Topical 8-Bromo-Cyclic GMP Lowers Intraocular Pressure in Rabbits

Bernard Becker

The topical application of 8-bromo-3',5'-cyclic guanosine monophosphate (8BrcGMP) produces significant decreases in intraocular pressure (IOP) in rabbit eyes. Maximum effects are obtained with a 4% concentration, and IOP is reduced significantly ($P < 0.001$) between 30 and 240 min after administration of the agent. The decrease in IOP occurs without significant change in tonographic outflow facility. A significant further decrease in IOP can be induced by topical 8BrcGMP in rabbits whose IOP has been lowered by the systemic administration of the carbonic anhydrase inhibitor acetazolamide. Invest Ophthalmol Vis Sci 31:1647-1649, 1990

Atrial natriuretic peptides (AP) are released from cardiac myocytes and may play important roles in fluid and electrolyte homeostasis. AP receptors are often associated with the membrane-bound form of the enzyme guanylate cyclase and are found in high concentrations in rabbit ciliary epithelium. When stimulated by AP, ciliary epithelium in vitro produces increased amounts of cyclic guanosine monophosphate (cGMP). In vivo, 4-48 hr after the injection of AP into the vitreous cavity of the rabbit eye, significant decreases in IOP (5-8 mmHg) and increases in anterior chamber aqueous humor concentration of cGMP (15-30-fold) are observed (unpublished observations). The decrease in intraocular pressure (IOP) is associated with a decrease in aqueous flow as estimated by fluorophotometry, tonography, and changes in aqueous humor ascorbate concentrations. The topical application of nitrovasodilators, such as nitroglycerine and sodium nitroprusside, which activate the soluble form of the enzyme guanylate cyclase, also increase aqueous humor cGMP and lower IOP (unpublished observations).

8BrcGMP is an analog of cGMP, which is more resistant to hydrolysis by phosphodiesterases. It mimics the effects of AP in several secretory sites. In the current study, topical 8BrcGMP is shown to lower IOP significantly in rabbit eyes.

Materials and Methods. Adult New Zealand albino rabbits (2-4 kg) of both sexes were housed and handled in accordance with the ARVO Resolution on the Use of Animals in Research. Animals were exposed to 12-hr light–dark cycles in a constant-temperature isolated environment for at least 2 weeks before use. They had free access to water and food and were handled repeatedly by only one trained technician. After application of topical 0.5% proparacaine (Alcon Laboratories Inc., Fort Worth, TX), intraocular pressures were measured on unrestrained rabbits with a manometrically calibrated pneumotonometer (Digilab, Bio-Rad Ophthalmic Division Cambridge, MA). Preliminary IOP measurements were made to accustom the animals to the procedure. Tonography was carried out continuously for 4 min with a mechanical Schiotz tonometer using the 5.5-g weight. Readings were made every 15 sec. Outflow facility values (C) were estimated from tables calculated for human eyes.

8BrcGMP was purchased from Sigma (St. Louis, MO). For topical use fresh solutions were made daily in sterile 1% hydroxypropyl methylcellulose solution (2.5% hydroxypropyl methylcellulose [Smith, Miller and Patch] diluted with 0.9% NaCl solution). A single 50-μl drop was administered to the experimental eye at time 0 and repeated at 5 min. Vehicle was used in contralateral control eyes. All testing was done between 10 AM and 4 PM, and animals were not retested for at least 48 hr. IOP was measured at times 0, 30, 60, 120, 180, 240, and 300 min after topical administration. Dose–response studies were carried out on separate days for each concentration. Sodium acetazolamide (American Cyanamid, Pearl River, NY) was injected in doses of 50 mg/kg intravenously and 50 mg/kg subcutaneously. A two-tailed paired t-test was used to compare experimental and control eyes (each corrected for its baseline value).

Results. IOP and Tonography: After the topical administration of 8BrcGMP 4%, IOP was significantly decreased ($P < 0.001$) at all time intervals between 30 and 240 min in the experimental eye, without significant effect on the contralateral control eye (Fig. 1). No significant irritation or inflammatory response was noted. Maximum effect (approximately 23-25% decrease in IOP) occurred between 60 and 180 min with recovery to baseline levels by 5-6 hr. Dose–response studies for the same time intervals after applications of 0.5-8.0% concentrations of 8BrcGMP revealed that maximum pressure lowering was accomplished with 4.0% concentration; higher concentrations produced no greater effect on IOP but tended to prolong the effect beyond 4 hr (Table 1). Tonographic data 2 hr after repeated doses of topical 8BrcGMP 2% demonstrated significant decreases in IOP without change in outflow facility (Table 2).
Fig. 1. Effect of topical 8-BrcGMP 4% (50 μl x 2) on IOP. Actual IOP plotted as mean ± SEM for ten rabbits. Differences in IOP between treated experimental and contralateral control eyes (corrected for differences at time 0) are significant ($P < 0.001$) at times 30–240 min.

Acetazolamide: The administration of acetazolamide to ten rabbits 30 min before the topical application of 8BrcGMP 4% produced a significant ($P < 0.001$) and almost identical decrease in IOP, of 4.5 ± 0.7 mmHg, in both eyes at time 0 (Figure 2). The subsequent application of 8BrcGMP 4% to one eye and diluent to the contralateral control eye resulted in a significant ($P < 0.001$) further decrease in IOP in the treated eye and no change in the control eye. The decrease was slightly less in absolute magnitude but similar in relative change (approximately 20–23%) when compared with animals not pretreated with acetazolamide.

Discussion. Cyclic nucleotides are postulated to play a significant role in the regulation of IOP. Most studies concern cyclic AMP and its effects on both aqueous humor secretion and outflow facility.9–11 The finding of AP receptors in the ciliary epithelium and the lowering of IOP by intravitreal injections of AP raises the question of a possible role of cGMP in the regulation and induced alterations of aqueous humor secretion.5,6 Although the nitrovasodilators stimulate soluble rather than particulate guanylate cyclase, their ability to lower IOP when applied topically further suggests cGMP as a possible mediator.5,6 The significant decreases in IOP that follow the topical application of 8BrcGMP, an analog of cGMP that resists hydrolysis, is compatible with such a hypothesis.

The mechanism by which 8BrcGMP alters transport is not known. It inhibits NaCl co-transport in the teleost intestine7 and simulates the natriuretic action of AP by inhibiting renal Na+ and water transport by the inner medullary collecting duct.8–12 It is apparent that the rabbit eye with IOP lowered by systemic carbonic anhydrase inhibition can still respond to topical 8BrcGMP with additional significant lowering of IOP. This suggests at least two separate and relatively independent pathways for altering the secretion of aqueous humor.

Questions now are raised as to the role of cGMP and its production by endocrine (eg, AP) and other "endogenous nitrovasodilators" (eg, the endothelial derived relaxing factor [EDRF]13) in the regulation of IOP in the rabbit. Studies are planned in primates, especially in humans, to determine whether similar

### Table 1. IOP dose response to topical 8-BrcGMP

<table>
<thead>
<tr>
<th>Concentration of 8 BrcGMP (%)</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0 ± 0.5</td>
<td>-0.2 ± 0.3</td>
<td>-0.9 ± 0.7</td>
<td>-0.7 ± 0.2</td>
<td>-0.5 ± 0.2</td>
<td>-0.4 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>-0.9 ± 0.7</td>
<td>-1.1 ± 0.5$ $</td>
<td>-2.5 ± 0.5*</td>
<td>-2.3 ± 0.6*</td>
<td>-0.8 ± 0.4</td>
<td>-0.1 ± 0.3</td>
</tr>
<tr>
<td>2.0</td>
<td>-2.1 ± 0.6†</td>
<td>-4.1 ± 0.9*</td>
<td>-4.1 ± 0.6*</td>
<td>-4.3 ± 0.7*</td>
<td>-2.7 ± 0.7†</td>
<td>-1.1 ± 0.6</td>
</tr>
<tr>
<td>4.0</td>
<td>-2.3 ± 0.4*</td>
<td>-4.6 ± 0.7*</td>
<td>-5.1 ± 0.7*</td>
<td>-5.6 ± 0.9*</td>
<td>-3.3 ± 0.5*</td>
<td>-1.5 ± 0.6†</td>
</tr>
<tr>
<td>8.0</td>
<td>-1.8 ± 0.3*</td>
<td>-3.9 ± 0.5*</td>
<td>-5.3 ± 0.6*</td>
<td>-5.3 ± 0.7*</td>
<td>-4.8 ± 0.8*</td>
<td>-3.3 ± 0.9†</td>
</tr>
</tbody>
</table>

Values (expressed in mmHg) are differences in IOP (mean ± SEM) between experimental and contralateral control eyes (corrected for differences at time 0) at various times after topical administration of 8-BrcGMP. n = 10 rabbits at each concentration.

* $P < 0.001$.
† $P < 0.01$.
$P < 0.05$.

### Table 2. Tonography 2 hr after topical 8 BrcGMP 2%

<table>
<thead>
<tr>
<th>Eye</th>
<th>IOP (mmHg)</th>
<th>Outflow facility (μl/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.7 ± 0.8</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Experimental</td>
<td>15.1 ± 0.6*</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>

Topical 8 BrcGMP 2% (50 μl) to experimental eye at 0, 30, and 60 min. Values are mean ± SEM for seven rabbits.

* $P < 0.001$ for the difference between control and experimental eye.
Fig. 2. Effect of topical 8-BrcGMP 4% (50 μl x 2) on IOP (mean ± SEM) of ten rabbits treated with systemic acetazolamide (50 mg/kg IV + 50 mg/kg SC) administered 30 min before topical 8-BrcGMP 4%. Decreases in IOP in both eyes 30 min after acetazolamide are significant (P < 0.001). Differences in IOP between 8-BrcGMP-treated experimental and contralateral control eyes are significant after 30 min (P < 0.01) and at times 60-240 min (P < 0.001).

mechanisms apply. In addition to the possible clinical usefulness of 8BrcGMP itself, other pharmacologic agents that activate guanylate cyclase and produce cGMP or inhibit its hydrolysis by phosphodiesterases may offer exciting new approaches to the control of IOP.

Key words: atrial natriuretic peptides, cyclic GMP, 8-bromo-cGMP, intraocular pressure, acetazolamide

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References

Normal Square Wave Jerks in Differing Age Groups

Josephine Shallo-Hoffmann, Barbara Senderl, and Hermann Mühlenedyck

This report details the characteristics of normal square wave jerks (SWJ) over a broad age range. Sixty normally sighted subjects, categorized into three age groups (35–49 yr, 50–64 yr, and 65–89 yr) underwent electrooculographic