Reduction of intraocular pressure by prostaglandins applied topically to the eyes of conscious rabbits

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Topically applied prostaglandins (PG's) in the dose range of 25 to 200 μg/eye caused a prolonged (15 to 20 hr.) ocular hypotony (as much as 7 mm. Hg below control values) in the conscious rabbit following the well-known initial hypertensive phase. The aqueous humor protein concentration was elevated, and the ascorbic acid concentration was decreased during the initial hypertony, but both approached normal during the first half of the hypotensive phase. This biphasic intraocular pressure (IOP) response was dose-dependent and was also observed after intravitreal PG administration. A reduction of IOP (as much as 7 mm. Hg) lasting for 12 hr. or more was observed following the topical application of a very low dose (5 μg) of PGF2α which was insufficient to cause an initial increase in IOP. Neither pretreatment with indomethacin nor sympathetic denervation diminished the biphasic IOP effect of PGF2α, suggesting that neither de novo synthesis of PG's nor release of endogenous norepinephrine was responsible for the hypotony. Assay of aqueous humor for PG's showed a high level of PG activity after 30 to 60 min. and a small residual activity 6 to 18 hr. following PG application. The hypotensive phase was associated with a reduction in outflow resistance as measured in either anesthetized or freshly killed animals. The present experiments suggest that exogenous administration of low doses of certain PG's or their analogues may aid in the treatment of ocular hypertension and that endogenous PG synthesis may, in some cases, contribute to or actually mediate the profound hypotony that often follows ocular trauma and inflammation.

Key words: PGE2, PGF2α, prostaglandins, intraocular pressure, uveitis, inflammation (ocular), hypotony (ocular), glaucoma, sympathetic denervation, indomethacin, outflow resistance, aqueous humor, ascorbic acid.

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Prostaglandins (PG's), administered intracamerally, topically, or intravenously, have been shown to reproduce most of the characteristic signs of acute ocular inflammation, including entry of plasma proteins into the aqueous humor and a rise in intraocular pressure (IOP). Increased levels of PG's were found in the aqueous humor of patients with untreated acute anterior
uveitis and of rabbits at the height of the inflammatory response induced by the intravitreal injection of either bovine serum albumin (BSA) or bacterial endotoxin. These and other observations suggest that PG's are important mediators in the breakdown of the blood-aqueous barrier and the associated increase in IOP during the initial phase of ocular inflammation.

The ocular hypertensive phase of uveitis is followed by a more prolonged hypotensive phase that lasts for 1 to 2 weeks following endotoxin- or x-irradiation-induced uveitis and for 1 to 2 months following BSA-induced uveitis. Furthermore, ocular hypotony characteristically accompanies chronic uveitis in humans. In contrast to the initial hypertensive phase, the mechanism and the possible chemical mediators of the prolonged reduction in IOP have not been studied in detail.

The intracameral administration of PGE₁ or PGE₂ into rabbit eyes has been shown to produce a dose-dependent reduction of IOP following the initial hypertensive response. These experiments were carried out in cannulated eyes of anesthetized animals; thus the duration of the hypotension could not be determined. In the same study, it was observed that when the ocular hypertension caused by exogenous PGE, or PGE₂ was blocked by pretreatment with polyphloretin phosphate, the hypotensive action of these PG's remained. The possibility of a dual effect of PG's on intraocular fluid dynamics is also suggested by a PG-induced increase in total outflow facility during the initial hypertensive phase.

In the present experiments, the complete time course of PG-induced hypertension and hypotension was studied in conscious rabbits. The relation between the dose of topically applied PG's and the IOP effects was established to determine whether a dose of PG's could be selected which would reduce IOP without an initial hypertensive response. In addition, the time course of PG-induced alterations in PG, ascorbic acid, and protein concentrations in the aqueous humor was correlated with the time course of IOP effects. The IOP response to PG's was also studied on indomethacin-pretreated animals and on sympathetically denervated eyes in an attempt to elucidate the mechanism by which PG's reduce IOP.

Methods

New Zealand white (albino) rabbits (2 to 4 kg.) were placed in rabbit boxes in order to accustom them to handling and restraint before they were used in these experiments.

Measurement of IOP. Following topical application of 0.5 percent Ophthaine solution (E. R. Squibb & Sons, Inc., Princeton, N. J.), the IOP of the conscious animals was measured with a pneumatic floating-tip tonometer calibrated on the cannulated rabbit eye by the open-stopcock method. At least two IOP measurements were made on each eye within 1 hr. prior to PG administration. Following the PG administration, IOP was measured at varying intervals for up to 22 to 30 hr.

Drug administration

Prostaglandins. In the first series of experiments, the IOP effects of three different modes of PGE₁ administration were compared.

1. A 25 μg amount of PGE₁ (The Upjohn Co., Kalamazoo, Mich.) in 50 μl of phosphate buffer (50 mM, pH 7.4) was dropped onto the cornea of the experimental eye, the contralateral control eye receiving 50 μl of phosphate buffer.

2. A 25 μg amount of the sodium salt of PGE₁ (made by adding sodium carbonate to PGE₁ dissolved in ethanol according to the procedure provided by The Upjohn Co.) in 5 μl of saline (0.9 percent sodium chloride) was dropped onto the cornea of the experimental eye and rinsed off 3 to 4 min. later with 2 to 4 ml. of saline; 5 μl of the vehicle solution (ethanol and sodium carbonate in saline) was similarly applied to the contralateral control eye and rinsed.

3. A 10 μg amount of PGE₁ in 10 μl of 10 percent ethanol was injected into the center of the vitreous body of the experimental eye; 10 μl of 10 percent ethanol was similarly injected into the contralateral control eye. The globe was rinsed with saline after the needle was withdrawn.

Best results were obtained with the second mode of administration (see Results); therefore, in all subsequent experiments, PGE₁ or PGF₂a (obtained as the tromethamine salt; The Upjohn Co.) was applied topically in a 5 μl volume, followed 3 to 4 min. later by rinsing of the cornea and conjunctival cul-de-sac with 2 to 4 ml. of saline.

Indomethacin. The sodium salt was prepared immediately prior to use by adding sufficient sodium carbonate to a 20 mg./ml. indomethacin (Merck, Sharp & Dohme, Rahway, N. J.) suspension to obtain a clear solution; 50 mg./kg. free
acid equivalent was injected intraperitoneally 1 hr. prior to PG administration. The effectiveness of this pretreatment was demonstrated by the absence of an ocular hypertensive response to 50 µl of 1.0 percent sodium arachidonate applied topically to the contralateral control eye at least 6.5 hr. after indomethacin pretreatment.

Superior cervical ganglionectomy. Eight rabbits were anesthetized with Chloropent (3 ml./kg., intravenously; Fort Dodge Laboratories, Fort Dodge, Iowa) before unilateral removal of the superior cervical ganglion together with approximately 1 cm. of preganglionic sympathetic fiber. After 24 hr. all animals showed the characteristic ipsilateral iridial hyperemia and ocular hypotony (3 to 8 mm. Hg below control). Seven to 14 days later, the IOP was measured both before and up to 24 hr. after administration of 50 µg of PGE₂ to both eyes.

Aqueous humor composition. Forty-four rabbits were killed with an overdose of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.) 0.5 to 24 hr. after topical application of 50 µg of PGE₂ or PGF₂α; the aqueous humor was removed from the PG-treated and the contralateral control eyes. The protein and the ascorbic acid concentrations of individual control and experimental aqueous samples were determined. Pooled aqueous samples from two to six eyes were extracted for PG assay, and the PG content of these extracts was measured either with bioassay on the rat stomach strip preparation or with radioimmunoassay (Clinical Assays, Inc., Cambridge, Mass.) to determine PGF₂α levels.

Measurement of gross outflow resistance. Following topical application of 50 µg of PGE₂, gross outflow resistance was measured simultaneously on both the experimental and the contralateral control eyes by constant-rate infusion during the hypotensive phase (i.e., 5 to 12 hr. after application) or during recovery from hypotony (i.e., 17 to 19 hr. after application). The IOP's were monitored manometrically with Sanborn 267b pressure transducers in conjunction with Sanborn 500A carrier preamplifiers and a multichannel Model 350 rectilinear recorder. In these experiments, the outflow resistance was determined on animals anesthetized with Chloropent (3 ml./kg., intravenously) or, in some cases, on animals killed with an overdose of Chloropent or Nembutal.

Results

Topical application or intravitreal injection of PGE₂ resulted in a biphasic IOP response: a relatively short initial hypertensive phase followed by a prolonged hypotony (Fig. 1). The extent of the hypertensive response seemed to depend on the volume in which the PGE₂ was applied to the cornea, since topical application of 25 µg of PGE₂ in 50 µl of phosphate buffer (50 mM, pH 7.4) was applied topically to the experimental eye of 8 rabbits. The contralateral control eye received 50 µl of phosphate buffer. B, 25 µg of PGE₂ in 5 µl of normal saline was applied topically to the experimental eyes of four rabbits, which were then rinsed with saline. The contralateral control eye received 5 µl of phosphate buffer. C, 10 µg of PGE₂ in 10 µl of 10 percent ethanol was injected intravitreally into the experimental eye of six rabbits. Ten microliters of 10 percent ethanol was similarly injected into the contralateral control eye. The points represent means and the limits ± 1 S.E.M.
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Fig. 2. Effects of repeated topical PGE2 application on the IOP of rabbits. A, 25 μg of PGE, in a 5 μl volume was applied to both eyes of five rabbits. After 3.5 hr., a second dose of 25 μg of PGE2 was applied to the right eyes only of each animal. The left eye received 5 μl of vehicle solution at this time. B, 25 μg of PGE2 in a 5 μl volume was applied six consecutive times at hourly intervals to one eye of six rabbits. Five microliters of vehicle solution were applied to the contralateral control eyes at the same time. The points represent means or mean differences [(IOP of the PGE-treated eye) - (IOP of the contralateral control eye)] measured at the same time; the limits are ± 1 S.E.M.

hypertensive effect seen in the control eye following the first mode of PG application (Fig. 1, A vs. B). Although intravitreal injection of PGE2 produced a similar biphasic IOP response, the onset of the hypotony was more variable, as indicated by the larger standard errors during the slower transition from the hypertensive to the hypotensive phase (Fig. 1, C). For this reason, the second mode of PG application was used in all subsequent experiments.

When a second dose of PGE2 was applied to rabbit eyes during the hypotonic phase—3.5 hr. after the first PG application—there was a significant second rise in the IOP followed by an accentuated hypotony as compared with the contralateral eyes which received only a single dose of PGE2 (Fig. 2, A). When six identical doses (25 μg in 5 μl each) of PGE2 were applied at hourly intervals, the duration of the hypotonic phase was increased, and the ocular hypotony was accentuated (Fig. 2, B, vs. Fig. 1, B).

A biphasic IOP response was also observed following topical application of high doses of PGF2α (Fig. 3, C and D). At a dose of 50 μg, PGF2α produced a smaller initial rise in IOP than the same dose of PGE2 even though the hypotony produced by both PG's was similar both in extent and
duration (Fig. 3, C, vs. Fig. 4, A). At a dose of 5 μg of PGF₂α, a prolonged hypotony was obtained without any significant initial rise in IOP (Fig. 3, B).

For some of these experiments, the differences between the IOP's of the two eyes—as a function of time after the application of PG or the vehicle solution—are reported, since this expression eliminates animal-to-animal variations in the pre-existing IOP, changes in the physiological state of the animals, diurnal IOP variations, and tonometry-induced IOP effects. As in the case of PGE₂, when PGF₂α was applied at any of the doses in a small volume (5 μl) to one eye, the contralateral control eye did not show a significant initial increase in IOP. The possibility that the contralateral control eyes show small reductions in IOP after PG administration to the experimental eyes cannot be ruled out at this time. The study of such contralateral effects would require the use of much larger numbers of animals, including untreated controls, to distinguish among drug effects, circadian variations, and tonometry-induced changes in IOP.

The aqueous humor protein concentration was increased 30- to 50-fold 2 hr. after the administration of 50 μg of PGE₂ (Fig. 4, B), and the ascorbic acid concentration was decreased by 30 percent (Fig. 4, C). The onset of these changes approximated the initial rise in IOP. Both of these parameters began to return toward normal at the onset of the hypotensive phase and showed almost complete recovery during the first half of this period (Fig. 4).

PG levels in the aqueous humor were measured at varying time intervals following the topical application of 50 μg of PGF₂α (Fig. 5, A) or PGE₂ (Fig. 5, B). With the use of either radioimmunoassay for PGF₂α levels or bioassay for total PG-like activity, the PG concentrations were at their highest level 0.5 hr. after topical PG administration, corresponding in time to the maximal rise in IOP. At this time, small but clearly measurable amounts of PG's were also detected in the contralateral control eyes. The high levels of PG's in the PG-treated eyes decreased rapidly but were still slightly elevated during the onset of the hypotensive phase. At 12 hr. after administration of PGF₂α or PGE₂, the aqueous humor of the treated eyes contained clearly detectable amounts of PGF₂α or PGE₂ as determined by immunoassay or bioassay, respectively. On the other hand, PG activity could not be detected in the control eyes by either technique at 12 hr. or anytime thereafter. After 24 hr., PG's could not be detected in the aqueous humor of either the experimental or the control eyes.
Fig. 5. Levels of PG in the aqueous humor following topical PG application at a dose of 50 μg. A, PGF₂α levels at various times after PGF₂α application were measured by radioimmunoassay on aqueous humor samples pooled for each point from either the PG-treated or the contralateral control eyes of three rabbits. B, PG-like activity at various times after PGE₂ application was determined by bioassay on one or two aqueous humor samples pooled for each point from either the PG-treated or the contralateral control eyes of two to six rabbits.

Two experiments were carried out to determine whether endogenous PG's or catecholamines contribute to the ocular hypotensive effects of exogenously administered PG's. Neither indomethacin pretreatment (Fig. 6) nor chronic sympathetic denervation (Fig. 7) diminished the biphasic IOP response to 50 μg of PGE₂.

During the time of maximal hypotony, the gross outflow resistance of the PG-treated eye was 40 to 50 percent that of the contralateral control eye. Moreover, this decrease in outflow resistance persisted when the animals were killed prior to the measurement (Fig. 8). As the outflow pressure was returning to the control value 18 hr. after PG administration, the outflow resistance also approached the control value.

Discussion

The results presented here show that in addition to their well-known ocular hypertensive effects, topically applied PG's also cause a significant and prolonged reduction in IOP. This hypotony is not a compensatory response to the initial hypertensive phase, since it was also observed with a dose of PGF₂α too low to cause any significant initial rise in IOP. This finding is consistent with the observation that intracameral injection of PGE₁ or PGE₂ lowered IOP in cannulated eyes of anesthetized rabbits when the hypertensive effect was blocked by pretreatment of the eye with polypheoretin phosphate. Bhattacherjee and Hammond referred to preliminary results indicating that "250 ng. of PGE₁ do not raise but in fact reduced ocular tension by a small degree (approx. 1-2 mm. Hg)," but neither the route of administration nor the duration of the effect was stated.

The present observation that a low dose of topically applied PGF₂α can lower IOP by as much as 7 mm. Hg for 12 hr. or more without a significant initial rise in IOP suggests that suitable doses of certain PG's or their analogues may be useful in the therapeutic control of ocular hypertension. In
IOP reduction by topical prostaglandins

Fig. 7. Comparison of the IOP response to PGE₂ (50 μg) applied topically to both the sympathectomized and the contralateral normally innervated eyes of eight rabbits. The points represent means and the limits ± 1 S.E.M.

In this respect, it is interesting to note that PGF₂α, administered by intrauterine or intravenous injections to pregnant women to induce abortions, has been reported to cause a reduction in IOP. However, the duration of this PG-induced ocular hypotony was not determined.

It has been suggested that the decreased gross outflow resistance observed during PG-induced ocular hypertension reflected an increased pseudofacility due to the breakdown of the blood-aqueous barrier. The work of Masuda and Mishima showed that the increased rate of aqueous flow, which reached a peak at 1.5 hr. after the administration of a very high dose (100 μg) of PGE₂, returned to essentially normal by 4 hr. During this phase of increased aqueous formation, there was an increase of pseudofacility in the eyes of these urethane-anesthetized rabbits. None of these studies included measurement of outflow facility, flow, or pseudofacility more than 4 hr. after PG administration. Thus the conclusions of these authors refer only to the hypertonic phase which is clearly associated with a breakdown of the blood-aqueous barrier.

The PG-induced hypotony observed in the present experiments could not be accounted for by a maintained breakdown of this barrier, since the aqueous humor protein concentration returned toward normal during the first half of the hypotony. The fact that a significant increase in IOP resulted when a second dose of PGE₂ was applied to the eye during the hypotonic phase also suggests that the blood-aqueous barrier was functional. If the barrier were broken down, a second dose of PG’s would not show a further increase in IOP.

The long-term hypotony observed in these experiments cannot be accounted for by damage to the secretory mechanisms or reduced blood flow to the ciliary processes, since the aqueous humor ascorbic acid concentration, which depends on both secretory processes and ciliary blood flow, returned toward its normal level during maximal hypotony. These considerations

Fig. 8. Graphic comparison of calculated outflow pressure (IOP-Pv) with measured outflow resistance (R) during maximal hypotony (5 to 12 hr. after topical application of 50 μg of PGE₂ to one eye) and during recovery from hypotony (17 to 19 hr. after PG application). The outflow resistances of the PG-treated and the contralateral control eyes of 11 anesthetized (Ralive) or freshly killed (Rdead) rabbits were measured as described in the text. The percent change in outflow pressure, [(IOP - Pₐ)exp/(IOP - Pₐ)cont] × 100, was calculated with the mean IOP before PG treatment, during maximal hypotony, and during recovery (based on the 17 rabbits shown in Fig. 4, A); episcleral venous pressure (Pv) was assumed to be 9 mm Hg.
indicate that the reduction in IOP could not be due to alterations in secretory mechanisms or to pseudofacility, and hence they must be primarily attributed to a reduction in true outflow resistance. This conclusion was further supported by the fact that the decreased outflow resistance measured in the present study persisted in the eyes of dead rabbits.

The possibility that the hypotony depends on the de novo synthesis of PG's or other cyclo-oxygenase products such as endoperoxides, thromboxane A₃, or prostacyclin can be ruled out because the hypotensive phase was not diminished by systemic pretreatment with indomethacin, an effective inhibitor of cyclo-oxygenase. Furthermore, it is unlikely that the IOP effect of PG's is related to local sympathetic activity or to the release of endogenous catecholamines, since the time course of the IOP response was very similar in both normally innervated and sympathetically denervated eyes. In fact, the only difference was a faster transition from the hypertensive to the hypotensive phase in the denervated eye.

Since small amounts of PG activity could be detected in the aqueous humor by either immunoassay or bioassay for 6 to 18 hr. after topical PG application, the hypotensive phase may result from the continual presence of PG's in the eye. Although more detailed studies will be required to determine the statistical and physiological significance of these residual PG levels, it should be noted that the aqueous humor levels may underestimate true bioavailability of the exogenous PG's at the receptor sites, since the anterior uvea, for example, was shown to accumulate PG's against a several-fold concentration gradient.

We must also consider the possibility that the prolonged hypotonic effects of PG's is due to the accumulation of a second autocoid. PG's are known to exert their effects in many biological systems by altering intracellular cyclic AMP levels. PG's were, in fact, shown to increase the cyclic AMP content of several ocular tissues in vitro, including the sclera-trabecular ring, iris-ciliary body, and cornea, and intracameral injection of cyclic AMP was shown to increase outflow facility in the rabbit eye. Therefore, following exogenous PG administration, the prolonged residual PG levels in the eye may cause an even longer increase in the cyclic AMP content of intraocular tissues and/or fluids, which in turn may account for the long-term increase in outflow facility.

The release of a second messenger could also provide an explanation for the hypotonic effect of intravitreally injected PC. It was shown that intravitreally injected PG's do not effectively reach the anterior chamber as a result of the absorptive transport activity of the ciliary processes. Therefore it could be argued that PG's could not reach the chamber angle from the vitreous and hence could not have an effect on true outflow facility. On the other hand, accumulation of PG's in the ciliary processes may cause the release of cyclic AMP or other endogenous autocoids, which in turn could reach the trabecular meshwork region by bulk flow through the posterior and anterior chambers or by diffusion through the stroma of the ciliary body and the iris. In addition, PG's may reach the outflow angle by the latter route, since PG's, which are accumulated by the ciliary body, were shown not to be effectively metabolized by this tissue.

Severe trauma may cause irreversible changes in the secretory processes, resulting in prolonged and sometimes irreversible hypotony. However, the reversible hypotony that is observed following more moderate forms of uveitis, e.g., those induced by bacterial endotoxin or cataractogenic doses of x rays may be mediated by the continual release of PG's. It was shown that ocular inflammation or uveitis can damage the transport processes of the anterior uvea which normally remove PG's from the intraocular fluids. Interference with this active transport mechanism may contribute to the accumulation of PG's within the aqueous humor during uveitis, since PG's are not
effectively metabolized by intraocular tissues. In the absence of such absorptive transport processes, endogenously produced PG's could exert long-term hypotensive effects.

The long-term reduction of IOP induced by twice daily topical epinephrine treatment was recently shown to be blocked by pretreatment of the rabbits with indomethacin, suggesting that the epinephrine-induced hypotony may be dependent upon the endogenous synthesis of PCs. The present demonstration that PCs can cause a long-term reduction of IOP in normal conscious rabbits supports this interpretation and suggests that the use of very low doses of certain PG's or PG analogues may provide a more direct therapeutic approach to the control of ocular hypertension.

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REFERENCES


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