Effect of Prostaglandin $F_{2\alpha}$ on Aqueous Humor Dynamics of Rabbit, Cat, and Monkey

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Topical administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produced a reduction in intraocular pressure in eyes of rabbits, cats, and cynomolgus monkeys. In rabbit eyes at 5 or 6 hr, 50 μg, 100 μg, or 250 μg of $PGF_{2\alpha}$ caused a significant intraocular pressure reduction with a small miotic effect. Treatment with 500 μg, 750 μg, or 1000 μg of $PGF_{2\alpha}$ lowered intraocular pressure significantly in cat eyes for at least 24 hr with the development of profound pupillary constriction. Administration of 500 μg, 750 μg, or 1000 μg of $PGF_{2\alpha}$ produced a significant reduction of intraocular pressure in monkey eyes lasting at least 24 hr, with an initial hypertensive phase and a small decrease in pupillary diameter in the treated eyes. Tonography revealed an increased facility of outflow simultaneous with the reduction of intraocular pressure in the eyes of cats and monkeys. These increases of outflow facility could not explain completely the reductions in intraocular pressure. The aqueous humor flow measured by fluorophotometry was unaltered in both species, and possible reasons for this finding are discussed. Anterior chamber aqueous humor protein was significantly higher in cat eyes topically treated with 750 μg of $PGF_{2\alpha}$ than in the diluent-treated fellow eyes. Invest Ophthalmol Vis Sci 25:1087–1093, 1984

Early studies of the effect of prostaglandins (PGs) on intraocular pressure led to the general conclusion that PGs, administered topically or systemically, elevated intraocular pressure in rabbits, cats, and monkeys.[1–5] More recently, some studies have shown that topical application of either PGE_2 or $PGF_{2\alpha}$ effectively reduced the intraocular pressure in rabbits, cats, and monkeys.[6–8] Those studies suggested that PGs, especially $PGF_{2\alpha}$ and/or its analogues, may provide a new therapeutic approach to the clinical control of intraocular pressure and the treatment of glaucoma.

The present study was designed to investigate further the mechanism of the hypotensive effect of $PGF_{2\alpha}$ on rabbit, cat, and monkey eyes.

Materials and Methods

Adult, albino, unanesthetized rabbits, 2–3 kg, were restrained. Eleven adult cats, 2.5–3.5 kg, and eight, adult, cynomolgus monkeys, 4–5 kg, were lightly tranquilized with 5–10 mg/kg of ketamine. The cats were restrained, and the monkeys were kept in primate chairs throughout each experiment.

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Intraocular pressure was measured under 0.5% topical proparacaine hydrochloride anesthesia using a manometrically calibrated Alcon pneumatonometer. New animals were acclimated to the tonometer by undergoing several readings the day before they were to be used in an experiment. Two sets of baseline readings were taken each day before 9 AM.

Pupillary diameters were measured with a millimeter ruler in normal room light. In cats, the horizontal (shorter) diameter always was recorded.

The aqueous flare and cellular response in the anterior chamber were assessed by slit-lamp examination and rated from 0 to 3 (aqueous flare: 0 = no Tyndall effect; 1+ = slight Tyndall effect; 2+ = moderate to dense Tyndall effect; 3+ = dense Tyndall effect with fibrin clots; cellular response: 0 = no cells apparent; 1+ = few cells; 2+ = many cells; 3+ = cell clumps).

Following these baseline observations, a 5 mg per ml solution of $PGF_{2\alpha}$ (each ml of this solution contains prostaglandin $F_{2\alpha}$ tromethamine salt equivalent to 5 mg prostaglandin $F_{2\alpha}$, and benzyl alcohol, 9.45 mg, added as a preservative. The Upjohn Co. (Kalamazoo, MI), diluted with normal saline to various concentrations, was applied topically to one eye of each animal. As topical application of an aqueous solution containing 9.45 mg per ml of benzyl alcohol did not alter the intraocular pressure in our trials with cynomolgus monkeys, we used an equal volume of normal saline applied to the contralateral eye as the control. All the drugs were made up just prior to their administration. The following amounts of $PGF_{2\alpha}$ were applied: rabbits—1 μg in 1 μl, 5 μg in 1 μl, 25 μg in 5 μl, 50 μg

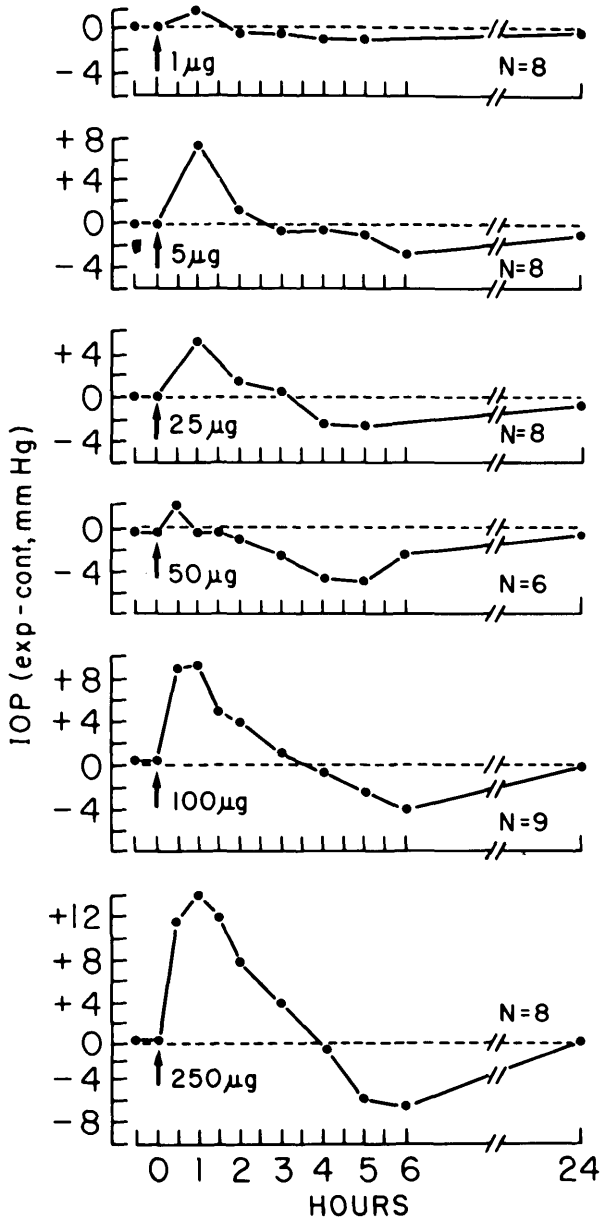

Fig. 1. Effects of topical application of 1–250 μg of $PGF_{2\alpha}$ on the intraocular pressure of rabbits. Points represent the mean pressure values. The greatest SE was ±2.1 mmHg.

in 50 μl, 100 μg in 50 μl, and 250 μg in 50 μl; cats and monkeys—250 μg, 500 μg, 750 μg, 1000 μg as 250 μg in 50 μl given one, two, three, or four times, respectively, 3–5 min apart. Repeat, intraocular pressure measurements were made at 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 24 hr after instillation of $PGF_{2\alpha}$.

Tonography was performed with an electronic tonometer (Alcon EDT-103) in 21 cats and 20 monkeys. Baseline outflow facility was determined at 8:30 AM–9 AM. $PGF_{2\alpha}$ (750 μg in cats and 500 μg in monkeys) was applied randomly to one eye and an equal volume of normal saline to the contralateral eye 2 hr after baseline measurements. The tonography results were obtained at 2 hr (cats) or 4 hr (monkeys) after instillation of $PGF_{2\alpha}$. Tonography values were approximated from the 1955 Friedenwald tables.

Aqueous humor flow was estimated using a fluorophotometric technique[9] on 14 cats and 10 monkeys. The fluorescein iontophoresis was done at 4 PM and fluorescence measurements were made from 9 AM–2 PM on the following day. The iontophoresis was carried out in the central 4 mm of the cornea with an electrode of 10% fluorescein in 2% agar. A current of 200 μA was used for 5 min. Fluorophotometric measurements of the cornea and anterior chamber were repeated at about 60-min intervals. Five to six such measurements were made. Following these baseline measurements, on another day, $PGF_{2\alpha}$ (750 μg in cats and 500 μg in monkeys) was topically applied to one eye of each animal at about 8:30 AM. An equal volume of normal saline was applied to the control eye. The iontophoresis was carried out at 4 PM on the preceding day as described above. Fluorophotometric measurements were taken from 1–6 hr after instillation of $PGF_{2\alpha}$. The cornea and anterior chamber readings were divided by the reference filter reading and the ratio (F) was recorded. For each animal, the natural logarithm of F was plotted versus time. The lines of best fit and their slopes were calculated by the least-squares method.

The value of aqueous flow was calculated by the mathematical assumptions of Yablonski and co-workers.[10] The value of A used for each eye was midway between the absolute values of the slopes of the anterior chamber and cornea lines of best fit. The value of Fc/Fa was determined from the corresponding lines of best fit at 2 hr (cats) or 4 hr (monkeys) after $PGF_{2\alpha}$ administration. Values of 853 μl for anterior chamber volume[11] and 296 μl for cornea volume[12] in cats were used in the calculations. Values of 106 μl[13] for anterior chamber volume and 50 μl (unpublished data, M. E. Yablonski and J. B. Serle) for cornea volume in monkeys were used in the calculations.

Seven hundred fifty micrograms of $PGF_{2\alpha}$ were instilled in one eye of awake, restrained cats, control solution in the other eye. Two hours later, under ketamine anesthesia, a 25-gauge needle was inserted through clear cornea and aqueous humor withdrawn. Care was taken to avoid the iris and lens. Aqueous humor protein concentrations were measured by the method of Lowry and co-workers.[14]

These experiments adhered to the ARVO resolution on the use of experimental animals in research.

Results

Intraocular Pressure

Rabbits. $PGF_{2\alpha}$ administered topically to rabbit eyes often induced a biphasic intraocular pressure response:

a relatively short, initial, hypertensive phase followed by prolonged hypotony. Dose–response relationships could be demonstrated (Fig. 1). Topical application of 100 μg or 250 μg of PGF$_{2\alpha}$ produced a significant ($P < 0.01$) initial increase in intraocular pressure. Topical application of all doses of PGF$_{2\alpha}$ produced a significant ($P < 0.05$) ocular hypotony at 5 or 6 hr. The greatest hypotensive response was observed in eyes given 250 μg of PGF$_{2\alpha}$.

Cats. Topical application of 500–1000 μg PGF$_{2\alpha}$ to the eyes of cats produced a significant ($P < 0.05$) decrease in intraocular pressure, as compared with the pressure of the control eyes, occurring between 30 min–24 hr after PGF$_{2\alpha}$ administration. The greatest hypotensive response was observed in eyes given 750 μg of PGF$_{2\alpha}$ at 2 hr ($P < 0.001$). There was no transient ocular hypertensive response in cats. Dose–response relationships could be shown (Fig. 2).

Monkeys. Topical application of 250 μg, 500 μg, 750 μg, or 1000 μg of PGF$_{2\alpha}$ to one eye of monkeys resulted in a biphasic intraocular pressure response: a relatively short initial hypertensive phase followed by a prolonged hypotony (Fig. 3). The maximum rise of

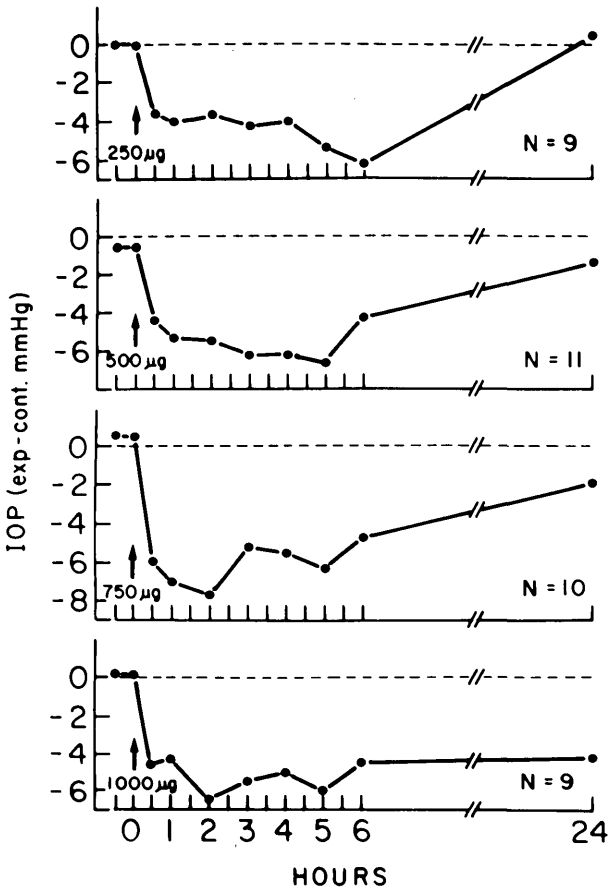

Fig. 2. Effects of topical application of 250–1000 μg of PGF$_{2\alpha}$ on the intraocular pressure of cats. Points represent the mean pressure values. The greatest SE was ±1.7 mmHg.

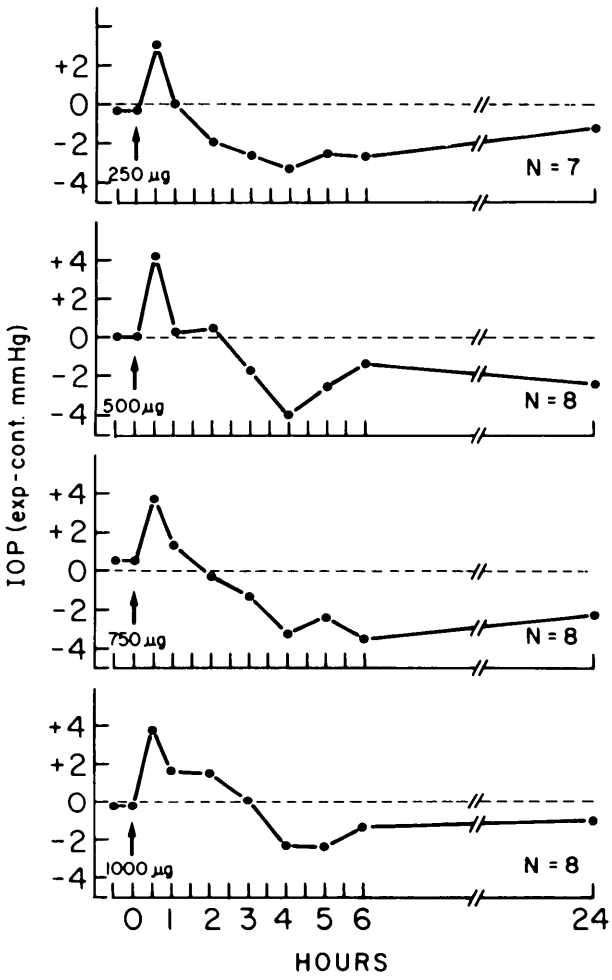

Fig. 3. Effects of topical application of 250–1000 μg of PGF$_{2\alpha}$ on the intraocular pressure of monkeys. Points represent the mean pressure values. The greatest SE was ±1.7 mmHg.

the pressure occurred at 30 min. The intraocular pressure then rapidly decreased. The maximum ocular hypotensive response occurred after topical application of 500 μg of PGF$_{2\alpha}$, with a significant ($P < 0.001$) decrease in intraocular pressure of 4 mmHg at 4 hr, as compared with the pressure of the control eyes. The intraocular pressure was significantly ($P < 0.05$) reduced up to 24 hr by 500–1000 μg PGF$_{2\alpha}$.

Miotic Response

Rabbits. Topical application of 50 μg or 100 μg of PGF$_{2\alpha}$ produced a miotic response ($P < 0.05$) of 1 mm at 1.5 hr, which returned to baseline values at 5 hr (Fig. 4).

Cats. Topical administration of 500 μg, 750 μg, or 1000 μg of PGF$_{2\alpha}$ caused significant ($P < 0.01$) miotic responses similar in magnitude (Fig. 5). A dose of 500 μg of PGF$_{2\alpha}$ produced an apparently maximum miotic response (9 mm decrease in pupillary diameter) at 1 hr, the pupillary diameter 7 mm less than the control

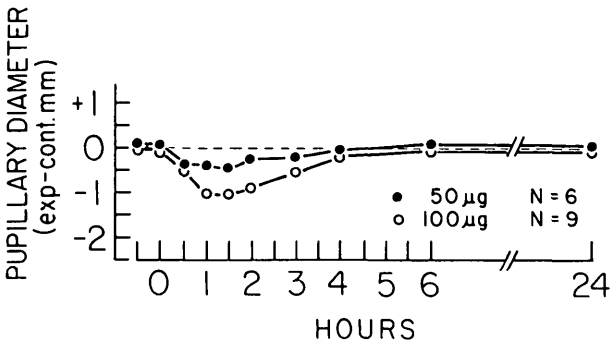

Fig. 4. Effects of topically applied $PGF_{2\alpha}$ on the pupillary diameter of rabbit eyes as compared with the pupillary diameter of control eyes. The greatest SE was ±0.4 mm.

eyes for 5 hr, followed by redilation to near baseline values at 24 hr.

Monkeys. Topical application of $PGF_{2\alpha}$ doses produced a small decrease in pupillary diameter in the treated eyes and an increase in the control eyes. The effects of topically applied $PGF_{2\alpha}$ on the pupillary size of monkeys occurred between 15 min and 4 hr after $PGF_{2\alpha}$ administration (Fig. 6). The miotic response of the treated eyes and the dilation of the pupil of the control eyes were significant ($P < 0.02$) as compared with the baseline values 0.5 hr after the application of 1000 µg of $PGF_{2\alpha}$. Topical application of $PGF_{2\alpha}$ in amounts of 250–1000 µg produced a significantly ($P < 0.05$) smaller pupil in treated eyes as compared with control eyes at various times.

Aqueous Flare and Cellular Response in the Anterior Chamber

Cellular response in the anterior chamber was not observed under slit-lamp examination in any of these animals at any time after the topical application of $PGF_{2\alpha}$.

Some aqueous flare was observed in the anterior chamber of the treated eye of most rabbits at 0.5–5 hr after the topical application of 50 µg or 100 µg of $PGF_{2\alpha}$ (Fig. 7). Topical application of $PGF_{2\alpha}$ in amounts of 50 µg at 1–3 hr ($P < 0.05$) and 100 µg at 0.5–6 hr ($P < 0.005$) produced a significant aqueous flare in treated eyes as compared with control eyes.

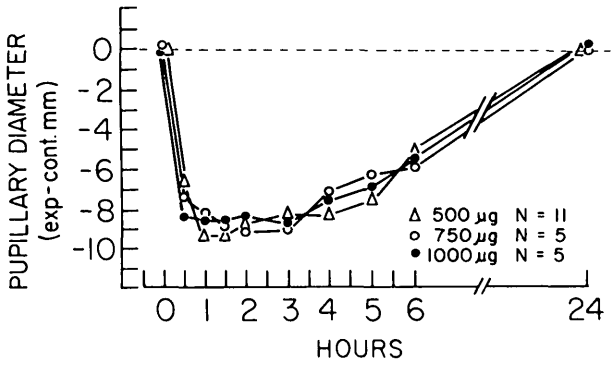

Fig. 5. Effects of topically applied $PGF_{2\alpha}$ on the pupillary diameter of cat eyes as compared with the pupillary diameter of control eyes. The greatest SE was ±1.2 mm.

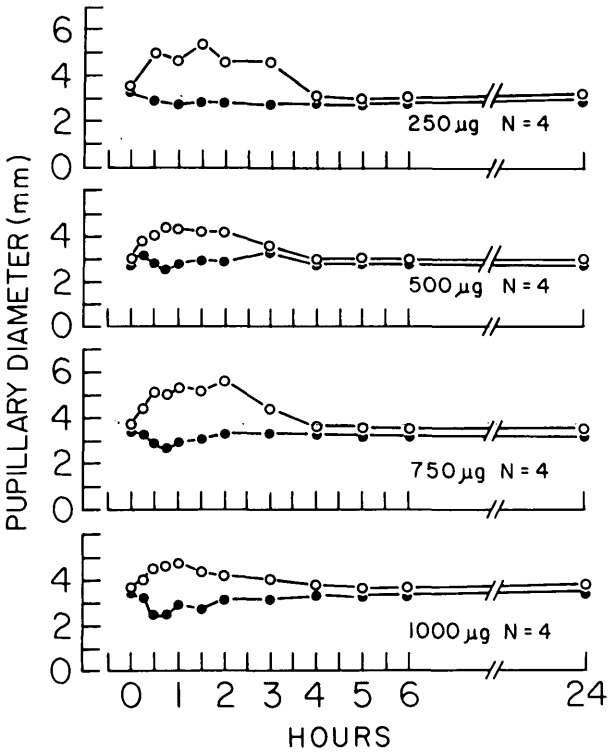

Fig. 6. Effects of topically applied $PGF_{2\alpha}$ on the pupillary diameter of PG-treated (-·-·-·-) and control (-O-O-O-) eyes of all observed monkeys. The greatest SE was ±0.6 mm.

Aqueous flare was observed in the anterior chamber of the treated eye of some cats at 2–6 hr after 500 µg, 750 µg, or 1000 µg of $PGF_{2\alpha}$ administration (Fig. 8). This was significant ($P < 0.05$) as compared with control eyes at 3 hr after 500–1000 µg of $PGF_{2\alpha}$ application.

Aqueous flare was not observed in any of the eyes of monkeys at any time after the topical application of 250–1000 µg of $PGF_{2\alpha}$.

Outflow Facility

In 21 cats, 2 hr after a topical dose of 750 µg of $PGF_{2\alpha}$, the intraocular pressure was ($P < 0.001$) reduced significantly in treated eyes as compared with baseline values and control eyes, and the mean outflow facility was increased significantly ($P < 0.001$) 48 ± 12% as compared with control eyes. In the control eyes, the outflow facility was not significantly altered, as compared with baseline values.

In monkey eyes 4 hr after administration of 500 µg of $PGF_{2\alpha}$, the intraocular pressure was significantly reduced as compared with baseline values ($P < 0.005$)

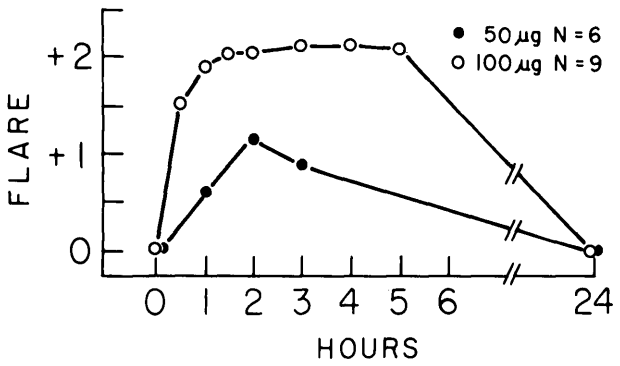

Fig. 7. Development of anterior chamber flare in all observed rabbit eyes after the topical application of 50 μg or 100 μg of PGF$_{2\alpha}$. The greatest SE was ±0.3 scale units.

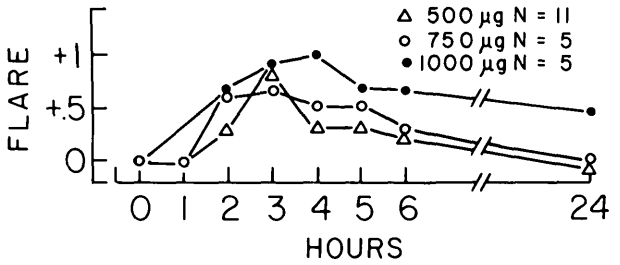

Fig. 8. Development of anterior chamber flare in all observed cat eyes after the topical application of 500 μg, 750 μg, or 1000 μg of PGF$_{2\alpha}$. The greatest SE was ±0.2 scale units.

and to control eyes ($P < 0.001$), and the outflow facility was increased significantly as compared with baseline values ($P < 0.05$) and control eyes ($P < 0.025$) (Table 1). In the control eyes, PGF$_{2\alpha}$ did not produce a contralateral alteration of outflow facility.

Aqueous Flow

Values of aqueous humor flow were not ($P > 0.4$) changed significantly by unilateral administration of 750 μg PGF$_{2\alpha}$ in cats and 500 μg PGF$_{2\alpha}$ in monkeys. Baseline aqueous humor flow (mean μl/min ± SE) in treated and control eyes, respectively, was 22.5 ± 2.1 and 22.7 ± 3.6 in nine cats. Two hours after unilateral administration of 750 μg PGF$_{2\alpha}$ aqueous humor flow in 14 cats was similar in the treated eyes, 18.7 ± 1.6, and control eyes, 20.9 ± 1.7. Aqueous humor flow in the treated eyes of 10 monkeys was 1.9 ± 0.1 prior to and 1.8 ± 0.1, 4 hr after treatment and was 1.9 ± 0.1 prior to and 1.8 ± 0.1 after diluent in control eyes.

Aqueous Humor Protein

The protein level in the aqueous humor of the treated eyes of 11 cats, 2.02 ± 0.33 mg/ml, 2 hr after 750 μg of PGF$_{2\alpha}$, was significantly ($P < 0.001$) higher than that of the control eyes, 0.45 ± 0.07 mg/ml.

Discussion

The results presented here show that topical application of PGF$_{2\alpha}$ can reduce effectively intraocular pressure in rabbits, cats, and cynomolgus monkeys. There are, however, significant species differences. Rabbit and cynomolgus monkey eyes have a similar tendency to an initial hypertension before the onset of PG-induced hypotension. Cynomolgus monkey eyes are less sensitive than rabbit eyes to the hypertensive effects of topically administered PGF$_{2\alpha}$. No initial hypertension occurs in the eyes of cats after the topical application of PGF$_{2\alpha}$ in doses that are highly effective in reducing intraocular pressure. Moreover, the duration of intraocular pressure reduction that follows topical PGF$_{2\alpha}$ application is much longer in cat or monkey eyes than that in the eyes of rabbits. Cat eyes are clearly more sensitive to the hypotensive effects of PGF$_{2\alpha}$ than the eyes of rabbits and monkeys. Our results are similar to previously reported findings on the effects of topically applied PGs on the eyes of rabbits,[6] cats,[7] and monkeys.[7,8] These species differences in the duration of the hypotensive effect of PGs may arise from differences between the ocular pharmacokinetics of PGs in these species.[7]

PGF$_{2\alpha}$ reduces intraocular pressure in various species of monkeys. Previous experiments show that topical application of a single dose of 1000 μg PGF$_{2\alpha}$ onto the cornea of five, trained, owl monkeys produces a prolonged and highly significant ocular hypotony. The intraocular pressure of the treated eye was 4.7 ± 0.9 mmHg below that of the control eye 18 to 24 hr after PGF$_{2\alpha}$ application and remained significantly reduced for over 72 hr.[8] Topical application of either PGF$_{2\alpha}$ or PGE$_2$ to the eyes of rhesus monkeys also causes significant dose-dependent reduction in intraocular pressure.[7] The present experiments indicate that topical application of 500 μg of PGF$_{2\alpha}$ administered to the eyes of cynomolgus monkeys causes significant reduction in intraocular pressure at 3–24 hr after application. The maximum decrease of 4 mmHg below

Table 1. The effect of 500 μg PGF$_{2\alpha}$ on the outflow facility of 20 monkeys

	Intraocular pressure		*Outflow facility*	
	Mean ± SE (mmHg)		*Mean ± SE (μl/min/mmHg)*	
Treatment	*0 min*	*4 hr*	*0 min*	*4 hr*
PGF$_{2\alpha}$	18.3 ± 0.4	14.9 ± 0.8*	0.48 ± 0.03	0.60 ± 0.04†
Control	17.8 ± 0.5	17.7 ± 0.8	0.50 ± 0.03	0.49 ± 0.04

* Significantly different as compared with 0 min ($P < 0.005$) and with control eyes ($P < 0.001$), paired t-test.

† Significantly different as compared with 0 min ($P < 0.05$) and control eyes ($P < 0.025$), paired t-test.

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