Comparison of the hypotensive and other ocular effects of prostaglandins E$_2$ and F$_{2\alpha}$ on cat and rhesus monkey eyes

F. A. Stern and L. Z. Bito

It is generally accepted that exogenous or endogenous prostaglandins (PGs) can give rise to acute increases in intraocular pressure (IOP) and to the development of flare and other signs of uveitis. It was recently shown, however, that low doses of PGE$_2$ and/or PGF$_{2\alpha}$ topically applied to rabbit or owl monkey eyes significantly reduce IOP. The present experiments show that topical application of 10 to 500 μg of PGE$_2$ also causes a highly significant IOP reduction in cat eyes lasting up to 48 hr with little or no development of flare or miosis, whereas similar application of PGF$_{2\alpha}$ causes, in addition to an IOP reduction, the development of profound pupillary constriction. Topical application of either PGF$_{2\alpha}$ or PGE$_2$ to the eyes of rhesus monkeys also causes significant dose-dependent reduction in IOP. The hypotensive response in the rhesus monkey is not associated with detectable flare or consistent pupillary constriction, although at higher PG doses, hypotension tends to be preceded in both species by a brief (15 to 30 min) hypertensive phase. It is concluded that the eyes of different species show different patterns of IOP, miotic, and flare responses to topically applied PGs, the only consistent effect being a reduction in IOP. In some species, most notably in primates, a reduction in IOP is the predominant effect of PGs. Thus PGs or their analogues may provide a new approach to the clinical control of IOP and the treatment of glaucoma. (INVEST OPHTHALMOL VIS SCI 22:588-598, 1982.)

Key words: intraocular pressure, miosis, blood-aqueous barrier, prostaglandin, PGE$_2$, PGF$_{2\alpha}$, cat, rhesus monkey, Macaca mulatta

Early experiments, based principally on the initial effects of large doses of topically or intracameral administered prostaglandins (PGs), led to the general conclusion that PGs are primary mediators of ocular inflammation.$^{1-4}$ Much attention has therefore been focused on the use of inhibitors of PG synthesis to prevent or reduce ocular inflammation.$^{5, 6}$ However, the possible involvement of these autacoids in normal anterior segment physiology and the therapeutic potential of PGs and/or their analogues has for the most part been ignored.

More recently, our laboratory has shown that in rabbits, PGE$_2$ and especially PGF$_{2\alpha}$ can reduce intraocular pressure (IOP) when applied topically in doses insufficient to cause hypertension or other signs of ocular inflammation.$^7$ In the rabbit there was a very narrow margin between the ocular hypotensive and inflammatory effects of these PGs. However, topical application of a very high dose of

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Fig. 1. Effects of topical application of 10 to 1000 μg of PGE2 on the IOP of cats. Points represent the mean IOP values obtained on four (A, B, and D) or five cats (C). The limits represent ±1 S.E.M.

PGF2α to the eyes of owl monkeys (Aotus trivirgatus) was found to cause a large IOP reduction of long duration, with negligible breakdown in the blood-aqueous barrier. Although the owl monkey is a readily available primate, very little is known about aqueous humor dynamics and its pharmacologic control in this species. Furthermore, on the basis of these two initial studies, it was impossible to determine which type of PG-
induced response may be regarded as typical of the mammalian eye in general.

The present investigation was therefore undertaken to study the ocular responses to topically applied PGs in rhesus monkeys, a species generally regarded as one of the best animal models for the human eye, and to compare the ocular effects of PGs in this macaque to those in the eyes of cats. Such a comparison was deemed necessary because the limited availability of rhesus monkeys or most other higher primates precludes their use as a general model for dose-response, drug-interaction, biochemical, and histochemical studies, which will be required for the elucidation of the basic mechanisms of PG-induced ocular hypotension.

Materials and methods

Fourteen cats of either sex (2.5 to 3.5 kg) and two female rhesus monkeys (Macaca mulatta; 3.8 and 4.0 kg) were lightly tranquilized with 5 to 10 mg/kg of ketamine (Ketaset; Bristol-Myers Co., Syracuse, N. Y.). 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, Tex.) was applied to each eye, and IOPs were measured with a Pneumotonograph (Alcon) calibrated on the eyes of several species, including rhesus monkeys. New animals were accustomed to the tonometer by undergoing several readings the day before they were to be used in an experiment.
Several sets of baseline readings were taken 30 min to 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 naso-temporal (shorter) diameter was always recorded. In several experiments the pupillary diameters of cats were remeasured in total darkness with infrared illumination and an infrared image converter.10 Anterior-chamber flare and cellular invasion were determined by slit-lamp examination and rated as previously described.11

A 50 μl aliquot of a solution containing one of several concentrations of PGE₂, converted to its soluble sodium salt with the addition of an equimolar amount of Na₂CO₃, or the tropemamine salt of PGF₂α (The Upjohn Co., Kalamazoo, Mich.), both dissolved in saline, was topically applied to one eye of each cat or monkey. An equal volume of physiologic 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 intraperitoneally at 24, 16, and 2 hr prior to the topical administration of the PG solution; two other animals received no such pretreatment. All the drugs were made up as previously described7 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) 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, as shown in Results. At least 7 days elapsed between any two applications of PG-containing solution to the same eye. Cats were reused to a much more limited extent; only one PG solution was tested on each eye of most cats, allowing at least 1 week between each test. In some cases, an eye that 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 2 weeks after it was first treated with a PG solution.

Results

Cats. Topical application of up to 1000 μg of PGE₂ to the cat eye produced a significant decrease in IOP with the maximum reduction, as compared with the IOP of the contralateral eye, occurring between 1 to 8 hr after PG administration (Fig. 1). The greatest and most prolonged hypotensive response was observed in eyes given 500 μg of PGE₂. In eyes that 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 (Fig. 2). In contrast, topical application of 1000 μg of PGE₂ (Fig. 1, D) produced a distinct initial ocular hypertension between 15 min 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 PGF₂α produced IOP responses similar in magnitude (Table I) and duration to those produced by PGE₂.

Topical administration of 1.0 μg of PGF₂α caused a threshold miotic response, decreasing the pupillary diameter by an average of 1.5 mm, from 11 to 9.5 mm at 1 hr (Fig. 3). An approximately one-half maximal miotic response occurred after the topical application of 5 μg of PGF₂α, with a decrease in pupillary diameter of over 5 mm at 2 hr. A dose of 100 μg of PGF₂α produced an apparently maximum miotic response (9.5 mm decrease in pupillary diameter) within 2 hr, which was not exceeded in extent or duration in eyes treated with a 10-fold greater dose.

Table I. Comparison of maximum IOP reduction 3 to 6 hr after unilateral topical application of various doses of PGE₂ or PGF₂α to cat eyes*

<table>
<thead>
<tr>
<th>Dose (μg/eye)</th>
<th>Mean difference (exp - cont) in IOP (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>PGE₂</td>
</tr>
<tr>
<td>10</td>
<td>−4.5 ± 2.1</td>
</tr>
<tr>
<td>100</td>
<td>−12.0 ± 1.4</td>
</tr>
<tr>
<td>500</td>
<td>−13.8 ± 0.8</td>
</tr>
<tr>
<td>1000</td>
<td>−11.8 ± 3.6</td>
</tr>
</tbody>
</table>

exp = experimental eye; cont = control eye.

*IOP was measured at 3, 4, and 6 hr after the topical application of the indicated dose of PGE₂ or PGF₂α. 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.
Fig. 3. Effects of topically applied PGF$_{2\alpha}$ (A) or PGE$_2$ (B) on the pupillary diameter of cat eyes as compared with the pupillary diameter of contralateral control eyes. For each curve, $n = 4$; limits represent ±1 S.E.M.

(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$_{2\alpha}$ (Fig. 4). The administration of similar doses of PGE$_2$ resulted in far more moderate miotic responses (Fig. 3). The threshold miotic dose of PGE$_2$ was 100 μg and even a 10-fold greater dose produced only a submaximal decrease in pupillary diameter (from 10 to 2.5 mm), followed by rapid redilation.

In one experiment in which two out of four 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 a result of the stimulation of the synthesis of PGs and/or related cyclooxygenase 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 with 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
Prostaglandin-induced ocular hypotension

Fig. 4. Miotic effect of topically applied PGF$_{2\alpha}$ on cat eyes pretreated with atropine. Both eyes of four cats were treated with 125 $\mu$L of 0.5% atropine solution 20 min before the topical application of PGF$_{2\alpha}$ to one eye. This dose of atropine was sufficient to block the pupillary light reflex in both eyes but did not have an observable effect on PGF$_{2\alpha}$-induced miosis. The limits represent ±1 S.E.M.

Fig. 5. Development of anterior-chamber flare in cat eyes after the topical application of PGE$_2$. Note that anterior-chamber flare was not observed at any time up to 24 hr after the topical application of 10 $\mu$g of PGE$_2$. For each curve, n = 4; limits represent ±1 S.E.M.

time after the topical application of up to 1000 $\mu$g of PGF$_{2\alpha}$. However, some flare was observed in the anterior chamber of most cats 2 to 18 hr after the topical application of 100 or 500 $\mu$g of PGE$_2$ (Fig. 2) but not after the application of 10 $\mu$g of PGE$_2$ (Fig. 5).

*Rhesus monkeys.* Topical application of 100, 500, or 1000 $\mu$g of PGF$_{2\alpha}$ to the eyes of rhesus monkeys produced a significant decrease in IOP within 2 hr (Fig. 6); application of a much lower dose (10 $\mu$g) did not have a similar effect. Although insignificant initial increases in IOP were observed after application of 100 or 500 $\mu$g of PGF$_{2\alpha}$, a dose of
Fig. 6. Effects of topically applied PGF$_{2\alpha}$ (A, B, and C) and PGE$_2$ (D) on the IOP of rhesus monkeys. All data presented were obtained from two animals over a 6 week period. Each eye of these two monkeys received two or three different PG doses at 2 week intervals; the contralateral eyes received, each time, an identical volume (50 µl) of saline. PG doses were applied randomly as indicated in Table I. Points represent means obtained from three (A, C, and D) or four (B) eyes. The limits represent ±1 S.E.M.

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 that received PGF$_{2\alpha}$. With both PGs, a reduction in IOP of more than 50% was maintained for 3 to 10 hr (Table II).

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 redilation to near baseline values.
Table II. Extent and duration of IOP reduction in rhesus monkeys induced by the topical application of PGF$_{2\alpha}$ or PGE$_2$

<table>
<thead>
<tr>
<th>Prostaglandin (dose/eye)</th>
<th>Animal No. (exp eye)</th>
<th>Date (1981)</th>
<th>IOP (mm Hg)</th>
<th>Baseline</th>
<th>Maximum reduction (exp – cont)</th>
<th>Duration of &gt;50% IOP reduction (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF$_{2\alpha}$</td>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>OS</td>
<td></td>
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<tr>
<td>100 µg</td>
<td>48 (OS)</td>
<td>3/24</td>
<td>23</td>
<td>24</td>
<td>–7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>48 (OD)</td>
<td>3/27</td>
<td>24</td>
<td>26</td>
<td>–5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>53 (OD)</td>
<td>3/27</td>
<td>27</td>
<td>28</td>
<td>–6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>48 (OD)</td>
<td>4/7</td>
<td>25</td>
<td>25</td>
<td>–8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>48 (OS)</td>
<td>4/28</td>
<td>24</td>
<td>25</td>
<td>–8</td>
<td>3</td>
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<tr>
<td></td>
<td>53 (OD)</td>
<td>4/7</td>
<td>26</td>
<td>26</td>
<td>–6</td>
<td>6</td>
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<tr>
<td></td>
<td>53 (OS)</td>
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<td>21</td>
<td>–9</td>
<td>3</td>
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<td>500 µg</td>
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<td>25</td>
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<td>–6</td>
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</tr>
<tr>
<td></td>
<td>53 (OS)</td>
<td>4/2</td>
<td>28</td>
<td>28</td>
<td>–2</td>
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</tr>
<tr>
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<td>53 (OD)</td>
<td>4/28</td>
<td>25</td>
<td>26</td>
<td>–7</td>
<td>5</td>
</tr>
<tr>
<td>1000 µg</td>
<td>48 (OS)</td>
<td>4/14</td>
<td>25</td>
<td>25</td>
<td>–7</td>
<td>5</td>
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<td></td>
<td>48 (OD)</td>
<td>4/21</td>
<td>24</td>
<td>25</td>
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<td>6</td>
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<tr>
<td></td>
<td>53 (OS)</td>
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<td>26</td>
<td>26</td>
<td>–4</td>
<td>4</td>
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<tr>
<td>PGE$_2$</td>
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<td></td>
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<td>4/14</td>
<td>26</td>
<td>26</td>
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exp = experimental eye; cont = control eye.

Discussion

Our results clearly demonstrate that topical application of either PGE$_2$ or PGF$_{2\alpha}$ can effectively reduce the IOP in both cats and rhesus monkeys. More importantly, significant IOP reduction was obtained in both of these species in response to PG doses that did not cause a breakdown in the blood-aqueous barrier or any other signs of ocular inflammation. Development of mild flare was observed only in cats and only when PGE$_2$ or PGF$_{2\alpha}$ was administered in doses 10- to 100-fold greater than that required to reduce IOP. It should be noted that in some experiments the observed flare may have been caused by the combined effects of PG application and the frequent tonometry, since reduced frequency of tonometry lessened the extent of flare. Furthermore, topical application of hypotensive doses of PGE$_2$ or PGF$_{2\alpha}$ to the rhesus eye or of PGE$_2$ to the cat eye caused only negligible miosis. These and previous findings support the conclusion that PGs or their synthetic analogues could provide a new approach to the clinical control of IOP and the treatment of glaucoma.

There are, however, significant species differences between rhesus monkeys and cats, as reported here, as well as between these species and previously reported findings on the effects of topically applied PGs on the eyes of rabbits and owl monkeys. Rabbit and cat eyes are clearly more sensitive to the ocular effects of PGs than the eyes of either rhesus or owl monkeys. It should be noted that the rhesus eye has also been reported to be less sensitive than the rabbit eye to the hypertensive effects of intracamerally administered PGs. Rabbit and rhesus eyes, however, have a similar tendency to yield a brief episode of ocular hypertension before the onset of PG-induced hypotension. No initial hypertension occurs in the eyes of owl monkeys or cats after the topical application of PG doses that were highly effective in reducing IOP. Moreover, the hypotensive period that follows topical PG application is much longer in cat or owl monkey (48 to 72 hr) eyes than that in the eyes of rabbits or rhesus monkeys (10 to 20 hr).

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. It has been shown that intraocular tissues of rabbits do not have an appreciable capacity to metabolically inactivate PGs. Instead, the termination of action of intraocular PGs is achieved by their rapid absorptive transport out of the eye across the blood-ocular barriers. Although some studies indicate that a similar transport function does exist in all species, species variation in the efficiency of this transport or in the capacity of intraocular tissues to metabolize PGs cannot be ruled out. Studies similar to those described here but using synthetic PG analogues, which are not substrates for PG dehydrogenase, or using animals that have been pretreated with PG transport inhibitors should help to resolve this question.

Although rabbit and rhesus eyes are somewhat similar with respect to the time course of PG-induced IOP changes, these two species are strikingly different with respect to their sensitivity to the generally assumed primary pathophysiologic effect of PGs, i.e., the breakdown of the blood-aqueous barrier. In the rabbit eye, topical application of as little as 50 μg of PGE₂ has been shown to cause a breakdown of the blood-aqueous barrier. In contrast, very careful slit-lamp examination revealed no detectable flare in rhesus eyes even after the topical application of 500 or 1000 μg of PGF₂α. It should also be noted that the response of cat and owl monkey eyes are somewhere between two extremes, with some development of flare but only at very high doses of PGs.

The sensitivity of the cat eye to PGF₂α-induced miosis is also noteworthy. Although PGs are generally regarded as effective miotics, the reason for this generalization is unclear. The present study and several previous studies suggest that PGs have little or no miotic effect on untraumatized rabbit or primate eyes in vivo or on isolated rat or golden hamster eyes in vitro. In fact, there is accumulating evidence that many biologically active peptides, some of which have been shown to be present in the eye and to be released into ocular compartments as a result of ocular irritation, are much more potent miotics than PGs. The cat eye may be a notable exception in this regard, although the possibility cannot yet be ruled out that the profound miosis induced in this species by PGF₂α, but not by PGE₂, is mediated by release of a second autacoid, possibly a polypeptide. The fact that PGF₂α-induced miosis was not inhibited by topical application of sufficient atropine to block the light reflex clearly indicates that this miosis is not mediated by a cholinergic mechanism.

These considerations suggest that there are no two species in which topical PG application results in the same ocular effects. The fact that PGs were found to have an ocular hypotensive effect on all four species studied so far indicates, however, that PGs reduce IOP by affecting some very basic process common to all species. Thus we can expect that PGs also have a hypotensive effect on the human eye. It should be noted that the ocular hypotensive effect of PGF₂α in rabbits was shown to be attributable to an increase in true outflow facility. However, the possibility that this ocular hypotension is not mediated by the same mechanisms in all species cannot be ruled out.

Considering the fact that the human eye is reputed to share with the eyes of other primates a resistance to the breakdown of the blood-aqueous barrier, it is reasonable to assume that in the human, as in other primates, reduction of IOP could be achieved by topical application of PGs and/or their analogues without the development of significant flare. Whether the ocular hypotensive effect of PGs in the human eye might be of relatively shorter duration (i.e., 6 to 12 hr), as in the rabbit or rhesus monkey, or last for several days, as is the case in cats or owl monkeys, cannot be predicted at this time. It has been reported that intravenous or intraterine administration of PG solutions results in an IOP reduction in women. However, the results presented and their statistical analyses were too scant to allow any definite conclusion concerning the extent or duration of this effect.
In summary, each species studied so far yields a distinctly different ocular response to the topical application of PGs. Such differences have been demonstrated with respect to the time course of IOP reduction and the occurrence and extent of other ocular effects, such as initial ocular hypertension, anterior chamber flare, and miosis. However, a significant reduction in IOP occurred in each of these species. In fact, ocular hypotension appears to be the only invariant ocular effect of these autacoids. Thus PGs should be regarded as potent ocular hypotensive agents. Although PGs, like any other drug or autacoid, may in high doses have other physiologic or pathophysiologic effects or side effects such as the development of miosis or flare, these effects appear to be more pronounced in lower vertebrates than those in primates. Intravitreal injection of very high doses of PGE_1 or PGE_2 have been shown to have only negligible effects on retinal function in rabbits, unless normal PG transport processes were blocked by prior systemic administration of a transport inhibitor. It should be noted, however, that similar or even higher doses of PGF_2a were found not to affect retinal function even when PG transport was blocked. These considerations suggest that PGs, especially PGF_2a and/or its analogues, or the specific stimulation of endogenous PG production, may provide a new therapeutic approach to the clinical control of IOP and the treatment of glaucoma.

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