreatment of cells with indomethacin reduced PGE₂ synthesis and [³H]thymidine incorporation. The human and rabbit cells responded in a similar manner to changes in DNA synthesis after UVB exposure. The addition of IBMX or dbcAMP to indomethacin-treated, UVB-exposed cells restored DNA synthesis toward the levels observed in the UVB-exposed cells. An increase in the concentration of cAMP was observed in lens epithelial cells exposed to exogenous PGE₂. PGE₂ synthesis in intact human lenses also increased two fold 24 hours after UVB exposure. Exposure of the rabbit eye in vivo to an optimal dose of UVB produced an increase in the PGE₂ levels of the lens and the aqueous humor. Measurements of the intraocular pressure (IOP) of the animals showed a decrease in IOP by 2.21 ± 0.66 and 6.45 ± 0.79 mm Hg (mean ± SEM, P = 0.004, t-test) at 6 and 24 hours after UVB exposure, respectively. The decrease in IOP was prevented by pretreatment with indomethacin. Exposure of the rabbit lens to UVB radiation in vivo enhanced [³H]thymidine incorporation twofold into the lens. Pretreatment of rabbits with indomethacin before exposure reduced this response.

Conclusions. Results indicate that UVB exposure enhances PGE₂ synthesis in HLE cultures as well as in rabbit lenses irradiated in vivo. This increased PGE₂ synthesis is related to the increase in DNA synthesis observed after UVB treatment. The modulation of DNA synthesis in cultured lens epithelial cells after UVB exposure may be mediated by a cAMP-dependent mechanism. Invest Ophthalmol Vis Sci. 1996;37:142-153.

Prostaglandins are local hormones that function at or near their site of synthesis and regulate diverse cellular processes.¹ A number of stimuli, such as membrane perturbation by trauma, inflammation, Ca²⁺ ionophore treatment, or ultraviolet (UV) radiation exposure may cause the release of arachidonic acid from esterified membrane lipids through the action of phospholipases, resulting in enhanced production of prostaglandins.²-⁴ The newly mobilized arachidonate is enzymatically converted to an endoperoxide inter-

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mediate by prostaglandin PGG/H synthase (cyclooxygenase), which is then converted into biologically active prostaglandins. The formation and release of prostaglandins in the eye has been demonstrated in a number of models of ocular trauma or injury.5,6 The lens has low levels of cyclooxygenase activity, whereas the iris-ciliary body has extensive prostaglandin synthesis.13 This enhanced PGE2 synthesis by cyclooxygenase and lipooxygenase activities. 89 The presence of cyclooxygenase has been demonstrated immunohistochemically in the rat lens epithelium.10 Cyclooxygenase concentration appears to be highest in the germinative and transitional zones and meridional rows, suggesting that it plays a role in lens cell differentiation. Synthesis of prostaglandin PGE2 in the lens is enhanced in experimental ocular inflammation,11 by exposure to YAG laser,12 or to UVB radiation.13 Ocular tissue, such as cornea and lens, absorb UVB and UVA radiation and undergo detrimental changes in their structure and function. Epidemiologic and experimental studies suggest that exposure to UV radiation in vivo increases the risk of human cortical and posterior subcapsular cataracts.14,15 The lens epithelium is an important target of UV radiation in vivo.10 Recently, we demonstrated that exposure of cultured rabbit lens epithelial cells and intact calf lenses to low levels of UVB radiation enhances prostaglandin synthesis.13 This enhanced PGE2 synthesis by cultured rabbit lens epithelial cells specifically regulates DNA synthesis, as measured by [3H]thymidine incorporation after UVB exposure. The synthesis of PGE2 by UVB exposure of the lens in vivo has not been investigated. It has been reported that secondary aqueous humor formed after ocular trauma, which may increase prostaglandin synthesis, triggers mitosis of lens epithelial cells.17 Prostaglandin synthesis has been reported in cultured human lens capsule-epithelial tags obtained during cataract surgery.18 Most of the studies relating ocular inflammation and prostaglandin synthesis have been performed in the rabbit eye.19 Low doses of prostaglandins lower intraocular pressure, whereas high doses cause an initial increase.20 Topical prostaglandins in low doses cause a release of polymorphonuclear cells into the tear fluid of rabbits.21 In the smooth muscle cells of the iris-ciliary body, prostaglandin receptors can mobilize Ca2+, causing contraction, and can raise cyclic adenosine monophosphate (cAMP), which generally is associated with relaxation in the smooth muscle.22

The purposes of this study were to determine whether UVB radiation in vivo alters lens and aqueous humor PGE2 and alters intraocular pressure; determine whether these effects can be prevented by indomethacin; compare UVB-induced PGE2 synthesis in rabbit and human lens epithelial cells and understand the role of UVB-induced PGE2 synthesis in lens epithelial cells.

MATERIALS AND METHODS

All studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Tenets of the Declaration of Helsinki were followed for research on human tissue, and approval from the Human Studies Committee at Washington University was obtained.

Cell Culture

Human lens epithelial cells (HLE B-5) were cultured in minimum essential medium (MEM; Sigma, St. Louis, MO) containing 20% fetal calf serum as described previously.23 This infant human lens epithelial cell line was immortalized by infection of cells with Ad12-SV40 hybrid virus, has a stable epithelial morphology, has an aneuploid karyotype, and maintains lens-specific function such as synthesis of β-crystallin. Cultures were maintained at 37°C in a water-saturated air atmosphere containing 5% CO2. Medium was changed twice weekly. Cells (passages 10 to 20) were subcultured using trypsin–EDTA (Sigma) into 12-well tissue culture plates (Corning, Corning, NY) and grown to confluence.

Rabbit lens epithelial cells (cell line N/N1003A) were cultured in MEM containing 8% rabbit serum and 50 µg/ml gentamicin as described previously.13 This is a spontaneously immortalized, nontransformed cell line that maintains a diploid karyotype. These experiments were performed on cells between passages 20 to 30.

The effect of indomethacin, a cyclooxygenase inhibitor, on prostaglandin synthesis in lens epithelial cells was investigated. Six µg/ml indomethacin (10 mg/ml stock solution in ethanol; Sigma) was added to the cells 45 min before UV exposure. Immediately before UVB exposure, the cell monolayer was rinsed with phosphate-buffered saline (PBS) containing Ca2+ and Mg2+, and a thin layer of PBS was placed over the cultures to prevent drying during UV exposure. After
irradiation, cells were incubated in MEM containing 10% fetal calf serum (HLE B-3) or 8% rabbit serum (N/N1003A) with or without 3 μg/ml indomethacin. In some wells, the appropriate prostaglandin solution (100 nM) was added to the indomethacin-treated cultures.

Irradiation

Cell cultures were exposed to a bank of eight FS-20 T12-UVB lamps (National Biological Corporation, Twinsburg, Ohio), which emit radiation predominantly in the UVB region (290 to 320 nm) but also emit some radiation in the UVA and UVC regions. The emission maximum of the source is at 310 nm. A Corning CS 0–53 filter (1 mm thick) was used to filter out UVC radiation. Irradiance was 10 W/m², as measured using a research radiometer (IL1700; International Light, Newburyport, MA). Fluence was varied by the length of irradiation ranging from 0 to 60 seconds. A fluence of 250 J/m² (delivered in 10 seconds) was used in experiments in which DNA synthesis was measured.

New Zealand white rabbits were used for in vivo exposure of eyes to UV radiation. For pretreatment with indomethacin, animals were administered 50 mg/kg indomethacin intraperitoneally 45 minutes before UV exposure. The left eye was patched (control), and the right eye was irradiated at a distance of 30 cm from the bank of UV lamps at a fluence rate of 6.45 W/m². Fluence of radiation at the cornea was 3483 J/m² delivered in 9 minutes, unless indicated otherwise. The left eye of each rabbit was exposed to approximately 150 J/m² under these conditions. This fluence is equivalent to a few hours of solar UVB exposure of the lens epithelium in vivo,24-26 it is estimated that lens is exposed to approximately 150 J/m² under these conditions.

Measurement of Intraocular Pressure

Intraocular pressure was measured on awake rabbits after topical anesthesia with 0.5 percent proparacaine using a Digilab Modular One PneumaTonometer (Mentor O and O, Cambridge, MA). A baseline measurement of intraocular pressure was made before the beginning of each experiment. One eye of the rabbit was exposed to UVB radiation, and the other eye was patched. Intraocular pressure of control and irradiated eyes was measured immediately after UVB exposure, and the measurements were repeated 30 minutes and 1, 2, 3, 4, 5, 6, and 24 hours thereafter. Indomethacin solution was prepared by dissolving 500 mg of the drug in 2 ml sodium hydroxide (1 N) and 8 ml water. The pH of the indomethacin solution was 7.9.

Forty-five minutes before UVB exposure, indomethacin was administered intraperitoneally at a concentration of 50 mg/kg. In animals monitored for 24 hours, the indomethacin treatment was repeated 6 hours after UV exposure. No adverse effects (pain, peritonitis, toxicity) of indomethacin treatment were observed during the 24-hour period of this study. Aqueous humor was removed from rabbit eyes at 6 and 24 hours after UVB exposure and analyzed for PGE₄ concentration by radioimmunoassay.

Radioimmunoassay

PGE₄ was assayed using rabbit antisera as previously described.13,28 Culture medium from control and UVB-irradiated cells was collected 24 and 48 hours after UV exposure. The assay mixtures contained [³H]PGE₂, 2,500 cpm (100 Ci/mmol; New England Nuclear, Boston, MA), 0.05 M potassium phosphate, pH 7.4, containing 0.1% bovine serum albumin, antimouse 1:16,000 dilutions, and culture medium containing 2.0 to 200 pg PGE₂, in a total volume of 0.2 ml. The tubes were incubated for 16 hours in the cold, and then dextran-coated charcoal (0.025% dextran, 0.15% charcoal in saline) was added to all the tubes, which were centrifuged to pellet the charcoal. The supernatant was decanted into scintillation fluid and counted. Prostaglandin levels were expressed per microgram of cell protein.

Cyclic adenosine monophosphate was assayed using rabbit antisera (a generous gift of Dr. Adolph Cohen).29 Cells were grown to confluence in 60-mm plates and exposed to 100 nM of PGE₂. After treatment, 10% fetal bovine serum-MEM containing 1 mM IBMX (Sigma) was added to the cultures, and they were incubated for 15 minutes. To measure the concentration of intracellular cAMP, cells were washed with PBS and extracted with 10% trichloroacetic acid (0.2 ml) on ice. An aliquot (0.025 ml) of the trichloroacetic acid extract was saved for protein analysis, and the remainder (0.175 ml) was washed with water-saturated ether three times. The aqueous phase was dried with N₂, suspended in 0.5 ml of 50 mM sodium acetate buffer (pH 5.95), and acetylated with 15 μl of a 1:2 mixture of acetic anhydride and triethylamine. The assay mixture for RIA contained 5 to 20 μl of the cell extract, cAMP standard at concentrations of 10 to 160 fm, 50 μl of ¹²⁵I-cAMP-tyrosine methyl ester, 15,000 cpm (Amersham, Arlington Heights, IL), 50 mM sodium acetate buffer, pH 5.95, primary antiserum at a dilution of 1:30,000, and secondary antibody (goat anti rabbit IgG) at a dilution of 1:50 in a total volume of 2.5 ml. The reaction mix was incubated overnight, 2 ml cold H₂O was added, and then it was centrifuged and counted in a gamma counter. cAMP concentrations were expressed per milligram of cell protein.
DNA Synthesis ([3H]Thymidine Incorporation) and Proliferative Autoradiography

To measure DNA synthesis, lens epithelial cells were labeled with 2 μCi/ml of [3H]thymidine (Amersham) from 24 to 30 hours after UVB radiation as described previously. Cultures were then rinsed twice with PBS at 37°C and harvested by the addition of 1 ml of 6% trichloroacetic acid. After centrifugation, the pellet was resuspended in 200 μl of 3% perchloroacetic acid, heated to 95°C for 15 minutes, and centrifuged for 5 minutes. A 40-μl aliquot of the supernatant was counted in the scintillation counter to determine thymidine incorporation. The pellet was dissolved in 0.62 M NaOH and used for protein assay. Measurements were made in triplicate under each condition.

To measure DNA synthesis in rabbit lenses irradiated in vivo, control and UVB-treated rabbit lenses were incubated in culture medium containing 2 μCi/ml of [3H]thymidine between 24 and 30 hours after UVB exposure. The unincorporated radiolabel was removed, and the lenses were washed carefully with medium and fixed in 3:1 ethanol:acetic acid. The lens epithelium was carefully removed, scintillation fluid was added, and the incorporation of radioactivity was counted using a scintillation counter.

Proliferative autoradiography on rabbit lenses irradiated in vivo was performed by incubating the isolated lenses in culture medium containing 1 μCi [3H]thymidine from 24 to 28 hours after UVB exposure. The contralateral unirradiated eye of the same animal was used as a control. After incubation, the radiolabel was rinsed, lenses were fixed with 3:1 ethanol:acetic acid, and wholemounts of epithelial explants were prepared by adding equal volumes of 35% pentafluorobenzyl bromide in acetonitrile and diisopropylethylamine; the solution was heated to 45°C for 15 minutes. Samples were evaporated to dryness under N2 and derivatized to the trimethylsilyl ether with 20 μl of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane and 20 μl of pyridine, vortexed, and warmed to 45°C for 15 minutes. Samples were evaporated to dryness under N2 and resuspended in 25 μl n-heptane.

The pentafluorobenzyl ester, methoxime trimethylsilyl ether, of each putative prostaglandin was measured by mass ion detection using a Nermag 1010H mass spectrometer interfaced with a Delsi 200 gas chromatograph. The column used was a 25-m Ultra2 crosslinked OV-1 capillary column (Hewlett-Packard). Injection temperature was 250°C, and interface temperature was 280°C. The column was programmed from 150 to 270°C at 25°C/minute. Data were collected on Spectral 30 software (Delsi). Characteristic mass ions detected were monitored at m/z 524 (PGE2) and m/z 569 (PGF2α).

Protein Assays

Cells were washed twice with phosphate-buffered saline (50 mM potassium phosphate, pH 7.4); 0.62 M NaOH was added to each well to solubilize the protein. Protein concentration was determined using Pierce (Rockford, IL) BCA protein assay with bovine serum albumin as the standard.

RESULTS

Effect of Ultraviolet B Radiation on the Rabbit Eye In Vivo

Rabbit eyes were exposed to varying fluences of UVB radiation to determine its in vivo effects on the PGE2...
Arachidonic Acid Metabolism Products by Lenses Exposed to UVB Radiation

**TABLE 1. Quantitative Analysis of Arachidonic Acid Metabolism Products by Mass Spectrometry**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal Number</th>
<th>Condition</th>
<th>PGE₂</th>
<th>PGF₂α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit lenses</td>
<td>6 hours</td>
<td>C</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV</td>
<td>1.48</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C + I</td>
<td>0.13</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV + I</td>
<td>0.39</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>C</td>
<td>0.10</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV</td>
<td>0.44</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C + I</td>
<td>0.12</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV + I</td>
<td>0.18</td>
<td>0.06</td>
</tr>
</tbody>
</table>

C = control; C + I = indomethacin-treated control; UV = exposed to UVB radiation; UV + I = exposed to UVB radiation after pretreatment with indomethacin; ND = not detected; PGE₂ = prostaglandin E₂; PGF₂α = prostaglandin F₂α.

* All values are expressed in nanograms. Results of a representative experiment are shown. The experiment was repeated with similar results. UVB fluence was 3483 J/m² for rabbit lenses irradiated in vivo. The internal standards PGE₂ and PGF₂α were added at a concentration of 25 ng to the extracts before analysis.

Concentration of the lenses. Analysis of arachidonic acid metabolism products by mass spectroscopy of rabbit lenses is shown in Table 1. PGE₂ was the major cyclooxygenase product of arachidonic acid metabolism in rabbit lenses irradiated in vivo, with minor amounts of PGF₂α. The concentration of PGE₂ in the lens decreased to control levels in irradiated animals pretreated with indomethacin.

Table 2 shows the change in the concentration of PGE₂ in the aqueous humor of rabbit eyes exposed to 3483 J/m² of UVB radiation in vivo. Compared to control eyes, the concentration of PGE₂ in the aqueous humor increased twofold to threefold in the exposed eyes of animals when measured 6 and 24 hours after exposure. Six hours after UVB exposure, this increased PGE₂ was eliminated in the eyes pretreated with indomethacin. Pretreatment with a single dose of indomethacin appeared to be less effective in preventing a UVB-induced increase of aqueous humor PGE₂ at the 24 hours observation time. Figure 1A shows the time course of the change in IOP of rabbit eyes exposed to 3483 J/m² of UVB radiation in vivo. Lower doses of UVB did not have a significant effect on IOP. A decrease in IOP was observed at 5 to 6 hours after UV treatment, continued for the 24-hour period of study, and was abolished or reduced in animals pretreated with indomethacin (Fig. 1B).

DNA Synthesis After Ultraviolet B Exposure

Figures 6 and 7 show the regulation of DNA synthesis by PGE₂ after UVB exposure for human and rabbit cells, respectively. UVB exposure enhanced [³H] thymidine incorporation 4.5-fold in rabbit cells and 2.4-fold in human cells. In UVB-exposed rabbit cells, the enhanced DNA synthesis is eliminated in the presence of PGE₂ as measured by proliferative autoradiography.
TABLE 2. Prostaglandin E\(_2\) (PGE\(_2\)) Concentration (pg/\(\mu\)l) of Aqueous Humor of Rabbit Eyes Irradiated In Vivo

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>UV</th>
<th>UV + I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>UV</td>
<td>C + I</td>
</tr>
<tr>
<td>6 hours</td>
<td>0.05</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>0.25</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td>0.68</td>
<td>0.06</td>
</tr>
<tr>
<td>0.18 ± 0.08</td>
<td>0.38 ± 0.15*</td>
<td>0.05 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>0.04</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.24</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td>1.94</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>0.51</td>
<td>1.53</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.63</td>
<td>0.06</td>
</tr>
<tr>
<td>0.28 ± 0.13</td>
<td>0.88 ± 0.37</td>
<td>0.14 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

C = unexposed, left eye of animal not treated with indomethacin; UV = right eye of same animal exposed to 9 minutes of UV radiation in vivo; C + I = unexposed, left eye of animal pretreated with indomethacin; UV + I = right eye of same animal exposed to 9 minutes of UV radiation in vivo.

PGE\(_2\) was assayed by radioimmunoassay. Values are expressed in pg/\(\mu\)l. Sixteen rabbits were used for this set of experiments. Each experiment represents two animals, one of which was exposed to indomethacin and the other was not. The right eye of each of the animals was exposed to UV radiation for 9 minutes, and the left eye was patched. Indomethacin (50 mg/kg) was given 45 minutes before UV exposure. Animals studied for 24 hours were given a second dose of indomethacin 6 hours after UV exposure.

The current study shows that UVB exposure of rabbit lenses irradiated in vivo causes an increase in PGE\(_2\) content of the aqueous humor as well as a decrease of the intraocular pressure. These responses are effectively reduced by pretreatment of the animal with indomethacin. The current study focused on albino rab-
FIGURE 1. (A) Time course of the change in intraocular pressure of rabbit eyes exposed to a fluence of 3483 J/m² of ultraviolet (UV) B radiation in vivo. A representative experiment is shown in A and B. (filled squares) UV-exposed eye. (upward triangles) Unexposed eye. (B) Time course of the change in intraocular pressure of rabbit eyes exposed to UVB radiation in vivo. Conditions were the same as in A except that the animal was pretreated with indomethacin (50 mg/kg) 45 minutes before UV exposure. (filled circles) UV-exposed eye. (downward triangles) Unexposed eye.

bits, but a limited study of the effect of UVB radiation on pigmented rabbit eyes in vivo showed that UVB radiation (3500 J/m²) causes an initial rise in IOP, followed by a decrease at 24 hours after exposure (data not shown). The actions of PGE₂ on IOP have been well investigated, and it effectively lowers IOP in animals. Changes in IOP of rabbits by topical arachidonic acid and prostaglandins have been attributed to damaged iris-ciliary processes. It is unknown whether the changes in IOP reported in this study were caused by PGE₂ alone or whether other cyclooxygenase products contribute to it because PGF₂α is synthesized in small amounts and is known to be a potent hypotensive agent. It is well documented that the rabbit eye is particularly sensitive to topical prostanoids, and the UV injury model of the rabbit eye may not be entirely representative of the human response. Another issue is that the human eye is exposed to lower levels of repeated exposures to environmental UV radiation, whereas a single, acute exposure to UV radiation was used in the current study. Nevertheless, the current study shows that in an in vivo model of UV injury, the concentration of PGE₂ of the lens, and aqueous humor are altered by UV exposure, and that indomethacin prevents these changes. The current study further shows that a decrease in IOP of the rabbit eye occurs concomitant with UV-induced increase in PGE₂ of aqueous humor.

The iris–ciliary body has a higher activity of cyclooxygenase enzyme than the lens and may contribute to PGE₂ concentration in the aqueous humor. Rabbit iris–ciliary body produces large amounts of PGE₂, PGF₁α, and PGD₂. The ciliary epithelium, in contrast, synthesizes mainly lipoxygenase products (HETES).³³ Because iris cells overlay the germinative zone of the lens, the increased synthesis of PGE₂ in the iris may affect lens epithelial cells in this region and exert effects on lens cell differentiation or proliferation in the germinative zone. Results of the current study suggest that the presence of prostanoids in the aqueous humor may modulate lens epithelial proliferation. Although the aqueous humor contains several prostanoids, our previous study showed that the effect on lens epithelial proliferation is specific to PGE₂ and that PGD₂ or PGF₂α are not effective in restoring UV-induced cell proliferation in indomethacin-treated rabbit lens epithelial cells.¹³ The relationship between concentration of PGE₂ and increased rates of cell replication has been reported in other

FIGURE 2. Difference in the intraocular pressure of the ultraviolet B (UVB)-exposed eye and unexposed eye 6 and 24 hours after UVB exposure. (filled bars) In the absence of indomethacin. (unfilled bars) In the presence of indomethacin. Mean ± SEM; n = 14 at 0 and 6 hours, and n = 11 at 24 hours. Asterisks indicate that the differences in intraocular pressure between unexposed eyes and UVB-exposed eyes at 6 and 24 hours after UVB exposure were statistically significant (P = 0.004, unpaired *t* test).
cell types. Exogenous PGE₂ also has been shown to retard replication and differentiation of cells in vitro. Rabbit lenses irradiated in vivo had a higher level of PGE₂ and of thymidine incorporation than lenses of contralateral control eyes from the same animal (Table 1, Fig. 3). Although less likely, it is conceivable that arachidonic acid metabolites mediate their effects on lens epithelial cells through the production of free radicals produced during the activation of cyclooxygenase by UVB radiation.

The current work shows that in both cultured rabbit and human lens epithelial cells, the synthesis of PGE₂ is enhanced by UVB exposure, and this enhancement is eliminated in cells pretreated with indomethacin. However, the amount of PGE₂ synthesized by the rabbit cell cultures is 2000-fold greater than the human epithelial cultures. Differences between rabbit and human cell cultures may exist because of species and biochemical differences and because an SV40-immortalized cell line is used for the human cell cultures. In cells of both species, this increased PGE₂ synthesis has a specific function in regulating DNA synthesis after UV injury. Stimulation of cell proliferation in lens epithelial cells is modulated by the synthesis of PGE₂ after UV exposure. Indomethacin pretreatment eliminated PGE₂ synthesis and decreased DNA synthesis. Lack of complete inhibition of UV-induced DNA synthesis by indomethacin in the human cells suggests that there is an alternative, prostaglandin-independent pathway enhancing DNA synthesis in these cells. IBMX and dbcAMP, the phosphodiesterase inhibitor and cAMP analog, enhanced DNA synthesis in unirradiated cells, indicating that elevated cAMP levels enhance DNA synthesis. In irradiated cells, IBMX and dbcAMP enhanced DNA synthesis to levels greater than that with UVB irradiation alone. Indomethacin pretreatment lowered PGE₂ synthesis as well as DNA synthesis in irradiated cells. Because it is possible that indomethacin itself may be an inhibitor for DNA synthesis and, therefore, reduces thymidine incorporation, the relationship between PGE₂ synthesis and DNA synthesis was investigated further. Cell cultures were pretreated with indomethacin and exposed to UVB radiation, and irradiated cells were treated with IBMX or dbcAMP. The level of DNA synthesis...
Figure 5. Increase in prostaglandin E₂ synthesis with fluence of radiation in rabbit lens epithelial cells (cell line N/N1003A). All conditions were the same as in Figure 4, except that cells were incubated in minimum essential medium containing 8% rabbit serum. (●●●), 24 hours; (■●■), 48 hours; (▲) in the presence of indomethacin, 24 hours.

Figure 6. (top) Increase in basal prostaglandin E₂ synthesis of cultured human lens epithelial cells (cell line HLE B-3) 24 hours after ultraviolet (UV) exposure. Cells were pretreated with 6 μg/ml indomethacin (I) where indicated. After exposure to a UVB fluence of 250 J/m², IBMX (0.45 mM) or dibutyryl cyclic adenosine monophosphate (1 μg/ml) was added to the medium where indicated. (bottom) Increase in DNA synthesis as measured by [³H] thymidine incorporation into DNA after UV exposure of human lens epithelial cells. Twenty-four hours after exposure to a UVB fluence of 250 J/m², cells were treated with 2 μCi [³H] thymidine for 6 hours, washed to remove free radiolabel, DNA was extracted, and radioactivity incorporated was counted in a scintillation counter. Mean ± SEM, n = 4, P < 0.001, paired t-test for the following: control (C) versus C + I; UV versus UV + I; C versus UV; UV + I versus UV + I + IBMX; UV + I versus UV + I + dbcAMP.

Table 3. Increase in the Concentration of Prostaglandin E₂ (PGE₂) in Culture Medium Containing Human Lenses

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Time (hours)</th>
<th>Control</th>
<th>Ultraviolet</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>5</td>
<td>499 ± 50</td>
<td>1359 ± 620</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>1277 ± 235</td>
<td>2648 ± 58</td>
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<tr>
<td>29</td>
<td>5</td>
<td>885 ± 79</td>
<td>4069 ± 81</td>
</tr>
<tr>
<td>37</td>
<td>5</td>
<td>531 ± 14</td>
<td>972 ± 12</td>
</tr>
<tr>
<td>47</td>
<td>24</td>
<td>132 ± 456</td>
<td>475 ± 54</td>
</tr>
<tr>
<td>66</td>
<td>5</td>
<td>3220 ± 122</td>
<td>20,145 ± 1896</td>
</tr>
</tbody>
</table>

Human lenses were incubated in minimum essential medium containing 10% fetal calf serum for 5 or 24 hours after exposure to UVB fluence of 1200 J/m² to the lens epithelium. An aliquot of the medium was removed at the indicated times and assayed for PGE₂ by radioimmunoassay. Values are expressed as pg/lens, and represent the mean and standard deviation of triplicate measurement of each sample.
Ultraviolet B-Induced Lens Prostaglandin Synthesis

FIGURE 7. (top) Increase in prostaglandin E2 synthesis and (bottom) DNA synthesis of cultured rabbit lens epithelial cells (cell line N/N1003A). Conditions were the same as in Figure 6. Mean ± SEM, n = 4, P < 0.001, paired t-test for the following: control (C) versus C + I; UV versus UV + I; C versus UV; UV + I versus UV + I + IBMX; UV + I versus UV + I + dbcAMP

that the PGE2 receptors in lens epithelial cells may be of the EP2 subtype, that is, they may be coupled to the stimulation of adenylyl cyclase. Recent reports suggest that EP3 receptor subtype may also stimulate cAMP.

Current studies show that arachidonic acid metabolism may play an important role in the response of lens epithelial cells stimulated with low fluences of UVB radiation and suggest that enhanced PGE2 synthesis modulates UVB-induced DNA synthesis in the cells through a cAMP-dependent mechanism. They do not rule out alternative, nonprostaglandin-dependent pathways for regulating DNA synthesis in lens epithelial cells. For example, recent studies indicate that the synthesis of a lipoxygenase product of arachidonic acid metabolism, 12(S)-HETE, is elevated during rapid lens growth of neonatal rat lenses and declines as the cells differentiate. It has been reported that inhibition of 12-lipoxygenase decreases the levels of 12-HETE and simultaneously lowers DNA synthesis and expression of c-myc mRNA in the neonatal rat lens epithelium. Furthermore, stimulation of human lens epithelial cell DNA synthesis by EGF and insulin is prevented by inhibiting 12-lipoxygenase with cinna

TABLE 4. Concentration of Intracellular cAMP (pg/mg protein) in Cultured Lens Epithelial Cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Human Cells</th>
<th>Rabbit Cells N/N1003A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.22 ± 0.35 (5)</td>
<td>11.0 ± 1.30 (3)</td>
</tr>
<tr>
<td>IBMX</td>
<td>14.16 ± 0.89 (5)</td>
<td>19.84 ± 2.27 (3)</td>
</tr>
<tr>
<td>IBMX + PGE2</td>
<td>56.62 ± 8.94 (4)</td>
<td>40.45 ± 6.55 (3)</td>
</tr>
</tbody>
</table>

* Cultures were incubated under the indicated condition for 15 minutes, cells extracted with TCA, dried and acetylated; cAMP was determined by radioimmunoassay. Values are mean ± SEM, and the number of independent experiments is indicated in parentheses. Statistical significance was tested using Student's t-test, and the results are given as follows: Human cells: Control versus IBMX, P < 0.001, paired t-test; IBMX vs. IBMX + PGE2, P < 0.001, unpaired t-test. Rabbit cells: Control vs. IBMX, P < 0.05, paired t-test; IBMX vs. IBMX + PGE2, P < 0.05, paired t-test.
standing the role of prostaglandins in the UV-induced cataract formation. Ongoing studies using doses of UVB which produce permanent lens opacities will clarify whether prostaglandins have any role in obviating or enhancing UV cataractogenesis. Finally, future studies using specific inhibitors and potential mediators of the cyclooxygenase pathway will define localized mechanisms in the response of lens epithelial cells to UV radiation.

Key Words
cyclic adenosine monophosphate, DNA synthesis, human lens, intraocular pressure, lens epithelial cells, rabbit eyes

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References
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