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[54] **USE OF EICOSANOIDS AND THEIR DERIVATIVES FOR TREATMENT OF OCULAR HYPERTENSION AND GLAUCOMA**

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[58] **Field of Search** 424/305, 317; 514/530

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[57] **ABSTRACT**

Ocular hypertension and glaucoma can be effectively controlled in primates through topical application of an effective amount of an eicosanoid or an eicosanoid derivative to the surface of an afflicted eye. Eicosanoids, particularly the prostaglandins PGE_2 and $PGF_{2\alpha}$, and derivatives thereof, have been found effective in quantities less than about 1000 μg per eye. Ophthalmic compositions containing C_1 to C_5 alkyl esters of $PGF_{2\alpha}$ are presently preferred for use in treating ocular hypertension and glaucoma in primates, including man.

19 Claims, No Drawings

USE OF EICOSANOIDS AND THEIR DERIVATIVES FOR TREATMENT OF OCULAR HYPERTENSION AND GLAUCOMA

The invention described herein was made in the course of work under U.S. Public Health Service Research Grant Numbers EY 00333 and EY 00402 from the National Eye Institute, Department of Health and Human Services.

BACKGROUND OF THE INVENTION

In primates, intraocular pressure is measured with a tonometer. A normal reading for a healthy, adult primate eye would be in the range 14 to 24 mm Hg. [See generally DeRousseau, C. J. and Bito, L. Z., EXP. EYE RES. 32:407–417 (1981); Kornblueth, W., et al., ARCH. OPHTHALMOL. 72: 489–490 (1964).] An increase of about 4 to 7 mm Hg. above the average reading for a specific subject would be indicative of ocular hypertension.

Glaucoma, an eye disorder afflicting various mammals, including primates, is characterized by increased intraocular pressure (ocular hypertension). In man, such ocular hypertension results from an imbalance between the rate of secretion of aqueous humor by the ciliary epithelium into the anterior and posterior chambers of the eye and the rate of outflow or drainage of the aqueous humor from the anterior and posterior chambers, primarily via the canal of Schlemm. It is generally believed that obstruction of aqueous humor drainage is the primary cause of the imbalance.

Chronic glaucoma typically results in slow, progressive loss of visual fields, and, if not controlled, ultimately in blindness. Initial treatment usually involves topical application of miotics, particularly pilocarpine and carbachol. If treatment with miotics is not effective, systemic administration of carbonic anhydrase inhibitors may be employed. If such approaches are unsuccessful, the glaucoma may have to be treated by surgery.

The treatment of human glaucoma with miotics is unsatisfactory for several reasons. The miotics may destroy a patient's night vision or cause ciliary muscle spasms. Moreover, long-term use of miotics may result in the development of tolerance (tachyphylaxis) to the miotics, necessitating the use of progressively higher doses. Finally, miotics may cause discomfort or other undesirable side effects. Long-term use of carbonic anhydrase inhibitors has likewise been found unsatisfactory. Such use may produce adverse systemic results or lead to the development of cataracts.

Eicosanoids and their derivatives include numerous biologically useful compounds. For example, the prostaglandins (PGs), a group of eicosanoids which contain cyclical fatty acids, are known to possess diverse biological activities. Originally isolated as lipid-soluble extracts from sheep *seminal vesicles* and human seminal fluid, prostaglandins have now been found in most mammalian tissue, although in lesser concentrations.

Activities of prostaglandins include stimulation of smooth muscle, dilation of small arteries, bronchial dilation, lowering of blood pressure, inhibition of gastric secretion, of lipolysis and of platelet aggregation, and induction of labor, abortion and menstruation.

It has been previously believed that administration of PGs, particularly PGE_2, increases intraocular pressure based upon the results of studies involving intracameral and intravitreal injection of PGs into mammalian eyes. Accordingly, most research in this area focused on the use of prostaglandin antagonists rather than prostaglandins per se in the treatment of glaucoma.

More recently, studies of the effect of exogenous administration of PGs in cannulated and uncannulated rabbit eyes showed that topical aand intravitreal application of about 25 to 200 μg. PGE_2 or $PGE_{2\alpha}$ per eye produced a short hypertensive phase, followed by hypotony. [Camras, C. B., Bito, L. Z. and Eakins, K. E., INVEST. OPHTHALMOL. VIS. SCI., 16:1125–1134 (1977)] However, a small dosage of $PGF_{2\alpha}$, about 5 μg, topically applied on rabbit eyes, produced a long period of hypotony, without any significant initial rise in intraocular pressure. Id. Other studies have shown that rabbits produce tolerance or tachyphylaxis to intracamerally or topically administered PGs. [Eakins, K. E., EXP. EYE RES., 10:87 (1970); Beitch, B. R. and Eakins, K. E., BRIT. J. PHARM., 37:158 (1969); Bito, L. Z. et al., ARVO, 22(No. 3):39 (1982)]

In addition, studies on species variations in ocular irritative and inflammatory response have shown that vertebrates such as primates and birds, which depend primarily on vision for sensory input, have more complex eye structures than rabbits, including more sophisticated ocular defense mechanisms. Accordingly, the eyes of primates and birds respond to topical application of chemical irritants in a manner unlike those of rabbits. This phenomenon may be due to the fact that the ciliary processes in rabbits are morphologically different from those of other species. In rabbits, there are abundant iridial ciliary processes which are uniquely susceptible to breakdown, e.g., by neuronal irritation or paracentesis, and deterioration of the blood-aqueous barrier. This propensity for breakdown appears to have an important protective function for rabbits which have highly exposed eye globes. Because of its exaggerated ocular irritative response, the rabbit has been widely used in studies of the role of PGs in ocular inflammation. In contrast, primates show a qualitatively different response to paracentesis: protein entry through the canal of Schlemm rather than breakdown of the ciliary processes. [Raviola, EXP. EYE RES. 25 (Supp.):27 (1977)]. Accordingly, use of the rabbit eye as a model for primates has been discredited except in ocular inflammation studies. [Bito, L. Z. and Klein, E. M., EXP. EYE RES. 33:403–412 (1981); Klein, E. M. and Bito, L. Z., PROC. INT. SOC. EYE RES. 1:65; Klein, E. M. and Bito, L. Z., INVEST. OPHTHALMOL. VIS. SCI. 20 (Supp.):33 (1981)].

SUMMARY OF THE INVENTION

A method for treating glaucoma and ocular hypertension in primates is disclosed comprising topical administration of an effective amount of an eicosanoid to the afflicted eye. Repeated application, preferably daily, provides long-term reduction of intraocular pressure, without development of tachyphylaxis. Eicosanoids which may be employed for purposes of the present invention include prostaglandins and their derivatives, for example, PGE_2, $PGF_2\alpha$ and their derivatives. C_1 to C_5 alkyl esters of $PGF_{2\alpha}$, particularly $PGF_{2\alpha}$-methyl-ester, are presently preferred.

Pharmaceutical preparations in accordance with the present invention comprise effective amounts of eicosanoids and an ophthalmically acceptable carrier. Suitable carriers include sterile saline solution, peanut oil and mineral oil.

DETAILED DESCRIPTION OF THE INVENTION

Ocular hypertension and glaucoma can be controlled in afflicted primates by topical application of effective amounts of eicosanoids. Periodic application of eicosanoids reduces elevated intraocular pressure levels to normal values which continue during the course of treatment without development of tachyphylaxis. Treatments are preferably applied daily.

Of the family of eicosanoids, prostaglandins (PGs) have been found particularly effective. In particular, PGE_2 and $PGF_{2\alpha}$, and derivatives thereof have provided long-term effectiveness. Daily application of PGE_2 and $PGF_{2\alpha}$ or their derivatives in amounts varying from about 0.01 μg to about 1000 μg, per eye has been found effective In monkeys the preferred ranges would be 0.1–500 μg; in man the preferred ranges would be 0.1–1000 μg.

Lipid soluble PGE_2 and $PGF_{2\alpha}$ derivatives are particularly preferred for use in treatment of ocular hypertension. Such lipid solubility permits more ready penetration of the protective layers of the primate eye and it has been found that smaller quantities of such compounds can be used than non-liquid soluble PGs. In particular, C_1 to C_5 alkyl esters of $PGF_{2\alpha}$, such as $PGF_{2\alpha}$ methyl ester, $PGF_{2\alpha}$ ethyl ester, $PGF_{2\alpha}$ isopropyl ester, and $PGF_{2\alpha}$ isobutyl ester, would be suitable liquid soluble $PGF_{2\alpha}$ derivatives. Such liquid soluble compounds are effective in lower amounts, e.g. from about 0.01 to about 100 μg per eye. In man the preferred range would be from about 0.1 to 100 μg, particularly between about 1 μg to 50 μg.

Physiologically acceptable salts of $PGF_{2\alpha}$ and PGE_2 or their derivative can also be employed. In particular, $PGF_{2\alpha}$ tromethamine would be suitable for use in treatment of intraocular hypertension. Other suitable salts would include $PGF_{2\alpha}$ in sodium carbonate.

Compositions according to the present invention would generally comprise effective amounts of an eicosanoid or an eicosanoid derivative and an ophthalmically compatible carrier. Suitable ophthalmically acceptable carriers include sterile saline solution, an anhydrous peanut oil or a mineral oil. If prostaglandins and their derivatives are used, as noted above, the quantities topically applied to the primate eye are relatively small. Accordingly, compositions according to the present invention will generally be about 0.01% to 2.0% solutions of PGs (or PG equivalents if PG derivatives are used). Compositions according to the present invention containing $PGF_{2\alpha}$ and $PGF_{2\alpha}$ tromethamine, and sodium salts of $PGF_{2\alpha}$ may be employed in sterile saline solutions. The hyrophobic esters of $PGF_{2\alpha}$ (methyl ester, ethyl ester, etc.) may be employed in sterile anhydrous peanut oil.

EXPERIMENT 1

The first experiment is also reported in Camras, C. B. and Bito, L. Z., CURRENT EYE RESEARCH 1:205–209 (1981), the disclosure of which is hereby incorporated by reference into the present application.

Five normal owl monkeys (*Aotus trivirgatus*), 3 males and 2 females; 0.8 to 1.0 kg, and one female with unilateral angle recession glaucoma were conditioned to accept handling, restraint, and tonometry without anesthesia. The intraocular pressure (IOP) of both eyes was measured over a one-year period at random intervals, but at least once each month. One drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon Corp., Forth Worth, TX) was applied to the eye before IOP was measured with a floating tip pneumatic tonometer probe attached to a pressure transducer and a recorder. Each animal was placed in the supine position on the lap of the investigator and 2 or 3 IOP measurements, each several seconds in duration, were taken. The best steady-state segments of the IOP tracings were read and averaged. Pupillary diameter was measured in normal room light with a pupil gauge. Anterior chamber flare and cellular invasion were determined by slit lamp examination.

The tromethamine salt of $PGF_{2\alpha}$ was dissolved in physiological saline to yield $PGF_{2\alpha}$ concentrations of 20, 40, 80 or 200 mg/ml. In each experiment 5 μl of one of these solutions was applied to one eye of each monkey. The eyes were rinsed 3 to 5 min. later with 2 to 4 mls of saline. An equal volume (5 μl) of saline was similarly applied to the contralateral control eyes followed by rinsing. Measurements of IOP, pupillary diameter, and slit lamp evaluation of aqueous flare and cellular content of the anterior chamber were made at various intervals after $PGF_{2\alpha}$ application.

RESULTS

Normal Owl Monkey

Topical application of 0.2 mg of $PGF_{2\alpha}$ to one eye (left eye in 2 and right eye in 3 animals) of the 5 normal owl monkeys did not result in significant effects on the IOP as compared to the baseline IOP of the treated eye or the simultaneously measures IOP in the contraleteral eye. However, topical application of 1 mg of $PGF_{2\alpha}$ to the left eye of these animals 4 to 14 days after the first trial resulted in a prolonged hypotony in the treated eye compared with the contralateral eye. In 3 of the 5 eyes this hypotony was preceded by a 2–3 mm Hg rise in IOP occurring 15 min after treatment and showing borderline significance compared with the contralateral eye. A prolonged hypotony was also observed when the same dose of $PGF_{2\alpha}$ was applied 6 days later to the contralateral (right) eyes of these monkeys, or when it was applied 18 days later to the originally treated eyes. Although the extent of ocular hypotension in the treated eye was about the same after each application of 1.0 mg of $PGF_{2\alpha}$, the significance of the IOP differences between treated and contralateral eyes was reduced on subsequent PG application because of an apparent contralateral hypotensive effect. These IOP effects on the untreated contralateral eyes were not due to diurnal variations since tonometry done over a 24-hr period on the eyes of these same animals after bilateral saline-treatment or after unilateral treatment with a low dose of (0.2 mg) $PGF_{2\alpha}$ did not show significant lowering of IOP.

One half hour after topical application of 1.0 mg $PGF_{2\alpha}$, there was an average of 2.0±0.3 mm pupillary miosis compared to the contralateral control eyes. A gradual return to normal pupil size (4.8±0.2 mm) occurred over the next 18 hr. Slight aqueous flare was present in 4 of 5 eyes between 2 and 12 hr after the topical application of 1.0 mg of $PGF_{2\alpha}$. At 48 hr, a few cells were observed in 3 of the 5 treated, but in none of the control eyes. There was no apparent correlation between IOP reduction and the presence of flare and cells in the anterior chamber, i.e., the ocular hypotension was not associated with a notable inflammatory response.

Glaucomatous Owl Monkey

When purchased, one female monkey had eyes exhibiting a marked anisocoria with the right pupil being a consistent 2 mm larger than the left. Gonioscopic examination of the right eye revealed angle recession. The mean of 46 IOP measurements taken over a period of one year was 47.2±0.7 and 24.5±0.6 mm Hg for the right and left eyes respectively. Eleven months before this study on the effects of $PGF_{2\alpha}$, topical application of 1% pilocarpine reduced the IOP by 4 mm Hg in the left eye, but raised the IOP of the right eye by 16 mm Hg. Oxotremorine (0.05%) also increased the IOP of the right eye.

Within 20 min after application of 1.0 mg of $PGF_{2\alpha}$ to the right eye of this owl monkey, IOP dropped from an average pretreatment value of 50 mm Hg to 32 mm Hg, followed by a more gradual decline during the next 12 hr, ultimately reaching a value similar to that of the control eye and as low as 14 mm Hg. The IOP of the two eyes then remained similar for about 3 days, followed by a gradual return in the right eye to pretreatment IOP levels of 50 mm Hg.

During this period of normotension, there was marked clearing of the corneal haze of the right eye, but this haze reappeared as the IOP rose to its baseline values in the 40–50 mm Hg range. However, for several weeks thereafter, the IOP of this eye appeared to be much more labile than it was before the $PGF_{2\alpha}$ application.

EXPERIMENT 2

Fourteen cats of either sex (2.5 to 3.5 kg) and two female rhesus monkeys (*Mucaca mulatta;* 3.8 and 4.0 kg) were lightly tranquilized with 5–10 mg/kg of ketamine (Ketaset; Bristol-Myers Co., Syracuse, NY). Such doses of ketamine were found to tranquilize rhesus monkeys without significantly altering their IOP. The monkeys were kept in primate chairs throughout each experiment.

One drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon Corp., Fort Worth, TX) was applied to each eye and IOPs were measured with a Pneumontonograph (Alcon Corp.) which was calibrated on the eyes of several species, including rhesus monkeys. New animals were accustomed to the tonometer by taking several readings the day before they were to be used in an experiment. Several sets of baseline readings were taken 0.5–1 hr before each experiment and the best steady state readings were averaged. Pupillary diameters were measured in normal room light with a pupil gauge. In cats, the nasotemporal (shorter) diameter was always recorded. In several experiments, the pupillary diameters of cats were re-measured in total darkness, using infrared illumination and an infrared image converter. Anterior chamber flare and cellular invasion were determined by slit lamp examination. A 50-μl aliquot of a solution containing one of several concentrations of prostaglandin E_2 (PGE_2), converted to its soluble sodium salt with the addition of an equimolar amount of Na_2CO_3, or the tromethamine salt of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; The Upjohn Co., Kalamazoo, Mich.), both dissolved in saline, was topically applied to one eye of each cat or monkey. An equal volume of physiological saline was applied to the contralateral eye. In one set of experiments, two cats were pretreated with 10 mg/kg of indomethacin (Sigma Chemical Co., St. Louis, MO) injected i.p. at 24, 16 and 2 hrs. prior to the topical administration of the PG solution; two other animals received no such pretreatment. All of the drugs were made just prior to their administration. In another experiment, both eyes of a set of four cats were treated with 125 μl of 0.5% atropine (Isopto atropine, Alcon Corp.) 20 min. prior to administration of the PG solution. In all cases, measurements of IOP, pupillary diameter and slit lamp examinations for flare and cellular invasion of the anterior chamber were made at various intervals up to 72 hr after the application of PGs.

Because of the limited availability of rhesus monkeys, different doses of PGs were tested on each eye of two animals in a random sequence. At least seven days elapsed between any two applications of PG-containing solution to the same eye. Cats were re-used to a much more limited extent; only one PG solution was tested on each eye of most cats, allowing at least one week between each test. In some cases, an eye which showed no observable response or only a moderate response to a low dose of PG was used for a second time, but not less than two weeks after it was first treated with a PG solution.

Cat Results

Topical application of up to 1000 μg of PGE_2 to the cat eye produced a significant decrease in IOP with the maximum reduction, as compared to the IOP of the contralateral eye, occurring between 1 to 8 hr after PG administration. The greatest and most prolonged hypotensive response was observed in eyes given 500 μg of PGE_2. In eyes which were subjected to less frequent tonometry, the IOP remained 6 mm Hg below baseline for 48 hr; this hypotension was not preceded by an initial hypertensive phase. In contrast, topical application of 1000 μg of PGE_2 produced a distinct initial ocular hypertension between 0.25 and 2 hr followed at 6 hr by a maximum decrease of 11.7 mm Hg below the IOP of the contralateral control eye. Topical application of the same doses of $PGF_{2\alpha}$ produced IOP responses similar in magnitude and duration to those produced by PGE_2.

Topical administration of 1.0 μg of $PGF_{2\alpha}$ caused a threshold miotic response, decreasing the pupillary diameter by an average of 1.5 mm, from 11 mm to 9.5 mm at 1 hr. An approximately one-half maximal miotic response occurred after the topical application of 5 μg of $PGF_{2\alpha}$, with a decrease in pupillary diameter of over 5 mm at 2 hr. A dose of 100 μg of $PGF_{2\alpha}$ produced an apparently maximum miotic response (9.5 mm decrease in pupillary diameter) within 2 hr, which not exceeded in extent or duration in eyes treated with a ten-fold greater dose (1000 μg) of $PGF_{2\alpha}$. Topical pretreatment of cat eyes with 0.5% atropine, which was sufficient to block the pupillary light reflex, did not affect the miotic potency of topically applied $PGF_{2\alpha}$. The administration of similar doses of PGE_2 resulted in far more moderate miotic responses. The threshold miotic dose of PGE_2 was 100 μg and even a 100-fold greater dose produced only a sub-maximal decrease in pupillary diameter (from 10 mm to 2.5 mm), followed by rapid re-dilation.

In one experiment, in which 2 out of 4 cats were pretreated with indomethacin (10 mg/kg i.p.) prior to the topical application of PGE_2, no difference in either the miotic or IOP response was observed between indomethacin-pretreated and control cats, indicating that the IOP lowering effect of PGE_2 was not due to the stimulation of the synthesis of PGs and/or related cyclo-oxygenase products from endogenous precursors.

Several sets of cats had their pupillary diameters measured in both normal room light and complete darkness (with the aid of an infrared image converter) at the time when they showed a maximum pupillary constriction. The pupils of both eyes dilated slightly in complete darkness (by 1 to 3 mm) as compared to their diameters in room light, but the difference between the pupillary diameters of the PG-treated and the contralateral control eyes was only minimally affected.

Flare was not observed under careful slit lamp examination in any of these cats at any time after the topical application of up to 1000 μg of $PGF_{2\alpha}$. However, some flare was observed in the anterior chamber of most cats 2–18 hr after the topical application of 100 or 500 μg of PGE_2, but not after the application of 10 μg of PGE_2.

Rhesus Monkey Results

Topical application of 100, 500, or 1000 μg of $PGF_{2\alpha}$ to the eyes of rhesus monkeys produced a significant decrease in IOP within 2 hr; application of a much lower dose, 10 μg, did not have a similar effect. While insignificant initial increases in IOP were observed following application of 100 or 500 μg of $PGF_{2\alpha}$, 1000 μg of $PGF_{2\alpha}$ produced a brief (<30 min) initial IOP increase of 8 mm Hg, followed by a more prolonged decrease in IOP to 5 mm Hg below baseline. The application of 100 μg of PGE_2 or $PGF_{2\alpha}$ produced very similar IOP effects, with maximum decreases of 5 and 6 mm Hg, respectively. The IOP of eyes treated with PGE_2, however, returned to baseline values more gradually than eyes which received $PGF_{2\alpha}$. With both PGs, some reduction in IOP was maintained for 3 to 10 hr.

No miosis was observed in rhesus eyes after the topical application of any of the $PGF_{2\alpha}$ doses used here. However, 100 μg of PGE_2 produced a small but significant and brief decrease (3 mm) in pupillary diameter, followed by re-dilation to near baseline values by 2 hr after PG administration. No flare or cellular invasion of the anterior chamber of this species was detectable by careful slit lamp examination at any time after the topical application of 100 μg of PGE_2 or up to 1000 μg of $PGF_{2\alpha}$.

Tables 1 and 2 summarize results obtained in Experiment 2.

TABLE 1

Comparison of maximum IOP reduction 3 to 6 hr after unilateral topical application of various doses of PGE_2 or $PGF_{2\alpha}$ to cat eyes.*

Dose μg/eye	Mean difference (exp-cont) in IOP (mm Hg) PGE_2	$PGF_{2\alpha}$
10	−4.5 ± 2.1	−4.8 ± 1.1
100	−12.0 ± 1.4	−8.8 ± 0.8
500	−13.8 ± 0.8	−9.7 ± 0.3
1000	−11.8 ± 3.6	−11.3 ± 2.4

*IOP was measured at 3, 4 and 6 hr after the topical application of the indicated dose of PGE_2 or $PGF_{2\alpha}$. The largest negative value ($IOP_{exp} - IOP_{cont}$) observed for each animal during these three measurements was used in all cases to calculate the means.

TABLE 2

Extent and duration of IOP reduction in rhesus monkeys induced by the topical application of $PGF_{2\alpha}$ or PGE_2.

Prostaglandin dose/eye	Eye	Intraocular Pressure (mm Hg) Baseline (OD)	Baseline (OS)	Max. Reduction (exp−cont)	Duration of >50% IOP reduction (hr.)
$PGE_{2\alpha}$					
100 pg	A*(OS)	23	24	−7	3
	A (OD)	24	26	−5	3
	B (OD)	27	28	−8	4
500 pg	A (OD)	25	25	−6	5
	A (OS)	24	25	−8	3
	B (OD)	26	26	−8	6
	B (OS)	21	21	−8	5
1000 pg	A (OS)	25	25	−9	5
	B (OS)	28	28	−6	5
	B (OD)	25	26	−2	5
PGE_2					
100 pg	A (OS)	25	25	−7	5
	A (OD)	24	25	−7	6
	B (OS)	26	26	−4	4

*A and B refer to the two monkeys used in this experiment

EXPERIMENT 3

Fourteen cats of mixed breeds and of either sex (2.5 to 3.5 kg) were trained daily for 4–7 days to accept handling, periodic restraint in animal boxes and tonometry without the use of general anesthesia. One drop of 0.5% proparacaine hydrochloride (Alcaine, Alcon Corp., Fort Worth, TX) was applied topically to each eye and IOPs were measured using a floating-tip pneumatic tonometer (pneumotonograph; Alcon Corp.). Pupillary diameters (naso-temporal) were measured in normal room light and/or in dim light with a millimeter ruler. All eyes were examined with a slit-lamp and only animals which showed no signs of ocular inflammation were included in this study.

A 50-μl aliquot of 0.2 mg/ml Na_2CO_3 in saline or a saline solution containing 100 or 500 μg of prostaglandin E_2 (PGE_2) or F_2 ($PGF_{2\alpha}$) was topically applied to one eye of each animal typically at 24-hr intervals, but in some cases at 12-, 48-, or 72-hr intervals. An equal volume of vehicle solution was applied to the contralateral eye. Based on the prior experiment (Experiment 2), the dose of PGE_2 applied at each treatment throughout the 7-month period was 100 μg/eye, with the exception of the 100th day of treatment when 500 μg was applied to the experimental eyes of these animals. This high PGE_2 dose, however, resulted in the development of pronounced flare in the anterior chamber of every treated eye and was therefore not applied again. Another set of 6 cats received unilateral topical application of 100 or 500 μg/eye of $PGF_{2\alpha}$ for shorter time periods. IOPs and pupil diameters were measured, in most cases, every day at approximately 9 AM (just before the morning PG treatment), and on most days at 1, 3, 4 and 6 hr after the morning treatment. When treated twice daily, the second treatment was given between 9 and 10 PM. The protocol included rinsing of the tonometer probe in saline solution between each IOP reading in order to minimize the chances of transferring topically applied PGs from the experimental to the control eyes of these animals. Slit-lamp examinations were performed 4 to 5 hr after some PG applications and anterior chamber flare and cellular invasion were rated.

Similar experiments were also performed on two 5- to 7-year-old female rhesus monkeys. Both of these ani-

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