Stimulation of Cyclic Adenosine Monophosphate Accumulation Causes Breakdown of the Blood–Retinal Barrier

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Pigmented rabbits were given an intravitreous injection of 0.1 ml of various concentrations of test drug, and vitreous fluorophotometry was done 6 and 24 hr after injection. Dibutyryl cyclic adenosine monophosphate (AMP) and 8-bromo-cyclic AMP caused reversible intravitreous fluorescein leakage only at relatively high concentrations. Adrenergic agents that are effective stimulators of adenylate cyclase (epinephrine, isoproterenol, and norepinephrine) caused transient intravitreous fluorescein leakage (2.3–3.1-fold above baseline) that was significantly greater than that caused by phenylephrine (1.1-fold above baseline), an adrenergic agent that is a poor stimulator of adenylate cyclase. Prostaglandins E₁ and E₂, which are good stimulators of adenylate cyclase, caused striking disruption of the blood–ocular barriers, and prostaglandins that are not good stimulators of adenylate cyclase had little or no effect on these barriers. The magnitude of the prostaglandin E₁ effect (9.3-fold above baseline) was similar to that of N-ethylcarboxamidoadenosine (NECA), the most potent adenosine agonist, and was greater than one would predict based on its effect on adenylate cyclase in vitro. Prostaglandin E₁, like NECA, also caused retinal vasodilation and hemorrhages. These data suggest that stimulation of intracellular cyclic AMP accumulation may be a common feature of mediators that cause breakdown of the blood–retinal barrier, but there may be another as yet unexplained feature shared by PGE₁ and NECA that makes them particularly effective and capable of causing retinal vasodilation and hemorrhages. Invest Ophthalmol Vis Sci 32:2006–2010, 1991

Macular edema is a major cause of visual loss. It can occur after almost any type of intraocular surgery and complicates several disease processes. It is characterized by leakage and collection of fluid in the macula that, when chronic, results in cyst formation. In some instances, the source of the leakage is easily identified as focal or diffuse structural abnormalities of the paramacular blood vessels such as microaneurysms. However, macular edema occurring after intraocular surgery or in the setting of ocular inflammatory disease is not associated with identifiable structural abnormalities. It is likely that, in these settings, leakage occurs through some type of interaction between chemical mediators and cells that make up the blood–retinal barrier. We recently postulated that adenosine might be such a mediator in diffuse macular edema complicating ischemic retinopathies for a number of reasons. Adenosine is a potent vasodilator frequently released by ischemic tissue. It and its agonists cause retinal vasodilation and hemorrhages similar to that seen in ischemic retinopathies. Adenosine agonists cause breakdown of the blood–retinal barrier through specific interaction with A₂ adenosine receptors, and A₂ adenosine receptors occur on both vascular endothelium and retinal pigment epithelium. Since A₂ receptors are coupled to adenylate cyclase, we wondered if stimulation of cyclic adenosine monophosphate (AMP) accumulation might be characteristic of other mediators of blood–retinal barrier breakdown. In this study, we examined cyclic AMP analogues and agents that commonly act on receptors coupled to an adenylate cyclase for their effect on the blood–retinal barrier.

Materials and Methods

All drugs were obtained from Sigma (St. Louis, MO). Concentrated stock solutions of drugs were made in phosphate-buffered saline (PBS), dimethyl sulfoxide, or ethanol depending on solubility. They were then filter sterilized and diluted to desired concentrations in PBS.

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Pigmented rabbits were used in a manner that conformed to the ARVO Resolution on the Use of Animals in Research. They were anesthetized with subcutaneous injection of 5 mg/kg of xylazine hydrochloride and 25 mg/kg of ketamine hydrochloride, and their pupils were dilated with 2% phenylephrine hydrochloride eye drops. Baseline vitreous fluorophotometry (VFP) measurements were made before intravitreous injections. For injections, a 30-gauge needle was inserted 3 mm posterior to the limbus, and 0.1 ml of vehicle or test drug was injected slowly into the vitreous cavity under visualization by indirect ophthalmoscopy. The VFP measurements were done 6 and 24 hr after injections.

The equipment and technical aspects of our VFP technique were described previously. Briefly, the rabbits received an intravenous injection of 14 mg/kg of fluorescein sodium, and scans were made exactly 60 min later. Each scan was initiated just posterior to the retina 1 mm inferior to the optic nerve. The reading point was set 2 mm anterior to the retina, and fluorescence values obtained were entered directly into a computer for data processing and recording. The fluorescein concentration at the reading point was then calculated by the computer and printed on its display screen. Student unpaired t-test was used for all statistical comparisons.

Results

Cyclic AMP is poorly permeable through cell membranes. Two analogues of cyclic AMP that have greater membrane permeability, dibutylryl cyclic AMP and 8-bromo-cyclic AMP, cause breakdown of the blood–retinal barrier when injected into the vitreous cavity at concentrations above 10^{-3} M in 0.1 ml of vehicle (Table 1). Butyrate alone has no effect on the blood–retinal barrier (Table 1).

Adrenergic receptors coupled to an adenylyl cyclase were found on both retinal pigment epithelium and retinal microvessels. When injected into the vitreous cavity at a concentration of 10^{-5} M in 0.1 ml, epinephrine, isoproterenol, and norepinephrine each cause significant vitreous fluorescein leakage 6 hr after injection (Fig. 1). Each of these agents are effective stimulators of cyclic AMP accumulation in retinal pigment epithelial cells. Phenylephrine is less effective and causes significantly less fluorescein leakage when injected into the vitreous (Fig. 1). Injection of 10^{-4} M epinephrine did not result in greater fluorescein leakage at 6 hr than occurred after injection of 10^{-3} M epinephrine (19.1 ± 3.5 ng/ml versus 25.3 ± 4.6 ng/ml). This was also true for isoproterenol and norepinephrine (data not shown).

In retinal pigment epithelial cells, prostaglandins E1 and E2 are effective stimulators of cyclic AMP accumulation; prostaglandin F1α is not. When injected into the vitreous cavity, prostaglandin E1 causes extensive breakdown of the blood–retinal barrier, and prostaglandin F1α has no effect (Table 2). Prostaglandin F1α causes a very small but statistically significant increase in vitreous fluorescein leakage compared with vehicle alone. Prostaglandin E2 has a much greater effect on the blood–aqueous barrier than prostaglandin E1, making it difficult to assess its effect on the blood–retinal barrier, because the absorption of the excitation light beam by the high concentrations of fluorescein in the anterior chamber precludes accurate fluorophotometry readings in the vitreous. However, even though accurate quantitation could not be done, there were increased levels of fluorescein present in the vitreous, suggesting that prostaglandin E2 has an effect on the blood–retinal barrier. In addition, intravitreous injection of prostaglandin E1 or E2 produces retinal vasodilation and some hemorrhage, resulting in a fundus appearance similar to that seen after injection of adenosine agonists.

This made us consider the possibility that prostaglandins E1 and E2 might act through release of endogenous adenosine, or adenosine agonists might act through release of endogenous prostaglandins. To test these possibilities, we did two experiments. In the first, we co-injected 10^{-4} M prostaglandin E1 and 10^{-4} M BW1433 (a gift from Dr. Susan Daluge of the Burroughs Wellcome Company, Research Triangle Park, NC), an adenosine antagonist. Although the vitreous fluorescein leakage 6 hr after injection was somewhat less than when prostaglandin E1 was injected alone, the difference was not statistically significant (53.7 ± 7.8 ng/ml versus 72.6 ± 14.8 ng/ml, P = 0.29). This contrasts with our previously reported data demonstrating that BW1433 is an effective inhibitor of adenosine-induced breakdown of the

Table 1. Effect of intravitreous injection of cyclic AMP analogs on vitreous fluorophotometry in rabbits

<table>
<thead>
<tr>
<th></th>
<th>Intravitreous fluorescein 6 hr after injection (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>Dibutylryl cyclic AMP 10^{-5} M</td>
<td>36.5 ± 4.0*</td>
</tr>
<tr>
<td>5 × 10^{-5} M</td>
<td>21.8 ± 2.1*</td>
</tr>
<tr>
<td>10^{-3} M</td>
<td>8.3 ± 2.0</td>
</tr>
<tr>
<td>8-Bromo-cyclic AMP 10^{-2} M</td>
<td>26.2 ± 1.7*</td>
</tr>
<tr>
<td>10^{-3} M</td>
<td>13.5 ± 0.1*</td>
</tr>
<tr>
<td>Butyrate</td>
<td>8.7 ± 0.2</td>
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<tr>
<td>10^{-2} M</td>
<td></td>
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</tbody>
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Vitreous fluorophotometry was performed in rabbits 6 hr after intravitreous injection of various concentrations of the listed agents or vehicle alone (control).

* P < 0.01 for difference from control by the student unpaired t-test.
blood–retinal barrier. In the second experiment, we pretreated some rabbits with subcutaneous injections of indomethacin 6 mg/kg three times a day for 3 days and compared vitreous fluorescein leakage 6 hr after intravitreous injection of $10^{-2}$ M adenosine in these rabbits with those that were not pretreated. Pretreated rabbits had a mean VFP reading of 48.3 ± 8.0 ng/ml compared with 41.6 ± 2.7 ng/ml in rabbits that were not pretreated ($P = 0.48$), demonstrating that inhibition of endogenous prostaglandin synthesis has no significant effect on adenosine-induced breakdown of the blood–retinal barrier.

Table 2. Effect of intravitreous injection of prostaglandins on vitreous fluorophotometry in rabbits

<table>
<thead>
<tr>
<th></th>
<th>Intravitreous fluorescein sodium (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 hr after injection</td>
</tr>
<tr>
<td>Control</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>PGE$_1$ 10$^{-4}$ M</td>
<td>72.6 ± 14.8*</td>
</tr>
<tr>
<td>10$^{-3}$ M</td>
<td>27.8 ± 3.1*</td>
</tr>
<tr>
<td>10$^{-4}$ M</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>PGF$_{2a}$ 10$^{-4}$ M</td>
<td>11.3 ± 0.8*</td>
</tr>
<tr>
<td>PGF$_{2a}$ 10$^{-4}$ M</td>
<td>8.1 ± 0.6</td>
</tr>
</tbody>
</table>

Vitreous fluorophotometry was performed in rabbits 6 and 24 hr after intravitreous injection of the listed prostaglandins or vehicle alone (control).

N.D. = not done.

* $P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ for difference from control by student unpaired t-test.

Discussion

Macular edema occurring after intraocular surgery or in the setting of ocular inflammatory disease is probably due to breakdown of the blood–retinal barrier from interaction of chemical mediators with cells that make up this barrier: retinal vascular endothelial cells and retinal pigment epithelium cells. Prostaglandins have been considered the most likely candidates for this role, based on clinical studies that show that prostaglandin synthetase inhibitors decrease the incidence of postoperative fluorescein leakage in cataract patients.16-19 Direct experimental evidence to support this hypothesis is lacking, and no specific product of arachidonic acid metabolism has been implicated conclusively. Wallenstein and Bitot20 showed that intravitreous injection of large amounts (700 μg) of prostaglandin E$_1$ resulted in a decrease in the b-wave of the electroretinogram, but only when the animals were pretreated with an organic acid transport inhibitor. Peyman et al21 examined the effect of several prostaglandins on the retinal vasculature in rabbits by intravitreous injections of concentrations ranging from 10–1000 μg. They assessed the vasculature with fluorescein angioscopy at 2 hr, 24 hr, and 10 days after injection. No changes were observed for any of the prostaglandins when less than 200 μg was injected, but doses greater than or equal to 200 μg were associated with dye leakage, vascular occlusion, hemorrhages, and retinal detachments. This probably represents nonspecific toxicity because 200 μg is a...
very large dose and the effects were similar for each of
the prostaglandins. The results we report are not
likely to represent nonspecific toxicity because the ef-
fects occurred after intravitreous injection of much
smaller doses (0.33–3.3 µg), they differed for each of
the prostaglandins tested, and they were reversible
within 24 hr. Our ability to detect these changes is
probably due to the more sensitive technique that we
used to assess leakage (VFP) and the times selected.
Because VFP does not distinguish between vascular
and retinal pigment epithelium leakage, our measure-
ments of fluorescein leakage may be due to either or
both of these. Our results support the work of Pour-
naves et al.22 who showed that prostaglandin E₁ in-
jected close to the retinal vessels by iontophoresis in
miniature pigs causes vasodilation. Their calculated
concentration of 5.5 µM of prostaglandin E₁ at the
vessel wall is similar to what would be expected in
some of our experiments.

In general, the results of clinical trials examining
the effect of prostaglandin synthetase inhibitors on
the treatment and prophylaxis of macular edema
have been disappointing.19 The reason for this is un-
certain, but a possible explanation is that prostaglan-
dins are not the sole mediators involved. Our studies
support such a contention by showing that, although
prostaglandin E₁ can cause breakdown of the blood-
retinal barrier, other agents also share this ability. We
found that intravitreous injection of each of two ana-
logues of cyclic AMP, dibutyryl cyclic AMP and 8-
bromo-cyclic AMP, that can permeate cell mem-
branes, cause breakdown of the blood–retinal barrier
as measured by VFP. The need for these agents to
enter cells to exert their effect may be responsible for
the relatively high concentrations that are required.
Some adrenergic agents and prostaglandins act at cell
surface receptors to activate an adenylate cyclase and
increase intracellular cyclic AMP, and they cause
breakdown of the blood–retinal barrier after intra-
vitreous injection of much smaller concentrations.
The adrenergic agents, epinephrine, isoproterenol,
and norepinephrine, are effective stimulators of ade-
nylate cyclase,14 and each causes significant break-
down of the blood–retinal barrier. Phenylephrine, a
much less effective stimulator of adenylate cyclase,14
causes significantly less breakdown of the blood–reti-
nal barrier. Prostaglandins E₁ and E₂ are also effective
stimulators of adenylate cyclase in the retinal pigment
epithelium14 and cause marked disruption of the
blood–ocular barrier. Prostaglandin F₁α has little or
no effect on adenylate cyclase in the retinal pigment
epithelium14 and little or no effect on the blood–reti-
nal barrier.

These results suggest that one possible common
link for agents that cause blood–retinal barrier disrup-
tion is the ability to enhance intracellular cyclic AMP
levels. It is probable that this is not the only factor,
however. Although it can explain the relative differ-
ences in effectiveness among certain drug types, such
as the adrenergic agents or the prostaglandins, it does
not explain why prostaglandin E₁ and adenosine ana-
logues are more effective than adrenergics. Interest-
ingly, prostaglandin E, and adenosine analogues, but not adrenergics, are able to cause vasodilation and retinal hemorrhages, and they do so independently rather than by one agent increasing endogenous release of the other. The relationship between these phenomena and breakdown of the blood–retinal barrier has not yet been determined.

The mechanism by which cyclic AMP exerts its effect on the blood–retinal barrier is unknown, although previous studies suggest blood–retinal transport processes. Cyclic AMP inhibits fluid transport from the apical to the basal surface of retinal pigment epithelium in isolated frog retinal pigment epithelium–choroid preparations.23 It also inhibits the resorption of subretinal fluid in rabbits.24 Perhaps the outward pumping of fluid by the retinal pigment epithelium is a necessary component of the blood–retinal barrier and by antagonizing such pumping, cyclic AMP compromises the barrier. Additional work is needed to further define the effect of cyclic AMP on the blood–retinal barrier and to determine if persistent elevation of cyclic AMP plays a role in the development of macular edema.

Key words: blood–retinal barrier, adenylate cyclase, cyclic AMP, macular edema, prostaglandins

Acknowledgments

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References