Multiple Dosing of Prostaglandin $F_2\alpha$ or Epinephrine on Cynomolgus Monkey Eyes

I. Aqueous Humor Dynamics

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After obtaining baseline intraocular pressure (IOP) measurements for 1 week, prostaglandin (PG) $F_2\alpha$ (250 μg in 50 μl saline) or epinephrine 2% solution (50 μl) was topically applied twice daily for 2 weeks to one eye of six cynomolgus monkeys for each agent tested. Contralateral control eyes received their respective vehicles. $PGF_2\alpha$ significantly reduced IOP beginning 2 to 3 hr after the first dose, persisting thereafter. A significant ($P < 0.05$) hypotensive effect remained for at least 10 hr after the first dose and at least 14 hr after the sixth dose. At 4 hr after the seventh dose, the mean reduction was 10.2 ± 3.5 (±SD) mmHg below baseline ($P < 0.0025$). At this time, there was also a significant ($P < 0.01$) mean reduction of IOP in the contralateral vehicle-treated eyes of 6.0 ± 3.3 (±SD) mmHg below baseline, which did not appear to be secondary to diurnal fluctuations, repeated tonometry, experimental manipulation, or inadvertent drug transfer. Epinephrine significantly ($P < 0.05$) reduced IOP beginning 3 hr after the first dose, but this reduction was minimal and not consistent. Neither $PGF_2\alpha$ nor epinephrine altered aqueous flow as measured by fluorophotometry 2 to 6 hr after the fifth dose. Outflow facility could not be assessed by indentation tonography because IOP was often too low at the time of measurement. Whereas $PGF_2\alpha$ did not alter pupil size, epinephrine caused significant pupillary dilation. The results of this study demonstrate that multiple topical dosing with $PGF_2\alpha$ in normal subhuman primate eyes effectively produced a maintained reduction of IOP without evidence of tachyphylaxis or tolerance. These results suggest that $PGF_2\alpha$, or one of its analogues, warrants a clinical trial in glaucoma patients. Invest Ophthalmol Vis Sci 28:463–469, 1987

Although early studies showed that exogenously applied prostaglandins (PGs) caused an initial rise in intraocular pressure (IOP), especially when applied in high doses in rabbits,[1] recent studies have demonstrated that the predominant effect of topically applied PGs is to reduce IOP, especially in cats and primates.[2]

Topical application of relatively low doses of several PGs in single-dose studies resulted in a highly significant and prolonged reduction of IOP in rabbit,[3-5] cat,[4,6,7] and primate[4,6,8,9] normotensive eyes with no adverse effect, or with only minimal inflammation. Also, a single dose of topically applied $PGF_2\alpha$ reduced

IOP in glaucomatous subhuman primate eyes.[8,10] Whereas multiple dosing of PGE_2 in cats produced a maintained reduction of IOP for at least 9 months when applied once or twice daily, multiple dosing with $PGF_2\alpha$ in rabbits showed rapid development of tachyphylaxis, with failure to maintain a reduced IOP after the third dose when administered at 8 or 12 hr intervals.[11] Although some evidence was reported that multiple dosing in rhesus monkeys may be free of tachyphylaxis, this data was not conclusive, since it was based on results from only two animals.[11]

Some evidence exists suggesting that topically applied epinephrine may reduce IOP by stimulating endogenous PG synthesis. Alpha adrenergic activity has been shown to stimulate PG synthesis in rabbit and bovine ocular tissues.[12,13] Topical application of indomethacin solution, an effective inhibitor of PG biosynthesis,[14,15] has been shown to inhibit long-term epinephrine-induced ocular hypotony in rabbits.[16] Another study suggested that prostacyclin mediated the alpha adrenergic-induced reduction of IOP in rabbits.[17] A recent study demonstrated that systemically administered indomethacin inhibited the epinephrine-in-

duced reduction of IOP in patients with glaucoma or ocular hypertension.[18]

The purpose of the present study was twofold. First, the effect of multiple dosing of $PGF_2\alpha$ on the reduction of IOP in cynomolgus monkeys was evaluated to determine whether or not tachyphylaxis develops. Secondly, epinephrine was topically applied in a similar manner to determine whether it reduced IOP by a mechanism similar to that of $PGF_2\alpha$. A comparison of other pathophysiological effects of multiple dosing of $PGF_2\alpha$ or epinephrine on these monkeys is described elsewhere.[19]

Materials and Methods

Twelve adult female cynomolgus monkeys, 3–5 kg, were used in this study. They were secured in specially designed primate chairs in a sitting position during the experiments. Most IOPs were determined without any sedation to these restrained animals accustomed to handling. Occasionally, ketamine hydrochloride (50 mg/ml; Ketalar; Parke-Davis, Morris Plains, NJ) 1–5 mg/kg was required intramuscularly 5 min prior to tonometry for adequate sedation. IOP was measured with a calibrated pneumatonometer (Model 30R; Digilab, Inc., Cambridge, MA) following topical application of one drop of proparacaine hydrochloride 0.5% (Alcaine, Alcon, Humacao, Puerto Rico) as previously described.[10] Since tonometry tracings could not be consistently obtained when IOP dropped below 5 mmHg, these measurements were assumed to be 5 mmHg. Pupillary diameters were measured with a millimeter ruler under standard room illumination which was not varied.

$PGF_2\alpha$ tromethamine salt (Sigma Chemical Co., St. Louis, Mo.) 250 μg (in terms of the free acid equivalent, which is equivalent to 334 μg of the salt) in 50 μl of normal saline was topically applied to the cornea of one eye of six cynomolgus monkeys. The saline vehicle solution was applied to the contralateral control eyes of these six animals. Six additional monkeys received 50 μl of epinephrine hydrochloride 2% (Epifrin, Allergan Pharmaceuticals, Irvine, CA) to the cornea of one eye, with the contralateral control eye receiving vehicle solution (Allergan Pharmaceuticals). Each cornea and conjunctival cul-de-sac was thoroughly rinsed with approximately 2 ml of normal saline 1–2 min following topical application of the drug or its vehicle. These solutions were applied twice daily 5 days each week for 2 weeks at approximately 0800 and 1800 hr.

Seven days prior to the start of drug application (day −7) and on the day that treatment was begun (day 0), IOP and pupillary diameters were determined at 0745, 0830, 0900, 1000, 1100, 1200, 1400, 1600, and 1800

hr. On day −4 and again on day +3, these measurements were repeated at 0745, 0830, 0900, 1000, and 1200 hr. The term "baseline measurements" used subsequently in this paper refers to IOPs measured 7 days previously in the same eye of the same monkey at the same time of day.

Iontophoresis with 10% fluorescein in 2% agar was performed just after the fourth consecutive dose using a current of 200 μA for 7 min as previously described.[4] Under adequate ketamine sedation (3–8 mg/kg intramuscularly 5 min beforehand), 3–6 aqueous humor fluorophotometry measurements were perfomed at 60–90 min intervals within 2–6 hr after the fifth consecutive dose. These measurements were made using a scanning, computerized fluorophotometer (Fluorotron Master, Coherent Medical Division, Palo Alto, CA) with a software program designed by Yablonski based on previous studies,[20,21] which was shown to yield similar aqueous flow measurements when compared with a standard slit-lamp fluorophotometer.[22,23] Values of 102 μl for anterior chamber volume and 40 μl for corneal volume were used, based on previous measurements.[24]

Outflow indentation tonography was performed 4 hr after the seventh dose using an electronic tonography unit (Model EDT-103, Alcon Laboratories, Fort Worth, TX) with calculations approximated from standard human tonography tables[25] based on work by Friedenwald.

This investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Results

Twice daily topical application of $PGF_2\alpha$ to cynomolgus monkey eyes produced a highly significant and prolonged reduction of IOP which began 2–3 hr after the first dose when compared with either baseline measurements (Fig. 1) or the contralateral vehicle-treated eyes (Fig. 2). This significant ($P < 0.05$) reduction of IOP lasted at least 10 hr after the first dose. Fourteen hr following the sixth consecutive dose, IOP remained significantly ($P < 0.0025$ vs baseline; $P < 0.05$ vs contralateral control) reduced in the $PGF_2\alpha$-treated eyes. The mean reduction of IOP was as much as 10.2 ± 3.5 (±SD) mmHg below baseline ($P < 0.0025$) 4 hr after the seventh consecutive dose (Fig. 1).

In the $PGF_2\alpha$-treated monkeys, comparing the IOP of the contralateral vehicle-treated eyes with baseline measurements obtained in these same eyes 1 week previously, there was no significant contralateral effect following the first application of $PGF_2\alpha$ as measured throughout the day (Fig. 3). However, following the seventh consecutive dose of $PGF_2\alpha$, the IOP of the
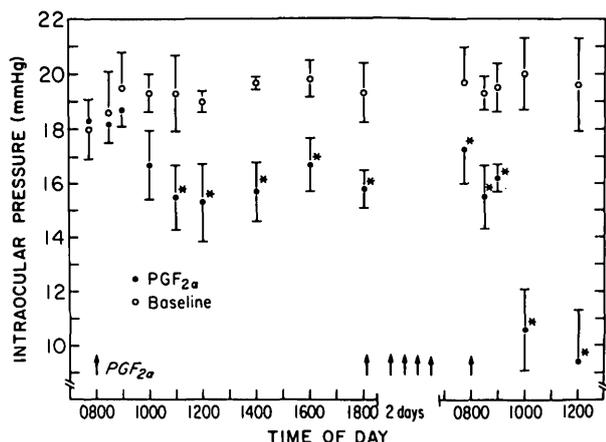
Fig. 1. Effects of multiple dosing with PGF$_2\alpha$ (●; 250 μg in 50 μl) on IOP compared with baseline measurements (O) taken one week previously in six cynomolgus monkey eyes. In all figures, time of day is indicated for the first and fourth day of treatment separated by 2 days of treatment without measurements. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly (*P* < 0.05) different from controls using one-tailed, paired student t-test.

Fig. 3. Effects on IOP in the contralateral control eyes (●) of six cynomolgus monkeys receiving multiple doses of PGF$_2\alpha$ to one eye compared with baseline measurements (O) taken 1 week previously in these same eyes. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly (*P* < 0.05) different from controls using one-tailed, paired student t-test.

contralateral vehicle-treated eyes progressively fell compared with baseline measurements. Four hr following this dose, IOP was reduced in the contralateral vehicle-treated eyes by 6.0 ± 3.3 (±SD) mmHg below baseline (*P* < 0.01) (Fig. 3).

Twice daily topical application of epinephrine 2% produced a minimal reduction of IOP, which was not consistent and did not persist throughout the experiment. With one exception, no statistically significant difference in IOP between the epinephrine-treated eyes and their baseline measurements was observed
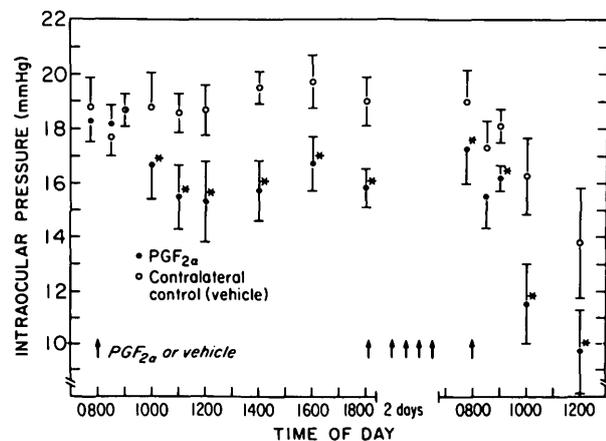
throughout the experiment, although there was a tendency for the IOP to be lower after treatment began (Fig. 4). Differences in IOP between epinephrine and contralateral vehicle-treated eyes reached statistical significance (*P* < 0.05) only when measured 3 or 8 hr after the first application or 1 hr after the seventh application, and were never greater than 1.7 ± 1.8 (±SD) mmHg (Fig. 5). In the epinephrine-treated monkeys, the IOPs of the contralateral vehicle-treated eyes were never significantly different from their baseline measurements (Fig. 6).

Fig. 2. Effects on IOP of multiple dosing with PGF$_2\alpha$ (●) to one eye and its vehicle (O) to the contralateral control eye of six cynomolgus monkeys. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly (*P* < 0.05) different from controls using one-tailed, paired student t-test.
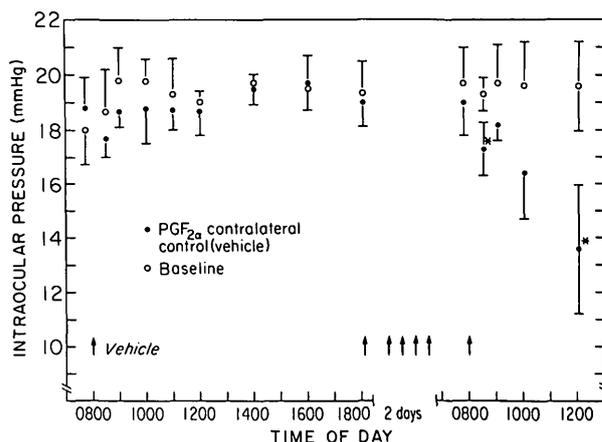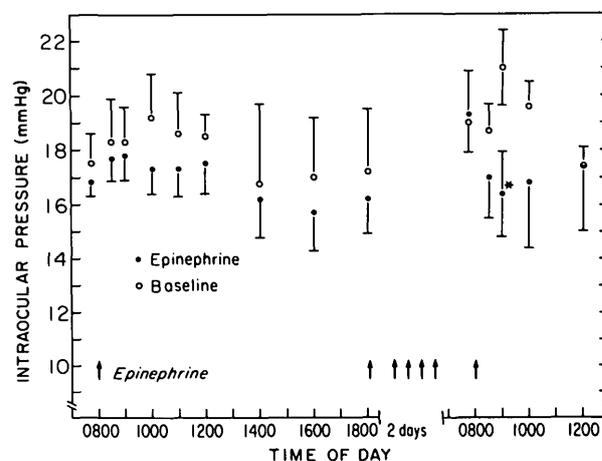

Fig. 4. Effects of multiple dosing with epinephrine 2% (●; 50 μl) on IOP compared with baseline measurements (O) taken 1 week previously in 6 cynomolgus monkey eyes. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly (*P* < 0.05) different from controls using one-tailed, paired student t-test.

Fig. 5. Effects on IOP of multiple dosing with epinephrine 2% (●) to one eye and its vehicle (O) to the contralateral control eye of six cynomolgus monkeys. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly ($P < 0.05$) different from controls using one-tailed, paired student t-test.
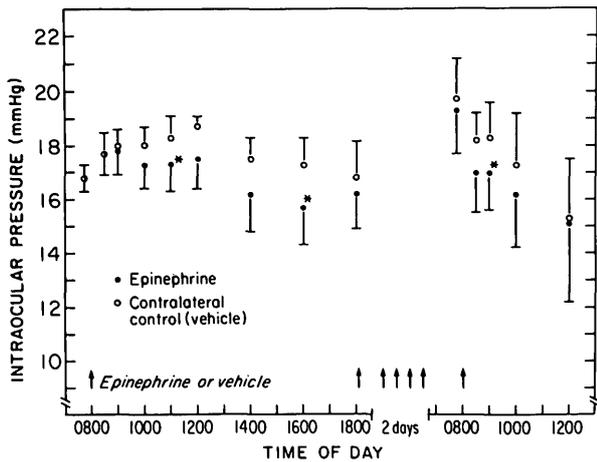
Neither $PGF_2\alpha$ nor epinephrine altered aqueous humor flow as measured by fluorophotometry 2–6 hr after the fifth consecutive dose (Table 1). Although an attempt at measuring outflow facility by indentation tonography was made 4 hr after the seventh consecutive dose, the IOPs of most eyes was too low to provide meaningful tracings.

$PGF_2\alpha$ did not significantly alter pupil size throughout the experiment (Fig. 7). On the other hand, epinephrine caused a significant pupillary dilation beginning within 30 min following topical application and peaking at 3.8 ± 1.6 (±SD) mm larger than the con-

Table 1. The effect of $PGF_2\alpha$ or epinephrine on aqueous humor flow as measured by fluorophotometry

	Aqueous flow (µl/min)*	
	Expt.	Vehicle
$PGF_2\alpha$	1.94 ± 0.16	1.84 ± 0.19
Epinephrine	1.86 ± 0.20	1.91 ± 0.22

* Each value represents mean ± 1 SEM for six eyes in each group as determined 2–6 h after the fifth consecutive dose.

tralateral control eye 1 hr following the first dose ($P < 0.0025$) (Fig. 7). The pupil size returned to baseline measurements 6–8 hr following the first application. One hr following the seventh consecutive dose of epinephrine, the pupil diameter increased by 2.4 ± 1.3 (±SD) mm in the epinephrine-treated eye ($P < 0.005$). Both the extent and the duration of the epinephrine-induced mydriasis was less after the seventh dose compared with the first dose (Fig. 7). There was no change in the pupillary diameters of any contralateral vehicle-treated eyes.

Discussion

The results of this study conclusively demonstrate that multiple dosing of $PGF_2\alpha$ twice daily on cynomolgus monkey eyes can maintain a reduction of IOP. Not only was there no evidence of tachyphylaxis or tolerance, but repeated dosing results in a more pronounced reduction. The dramatic ocular hypotensive effectiveness of multiple dosing with $PGF_2\alpha$ is dem-
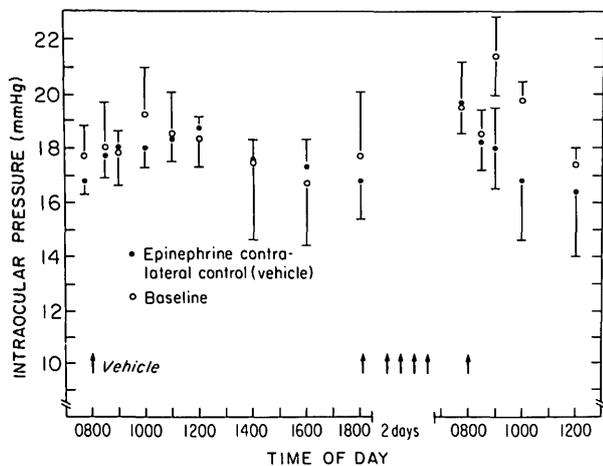

Fig. 6. Effects on IOP in the contralateral control eyes (●) of six cynomolgus monkeys receiving multiple doses of epinephrine to one eye compared with baseline measurements (O) taken 1 week previously in these same eyes. Points represent means and the limits ± 1 SEM. No measurements are significantly ($P < 0.05$) different from controls using one-tailed, paired student t-test.
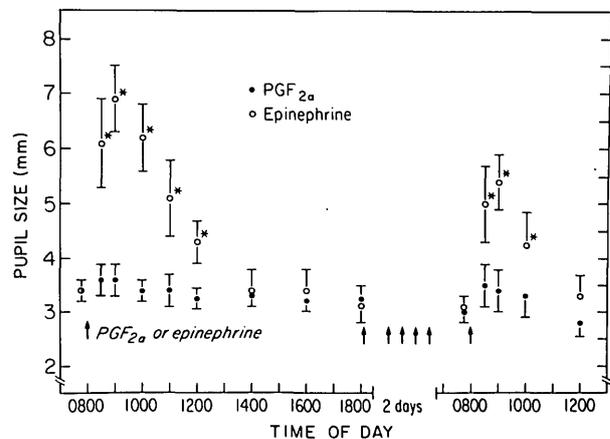

Fig. 7. Effects on pupillary diameter of multiple dosing with $PGF_2\alpha$ (●) or epinephrine 2% (O) to one eye of six cynomolgus monkeys for each agent. Points represent means and the limits ± 1 SEM. Asterisk (*) indicates measurements significantly ($P < 0.05$) different from controls using one-tailed, paired student t-test.

onstrated by its ability to reduce IOP by as much as 10 mmHg in previously normotensive monkey eyes. Its long-lasting effect is demonstrated by the persistent hypotensive response lasting at least 10 hr after the first dose, and at least 14 hr after the sixth dose. Notably, the ocular hypotensive response produced by the 250 μg dose used in the present study may have been somewhat reduced as a result of the ocular surface rinsing following each dose,[26] although a previous study using PGE$_2$ in rabbits failed to reveal a diminished effect with rinsing.[3] Another recent study[27] confirms that tachphylaxis does not develop after multiple dosing with PGF$_2\alpha$ in argon laser-induced glaucomatous eyes of cynomolgus monkeys.

PGF$_2\alpha$ produced a contralateral reduction of IOP after multiple dosing. A contralateral effect of unilaterally administered PGs has been demonstrated in other studies concerning both the hyper- and hypotensive response.[3,8,11,28,29] In the present study, transfer of the drug from one eye to the other by the animal itself or by the tonometer probe was unlikely since the eyes were thoroughly rinsed after each topical application. Furthermore, the monkeys were either well-sedated or had their extremities securely restrained in the primate chairs during drug administration. The appropriateness of the experimental rinsing design to control inadvertent drug transfer to the control eye was demonstrated by the lack of a contralateral effect of epinephrine, especially the absence of any contralateral mydriasis. Other studies have also demonstrated a contralateral effect after intracameral[28,29] or intravitreal[3] injection, modes of administration which make inadvertent contamination of the contralateral eye less likely. The possibility of diurnal fluctuations or repeated tonometry accounting for the contralateral effect was eliminated by the experimental design. Multiple IOP measurements in the week prior to drug administration failed to demonstrate significant diurnal fluctuations or variations due to repeated tonometry.

Systemic effects of the PGF$_2\alpha$ administered unilaterally are thought to be unlikely in view of the known highly effective enzymatic degradation of PGs after a single passage through the lungs.[30] It is unlikely that 15-keto-PGF$_2\alpha$, the initial metabolite, produced the contralateral effect since its ocular hypotensive potency is negligible when topically applied to the cat eye.[7] Whereas a direct interophthalmic communicating artery has been demonstrated in rabbit,[31] no such connection is known to exist in other species. Further evidence against a systemic circulatory effect is the failure to demonstrate elevated PG levels in the contralateral eyes.[19]

On the other hand, a previous study suggests that applying the same topical dose of PG in a smaller volume reduced the contralateral effect, which is consistent

with a systemic cross-over effect.[3] Other evidence supporting a systemic circulatory contralateral effect include a bilateral ocular hypertensive response following intravenous PG administration,[29,32-35] and a reduction in systemic blood pressure following intracameral administration of PGs in rabbits.[29] Although the possibility of some type of neurogenically-mediated contralateral effect has not been completely eliminated, one study reports that intracranial transection of the optic nerve, trigeminal nerve, and oculomotor nerve did not reduce the contralateral effect.[29] On the other hand, preliminary evidence indicates that the contralateral miotic effect of topically applied PGE$_2$ in cat eyes was blocked by atropine.[36] Therefore, it is conceivable that the contralateral hypotensive response to unilaterally administered PGs may be on the basis of a muscarinic mechanism. This possibility deserves further investigation.

The failure to demonstrate alterations in aqueous humor flow by fluorophotometry after multiple dosing is consistent with the results of a previous single-dose study,[4] but somewhat different from results found in a preliminary study.[37] However, possible aqueous flow alterations may be dependent on dosing and the time at which measurements are obtained. Furthermore, it must be noted that interpretation of these aqueous flow measurements may be influenced by protein binding of the fluorescein molecule, since the PGF$_2\alpha$-treated eye had a slightly elevated protein concentration in the aqueous humor, compared with their contralateral control eyes.[19] This binding may lead to underestimation of the true aqueous flow rate. On the other hand, a small breakdown of the blood-aqueous barrier may also result in increased loss of fluorescein through the iris stroma, which may cause overestimation of flow.[38] If PGs increase corneal epithelial permeability, resulting in some loss of fluorescein from the corneal stroma into the tear film rather than the anterior chamber, there again may be some overestimation of true flow. Although no direct evidence exists demonstrating that PGs alter corneal epithelial permeability, they have been reported to stimulate chloride transport in the corneal epithelium.[39] Unfortunately, since it is very difficult to accurately assess the relative influence of these variables on flow measurements, it is difficult to be certain whether our measurements accurately reflect true flow rates. Therefore, aqueous flow rates which are reported in this study reflect measurements made by the standard state-of-the-art technique, using assumptions which may or may not be applicable to PGs. Also of note is that a small effect on flow may have been missed using a relatively small number of animals. However, it is clear that the extent of the IOP reduction cannot be primarily attributed to a reduction in aqueous flow.

Unfortunately, no conclusions can be made about any possible effects on outflow facility in the present study. Unaltered aqueous flow measurements are consistent with the suggestion that PGs act in some way on total outflow, whether it be by conventional or unconventional pathways, on episcleral venous pressure, and/or conceivably on ocular blood volume. The extent of the IOP reduction down to 10 mmHg may best be explained by a marked increase in unconventional outflow. Several studies demonstrate significant increases in outflow facility after PG administration in rabbits[35,40–43] and monkeys[44] during the initial hypertensive response, and in rabbits,[3] cats, and monkeys[4] during the hypotensive phase. However, the increased outflow facility cannot totally account for the full extent of the IOP reduction.[4] Preliminary data from another study fail to demonstrate an increase in outflow facility.[37] Again, this apparently conflicting data may be secondary to differences in dosing, time of measurement, and/or technique of measurement.

Episcleral venous pressure was not measured in this study. The pigmented conjunctiva and orbital anatomy of cynomolgus monkeys prevent accurate measurements. However, no other ocular hypotensive agent has been shown to act primarily by reducing episcleral venous pressure.

The failure of $PGF_2\alpha$ to appreciably alter pupil size is consistent with previous observations in primates.[4,6,9] Mild pupillary constriction is noted only using very high doses (1 mg per eye) in owl monkeys weighing 0.8 to 1.0 kg.[8] The results presented here confirm that PGs are not potent miotic agents in the primate eye. Of the various PGs which have been tested in several animal species, only $PGF_2\alpha$ topically applied to cat eyes has a potent miotic effect.[4,6,7,11]

The lack of a consistent hypotensive effect of epinephrine after multiple dosing is surprising. A previous multiple-dose study in monkeys could not be found in the literature. However, in humans, it is known that the higher the baseline IOP, the more pronounced the epinephrine-induced hypotensive effects.[45] Therefore, normotensive eyes, as in the case of the monkeys used in the present study, may not exhibit a dramatic hypotensive response to most agents. The possibility that the epinephrine used was inactive is eliminated by its pronounced mydriatic effect. It is unlikely that a higher concentration of epinephrine would produce a greater reduction of IOP since studies in humans, having larger eyes and considerably greater body weights, seem to show that a 1–2% solution is at the peak of the dose-response curve.[46,47]

There is evidence in rabbits[16] and humans[18] that epinephrine reduces IOP in part by stimulating endogenous PG synthesis. Unfortunately, since epinephrine was only minimally effective in reducing IOP in

the present study, no definite conclusions about possible similar mechanisms of action can be made. However, like $PGF_2\alpha$ in this study, epinephrine did not alter the rate of aqueous humor flow, differing from results in human studies which demonstrated an increase in aqueous humor flow after single or multiple doses.[48,49] Again, a small change in flow could have been missed due to the relatively small number of animals.

These results demonstrate that multiple dosing with $PGF_2\alpha$ can effectively maintain an ocular hypotensive response in cynomolgus monkey eyes, and suggest that $PGF_2\alpha$, or one of its analogues, may be useful in the management of chronic glaucoma. Other pathophysiological effects of $PGF_2\alpha$ and epinephrine are described in companion studies.[19]

Acknowledgments

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