Localization of the Prostaglandin F$_{2 \alpha}$ Receptor Messenger RNA and Protein in the Cynomolgus Monkey Eye

Anette Ocklind,* Staffan Lake,† Parri Wentzel,* Monica Nister,‡ and Johan Stjernschantz*  

Purpose. To determine the distribution of the prostaglandin F$_{2 \alpha}$ (FP) receptor within the monkey eye.

Methods. The expression and localization of the FP receptor was studied by in situ hybridization and immunohistochemistry. Cryosections of the eye were hybridized with a $^{35}$S-labeled FP receptor riboprobe, and paraffin sections were immunostained with polyclonal antibodies against an FP receptor peptide. Reverse transcriptase–polymerase chain reaction (RT–PCR) was performed on cultured cell populations from the eye.

Results. Results of the three methods largely correlated with each other. The highest expression of FP receptor mRNA and protein was found in the corneal, conjunctival, and iridal epithelium, the ciliary muscle, and ciliary processes. Iridial and choroidal melanocytes, the retina, and the optic nerve expressed lower levels of both FP receptor message and protein. Using immunohistochemistry, the FP receptor protein was found in connective tissue fibroblasts, the corneal endothelium, and the vasculature; however, FP receptor expression was not detected using in situ hybridization. With RT–PCR, cultured retinal pigment epithelial and ciliary muscle cells from the cynomolgus monkey eye were found to express the FP receptor.

Conclusions. The FP receptor was found to be distributed widely in the ocular tissues, suggesting an array of autocrine and paracrine functions of PGF$_{2 \alpha}$ in the eye. Invest Ophthalmol Vis Sci. 1996;37:716–726.

Prostaglandin (PG) F$_{2 \alpha}$ is synthesized in several ocular tissues in a variety of species, including humans. The pharmacologic effects of PGF$_{2 \alpha}$ are species dependent and include, for example, contraction or relaxation of smooth muscle and a decrease in intraocular pressure. The decrease in intraocular pressure by topically administered PGF$_{2 \alpha}$ and PGF$_{2 \alpha}$ analogs in primates and humans has been shown to be mediated mainly by an increase in the uveal scleral outflow. The physiologic effect of PGF$_{2 \alpha}$ appears to be mediated primarily by the PGF$_{2 \alpha}$ (FP) receptor.

The FP receptor cDNA from mouse, human, and rat was recently cloned, and a high nucleotide sequence homology between the species was found. The FP receptor is a member of the seven transmembrane-receptor family and is linked to a guanosine triphosphate-binding protein. Stimulation of the receptor induces intracellular signaling by phospholipase C-mediated phosphoinositol turnover, which leads to the generation of the second messengers, inositoltriphosphate and diacylglycerol.

The distribution of the FP receptor in the eye is not well known. To understand and evaluate better the effects of PGF$_{2 \alpha}$ and analogs in the eye, we have studied the distribution of the FP receptor mRNA and protein in ocular tissues by in situ hybridization and immunohistochemistry. We have also used reverse transcriptase polymerase chain reaction (RT–PCR) to evaluate the expression of the FP receptor mRNA in cultured cells originating from the monkey eye.
Localization of the Prostaglandin F$_{2\alpha}$ Receptor

**TABLE 1.** Prostaglandin F$_{2\alpha}$ Receptor-specific Primers Corresponding to Positions 57–79 (Primer 1), 569–590 (Primer 2), and 547–568 (Secondary Primer 2) of the Human cDNA

<table>
<thead>
<tr>
<th>Primer</th>
<th>Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary primer 1</td>
<td>CAC AAG CTG CCA GAC GGA AAA C</td>
</tr>
<tr>
<td>Primary primer 2</td>
<td>CCA GTC TTT GAT GTC TTC TGT G</td>
</tr>
<tr>
<td>Secondary primer 2</td>
<td>TTG TAG AAA CAC CAG GTC CTC G used together with primary primer 1</td>
</tr>
</tbody>
</table>

**MATERIALS AND METHODS**

**Animals**

All animals involved in this study were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Tissue Preparation**

Four adult cynomolgus monkeys (*Macaca fascicularis*) were anesthetized with a mixture of ketamine (20 mg/kg body weight) and xylazine (1.5 mg/kg body weight) and perfused intracardially with 4% neutral buffered paraformaldehyde. For in situ hybridization, the eyes were enucleated and bisected equatorially, the lens and vitreous were discarded, and the hemispheres were further fixed for 4 hours at 4°C. After extensive washing in 15% sucrose in phosphate-buffered saline (PBS), pH 7.4, the anterior and posterior segments were subdivided into sectors. Tissue samples in OCT (Miles, Elkhart, IN), were frozen in liquid nitrogen-cooled isopentane and stored at −80°C for less than 2 weeks. Tissues were cryosectioned at 4 to 7 μm, mounted on Superfrost Plus slides (Gerhard Menzel GmbH KB, Braunschweig, Germany), refrozen at −80°C, and analyzed within 1 month. For immunohistochemistry, enucleated eyes were further fixed for 7 hours at room temperature. After 4 hours, a calotte was cut from each eye to facilitate the penetration of the fixative. After washing in PBS overnight at 4°C, the eyes were paraffin embedded and sectioned at 4 to 5 μm according to standard methods.

**Preparation of Probes**

Riboprobes were synthesized from PCR II (Invitrogen, San Diego, CA) plasmid vectors containing dual SP6 and T7 promoters and nucleotides 56 to 566 of the coding region of the human FP receptor, that is, the transmembrane regions 1 to 4 (see Fig. 4). Antisense and sense probes were transcribed from plasmids linearized with BamH1 using an RNA transcription kit (Ambion MAXI Script, Austin, Texas) in the presence of $^{35}$S-UTP (uridine 5’-$^{35}$S)triophosphate; Amersham, Solna, Sweden). The transcription reaction was carried out with SP6 (antisense) and T7 (sense) polymerases (Promega Biotech, Madison, WI) essentially according to the manufacturer’s instructions, with the exception that the labeling was performed for 75 minutes at 37°C. Approximately 70% to 90% of the $^{35}$S-UTP was incorporated, to yield a final specific activity of $1 \times 10^9$ cpm/μg RNA.

**In Situ Hybridization**

For in situ hybridization, cryosections were rehydrated and permeabilized in 0.2% Triton X-100 (Life Technologies, Louisville, IL) in PBS, washed in PBS, digested with Proteinase K (Boehringer Mannheim, Mannheim, Germany; 1 μg/ml in 10 mM Tris–HCl with 5 mM ethylenediaminetetraacetic acid) for 15 minutes at 37°C, treated with 0.1 M glycine in PBS, postfixed in 4% paraformaldehyde for 3 minutes, and rinsed in PBS. Sections were acetylated in 0.25% (vol/vol) acetic anhydride with 0.1 M triethanolamine, pH 8, for 10 minutes, rinsed in distilled water, and dried. Hybridization was performed in 50% formamide, 10% dextrane sulfate, 5 × Denhardt’s solution, 10 mM sodium pyrophosphate, 100 μg/ml denatured salmon sperm DNA, 10 μM dithiothreitol (DTT), and $5 \times 10^8$ cpm probe/μl. Twenty microliters of the hybridization mixture was applied to each tissue section, which was coverslipped and incubated for 4 hours at 48°C. Slides were rinsed in five changes of 2 × 0.15 M NaCl and 2 × 15 mM sodium citrate (2 × SSC) with 0.1% (vol/vol) sodium dodecyl sulfate and 10 mM DTT at room temperature and in four changes of 0.1 × SSC/0.1% sodium dodecyl sulfate per 10 mM DTT at 48°C, further rinsed in 2 × SSC, treated with 10 μg/ml RNase in 2 × SSC for 15 minutes at 37°C, and finally rinsed in 2 × SSC. Tissue sections were dehydrated in ethanol with 0.3 M ammonium acetate and were air dried overnight. Hybridized preparations were autoradiographed with NTB-2 emulsion (Eastman Kodak, Rochester, NY) diluted 1:1 in distilled water. After exposure during 11 to 14 days at 4°C, the slides were developed in Dektol (Kodak), counterstained with hematoxylin-eosin, mounted, and examined with combined epipolarization and bright-field microscopy.

**Cell Cultures**

Cynomolgus monkey retinal pigment epithelial cells were isolated from four freshly enucleated eyes essen-
FIGURE 1. In situ hybridization of cynomolgus monkey eye tissues with $^{35}$S-labeled prostaglandin F$_2$ (FP) receptor riboprobes. Photomicrographs showing in situ hybridization with antisense riboprobe (left panels) and sense riboprobe (right panels). The hybridization signal obtained with the sense probe is very low. (A,B) Anterior cornea. Corneal epithelium shows specific hybridization. (C,D) Longitudinal portion of ciliary muscle. (E,F) Ciliary process. Specific hybridization is shown on both the nonpigmented (arrow) and pigmented (arrowhead) epithelium. (G,H) Iris. FP receptor mRNA is visualized on the pigment epithelium (pe) and iris stromal melanocytes (arrow). (I,J) Inner retina. Specific hybrids are shown on the ganglion cell (gc) layer. (K,L) Outer retina. FP receptor mRNA is shown on retinal pigment epithelium (arrow), inner segments, and outer nuclear layer of photoreceptors (on). (M,N) Cross-section of optic nerve fiber bundles exhibiting specific hybridization. Photomicrographs are taken in simultaneous epipolarized and bright-field illumination. Magnification, ×400.
Localization of the Prostaglandin F$_2$ Receptor

FIGURE 1. (Continued)
FIGURE 2. Fluorescence photomicrographs of cynomolgus monkey eye sections stained with antibodies to the human prostaglandin F\textsubscript{2a} (FP) receptor. (A) FP receptor immunoreaction is localized to the corneal epithelium and, to a lesser extent, to the stromal fibroblasts (arrow). (B) Preabsorbed antibodies block the immunoreaction, as shown in the cornea. (C) Preimmune antibodies show little binding, as shown in the cornea. (D) Corneal endothelium and (E) lens epithelium are immunopositive. (F) The entire ciliary muscle is immunopositive, as shown in the longitudinal muscle portion. (G) Positive immunoreaction is seen in the ciliary processes (arrow). (H) In the retina, the outer nuclear (on) and inner nuclear (in) layers, as well as the ganglion cell (gc) layer, show positive immunoreaction. For the retinal pigment epithelium, see Figure 3. (I) Immunoreactive optic nerve fiber bundles are in longitudinal section visualized as four broad horizontal bands next to lamina cribrosa (lc). (J) Blood vessels (arrow), as exemplified in the limbal region, are immunopositive. (K) The lateral rectus extraocular muscle (cross-section through muscle bundles) is immunopositive. Magnification, X200.
Localization of the Prostaglandin F<sub>2</sub> Receptor

FIGURE 2. (Continued)

Two animals were killed by an intracardiac injection of pentobarbital (100 mg/kg body weight) under ketamine (20 mg/kg body weight) and xylazine (1.5 mg/kg body weight) anesthesia, and the eyes were enucleated. Each eye was bisected along the equator, and the vitreous was removed. The posterior eye cup was rinsed in PBS, pH 7.4, supplemented with 25 mM Hepes and 1% antibiotic-antimycotic solution (Life Technologies, Paisley, Scotland), and the neural retina was removed gently. After rinsing, the retinal pigment epithelial cells were released and collected from Bruch’s membrane by gently pipetting several changes of the rinsing solution in the eye cup. Cells were cultured in equal concentrations of Dulbecco’s modified Eagle medium (DMEM) and Ham’s F-12 supplemented with 15% fetal calf serum (FCS), 2 mM L-glutamine, 50 μg/ml gentamicin (all from Life Technologies), and 1 ng/ml human recombinant basic fibroblast growth factor (Sigma Chemical, St. Louis, MO) for approximately 1 week at 37°C, 5% CO<sub>2</sub> humidified air, after which the concentration of FCS was reduced to 10%. Confluent cultures were subcultured by trypsinization. In confluent cultures without basic fibroblast growth factor, the identity of the retinal pigment epithelial cells was confirmed by their flat cuboidal appearance and by the presence of pigment granules. Immunocytochemical staining with monoclonal antibodies (Clone KL 1; Immunotech, Marseilles, France) against the cytokeratins 1, 2, 5, 6, 7, 8, 11, 14, 16, 17, and 18 always was found in more than 95% of the cells in culture. Cultured human retinal pigment epithelial cells are reported to express the cytokeratins 7, 8, 18, and 19.

Cynomolgus monkey ciliary muscle cells were isolated and cultured from four freshly enucleated eyes essentially according to a previously published method with some adaptations. Briefly, each eye was divided by cutting between the ora serrata and the equator, and the lens and the iris were removed. The pars plana region of the ciliary body was cut out and divided radially into approximately four segments. The segments were incubated in 2.4 U/ml dispase (Boehringer Mannheim) in DMEM supplemented with 10% FCS for approximately 15 minutes at 37°C, 5% CO<sub>2</sub>, air, after which the epithelial layers and vessels on the posterior side and choroidal melanocytes on the anterior side were scraped from the ciliary muscle region. Originating from the longitudinal and radial ciliary muscle, cells were dissociated by cutting the tissue into 1-mm³ pieces and incubating in 0.1% collagenase (Sigma) in DMEM supplemented with...
10% FCS for 2 hours at 37°C. Cells were collected and cultured in the medium used for the retinal pigment epithelial cells with the further addition of 10 ng/ml human recombinant platelet-derived growth factor BB (Sigma). At confluence, the cells were subcultured by trypsinization. In confluent cultures without growth factors, the identity of the ciliary smooth muscle cells was apparent by their “hill-and-valley” growth pattern. Immunocytochemically, more than 95% of the cells always stained brightly for smooth muscle α-actin (antibodies from Sigma).

Reverse Transcriptase—Polymerase Chain Reaction

Specific primers were designed for the human FP receptor as presented in Table 1. The design of the primers was based primarily on the more conserved homology between species in the transmembrane regions and in certain regions conserved in the family of seven transmembrane receptors—in this case, the tryptophan, cysteine, phenylalanine motif present in most, if not all, seven transmembrane receptors. From a set of combinations of primers in these regions, the presented primers were shown to hybridize and amplify cynomolgus monkey FP cDNA. RT-PCR was performed on mRNA isolated from cell cultures of cynomolgus monkey retinal pigment epithelial and ciliary muscle cells. The RT-PCR products were analyzed on agarose gel, and bands of the expected sizes were cloned and sequenced. Deduced amino acid sequences were analyzed for homology to the human FP receptor sequences.

For the experiments, cells at passages 2 to 4 were grown to confluence and harvested. mRNA was isolated using Dynal mRNA Direct system (Dynal A/S; Oslo, Norway) according to the manufacturer’s recommendations. Approximately 500,000 cells of each finite cell line were used in the enrichment, where mRNA is covalently bound to an oligo-dT labeled Dynabead. Using reverse transcriptase, the first-stranded cDNA was amplified with receptor-specific nested primers for PCR-amplifying cDNA, according to the manufacturer’s recommendations. The FP receptor PCR reactions were performed in 50 μl final volume, including 5% dimethyl sulfoxide, 200 μM deoxyribonucleotides, and 20 pmol of each primer.

The PCR products from these reactions were analyzed on a 0.9% low melt preparative grade agarose gel (BioRad Laboratories, Melville, NY). DNA fragments of the expected size were thymidine–adenine cloned according to the manufacturer’s recommendations (PCR II; Invitrogen) and sequenced on an Applied Biomodel 373A DNA sequencing system (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol for their Taq Dye Dioxyl terminator cycle sequencing kit. The generated data were processed on a VAX computer using sequence analysis programs (Genetics Computer Group, Madison, WI).

Immunohistochemistry

A polyclonal antiserum against a synthesized peptide corresponding to the first extracellular loop (that is, between transmembrane regions 2 and 3) of the FP receptor was raised in rabbits and the immunoglobulin (Ig) G fraction was used. Specificity of the antibody was determined using stable FP receptor-transfected versus nontransfected host cells and by competition with its corresponding antigen. Specificity also was seen on Western blots of yeast-expressed FP receptor protein (data not shown). Preimmune serum was collected before immunization, and the immunoglobulin fraction was isolated.

Indirect immunofluorescence staining was performed on paraffin sections as previously described. Briefly, antigenic sites were exposed by incubating the sections in 0.1% trypsin (DAKO, Glostrup, Denmark) in 0.1% CaCl₂ for 5 minutes at 37°C. Autofluorescence and nonspecific binding were blocked with 0.15 M ethanolamine, pH 7.5. Nonspecific binding were blocked further with 5% normal goat serum, and possible endogenous biotin was blocked with avidin–biotin blocking solutions (DAKO). Sections were incubated with immune and preimmune antibodies at 4 μg/ml for 90 minutes and then with biotinylated goat-antirabbit IgG (1:250; Sigma) for 30 minutes. Binding sites were visualized with streptavidin-labeled Texas red (1:100; Amersham) and examined by epifluorescence microscopy.

In some experiments, gold-labeled goat anti-rabbit IgG (1:40; Amersham) were used instead of the biotinylated secondary antibodies. These sections were treated with Silver IntenSE (Amersham), counterstained with hematoxylin, and examined by combined epipolarization and bright-field microscopy. Control experiments were performed using preimmune IgG or PBS in place of the primary antibodies. In addition, control sections of all experimental tissues were incubated with primary antibodies that had been preabsorbed with the FP receptor peptide and the conjugation protein used for the immunization. Control experiments, antibodies were mixed with the peptide and its conjugation protein at a protein concentration ratio of 10:1, and the mixture was incubated for 30 minutes at 37°C. After centrifugation for 4 minutes at 10,000g, the supernatant was collected and used for the staining.
Localization of the Prostaglandin F\(_2\alpha\) Receptor

TABLE 2. Relative Expression of FP Receptor mRNA and of Immunoreactive FP Receptor in the Monkey Eye

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ISH</th>
<th>IF†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Stroma</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cornea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Stroma</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Endothelium</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Iris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Melanocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sphincter</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>Ciliary body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciliary muscle</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ciliary processes</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Trabecular meshwork</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Choroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanocytes</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganglion cells</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Photoreceptors</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Inner nuclear layer</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pigment epithelium</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Optic nerve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve fibers</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteries</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Veins</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Rectus muscle</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

* ISH = in situ hybridization. Hybridization is monitored as: − = no signal above background; + = weak positive signal; and ++ = strong positive signal. ND = not determined.
† IF = immunofluorescence. Immunoreactivity is monitored as: − = no signal; + = weak positive signal; and ++ = strong positive signal.

RESULTS

In Situ Hybridization

In situ hybridization results are shown in Figure 1 and are summarized in Table 2. Control hybridization (Fig. 1, right panels) with sense probe exhibited almost no labeling, as indicated by the sparse distribution of silver grains. However, pigmented cells and retinal nuclear layers generally displayed a slightly higher background level of silver grains than other cells. When the in situ hybridization procedure was performed without probe, no silver grains were seen on any cells (not shown).

In situ hybridization with the FP receptor antisense probe revealed a low, but specific, expression of mRNA on most cell types (Fig. 1, left panels), as detected by comparison with the sense probe hybridization (Fig. 1, right panels). The highest accumulations of silver grains were found throughout the corneal (Fig. 1A) and conjunctival epithelium, ciliary muscle (Fig. 1C), ciliary processes (Fig. 1E), iris pigment epithelium (Fig. 1G), choroidal melanocytes, and photoreceptors of the retina (Fig. 1K). Within these cell populations, the hybridization signals were homogeneously distributed with equal intensities. In the corneal epithelium (Fig. 1A), conjunctival epithelium, and iridial pigment epithelium (Fig. 1G), FP receptor mRNA appeared to be expressed in all cell layers. In the ciliary muscle (Fig. 1C), specific hybridization was seen in longitudinal, radial, and circular muscle portions. In the ciliary processes (Fig. 1E), FP receptor mRNA was shown on both epithelial cell layers, although the nonpigmented epithelium was less positive. In the photoreceptor layer (Fig. 1K), FP receptor mRNA was localized to the outer nuclear layer and the inner segments of rods and cones. Weak specific hybridization was found in the iridial melanocytes (Fig. 1G), iris sphincter muscle, trabecular meshwork, optic nerve fibers (Fig. 1M), retinal pigment epithelial cells (Fig. 1K), retinal inner nuclear layer and ganglion cell layer (Fig. 1I). In the optic nerve, most silver grains were seen to overlie the nerve fibers (Fig. 1M). No specific hybridization signal was detected in the corneal (Fig. 1A) and conjunctival fibroblasts, corneal endothelium, or vessels. The lens epithelium was not studied because the lens was removed before the cryopreservation.

Immunohistochemistry

Immunohistochemistry results are shown in Figures 2 and 3 and are summarized in Table 2. Indirect immunofluorescence staining with the polyclonal antibodies showed the FP receptor to be present in small amounts in all ocular tissues examined (Fig. 2). Preabsorbed antibodies reduced the signal intensity to near background levels (Fig. 2B). Preimmune serum showed little binding (Fig. 2C). The low nonspecific binding was achieved only by soaking the fixed specimens overnight in PBS to remove excess formaldehyde, by examination of the unreacted formaldehyde groups in ethanolamine, and by brief washings in PBS containing Triton X-100. Autofluorescence that could not be quenched occurred in the retinal pigment epithelium and in iridial melanocytes. Consequently, indirect immunogold staining and silver enhancement was used as a complimentary method to visualize binding sites in pigmented cells. The FP receptor protein was found predominantly in the conjunctival and corneal epithelium (Fig. 2A), notably in the basal layers, ciliary muscle (Fig. 2F), ciliary processes (Fig. 2G), iris pigment epithelium (Fig. 3C), lens epithelium...
In the ciliary muscle, staining appeared throughout all muscular portions. In the ciliary processes, most of the immunoreaction was located to the nonpigmented epithelium. The FP receptor seemed least abundant in fibroblasts from various connective tissues (Fig. 2A), corneal endothelium (Fig. 2D), iridal melanocytes (Fig. 3C), retinal neural and photoreceptor layers (Fig. 2H), extraocular muscle (Fig. 2K), and optic nerve (Fig. 2I). Blood vessels stained weakly. Occasionally, limbal vessels (Fig. 2J) showed more intense immunoreaction.

Reverse Transcriptase-Polymerase Chain Reaction

Retinal pigment epithelial and ciliary muscle cells in culture expressed the FP receptor, based on RT-PCR. One band corresponding to the expected 465-bp fragment was found. Cloning and sequence analysis confirmed the identity to the FP receptor. At the amino acid level, the homology between the cynomolgus and the human FP receptor was found to be 99% in the amplified TM1–TM4 region of the receptor (Fig. 4).

DISCUSSION

The purpose of the study was to investigate the distribution of the FP receptor in the monkey eye. Results obtained with an antiserum against the FP receptor correlate with immunohistochemical studies of the FP receptor in the rat eye in which the receptor was distributed in various tissues in the eye and in the rest of the body. Receptor binding as well as pharmacologic and molecular biologic studies have suggested the presence of the FP receptor in various tissues of the reproductive, renal, gastrointestinal, cardiovascular, digestive, respiratory, endocrine, and nervous systems in various species. Our study on the distribution of the FP receptor in the monkey eye seems to agree fairly well with published experiments on human cadaver eyes using ligand-binding techniques, in which the FP receptor was found in the ciliary muscle, iris pigment epithelium, iris sphincter muscle, and retina.

Overall, our results indicate a low abundance of FP receptor transcripts and protein. The signals were low in most ocular tissues, as evidenced by the low amount of silver grains despite an exposure time of 2 weeks. Longer exposure times increased the background level of grains and did not increase sensitivity. Detection of the autoradiographic silver grains was enhanced strongly with epipolarization microscopy. Under bright-field illumination, most grains were invisible. Furthermore, epipolarization microscopy permitted a clear-cut distinction between silver grains and
pigment granules derived from the pigmented cells. Only metal particles such as silver grains deflect polarized light, which is visualized as brightly shining spots against a dark background. With this technique, the signals were found to be specific. When the epipolarization was combined with bright-field illumination, cell morphology was clearly visible.

Our immunohistochemical studies of the distribution of FP receptor protein yielded results largely in agreement with our in situ studies. In addition, in this assay, the signals were low. Immunofluorescence studies required bright illumination, and photomicrography required long exposure times. In cell populations labeled by our antibodies, the strongest signals for each of the two techniques correlated with each other, except for the choroidal melanocytes and the photoreceptor layer. In these cell layers, FP receptor transcripts seemed to be relatively more abundant than the FP receptor protein. Retinal pigment epithelial cells, retinal ganglion cells, and nerve fibers in the optic nerve exhibited a slightly higher-than-background level of silver grains. The low abundance of the FP receptor in these tissues was, except for the retinal epithelium, confirmed by the immunohistochemical staining.

Interestingly, FP receptor transcripts appeared to be absent in vessels, corneal and conjunctival stroma, and scleral and corneal endothelium, whereas the FP receptor protein was detected in all these tissues. The differences between mRNA and protein distributions may be caused by different levels of sensitivity of the two methods. The antiserum against the FP receptor protein was produced against a small synthetic peptide corresponding to the first extracellular loop of the human FP receptor. Preabsorption experiments confirmed antibody specificity. Because of similarities between different PG receptors, it can be argued that antiserum cross-reacts with other PG receptors. However, we chose this particular peptide for the generation of antibodies because amino acid sequence differs most between PG receptors of different types but is well conserved between species. Cross-reactivity of the riboprobe with other PG receptor mRNA seems unlikely because of the considerable length of the probe. The reported overall amino acid identity between human PG receptors is only 27% to 42%. It cannot be excluded that the relative abundance of mRNA and protein might differ with respect to cell type because of synthesis, degradation, or secretion of protein in various cell types. In structures for which we can detect colocalization of mRNA and receptor protein, the FP receptor is present. If only receptor protein can be detected, however, it cannot be excluded that the immunoreaction is nonspecific.

To confirm the results obtained with in situ hybridization and immunohistochemistry, we took advantage of the possibility of analyzing cultured cells originating from the cynomolgus monkey eye. Here, we used RT-PCR as a different method to analyze the FP receptor mRNA. Taking into account the differentiation and possibly altered phenotype of cultured cells when compared to cells of intact tissues, our RT-PCR data showed good correlation with the results obtained with tissue sections. The high amino acid homology in the TM1–TM4 region implies that the monkey is a good model for humans.

These FP receptor results raise questions about the physiological role of PGF\(_{2\alpha}\) in the eye. Based on the widespread ocular distribution of the FP receptor, we can assume that, for a local hormone, PGF\(_{2\alpha}\) has a great variety of functions in the eye. Because of this widespread receptor distribution, we postulate the presence of cell type-specific intracellular signals to account for the many different biologic effects induced by PGF\(_{2\alpha}\).

**Key Words**

immunohistochemistry, in situ hybridization, localization, monkey eye, prostaglandin F\(_{2\alpha}\) (FP) receptor
References


