Prostaglandin E<sub>2</sub> Induces Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor mRNA Expression in Cultured Rat Müller Cells

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PURPOSE. To investigate the induction of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) gene expression by prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in cultured rat Müller cells and to study the mechanism of the induction.

METHODS. Müller cells were obtained from neonatal Sprague-Dawley rat retinas and cultured in essential modified Eagle’s medium supplemented with 10% fetal calf serum for up to four passages. Cells were treated with PGE<sub>2</sub>, protein kinase A (PKA) inhibitors H-89 or SQ 22536, protein kinase C (PKC) inhibitors calphostin C or GF 109203X, PKC activator phorbol 12-myristate 13-acetate (PMA), or the PKA activator forskolin. Northern blot analysis was performed to determine the levels of VEGF and bFGF mRNA.

RESULTS. PGE<sub>2</sub> induced VEGF and bFGF mRNA expression in a dose- and time-dependent manner. VEGF and bFGF mRNA reached peaks of 2- and 3.5-fold at 10 μM PGE<sub>2</sub>. No further increases were observed at 100 μM PGE<sub>2</sub>. When treated with 10 μM PGE<sub>2</sub>, the increases in VEGF and bFGF mRNA reached maximum by 2 hours, then slowly declined toward the control level within 24 hours of PGE<sub>2</sub> treatment. The inductions of VEGF and bFGF mRNA expression by PGE<sub>2</sub> were blocked by the specific PKA inhibitors H-89 (30 μM) or SQ 22536 (500 μM, 1000 μM). Forskolin (10 μM), a cyclic adenosine monophosphate activator, also stimulated VEGF and bFGF mRNA expression. However, the effects of forskolin and PGE<sub>2</sub> on VEGF gene expression were not additive, whereas forskolin enhanced the effect of PGE<sub>2</sub> on bFGF mRNA expression. The specific PKC inhibitors, GF 109203X (2 μM) and calphostin C (1 μM), did not inhibit PGE<sub>2</sub>-induced VEGF gene expression, whereas PGE<sub>2</sub>-induced bFGF expression was blocked by the PKC inhibitor GF 109203X. In addition, downregulation of PKC by PMA (0.8 μM) treatment did not block the induction of VEGF gene expression, whereas it did inhibit the induction of bFGF mRNA expression.

CONCLUSIONS. These results indicate that PGE<sub>2</sub> stimulates VEGF and bFGF mRNA expression in cultured rat Müller cells. The induction of VEGF seems to occur through activation of the PKA pathway, whereas that of bFGF occurs through PKA and PKC activation. These findings raise the possibility that endogenous PGE<sub>2</sub> stimulates VEGF and bFGF mRNA expression in Müller cells in vivo under conditions in which PGE<sub>2</sub> production is increased, such as in injury. (Invest Ophthalmol Vis Sci. 1998;39:581-591)

Prostaglandins, as pathogenic mediators, are produced in tissue, including the retina, in response to inflammation at the site of injury. In the acutely injured cat spinal cord or in cultures of cardiac myocytes and of nonmuscle cells, the release of prostaglandins is increased after injury. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is the major prostaglandin in the retina. Increases in PGE<sub>2</sub> were found in various pathologic conditions in the retina, including various retinal injuries, such as laser irradiation, optic nerve injury, and retinal detachment. Prostaglandins have also been found as mediators in retinal disorders with neovascularization, such as diabetic retinopathy, retrolental fibroplasia, and retinopathy of prematurity. Studies have shown that prostaglandins are capable of inducing angiogenesis. In addition to their well-known role as inflammatory mediator, there is increasing evidence to show that prostaglandins have cytoprotective properties on various tissue types against toxic chemicals. Cytoprotection of nerve tissue by prostaglandins has also been observed, but the mechanism mediating this cytoprotection is unknown. Because prostaglandins do not directly stimulate endothelial cell growth, it is speculated that their angiogenic effect may be fulfilled by the paracrine action of angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). On the other hand, because of their angiogenic effect, it is possible that the cytoprotective property of prostaglandins is also fulfilled by increased endogenous bFGF.

In the retina, VEGF is expressed by retinal glial cells, including Müller cells, astrocytes, and microglia. Among human retinal...
cells. Müller cells were found to have the most VEGF immunoreactivity. VEGF is involved in the development of vasculature in normal retina. Clinical studies have revealed a close correlation between active neovascularization induced by hypoxic injury and elevated VEGF concentrations in patients with diabetes mellitus, central retinal vein occlusion, and retinopathy of prematurity, suggesting a role for VEGF as an important angiogenic factor in these diseases. Experimental data from a mouse model of proliferative retinopathy showed a marked increase in VEGF expression in Müller cells before the development of neovascularization, and this neovascularization was suppressed by soluble VEGF-receptor chimeric proteins that inhibited VEGF, indicating the involvement of Müller cells and VEGF in neovascularization in proliferative retinopathy.

bFGF is one member of a family of nine heparin-binding proteins, thought to play key roles in angiogenesis. It is also known that bFGF exerts neurotrophic actions on a variety of neurons. Our previous studies have revealed that, in the Royal College of Surgeons rat with an inherited photoreceptor degeneration and in constant light-induced photoreceptor degeneration, intracranial injection of bFGF protected photoreceptors. More recently, it has been demonstrated that mechanical injury to the retina induced a substantial increase in bFGF expression. This elevation was concentrated in the vicinity of the lesion and localized mostly to the inner nuclear layer in which Müller cells bodies reside. We have also shown in a previous study that bFGF induces endogenous bFGF gene expression in cultured rat Müller cells through activation of the protein kinase C pathway. Our findings indicate that Müller cells may respond to injury by producing such growth factors as bFGF, and the stimulated production of these factors may be responsible for the photoreceptor protection in the presence of injury.

Recent studies showed that PGE2 stimulated VEGF expression in cultured osteoblast cells and synovial fibroblasts, providing evidence that PGE2 was capable of inducing VEGF expression. To the best of our knowledge, there is no such report in retinal Müller cells that PGE2 induces VEGF and bFGF gene expression. The importance of Müller cells in producing VEGF and bFGF and the involvement of prostaglandins in neovascularization and cytoprotection motivated us to use rat Müller cells in culture as a model system to explore the possible role of prostaglandins in regulating VEGF and bFGF mRNA expression. We found that PGE2 induced VEGF and bFGF expression in cultured Müller cells in a dose- and time-dependent fashion, whereas other inflammatory mediators, such as bradykinin, histamine, substance P, and vasoactive intestinal peptide, had no effect on VEGF and bFGF mRNA expression. Our results also provide evidence that the induction of VEGF by PGE2 was mediated by the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) and that the induction of bFGF by PGE2 was through the activation of pathways for PKA and protein kinase C (PKC). These findings raise the possibility that endogenous PGE2 stimulates VEGF and bFGF mRNA expression in Müller cells in vivo under conditions in which production of PGE2 is increased, such as injury.

**Materials and Methods**

**Animals and Cell Culture**

All animals used in this study were cared for and handled according to the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Müller cells were cultured and identified as described previously. Briefly, eyes from Sprague-Dawley rats of postnatal days 1 though 3 were enucleated and placed in essential modified Eagle's medium at room temperature overnight before transfer to a digesting medium (essential modified Eagle's medium containing 0.1% trypsin and 70 U/ml collagenase) for 30 minutes at 37°C. Retinas were gently dissected under a microscope. Cells were dissociated by trituration with a sterile Pasteur pipet, seeded into 10-cm culture dishes (Falcon, Oxnard, CA) containing growth medium (essential modified Eagle's medium, 10% fetal calf serum, 2 mM glutamine, and 1:1000 penicillin-streptomycin), and cultured at 37°C in a 5% CO2-95% air atmosphere in a humidified incubator. Neuronal cells and retinal debris were removed by forcibly pipetting the medium onto the culture dish three to five times when primary culture reached semi-confluence (5-7 days). Confluent cultures were passaged no more than four times. Cells were identified by immunocytochemical analysis using antibodies against the Müller cell markers, including vimentin, carbonic anhydrase II, and glutamine synthetase.

Cells were treated with PGE2, PKA inhibitors H-89 or SQ 22536, PKC inhibitors calphostin C or GF 109203X, PKC activator PMA, or the PKA activator forskolin. PGE2 and forskolin were from Sigma Chemical (St. Louis, MO); H-89, PMA, calphostin C, GF 109203X, and SQ 22536 were from Biomol (Plymouth Meeting, PA). We used phosphate-buffered saline for dissolving PGE2, forskolin, and SQ 22536 and used dimethyl sulfoxide solution for H-89, calphostin C, and GF 109203X. Addition of phosphate-buffered saline or dimethyl sulfoxide alone to the culture medium did not alter VEGF and bFGF mRNA levels.

**RNA Extraction and Northern Blot Analysis**

RNA extraction and northern blot analysis were performed as described previously. Cultured rat Müller cells were lysed and homogenized in 5.5 M guanidinium thiocyanate solution. Total RNA was isolated using a cesium trifluoracacetate gradient method (CsTFA; Pharmacia, Piscataway, NJ). Thirty micrograms total RNA from each sample was electrophoresed on 1% agarose formaldehyde gels and transferred to a nylon membrane (Hybond-N; Amersham, Arlington Heights, IL). Blots were UV irradiated to immobilize RNA and prehybridized for 4 hours at 50°C. Random-primed, 32P-labeled cDNA probes (VEGF, 930-bp human VEGF cDNA, gift of N. Ferrara; bFGF, 477-bp rat bFGF cDNA, gift of A. D. Baird; and 1.1-kb cDNA, gift of D. Schlessinger) were added to the hybridization buffer (104 cpm/ml) and hybridized at 50°C overnight. After the posthybridization wash, the blots were exposed to a storage phosphor screen (Molecular Dynamics, Sunnyvale, CA), and the data were digitized by scanning the phosphor screen with a phosphor imaging system (Molecular Dynamics). In all northern blots, VEGF mRNA was detected as a single band at approximately 3.8 kb, and bFGF mRNA was detected as a major band at approximately 7.0 kb, along with several minor bands at lower molecular weights. Data were digitized from this single band and analyzed using a phosphor imaging system (Molecular Dynamics). Hard copies of blots were obtained by exposing the blots to film (Hyper Film; Amersham). The blots were reprobed with the 18S rRNA probe, and the data from the 18S rRNA served as a control for RNA loading.
**Statistical Analysis**

Statistical differences were evaluated by one-way analysis of variance with the Newman-Keuls's test for significance.

**RESULTS**

In early experiments, we examined the effect of the inflammatory mediators, PGE\(_2\) (10 \(\mu\)M), bradykinin (10 \(\mu\)M), histamine (10 \(\mu\)M), substance P (10 \(\mu\)M), and vasoactive intestinal peptide (10 \(\mu\)M), on VEGF and bFGF gene expression in cultured rat Müller cells. Among these mediators, only PGE\(_2\) induced VEGF and bFGF gene expression when added to the culture medium (data not shown). Further experiments revealed that PGE\(_2\) induced VEGF and bFGF gene expression in cultured rat Müller cells in a dose- and time-dependent manner.

**Prostaglandin E\(_2\) Induces Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor mRNA Expression**

VEGF mRNA was detected as a band of approximately 3.8 kb (Fig. 1A) in all samples. Figure 1 shows VEGF mRNA expression when cells were stimulated with four concentrations of PGE\(_2\) for 2 hours. Quantitative data from three independent experiments are shown in Figure 1B. An increase in VEGF mRNA of 1.5-fold was observed at 0.1 \(\mu\)M PGE\(_2\). The increase was approximately 1.8-fold at 1 \(\mu\)M and reached a maximum of 2-fold at 10 \(\mu\)M. No further increase was observed at 100 \(\mu\)M PGE\(_2\).

Figure 2A shows the time course of VEGF mRNA expression induced by PGE\(_2\). When cells were treated with 10 \(\mu\)M PGE\(_2\), induction of VEGF mRNA expression was seen as early as 0.5 hours. It increased to 1.7-fold by 1 hour, reached a maximum of more than 2.3-fold by 2 hours, and then slowly declined to the baseline level by 24 hours.

The expression of bFGF mRNA (as 7.0-kb bands in northern blots) was relatively low in the control cells (Fig. 3). Induction of bFGF mRNA expression was first observed at a PGE\(_2\) concentration of 0.1 \(\mu\)M. The maximal induction was reached (3.5-fold) at 10 \(\mu\)M of PGE\(_2\). A further increase in PGE\(_2\) concentration (100 \(\mu\)M) did not increase bFGF mRNA.

Induction of bFGF mRNA expression by PGE\(_2\) was time dependent, (Figs. 4A, 4B). With 10 \(\mu\)M PGE\(_2\), induction was seen as early as 1 hour after PGE\(_2\) treatment. It reached a maximum of more than 3.5-fold at 2 hours and then slowly declined to the baseline level by 24 hours (Fig. 4A).

**Induction of Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor mRNA Expression by Prostaglandin E\(_2\) through the Protein Kinase A Pathway**

We used the adenylate cyclase activator forskolin, the selective PKA inhibitor H-89, and the adenylate cyclase inhibitor SQ 22536 to determine whether PGE\(_2\)-stimulated VEGF and bFGF mRNA expression are mediated by PKA in Müller cells. As shown in Figure 5, forskolin (10 \(\mu\)M, 2 hours) induced a 1.7-fold increase in VEGF mRNA (Fig. 5A; lane 2), and PGE\(_2\) (10 \(\mu\)M, 2 hours) stimulated a more than 2-fold increase in VEGF mRNA. Treatment with forskolin (10 \(\mu\)M, 2 hours) plus PGE\(_2\) (10 \(\mu\)M, 2 hours) stimulated a more than 2-fold increase in VEGF mRNA. Treatment with forskolin (10 \(\mu\)M, 2 hours) plus PGE\(_2\) (10 \(\mu\)M, 2 hours) resulted in no additional increase in VEGF mRNA (Fig. 5A, lane 4), suggesting that PGE\(_2\) and forskolin induced VEGF mRNA expression through the same pathway. However, forskolin (10 \(\mu\)M, 2 hours) induced a 2.9-fold increase in bFGF mRNA (Fig. 6A, lane 3).
Treatment with forskolin (10 μM, 2 hours) and PGE₂ (10 μM, 2 hours) resulted in an enhanced effect on bFGF mRNA expression (Fig. 6A; lane 4).

Pretreatment with H-89 (30 μM, 1 hour before adding PGE₂) inhibited the induction of VEGF (Fig. 5A; lane 6) and bFGF (Fig. 7A; lane 4) mRNA expression. Treating cells with H-89 (30 μM) alone for 3 hours had no effect on the baseline level of VEGF (Fig. 5A; lane 5) and bFGF (Fig. 7A; lane 3) mRNA.

In addition, SQ 22536 inhibited VEGF and bFGF mRNA production by PGE₂ (Figs. 8, 9). SQ 22536 is a cell-permeable, adenylyl cyclase inhibitor used in biologic systems to demonstrate that prostaglandins stimulate adenylyl cyclase activity. When the cells were pretreated with different concentrations of SQ 22536 (100, 500, and 1000 μM) for 1 hour, the PGE₂-induced VEGF and bFGF mRNA expressions were inhibited in a dose-dependent manner. SQ 22536 (1000 μM) alone had no effect on the baseline level of VEGF and bFGF mRNA (Figs. 8, 9). These results indicate that the induction of VEGF and bFGF mRNA expression by PGE₂ occurs through the adenylyl cyclase-protein kinase A pathway in cultured Müller cells.

Figure 2. Temporal expression of vascular endothelial growth factor (VEGF) mRNA in response to prostaglandin E₂ (PGE₂). (A) Müller cells were exposed to PGE₂ (10 μM) at different times in hours. Time after PGE₂ treatment is indicated at the top of each lane. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.05; **P < 0.01 versus the control.

Figure 3. Dose-response effects of prostaglandin E₂ (PGE₂) on the induction of basic fibroblast growth factor (bFGF) mRNA expression. (A) Müller cells were exposed to different concentrations of PGE₂ for 2 hours. The cultures without PGE₂ treatment served as the control. A major bFGF transcript was detected in all lanes (indicated at the left). Migration of 28S and 18S rRNA is indicated at the right (upper panel). The same blot was stripped of the bFGF probes and rehybridized with probes for 18S rRNA, which served as a control for RNA loading (lower panel). The concentration of PGE₂ is indicated at the top of each lane. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.05; **P < 0.01 versus the control.
Protein Kinase C and Prostaglandin E₂-Induced Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor mRNA Expression

We next investigated the possible involvement of PKC in PGE₂-induced VEGF and bFGF mRNA expression in cultured Müller cells. PMA is a PKC activator, although prolonged exposure to PMA downregulates intracellular PKC activity. PMA treatment (0.2 μM, 2 hours) induced a 6-fold increase in bFGF mRNA. Prolonged treatment with PMA (0.8 μM, 16 hours) blocked PGE₂-induced bFGF mRNA expression (Fig. 10A) and induction of bFGF mRNA expression by PMA (Fig. 10A; lane 5). But downregulation of intracellular PKC by PMA (0.8 μM, 16 hours) failed to show any inhibitory effect on PGE₂-induced VEGF mRNA expression (Fig. 12A; lane 4).

Figure 4. Temporal expression of basic fibroblast growth factor (bFGF) mRNA in response to prostaglandin E₂ (PGE₂). (A) Müller cells were exposed to PGE₂ (10 μM) at different times in hours. Time after PGE₂ treatment is indicated at the top of each lane. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.05; **P < 0.01 versus the control.

Figure 5. Effects of forskolin and the specific protein kinase A blocker H-89 on the induction of vascular endothelial growth factor (VEGF) mRNA. (A) Müller cells were treated with 10 μM forskolin (FKL) for 2 hours in the presence or absence of prostaglandin E₂ (PGE₂). H-89 (30 μM) was added to dishes 1 hour before PGE₂ exposure and was observed for an additional 2 hours. In addition, cells were treated for 3 hours with 30 μM H-89 or for 2 hours with PGE₂ (10 μM), respectively. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.01 versus the control; +P < 0.01 versus the PGE₂ group.
FIGURE 6. Effect of forskolin on the induction of basic fibroblast growth factor (bFGF) mRNA expression. (A) Müller cells were treated with 10 μM forskolin (FKL) for 2 hours in the presence or absence of prostaglandin E2 (PGE2). Cultures without treatment served as controls. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.01 versus the control.

We also used a specific PKC inhibitor, GF 109203X. Figure 11 shows that GF 109203X alone did not change the baseline level of bFGF mRNA (Fig. 11A; lane 5), but it blocked the effect of PGE2 on bFGF mRNA expression in a dose-dependent manner when pretreating Müller cells with different concentrations of GF 109203X (0.02, 0.2, and 2 μM) for 1 hour before PGE2 treatment. In addition, pretreatment with GF 109203X (2 μM, 1 hour before adding PGE2) failed to inhibit the PGE2-induced VEGF mRNA expression (Fig. 12B). Pretreatment with calphostin C (1 μM, 1 hour before

FIGURE 7. Effect of protein kinase A inhibitor H-89 on the induction of basic fibroblast growth factor (bFGF) mRNA expression by prostaglandin E2 (PGE2). (A) Müller cells were pretreated with 30 μM H-89 for 1 hour and subsequently treated with 10 μM PGE2 for 2 hours. Müller cells were also treated with 30 μM H-89 alone for 3 hours. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.01 versus the control; +P < 0.01 versus the PGE2 group.
FIGURE 8. Effect of SQ 22536 on the induction of vascular endothelial growth factor (VEGF) mRNA expression by prostaglandin E2 (PGE2). (A) Müller cells were pretreated with different concentrations of SQ 22536 (100, 500, and 1000 μM) for 1 hour and subsequently treated with 10 μM PGE2 for 2 hours. In addition, Müller cells were treated with 1000 μM SQ 22536 alone for 3 hours, PGE2 (10 μM) alone for 2 hours. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.01 versus the control; +P < 0.05; ++P < 0.01 versus the PGE2 group.

adding PGE2, a specific PKC inhibitor that interacts with the PKC regulatory domain, did not block the PGE2-induced VEGF mRNA expression either (Fig. 12A; lane 6).

These data suggest that the PKC pathway also is involved in PGE2-induced bFGF mRNA production but is not involved in VEGF mRNA expression.

FIGURE 9. Effect of SQ 22536 on the induction of basic fibroblast growth factor (bFGF) mRNA expression by prostaglandin E2 (PGE2). (A) Müller cells were pretreated with different concentrations of SQ 22536 (100, 500, and 1000 μM) for 1 hour and subsequently treated with 10 μM PGE2 for 2 hours. In addition, Müller cells were treated with 1000 μM SQ 22536 alone for 3 hours, PGE2 (10 μM) alone for 2 hours. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). *P < 0.01 versus the control; +P < 0.05; ++P < 0.01 versus the PGE2 group.

DISCUSSION
We have shown that, in cultured rat Müller cells, PGE2 induces the expression of VEGF and bFGF mRNA in a dose- and time-dependent fashion, but no such effect was seen with other inflammatory mediators, such as bradykinin, histamine, sub-
stance P, and vasoactive intestinal peptide. However, bradykinin has been shown to increase bFGF mRNA levels in rat dermal fibroblasts.43

Our results show that induction of VEGF mRNA expression by PGE2 in cultured Müller cells is mediated by the cAMP-PKA pathway, although the PKC pathway is not involved. These findings are consistent with those of studies of rheumatoid synovial fibroblast cells21 and osteoblasts36 in which it was shown that the cAMP-PKA pathway mediates the effect of PGE2. Our results also provide evidence that the PGE2-induced bFGF mRNA expression is mediated by either PKA or PKC activation in cultured rat Müller cells. Direct stimulation of adenylate cyclase with forskolin or of PKC with PMA induced bFGF mRNA expression (Figs. 6, 10).35 A study of bovine adrenal medullary cells44 showed that bFGF expression was induced through PKA and PKC pathways, and this induction was synergistic and mediated through different promoter regions.

Physiological functions of PGE2 are thought to be mediated through interactions with four distinct prostaglandin receptors, EP1, EP2, EP3, and EP4, which are associated with different signal transduction pathways.45-49 Binding with EP2 and EP4 stimulates adenylate cyclase and thus activates PKA.46,47 EP3 mediates the inhibition of adenylate cyclase.49 Interaction of PGE2 with EP4 activates phospholipase C, which hydrolyses polyphosphoinositide lipid, resulting in the second-messenger molecules inositol trisphosphate and diacylglycerol lipid. The latter activates PKC.49,50 Our results show that PGE2-induced VEGF mRNA expression in cultured Müller cells through the PKA pathway and imply that the effect of PGE2 is mediated through EP2, EP4 or both. Our results also reveal that the PKA and PKC pathways are involved in the PGE2-induced bFGF expression, implying that the effect of PGE2 is mediated through EP1 and EP2 or EP4. Further studies should determine the exact receptor involved.

Angiogenesis is the formation of new blood vessels and involves the proliferation and migration of endothelial cells. VEGF is a specific mitogen for endothelial cells and is considered a major angiogenic factor and a survival factor for newly formed vessels.51,52 In retinal neovascular diseases, such as diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration, uncontrolled proliferation of blood vessels is an important pathogenic component.42,53,54 VEGF is considered a common neovascularizing factor.55 The finding that higher VEGF concentrations are found in ocular fluid from subjects with active proliferative neovascularization suggests that VEGF mediates angiogenesis in these diseases.51 Evidence is accumulating that Müller cells are involved in inducing neovascularization by releasing VEGF. Müller cells are those retinal cells in humans that show the most VEGF immunoreactivity.23 In vitro studies showed that Müller cells stimulated endothelial cell growth or mediated the angiogenic effect of transforming growth factor-β on endothelial cells by releasing VEGF.56,57 Evidence supporting this finding comes from studies of a model of proliferative retinopathy induced by relative hypoxia in neonatal mice.25 Within 6 to 12 hours of relative retinal hypoxia, there was an increase in the VEGF mRNA level, which remained until neovascularization
**Figure 11.** Effect of the protein kinase C (PKC) inhibitor GF 109203X on the induction of basic fibroblast growth factor (bFGF) mRNA expression by prostaglandin E₂ (PGE₂). (A) GF 109203X (0.02, 0.2, and 2 μM) was added to dishes 1 hour before PGE₂ (10 nM, 2 hours) treatment. Müller cells were also treated for 3 hours with 2 μM GF109203X alone. (B) Data from three independent experiments were averaged and compared with the control level (mean ± SD, n = 3). GF 109203X attenuated the induction of bFGF by PGE₂ in a dose-dependent fashion. *P < 0.01 versus the control; +P < 0.01 versus the PGE₂ group.

**Figure 12.** Effects of protein kinase C (PKC) downregulation and of the specific PKC inhibitors, calphostin C and GF 109203X on the induction of vascular endothelial growth factor (VEGF) expression by prostaglandin E₂ (PGE₂). (A) Cells were pretreated with 0.8 μM phorbol 12-myristate 13-acetate (PMA) for 16 hours to downregulate PKC, then PGE₂ (10 μM) was added to the medium for an additional 2 hours. Cells were also treated with PMA (0.8 μM) alone for 16 hours or PGE₂ (10 μM) alone for 2 hours. In addition, we pretreated cells for 1 hour with the specific PKC inhibitors calphostin C (Cal) (1 μM) and (B) GF 109203X (GF) (2 μM) and then treated them with PGE₂. Cells were also treated with Cal (1 μM) for 3 hours and GF (2 μM) alone for 3 hours.
developed. In situ hybridization and immunohistochemical studies identified the predominant VEGF-expressing cells as Müller cells. This neovascularization was reduced by VEGF-receptor chimeric proteins injected into the eyes, providing evidence that VEGF was directly responsible for retinal neovascularization.

Mammalian retinas produce several oxygenated metabolites of arachidonic acid, including prostaglandins. Among them, PGE₂ is the major prostaglandin produced. In human vitreous, PGE₂ concentration is approximately 0.08 ng/ml. A dramatic increase in vitreous PGE₂ to 3.17 ng/ml (close to 0.01 μM) was found in patients with retinal detachment. The lowest concentration of PGE₂ tested in the present work was 0.1 μM (35.2 ng/ml), which induced a 50% increase in VEGF mRNA expression. It is tempting to speculate that Müller cells can be stimulated to produce more VEGF in human retina in pathologic conditions in which substantial PGE₂ is produced and that this may result in neovascularization. This suggestion must be tempered by the concentration differences, observed under in vivo and in vitro conditions, and by the lack of knowledge of PGE₂ concentration immediately surrounding Müller cells in vivo.

Increased in PGE₂ has been found in various pathologic conditions in the retina, including injuries to the retina, such as laser irradiation, optic nerve injury, and retinal detachment. Mechanical injury to the retina induces a dramatic increase in bFGF expression, which is localized to the inner nuclear layer in which Müller cell bodies reside. Our finding, that PGE₂ stimulates bFGF expression in cultured Müller cells, may point to the role of PGE₂ in mediating injury-induced bFGF expression in the retina.

In addition to their well-known role as inflammatory mediators, prostaglandins have the property of cytoprotection for gastrointestinal mucosa and other tissues. For example, prostaglandins protect rat liver injured with d-galactosamine, carbon tetrachloride, ethanol, and acetaminophen, and they are protective in experimental pancreatitis and myocardial ischemia. In addition, it has been found that PGE₂ affords cytoprotection to embryonal neuroectodermal tissue and embryonic neural retina cells from degeneration induced by actinomycin C. More recently, a study revealed that increased endogenous production of PGE₂ achieved by liposome-mediated combinator gene transfer of prostaglandin G and H synthase, protects rabbit lungs from endotoxin injury. The mechanisms that underlie cytoprotection have not been identified. Because of its well-known cytoprotective effects of bFGF, our finding, that PGE₂ induces bFGF expression, may provide insight into the mechanism of prostaglandin-induced cytoprotection.

We have reported that PGE₂ stimulates the expression of VEGF, an important angiogenic factor, in cultured rat Müller cells. Because bFGF is thought to be involved in angiogenesis, our finding that PGE₂ induces bFGF expression also suggests that PGE₂ has a role in inducing neovascularization in the retina.

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