The EP₂ receptor is the predominant prostanoid receptor in the human ciliary muscle

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Abstract

Prostaglandins can reduce intraocular pressure by increasing uveoscleral outflow. We have previously demonstrated that the human ciliary muscle was a zone of concentration for binding sites (receptors) for prostaglandin F_{2α} and for prostaglandin E₂. Here, we try to elucidate the types of prostanoid receptors in the ciliary muscle using competitive ligand binding studies in human eye sections and computer assisted autoradiographic densitometry. Saturation binding curves showed that the human ciliary muscle had a large number of binding sites with a high affinity for prostaglandin E₂ compared with prostaglandin D₂ and F_{2α}. The binding of tritiated prostaglandin E₂ and F_{2α} in the ciliary muscle was displaced most effectively by prostaglandin E₂ and 11-deoxy prostaglandin E₁ (a selective EP₂ prostanoid receptor agonist), whereas the binding of prostaglandin D₂ was displaced most effectively by prostaglandin E₂ and D₂. These results indicate that the dominant prostanoid receptor in the human ciliary muscle is the EP₂ subclass and that there is also a small number of DP receptors.

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Prostaglandins are known to reduce intraocular pressure in various species including rabbits, cats, monkeys, and humans, when given topically at low doses. The ability of prostaglandin F_{2α} to lower intraocular pressure has been well documented in normal subjects^{1–3} and also in patients with glaucoma.^{4,5} The reduction of intraocular pressure following prostaglandin E₂ administration has been observed in cats and monkeys,^{6–8} and the pressure lowering effect of prostaglandin D₂ has also been examined in cats and rabbits.^{9–11} However, their effects in humans have not been studied.

There are several lines of evidence indicating that prostaglandin F_{2α} lowers intraocular pressure by increasing uveoscleral outflow in monkeys.^{12–14} We have previously demonstrated, using an in vitro radioligand binding technique and autoradiography, that human ciliary muscle is a zone of concentration of binding sites (receptors) for prostaglandin F_{2α} and for prostaglandin E₂.¹⁵

A working classification of prostanoid receptors for five naturally occurring prostaglandins has been proposed based on pharmacological studies in which the agonist potencies of naturally occurring prostaglandins as well as other synthetic agonists and antagonists were compared.^{16,17} Receptors sensitive to thromboxane A₂, prostaglandin D₂, E₂, F_{2α}, and I₂ have been designated as TP, DP, EP, FP, and

IP prostanoid receptors, respectively. The EP receptor can be further classified into three subtypes, called EP₁, EP₂, and EP₃ receptors.^{18,19} The framework of the receptor classification has been supported in part, by cloning and expression of cDNA for a human thromboxane A₂ receptor.²⁰

It is important to know the types of prostanoid receptors located on the human ciliary muscle in order to understand its role in uveoscleral outflow, and to design new drugs with more potency and fewer adverse effects. In this study we tried to elucidate the type(s) of prostanoid receptors located on the human ciliary muscle by combining receptor autoradiography with competitive binding studies with various ligands on human eye sections.

Materials and methods

PREPARATION OF HUMAN EYE SECTIONS

Human cadaveric eyes were obtained within 24 hours after death from the Eye Bank of British Columbia. The eyes from two different individuals were used: a 45-year-old man who died of heart failure and a 52-year-old woman who died of colon cancer. The eyes used in this study had no documented ocular diseases. They were frozen in isopentane cooled to –80°C and stored at –20°C until use. Sections of 20 μm thickness were cut with a cryostat (Cambridge Instruments, Nussloch, Germany) and placed on glass slides coated with 1.7% gelatin.

IN VITRO LIGAND BINDING AND AUTORADIOGRAPHY

The sections were thawed at room temperature and preincubated in 50 mM TRIS-hydrochloride buffer (pH 7.4) containing 100 mM sodium chloride, 3 mM calcium chloride, and 5% (weight/volume) bovine serum albumin (Sigma, St Louis, MO) for 60 minutes at room temperature. The sections were then incubated in the same buffer containing a given concentration of tritiated prostaglandin D₂, E₂, or F_{2α} (Du Pont Canada, Mississauga, Ontario) for 90 minutes at room temperature, washed for 40 minutes in the ice-cold buffer containing 1% bovine serum albumin, and finally dried in an air stream. The sections were then apposed to tritium-sensitive films (Hyperfilm-³H; Amersham Canada, Oakville, Ontario) for 8 weeks in the dark.

SATURATION AND COMPETITION BINDING STUDIES
Sections were incubated with 1, 2, 5, 10, or 20 nM concentration of tritiated prostaglandin D₂,

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E₂, or F_{2α} to obtain a saturation binding curve for each prostaglandin. Specific binding at each concentration was obtained by subtracting non-specific binding (determined by addition of 10 μM corresponding unlabelled ligand) from total binding (determined without the unlabelled ligand). The density of binding sites in the ciliary muscle under different conditions was measured as described below.

For competitive binding, sections were

incubated with 10 nM of tritiated prostaglandin D₂, E₂, or F_{2α}, together with varying concentrations (10⁻⁹ to 10⁻³ M) of seven different unlabelled ligands including prostaglandin D₂, E₂, F_{2α}, U-46619, 11-deoxy prostaglandin E₁, 17-phenyltrior prostaglandin F_{2α} (all from Cayman Chemicals), or sulprostone (Schering AG, Berlin, Germany). The degree of competition for each ligand was expressed as the relative percentage between the binding of tritiated prosta-

Fig 1A

Fig 1D

Fig 1B

Fig 1E

Fig 1C

Fig 1F

Figure 1 Autoradiographic views of specific binding sites for 2 nM tritiated prostaglandin D₂ (A), E₂ (B), and F_{2α} (C) in sections of the human eye. The binding sites for the three prostaglandins are co-localised with concentrations in the ciliary muscle, iris sphincter muscle, iris epithelium, and retina. Non-specific binding for each prostaglandin determined by addition of the corresponding 10 μM unlabelled ligand is quite low. Nissl stained views of the same sections for the specific binding (A, B, and C) are given in D, E, and F, respectively.

Figure 2 Saturation binding curves (upper figure) and Scatchard plots (lower figure) for specific binding of prostaglandin D_2 (●), E_2 (○), and $\text{F}_{2\alpha}$ (■) in the human ciliary muscle. Each value represents an average of four different measurements in two eye sections. The dissociation constant (K_d) and maximum number of binding sites (B_{max}) for each prostaglandin are summarised in Table 1.

glandins obtained with a given concentration of each competitive ligand and the total binding obtained without the competitive ligand. Competitive binding curves for one of the unlabelled ligands versus the three tritiated prostaglandins (prostaglandin D_2 , E_2 , and $\text{F}_{2\alpha}$) were drawn by plotting relative autoradiographic density values (%) in the ciliary muscle against concentrations of each unlabelled ligand.

COMPUTER ASSISTED DENSITOMETRY

The autoradiographic images were trans-illuminated with a fluorescent light source which provided even illumination, captured by a video camera (Panasonic CCTV Camera, Model MW-BD400, Osaka, Japan) and then transmitted to a computer (Macintosh, Apple Computer, Cupertino, CA, USA) equipped with a frame grabber. The mean autoradiographic density in an area covering the ciliary muscle was measured with a densitometric program (IMAGE: Macintosh) and calibrated against the density of

Table 1 Dissociation constant (K_d) and maximum number of binding sites (B_{max}) for prostaglandin D_2 , E_2 , and $\text{F}_{2\alpha}$ in the human ciliary muscle

	K_d (nM)	B_{max} (fmol/mg tissue)
Prostaglandin D_2	5.2	22
Prostaglandin E_2	0.9	36
Prostaglandin $\text{F}_{2\alpha}$	1.4	21

Values were obtained from Scatchard analysis of the saturation curves shown in Figure 2.

Figure 3 Binding curves for 10 nM tritiated prostaglandin E_2 in the human ciliary muscle after competition with varying concentrations of unlabelled prostaglandin D_2 (●), E_2 (○), $\text{F}_{2\alpha}$ (■), U-46619 (□), 11-deoxy prostaglandin E_1 (▲), 17-phenyltrior prostaglandin $\text{F}_{2\alpha}$ (★), and sulprostone (△). Each value represents an average of four different measurements.

co-exposed ^3H -plastic standards ($[^3\text{H}]$ Microscales, Amersham). This enabled us to compare the density of images from different films and also to convert Gray scales to values of radioactivity per mg tissue. The density values in four different ciliary muscular areas from two eye sections studied under the same experimental conditions were averaged, as any one eye section contained two areas of the ciliary muscle (see Fig 1).

Results

Figure 1 shows autoradiographic views of specific binding sites for 2 nM concentrations of prostaglandin D_2 , E_2 , and $\text{F}_{2\alpha}$ in the human eye sections. Specific binding sites for prostaglandin D_2 , E_2 , and $\text{F}_{2\alpha}$ were concentrated in the same ocular tissues, namely, the ciliary muscle, iris sphincter muscle, iris epithelium, and retina. The binding of 2 nM concentrations of each tritiated prostaglandin was displaced completely by 10 μM concentrations of the corresponding unlabelled prostaglandin.

Figure 2 shows saturation binding curves for the specific binding of prostaglandin D_2 , E_2 , and $\text{F}_{2\alpha}$ in the ciliary muscle and Scatchard analysis of the saturation data. The dissociation constant (K_d) and the maximum number of binding sites (B_{max}) for each prostaglandin are summarised in Table 1. Values of B_{max} were expressed as fmol/mg tissue instead of the more usual fmol/mg protein because these values were obtained by densitometric analysis as described in Materials and methods. Levels of non-specific binding were always less than 20% those of the corresponding total binding. Prostaglandin E_2 had the largest number of binding sites and also the highest affinity binding sites among the three naturally occurring prostaglandins.

Figure 3 shows plots of prostaglandin E_2 binding in the human ciliary muscle after competition with various concentrations of the seven different ligands. Figures 4 and 5 show prostaglandin $\text{F}_{2\alpha}$ and D_2 binding curves after competition with the same seven unlabelled ligands, respectively. The concentrations (IC_{50}) of each ligand required to cause 50% inhibition of the binding of 10 nM tritiated prostaglandins are summarised in Table 2.

Table 2 The concentration (IC₅₀) of each ligand to cause 50% inhibition of the binding of 10 nM prostaglandin D₂, E₂, and F_{2α} in the human ciliary muscle

Ligand for displacement	Prostanoid receptor type	IC ₅₀ for binding of prostaglandin		F _{2α} (M)
		D ₂ (M)	E ₂ (M)	
Prostaglandin D ₂	DP	3.3 × 10 ⁻⁶	2.5 × 10 ⁻⁴	1.8 × 10 ⁻⁶
Prostaglandin E ₂	EP	1 × 10 ⁻⁶	5 × 10 ⁻⁸	1 × 10 ⁻⁹
Prostaglandin F _{2α}	FP	2.7 × 10 ⁻⁴	6 × 10 ⁻⁴	1.2 × 10 ⁻⁷
U-46619	TP	> 10 ⁻³	1.4 × 10 ⁻³	3.8 × 10 ⁻³
11-deoxy prostaglandin E ₁	EP ₂	> 10 ⁻³	7 × 10 ⁻⁶	1 × 10 ⁻⁴
Sulprostone	EP ₁ , EP ₃	> 10 ⁻³	8 × 10 ⁻³	1.8 × 10 ⁻⁷
17-phenyltrilor prostaglandin F _{2α}	FP	1 × 10 ⁻³	6.4 × 10 ⁻³	1 × 10 ⁻⁷

Values were obtained from curves in Figures 3, 4, and 5.

The order of potency to displace the binding of prostaglandin E₂ was: prostaglandin E₂>11-deoxy prostaglandin E₁ (EP₂ agonist)>prostaglandin F_{2α}>prostaglandin D₂>17-phenyltrilor prostaglandin F_{2α} (FP agonist)=sulprostone (EP₁, EP₃ agonist)>U-46619 (TP agonist). This order indicates that the binding of prostaglandin E₂ in the human ciliary muscle is predominantly to the EP₂ receptor subtype.

The order of potency to displace the binding of prostaglandin F_{2α} was: prostaglandin E₂>11-deoxy prostaglandin E₁ (EP₂ agonist)>17-phenyltrilor prostaglandin F_{2α} (FP agonist)=prostaglandin F_{2α}=sulprostone (EP₁, EP₃ agonist)>prostaglandin D₂>U-46619 (TP agonist). Thus prostaglandin E₂ and 11-deoxy prostaglandin E₁ displaced the binding of prostaglandin F_{2α} more effectively than did prostaglandin F_{2α} itself, indicating that prostaglandin F_{2α} binds to the EP₂ receptor and that the number of FP receptors in the ciliary muscle is limited.

The order of potency to displace the binding of prostaglandin D₂ was: prostaglandin E₂>prostaglandin D₂>>prostaglandin F_{2α}>17-phenyltrilor prostaglandin F_{2α} (FP agonist)>sulprostone (EP₁, EP₃ agonist)=11-deoxy

prostaglandin E₁ (EP agonist)=U-46619 (TP agonist). The binding of prostaglandin D₂ was effectively displaced by prostaglandin E₂ but not by 11-deoxy prostaglandin E₁ or sulprostone, implying that prostaglandin E₂ displaced prostaglandin D₂ bound to the DP receptor. This indicates that a smaller number of the DP receptor exists in the ciliary muscle in addition to the EP₂ receptor found in the other two displacement studies. Interestingly, the prostaglandin D₂ binding sites appear to have the same anatomical localisations as the EP₂ sites, namely, the ciliary muscle, iris sphincter muscle, iris epithelium, and retina.

The results described above were reproducible in the eyes from two different individuals.

Discussion

Competitive binding studies using various selective ligands are an important approach to elucidate receptor types in a given tissue. These studies are usually done with membrane preparations obtained by homogenisation of the tissue. In this study, we have used the methods of quantitative receptor autoradiography to accomplish the same end in the ciliary body of human eye sections. This method has an advantage of requiring only very small amounts of tissue – that is, eye sections with 20 μm thickness for each displacement experiment. This method is especially useful when we have to deal with rare human tissues and pathological materials such as glaucomatous eyes. In addition, this method enables us to compare displacement of binding sites in many different ocular tissues simultaneously in the same eye section.

There has been one report which describes prostaglandin E₂-binding studies using membrane preparations of the isolated bovine iris ciliary body.²¹ The binding sites for prostaglandin E₂ detected in this report would be heterogeneous with regard to their locations and receptor types, since the isolated iris ciliary body still contains many different structures as the ciliary muscle, ciliary and iris epithelium. It is also difficult to isolate purely the human ciliary muscle from other surrounding tissues to obtain membrane preparations in an amount suitable for binding studies. In the present experiments, we performed binding studies with eye sections and measured the density of autoradiographic images on the films by computer assisted densitometry to overcome the problems mentioned above.

Prostaglandins have the ability to bind to the ocular pigments,²² which gives rise to a high level of non-specific binding and hampers pharmacological binding studies done with membrane preparations or sections from ocular tissues. We were able to reduce the binding of prostaglandins to ocular pigments by adding a high concentration (5%) of bovine serum albumin in the incubation buffer and by washing sections for a long period (40 minutes in the present studies) as described previously.¹⁵

Saturable and displaceable binding sites for prostaglandin D₂, E₂, and F_{2α} were observed in the human ciliary muscle, indicating that these binding sites could be receptors for prosta-

Figure 4 Binding curves for 10 nM tritiated prostaglandin F_{2α} in the human ciliary muscle after competition with varying concentrations of unlabelled prostaglandin D₂ (●), E₂ (○), F_{2α} (■), U-46619 (□), 11-deoxy prostaglandin E₁ (▲), 17-phenyltrilor prostaglandin F_{2α} (★), and sulprostone (△). Each value represents an average of four different measurements.

Figure 5 Binding curves for 10 nM tritiated prostaglandin D₂ in the human ciliary muscle after competition with varying concentrations of unlabelled prostaglandin D₂ (●), E₂ (○), F_{2α} (■), U-46619 (□), 11-deoxy prostaglandin E₁ (▲), 17-phenyltrilor prostaglandin F_{2α} (★), and sulprostone (△). Each value represents an average of four different measurements.

glandins. The K_d values for prostaglandin D_2 , E_2 , and $F_{2\alpha}$ were in the nanomolar range. These values are in good agreement with dissociation constants for prostaglandins found in membrane preparations of the bovine iris-ciliary body²¹ and of the rat kidney,²³ or in rat brain synaptic membrane preparations,²⁴ suggesting that binding sites in these tissues represent similar populations of prostaglandin receptors.

The K_d values for prostaglandin E_2 (0.9 nM) and $F_{2\alpha}$ (1.4 nM) were similar to each other, while the K_d value for prostaglandin D_2 (5.2 nM) was apparently larger than the other two values. Furthermore, competitive displacement studies demonstrated that prostaglandin E_2 and $F_{2\alpha}$ bound to the same EP_2 receptor in the ciliary muscle. These results indicate that there are at least two different types of binding sites (receptors) for prostaglandins in the human ciliary muscle. Our conclusion is that these are the EP_2 and DP prostanoid receptors, based on the displacement studies as described in Results. There are more EP_2 receptors than DP receptors based on their B_{max} values. The FP receptor could be also present, though in small quantities, in the ciliary muscle, because B_{max} values for prostaglandin E_2 and $F_{2\alpha}$ are apparently different. More definite answers to these questions will be obtained by development of new ligands which are more sensitive and specific to each prostanoid receptor and by their use in the displacement experiments.

In rabbits, the EP_3 prostanoid receptor has been shown to be involved in reduction of intraocular pressure.^{25,26} In cats, prostaglandin-induced ocular hypotension was shown to be mediated by the EP_2 and DP prostanoid receptors.²⁷ There was a correlation between dose related relaxation of the isolated ciliary muscle and reduction of intraocular pressure in cats, which was obtained only with selective EP_2 or DP agonists.

Our findings have indicated that the EP_2 receptor predominates in the human ciliary muscle, as well as a small quantity of the DP receptor. At present it is unknown which prostanoid receptor, EP_2 or DP, plays a more important role in reduction of intraocular pressure. The receptor types in the human ciliary muscle were the same as those found in the cat's ciliary muscle, while different from those in the rabbit's muscle as mentioned above. This means that care must be taken to apply results obtained in animal experiments to humans.

Development of conjunctival and intraocular inflammation is the major adverse effect of prostaglandins used topically as ocular hypotensive drugs.¹⁻¹¹ In rabbits, the EP_2 prostanoid receptor subtype was involved in prostanoid induced breakdown of the blood aqueous barrier,²⁸ in contrast with the EP_3 receptor subtype related to ocular hypotension.²⁶ It is still unknown which subtype of the prostanoid receptor mediates development of intraocular

inflammation in humans. However, the identification of the prostanoid receptor types located on the human ciliary muscle may help to develop pressure-reducing drugs which do not cause such inflammation.

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- Giuffrè G. The effects of prostaglandin F2alpha in the human eye. *Graefes Arch Clin Exp Ophthalmol* 1985; 222: 139-41.
- Lee PY, Shao H, Xu L, Qu CK. The effect of prostaglandin F2alpha on intraocular pressure in normotensive human subjects. *Invest Ophthalmol Vis Sci* 1988; 29: 1474-7.
- Villumsen J, Alm A. Prostaglandin F2alpha isopropylester eye drops: effects in normal human eyes. *Br J Ophthalmol* 1989; 73: 419-26.
- Camras CB, Siebold EC, Lustgarten JS, Serle JB, Frisch SC, Podos SM, et al. Maintained reduction of intraocular pressure by prostaglandin F2alpha-1-isopropyl ester applied in multiple doses in ocular hypertensive and glaucoma patients. *Ophthalmology* 1989; 96: 1329-37.
- Villumsen J, Alm A, Soederstroem M. Prostaglandin F2alpha isopropylester eye drops: effect on intraocular pressure in open-angle glaucoma. *Br J Ophthalmol* 1989; 73: 975-9.
- Stern FA, Bito LZ. Comparison of the hypotensive and other ocular effects of prostaglandin E2 and F2alpha on cat and rhesus monkey eyes. *Invest Ophthalmol Vis Sci* 1982; 22: 588-98.
- Bito LZ, Draga A, Blanco J, Camras CB. Long-term maintenance of reduced intraocular pressure by daily or twice daily topical application of prostaglandins to cat or rhesus monkey eyes. *Invest Ophthalmol Vis Sci* 1983; 24: 312-9.
- Bito LZ, Srinivasan BD, Baroddy RA, Schubert H. Noninvasive observations on eyes of cats after long-term maintenance of reduced intraocular pressure by topical application of prostaglandin E2. *Invest Ophthalmol Vis Sci* 1983; 24: 376-80.
- Bito LZ. Comparison of the ocular hypotensive efficacy of eicosanoids and related compounds. *Exp Eye Res* 1984; 38: 181-94.
- Goh Y, Nakajima M, Azuma I, Hayaishi O. Prostaglandin D2 reduces intraocular pressure. *Br J Ophthalmol* 1988; 72: 461-4.
- Woodward DF, Hawley SB, Williams LS, Ralston TR, Protzman CE, Spada CS, et al. Studies on the ocular pharmacology of prostaglandin D2. *Invest Ophthalmol Vis Sci* 1990; 31: 138-46.
- Gabelt BT, Kaufman PL. Prostaglandin F2alpha increases uveoscleral outflow in the cynomolgus monkey. *Exp Eye Res* 1989; 49: 389-402.
- Nilsson SFE, Samuelsson M, Bill A, Stjernschantz J. Increased uveoscleral outflow as a possible mechanism of ocular hypotension caused by prostaglandin F2alpha-1-isopropylester in the cynomolgus monkey. *Exp Eye Res* 1989; 48: 707-16.
- Crawford K, Kaufman PL. Pilocarpine antagonizes prostaglandin F2alpha-induced ocular hypotension in monkeys. Evidence for enhancement of uveoscleral outflow by prostaglandin F2alpha. *Arch Ophthalmol* 1987; 105: 1112-6.
- Matsuo T, Cynader MS. Localisation of prostaglandin F2alpha and E2 binding sites in the human eye. *Br J Ophthalmol* 1992; 76: 210-3.
- Kennedy I, Coleman RA, Humphrey PPA, Levy GP, Lumley P. Studies on the characterization of prostanoid receptors: a proposed classification. *Prostaglandins* 1982; 24: 667-89.
- Coleman RA, Humphrey PPA, Kennedy I, Lumley P. Prostanoid receptors. The development of a working classification. *Trends Pharmacol Sci* 1984; 5: 303-6.
- Dong YJ, Jones RL, Wilson NH. Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues. *Br J Pharmacol* 1986; 87: 97-107.
- Eglen RM, Whiting RL. Characterization of the prostanoid receptor profile of enprostil and isomers in smooth muscle and platelets in vitro. *Br J Pharmacol* 1989; 98: 1335-43.
- Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R, Nakanishi S, et al. Cloning and expression of cDNA for a human thromboxane A2 receptor. *Nature* 1991; 349: 617-20.
- Bhattacharjee P, Csukas S, Paterson CA. Prostaglandin E2 binding sites in bovine iris-ciliary body. *Invest Ophthalmol Vis Sci* 1990; 31: 1109-13.
- Aula P, Kaila T, Huupponen R, Salminen L, Iisalo E. Prostaglandin F2alpha binding to bovine ocular and synthetic melanins in vitro. *Pharmacol Toxicol* 1989; 65: 100-3.
- Eriksson LO, Larsson B, Andersson KE. Biochemical characterization and autoradiographic localization of [3H]PGE2 binding sites in rat kidney. *Acta Physiol Scand* 1990; 139: 405-15.
- Shimizu T, Yamashita A, Hayaishi O. Specific binding of prostaglandin D2 to rat brain synaptic membrane. Occurrence, properties, and distribution. *J Biol Chem* 1982; 257: 13570-5.
- Woodward DF, Burke JA, Williams LS, Palmer BP, Wheeler LA, Woldemussie E, et al. Prostaglandin F2alpha effects on intraocular pressure negatively correlate with FP-receptor stimulation. *Invest Ophthalmol Vis Sci* 1989; 30: 1838-42.
- Waterbury LD, Eglen RM, Fautot GF, Cooper GF. EP3, but not EP2, FP, or TP prostanoid-receptor stimulation may reduce intraocular pressure. *Invest Ophthalmol Vis Sci* 1990; 31: 2560-7.
- Chen J, Protzman CE, Woodward DF. Comparison of the pharmacology of prostanoid induced ciliary muscle relaxation and ocular hypotension reveals a correlation. ARVO abstracts. Supplement to *Invest Ophthalmol Vis Sci*. Philadelphia: Lippincott, 1991: 870.
- Protzman CE, Woodward DF. Prostanoid-induced blood-aqueous barrier breakdown in rabbits involves the EP2 receptor subtype. *Invest Ophthalmol Vis Sci* 1990; 31: 2463-6.