The effects of intravitreally injected prostaglandin E₂ on retinal function and their enhancement by a prostaglandin-transport inhibitor. M. C. Wallenstein and L. Z. Bito.

In this study, the effects of intravitreally injected PG's on the bioelectrical activity of the retina were investigated on normal and BrCG-pretreated conscious rabbits by monitoring the electroretinogram (ERG) and the visually evoked response (VER).

Methods. Supradural electrodes were implanted in the skulls of female New Zealand white rabbits (2 to 3 kg) under general anesthesia (Equithesin; 2 to 3 ml/kg), as previously described. In addition, both nictitating membranes were removed (for better electrode-cornea contact). Five to 7 days after surgery, each rabbit was placed, without general anesthesia, in an animal box inside a grounded wire-mesh cage lined with reflective foil. Contact lenses containing chlorided silver surface electrodes were placed over the corneas. Retinal and cortical electrical activities were recorded monopolarly on a Grass 5 polygraph or a Beckman Dynograph. Light stimulation of 20 μsec duration was provided throughout each experiment at 2 sec intervals by a Flash-Tac Stroboscope (Electronic Applications Ltd., Hertfordshire, England) placed 35 cm in front of the animal and reflected by the foil and mirrors placed in front of each eye. A single high-intensity flash was delivered by holding a Bauer E16 (Robert Bosch Photokine, Germany) electronic flash (duration 1/800 sec at 40 watts/sec; color temperature 5600 K) about 4 cm from one eye of the rabbit.

After the recording of the normal evoked electrical activities, either 0.35 or 0.7 mg of PGE₁, was injected intravitreally into one eye of each rabbit at a rate of 1 μl/sec with a constant-rate clutch pump. The contralateral control eye was injected with 70% ethanol alcohol, and the recording of bioelectrical activity was resumed.

After at least 48 hr, each animal was pretreated with BrCG (10 mg/kg, intravenously); 10 min later, baseline VER and ERG were recorded, and the effects of intravitreally injected PG's on the bioelectrical activity of the retina could be explained by the PG-induced miosis. A moderate effect on some of these parameters. These effects could not be explained by the PG-induced miosis. PGF₂α (0.7 mg/eye) caused no significant changes in any of the parameters studied. These results indicate that exogenous PG's can have adverse effects on retinal function and that these effects are enhanced by BrCG, a PG-transport inhibitor. Presumably, this inhibitor blocks the PG-removal mechanisms across the blood-retinal barriers and hence allows the accumulation of PG's in the extracellular fluids of the retina.

Exogenous prostaglandins (PG's) have an adverse effect on the anterior segment of the eye, and endogenously released PG's have been shown to mediate some of the pathophysiological effects of intraocular inflammation. Intravitreally injected PG's were shown to affect retinal vasculature, but the effect of these autacoids on retinal function has not been studied.

Intravitreally injected [³H]PG's are lost from the eye with a half-time of about 3 hr, by means of facilitated, absorptive transport through the ciliary processes and apparently also across the blood-retinal barriers. This short half-time indicates that transport across the blood-retinal barrier of the normal animal can keep up with diffusion from the center of the vitreous to the retina and thereby prevent local accumulation of PG's in the extracellular space of this tissue.

It has already been shown that systemic administration of the PG-transport inhibitors, probenecid or bromocresol green (BrCG), renders the visual cortex, as well as the thermoregulatory centers of the brain, sensitive to supraocically applied PGE₁. Similarly, one could expect accumulation of endogenous or intravitreally injected PG's in the extracellular space of the retina to result from inhibition of PG transport and hence to have an adverse effect on the normal functioning of this neural tissue.

In this study, the effects of intravitreally injected PG's on the bioelectrical activity of the retina were investigated on normal and BrCG-pretreated conscious rabbits by monitoring the electroretinogram (ERG) and the visually evoked response (VER).

Table I. Effect of intravitreally-injected PG's on the ERG, VER, and pupil diameter of normal and BrCG-pretreated rabbits

<table>
<thead>
<tr>
<th>Pg</th>
<th>Pre-treatment</th>
<th>No. of animals</th>
<th>Average % decrease in amplitude*</th>
<th>Average % decrease in pupil area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>b-wave (ERG)</td>
<td>SNW (VER)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>of animals</td>
<td>of animals</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>6</td>
<td>11 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td>BrCG</td>
<td>6</td>
<td>9 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Ei (0.70)</td>
<td>None</td>
<td>6</td>
<td>24 ± 14</td>
<td>29 ± 10</td>
</tr>
<tr>
<td></td>
<td>BrCG</td>
<td>4</td>
<td>48 ± 4</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>Ei (0.35)</td>
<td>None</td>
<td>4</td>
<td>32 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>F2a (0.70)</td>
<td>BrCG</td>
<td>8</td>
<td>34 ± 5</td>
<td>49 ± 13</td>
</tr>
<tr>
<td>None</td>
<td>BrCG</td>
<td>3</td>
<td>5 ± 4</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

*Wave amplitude 20 to 30 min after intravitreal injection of PG or vehicle solution as a percentage of wave amplitude 10 min before injection.

1 Intravitreal injection of vehicle solution (20 μl of 70% EtOH) only.

then the eye that was previously used as a control was injected with 0.35 or 0.7 mg of PGE, or PGF2a while the contralateral eye was injected with an identical volume (20 μl) of vehicle solution (70% alcohol). The PG and BrCG (2 mg/ml in saline) solutions were prepared and stored as previously described.

The effects of these treatments were analyzed by measuring changes in (1) baseline patterns, (2) the amplitude of the VER slow negative wave (SNW), (3) the amplitude of the ERG b-wave, (4) the time required for the reappearance of ERG b-wave and the SNW following a single high-intensity light flash. Average amplitude of each component was derived from five consecutive evoked responses.

The effects of miosis on these parameters were studied on three rabbits which were prepared for recording as above. Instead of PG injection, one drop of 1.5% carbachol (Alcon Laboratories Inc., Fort Worth, Texas) solution sufficient to cause maximal pupillary constriction was applied to the corneal surface.

The miotic effects of intravitreally injected PG’s were studied on separate sets of rabbits. These animals were not fitted with electrodes, since the contact lens precluded accurate visualization of the iris. The pupillary diameter was measured by comparison with circles of known diameters. Measurements were made on a schedule similar to that used in the recording of electrical activity in the other set of experiments.

Results

Effect of intravitreal PG injection on normal rabbits. In rabbits which received no pretreatment with BrCG, intravitreal injection of 0.7 mg of PGE, resulted in a small but statistically significant decrease in the ERG b-wave amplitude when compared to the ERG of the same eyes before PGE, injection (Table I). A small reduction in the amplitude of the SNW of the contralateral VER (Fig. 1, b and c; Table I) was statistically significant (p < 0.05). Injection of PGE, into the eyes of 8 rabbits that were not fitted with corneal electrodes was found to produce a gradual miosis which reached a peak within 40 to 60 min (Table I).

A smaller dose of PGE, (0.35 mg/eye) had a similar effect on the amplitude of the ERG b-wave but had no significant effect on the SNW of the VER (Table I). Intravitreal injection of 0.7 mg of PGF2a did not have a significant effect on the ERG, VER, or pupillary diameter as compared to the preinjection values or to those measured after injection of the vehicle solution alone (Table I).

Effect of intravitreal PG injection on BrCG-pretreated rabbits. Intravitreal injection of PGE, (0.7 mg) 8 to 15 min after systemic pretreatment with BrCG (10 mg/kg) resulted in a significant (p < 0.001) decrease in ERG b-wave amplitude as compared to that observed following injection of the vehicle solution, but not statistically significant as compared to the decrease in b-wave amplitude following PGE, injection into the vitreous of normal rabbits (Table I). The inhibitory effect of intravitreally injected PGE, on the VER was, however, greatly enhanced by BrCG pretreatment (Table I; Fig. 1), which caused a significant (p < 0.002) decrease in the SNW amplitude when compared to the effect of 0.7 mg of PGE, without pretreatment. The SNW of the VER was absent in three of the four animals and partially lost in the fourth. The early negative component of the VER seemed unchanged (Fig. 1), but alterations in this
component were difficult to measure because of its small size. Periods of large amplitude irregular activity appeared in the electrocorticogram of all four animals.

A possible association between miosis and alterations in electrical activity after intravitreal injection of PGE₁ was also investigated by continuing measurements of the retinal and cortical activity of these four animals for 4 hr. The VER, but not the ERG b-wave, returned to control amplitude within 3 to 4 hr, but miosis was still present when the contact lens was removed at the end of the 4 hr period. Topical application of 1.5% carbachol, which caused maximum miosis, had no observable effect on the ERG or VER.

In contrast to PGE₁, intravitreal injection of 0.7 mg of PGF₂α into BrCG-pretreated rabbits did not have a significantly greater effect on the ERG, VER, or pupil size than injection of vehicle solution alone (Table I). Intravenous injection of BrCG (10 mg/kg) did not have a significant effect on the ERG or the VER in rabbits that received no subsequent PGE₁ injection.

**Effect of a single high-intensity flash on the ERG.** A single high-intensity light flash greatly reduced the amplitude of the ERG in all rabbits. The return of b-wave amplitude to the preflash level required 4 to 6 min (Table II; Fig. 2) in both the PGE₁-injected and control eyes of rabbits which were not pretreated with BrCG. However, in animals which were pretreated with 10 mg/kg BrCG before the intravitreal injection of 0.7 mg of PGE₁, the time required for the return of normal ERG b-wave amplitude following a single high-intensity flash was doubled (Table II; Fig. 2). The effect of the high-intensity flash on the VER of these BrCG-pretreated rabbits could not be established because in these animals the VER was either obscured or abolished following intravitreal injection of PGE₁.
be explained in terms of an action of PGE, on the rabbit eye are potentiated by systemic pretreatment with BrCG. Such potentiation was most clearly evident in the effect of exogenous PGE, on the rabbit eye is expected to occur in the retina as a result of inhibition of normal PG transport across the blood-retinal barriers, primarily across the pigment epithelium. It should be noted that in the rabbit, less than 5% of the retina is vascularized; thus passive diffusion or facilitated transport into retinal capillaries cannot be an important route of PG elimination. High levels of PGE, could effect retinal function by their effects on calcium binding, adenyl cyclase activity, metabolic activity, and/or ionic gradients.

Intravitreal injection of more than 0.2 mg of any of several PG’s was shown by Peyman et al. to cause vascular leakage and occlusion, hemorrhage, and retinal detachment. Their first observation, however, was made 2 hr after PG injection, and some of the changes described took days to develop. In our studies, alterations in retinal function showed a maximum at 30 to 40 min after PGE, injection, and recovery of the SNW, for example, was essentially complete within 3 to 4 hr. In addition, although Peyman et al. reported that PGE, and PGF2a were equally effective in producing angiographic signs of dye diffusion in the rabbit retina, in our studies, electrical activity of the retina was not affected by PGF2a. Furthermore, since fluorescence leakage was found to occur only in the central portion of the small vascularized region, vascular leakage could have a direct effect on only a small fraction of the retina and hence could not be expected to alter the ERG. Although we cannot rule out the possibility that injection of PGE, caused an increased retinal vascular permeability during the course of our experiments, it seems that vascular changes alone could not account for all the observed effects of intravitreally injected PGE, on retinal functions.

Although the actual mechanisms mediating the effects of PGE, on retinal function remain to be elucidated, the present results support the concept that the blood-ocular barriers have an absorptive PG transport system or a “PG-removal mechanism,” which serves a physiological function. When this transport mechanism is inhibited, the retina becomes more vulnerable to the pathophysiological effect of exogenous PG’s, and presumably also endogenous PG’s. The possibility that

<table>
<thead>
<tr>
<th>PGE, (mg/eye)</th>
<th>Pre-treatment</th>
<th>No. of animals</th>
<th>Duration of ERG loss (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>6</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>0.35</td>
<td>BrCG</td>
<td>6</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>0.70</td>
<td>None</td>
<td>4</td>
<td>4.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>BrCG</td>
<td>4</td>
<td>8.9 ± 0.6</td>
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**Discussion.** These experiments show that the pathophysiological effects of intravitreally injected PGE, on the rabbit eye are potentiated by systemic pretreatment with BrCG. Such potentiation was most clearly evident in the effect of exogenous PGE, on the SNW of the VER during low-intensity flash trials and on the duration of the ERG amplitude reduction following a single high-intensity light flash. Both of these effects can best be explained in terms of an action of PGE, on the retina itself and the enhancement of PG accumulation in this tissue by BrCG-induced inhibition of PG transport across the blood-retinal barriers.

The possibility that the attenuation of the ERG b-wave amplitude was due simply to PGE,-induced miosis may be considered, since ERG b-wave amplitude is dependent on the amount of light reaching the retina. The iris of the albino rabbit, however, is translucent to light; thus passive diffusional or facilitated transport into retinal capillaries cannot be an important route of PG elimination. High levels of PGE, could effect retinal function by their effects on calcium binding, adenyl cyclase activity, metabolic activity, and/or ionic gradients.

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**Table II.** Enhancement of the duration of ERG loss due to single high-intensity flash after intravitreal injection of PGE, into BrCG-pretreated rabbits

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<td></td>
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<td>8.9 ± 0.6</td>
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inflammation-induced inhibition of ocular PG transport may contribute to uveitis-induced photophobia also seems worthy of investigation.

We thank Dr. Kenneth E. Eakins for numerous discussions and suggestions, Roger A. Baroody for technical assistance, Dr. Peter Gouras for his helpful comments, and Ms. Susan Q. Merritt for editorial assistance during the preparation of this manuscript. We are indebted to Dr. John Pike of The Upjohn Co., Kalamazoo, Mich., for the supply of prostaglandins.

From the Research Division, Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, N. Y. This investigation was supported by Post-doctoral Training Grant EY 00029 from the National Institutes of Health, Bethesda, Md. Submitted for publication May 12, 1978. Reprint requests: Dr. Laszlo Z. Bito. Ophthalmology Research, 630 West 168th St., New York, N. Y. 10032.

Key words: prostaglandin, transport, retina, blood-retinal barrier, electroretinogram, visually-evoked response, bromocresol green

REFERENCES


Correlation of aqueous humor ascorbate with intraocular pressure and outflow facility in hereditary buphthalmic rabbits. PEI-FEI LEE, RICHARD FOX,* IRENE HENRICK, AND WILLIAM K. W. LAM.

The hypotensive effect of ascorbate on intraocular pressure has been reported following topical application, oral administration, or anterior chamber infusion in animals. The present report describes the correlation of aqueous humor ascorbate concentration with intraocular pressure as well as outflow facility in vivo. Low aqueous ascorbate level was seen in buphthalmic eyes with high intraocular pressure and low outflow facility. The opposite correlation was observed in normal eyes. Ascorbate concentration in the anterior chamber of the rabbit eye is apparently related to the alteration of outflow facility and the movement of fluid in the anterior chamber.

The possible role of ascorbate in regulating intraocular pressure has been suggested by a number of animal experiments. However, the precise role of ascorbate in aqueous humor dynamics is unclear and has not been fully evaluated. The recent observation of a drastic reduction of ascorbate concentration in the eyes of adult buphthalmic rabbits prompted the present study of the possible correlation of aqueous ascorbate concentration with intraocular pressure and outflow facil-