Do α-Adrenergic Receptors Participate in Control of the Circadian Rhythm of IOP?

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The α2-adrenergic antagonists, yohimbine and rauwolscine, and the α1-adrenergic antagonist, bunazosin, were used to explore the role of α-adrenergic receptors in the regulation of the circadian rhythms of intraocular pressure and aqueous flow in New Zealand white rabbits. Blockade of α2-adrenergic receptors with yohimbine or rauwolscine produced small decreases in intraocular pressure during both light and dark phases. Rauwolscine had no effect on aqueous flow during the light or dark, but it increased the concentration of norepinephrine in the aqueous during both light and dark. These observations are difficult to reconcile with earlier suggestions that increased sympathetic input to the eye increases intraocular pressure and aqueous flow during the dark. The role of α2-adrenergic receptors in the control of the circadian rhythm of intraocular pressure is unclear. Blockade of α1-adrenergic receptors with bunazosin produced a dose-dependent reduction of IOP during the dark phase of the circadian cycle, a smaller reduction during the light phase, and no reduction during either light or dark in rabbits after superior cervical ganglionectomy or preganglionic section of the cervical sympathetic trunk (decentralization). Bunazosin decreased pupil diameter during the dark phase but had no effect on aqueous flow. Because it is unlikely that α1-adrenergic blockade increased outflow facility or uveoscleral outflow, the mechanism for the role of α1-adrenergic receptors in the control of the circadian rhythm of intraocular pressure in rabbits remains to be identified. Invest Ophthalmol Vis Sci 33:3186–3194, 1992

Rabbits have circadian rhythms of intraocular pressure (IOP) and aqueous flow; both IOP and flow are lowest during the light phase and highest during the dark phase. Superior cervical ganglionectomy (CGX) or preganglionic section of the cervical sympathetic trunk (decentralization, DX) blunted the dark phase increases in IOP, aqueous flow, aqueous norepinephrine (NE) concentration, and aqueous cyclic adenosine monophosphate (cAMP). Therefore, we and others have argued that the increases in IOP and aqueous flow observed during the dark phase of the circadian cycle in rabbits result from elevated sympathetic input to the eye during the dark. The dark phase increases of IOP and aqueous flow appear to be mediated partly by stimulation of β-adrenergic receptors by NE. Both CGX and DX decreased IOP 5 mmHg early during the dark phase, approximately half the range of the circadian rhythm of IOP. Blockade of β-adrenergic receptors with timolol (TIM) during the dark produced small decreases of IOP and aqueous flow (<10%) and reduced the dark phase increase of aqueous cAMP. Because the effects of TIM on IOP and aqueous flow were not large enough to explain the entire dark-phase increases of either IOP or flow or even the dark phase increases that require intact sympathetic innervation to the eye, it seemed likely that NE stimulation of α-adrenergic receptors also may play a role in the regulation of the dark-phase increases of IOP and aqueous flow in rabbits. Recently, a study was published of the effects of α-adrenergic agents on the increases in IOP and aqueous flow between late light phase and early dark phase.

Although the major effect of CGX or DX on IOP in rabbits was to decrease IOP during the dark, both procedures produced small increases of IOP early in the light phase. Furthermore, CGX increased aqueous flow during the light, and although the changes were not statistically significant, there was a tendency toward increased flow during the light after DX. Both surgical procedures decreased aqueous NE during the light phase. Although blockade of β-adrenergic receptors with TIM decreased IOP and aqueous flow during the dark, it had no effect on IOP or aqueous flow during the light phase. Therefore, it is reasonable to think that input from the sympathetic nerves
to the eye has two effects on IOP and flow in rabbits. In addition to increasing IOP and aqueous flow during the dark (in part by stimulating β-adrenergic receptors), sympathetic input may decrease both IOP and flow by a different mechanism during the light. For this reason, we treated rabbits entrained to alternating 12-hr periods of light and dark with yohimbine (YOH) and rauwolscine (RWL), a2-adrenergic antagonists, and bunazosin (BNZ), an α1-adrenergic antagonist,14-16 during both the light and dark phases of the circadian cycle to explore the possibility that α-adrenergic receptors participate in control of the circadian rhythms of IOP and aqueous flow.

Materials and Methods

Animals and Animal Surgery

All experimental procedures using animals adhered to the ARVO Resolution on the Use of Animals in Research. Male New Zealand white rabbits (weight range, 2–3 kg) were maintained in a 12-hr light-dark cycle for at least 2 wk before their use in all experiments. Animals subjected to bilateral CGX or bilateral DX4°8 were allowed to recover in a room with a lighting schedule of alternating 12-hr light-dark periods. We confirmed CGX and DX2-3 wk after surgery as previously described.12

IOP Measurements

We measured IOP as previously described9 during the dark using the light of a Bright Lab Jr. red light (Delta 1, Dallas, TX), which produces visible light in the far red.3 A crossover protocol was used for IOP experiments with YOH HCl (Sigma, St. Louis, MO) and RWL HCl (Atomergic, Plainview, NY) each at a concentration of 0.3% in water. We applied 50 μl of the drug topically to one eye of one half of each group of animals; contralateral eyes were treated with water. The other half of the group received 50 μl of water to both eyes. No less than 3 days later, animals previously treated with YOH or RWL were treated with water, and those previously treated with water were treated with YOH or RWL. For experiments using a concentration of 0.1% of drug, the 0.3% solution was diluted to 0.1% with saline. Contralateral eyes were treated with one part water and two parts saline, and they were used as controls. Both YOH or RWL were applied either at 02:00 circadian time (CT, ie, lights on at 00:00 CT) or at 14:00 CT (lights off at 12:00 CT). We recorded IOP at -1, +0.5, +1, +2, +3, +4, and +6 hr.

A crossover protocol also was used in all IOP experiments with BNZ (Santen, Osaka, Japan). The drug was dissolved in physiologic saline, and control eyes were treated with physiologic saline. We applied BNZ (50 μl) at 00:00 or 12:00 CT; IOP was recorded at the same time intervals as in the experiments with YOH and RWL. Our data are expressed as the average difference between IOP in the treated (and contralateral) eye the day of treatment and the average IOP in both eyes on the day the animals were not treated with BNZ. Concentrations of BNZ, YOH, and RWL were expressed as percent of the base drug, ie, in grams of base per deciliter.

Pupillary Diameter

This was estimated by comparing the pupillary diameter of the rabbits to semicircles of known diameter on a clinical examination card after unilateral application of 0.3% BNZ at 13:00 CT (one dose of 50 μl) or at 12:30 and 12:45 CT (two doses of 25 μl each). Pupillary diameter was measured during the dark by the light of the same dim red light used for IOP measurement. Illuminance was measured at the same position as the rabbits’ eyes during pupillary diameter measurements with lights on and off (20–36 and 0.2–0.3 foot candles, respectively). Illuminance was measured with a J16 Digital Photometer fitted with a J6511 illuminance probe, which has a spectral sensitivity comparable to human cone photoreceptors (Tektronix, Beaverton, OR).

Aqueous Flow Measurements

Aqueous flow rates were estimated using modifications of the intravitreal depot method17 and the corneal depot method.18

Intravitreal depot method: Flow measurements using the intravitreal depot method were done as previously described11,14 using a crossover protocol. We applied BNZ (50 μl of 0.1% unilaterally) topically to one half of the rabbits at 12:00, 14:00, and 16:00 CT; the other half received saline in both eyes. On the following day, animals previously treated with saline received unilateral BNZ, and those previously treated with BNZ received saline in both eyes. Fluorescence was measured with a scanning fluorophotometer (Fluorotron Master; Coherent, Palo Alto, CA) at 11:00, 13:00, 15:00, 17:00, and 19:00 CT. We applied BNZ topically three times to ensure that, if aqueous flow was reduced by BNZ, it would be reduced long enough to maximize the probability that the change could be detected and also to reproduce the experimental protocol used earlier11 to measure aqueous flow after TIM treatment.

Corneal depot method: Fluorescein was applied to the cornea approximately 16 hr before flow measurements. Corneas were anesthetized with 0.5% proparacaine HCl (Ophthetic; Allergan, Irvine, CA). Then flu-
Aqueous flow was calculated from the equation:
\[
\text{flow} = \frac{-d \ln C_a}{dt} V_a + \frac{-d \ln M_c}{dt} \frac{M_{cave}}{C_{ave}}
\]
where: \(C_a\) is the concentration of fluorescein in the anterior chamber, \(M_c\) is the mass of fluorescein in the cornea, \(V_a\) is the volume of the anterior chamber (assumed to be 250 \(\mu l\)), \(M_{cave}\) is the average mass of fluorescein in the cornea during the time of fluorescence measurements, and \(C_{ave}\) is the average concentration of fluorescein in the anterior chamber during the time of fluorescence measurements.

\(M_{cave}\) was calculated from the equation:
\[
M_{cave} = \frac{M_c - M_{ef}}{\ln M_c - \ln M_{ef}}
\]
where \(M_c\) and \(M_{ef}\) are the mass of fluorescein in the cornea at the time of the initial and final measurements of fluorescence, respectively. \(C_{ave}\) was calculated from an equation analogous to that for \(M_{cave}\);
\(d\ln M_c/dt\) and \(d\ln C_c/dt\) were determined by regression analysis.

The following experiment was done to correct for the fact that the optical cuvette created by the Fluorotron Master is large relative to the thickness of the rabbit cornea and, therefore, underestimates corneal fluorescence. Fluorescein was applied iontophoretically to the corneas of rabbits and corneal fluorescence (Cc) measured approximately 16 hr later. The animals then were killed, and both corneas were removed. The corneas were extracted with 4 ml of phosphate-buffered saline for 5 days at 4°C, the concentration in the extract was measured with the Fluorotron Master, and the mass of fluorescein was calculated from the volume of the extract. These data were used to construct a standard curve for converting \(C_c\) measured with the Fluorotron Master to \(M_c\) (\(M_c\) [ng] = 0.157 \(C_c\) [ng/ml] + 6.3 [ng]).

Contralateral eyes were used as controls for flow measurements after topical RWL or BNZ. We applied RWL (0.3% in water, 50 \(\mu l\)) or BNZ (0.1% in saline, 50 \(\mu l\)) unilaterally at 00:00 and 03:00 CT (RWL, light phase) or at 12:00 and 15:00 CT (RWL or BNZ, dark phase). Corneal and anterior chamber fluorescence were measured at 00:30, 02:00, 03:30, and 05:00 (RWL) or 12:30, 14:00, 15:30, and 17:00 CT (RWL or BNZ). Contralateral eyes were treated with water (RWL) or saline (BNZ). A crossover protocol was used for flow measurements after topical TIM (Merck Sharp & Dohme, West Point, PA). We applied TIM (0.1% in saline, 50 \(\mu l\)) unilaterally at 11:00, 12:00, and 13:00 CT to one half of the animals; the other half was treated with saline. Corneal and anterior chamber fluorescence were measured at 12:30, 13:30, 14:30, 15:30, and 16:30 CT. Nine or 11 days later, TIM was applied to those animals previously treated with saline, and saline was applied to those animals previously treated with TIM. We applied TIM, BNZ, or RWL topically more than once to ensure that, if any one of them changed aqueous flow, the flow rate would be changed long enough to maximize the probability that the change could be detected. To reproduce more nearly the protocol reported earlier in which the intravitreal depot method was used to measure aqueous flow after TIM treatment, this drug was applied three times.

**Aqueous NE and cAMP**

Rabbits were treated unilaterally with 50 \(\mu l\) of 0.3% RWL 3 hr and 1 hr before being killed at 03:00 or 15:00 CT with T-61 Euthanasia solution (contains 200 mg/ml embutramide, 50 mg/ml mebezonium iodide, 5 mg/ml tetracaine HCl; Hoechst-Roussel, Somerville, NJ) delivered through the marginal ear vein; contralateral eyes were treated with water. This was the same protocol for RWL application used for the aqueous flow measurements after RWL treatment. Aqueous was removed quickly from both eyes, chilled on ice, frozen, and stored at \(-70°C\). Aqueous catecholamines were assayed as previously described using CAT-A-KITs (Amersham, Arlington Heights, IL) by separating \(^3\)H-O-methylated derivatives of the catecholamines on silica gel thin-layer plates (Analtech Uniplate; Newark, NJ). We assayed cAMP by radioimmunoassay as previously described using kits from Biomedical Technologies (Stoughton, MA).

**Statistical Analysis**

The data in the tables and figures are expressed as the mean ± the standard error of the mean; \(n\) indicates the number of animals. Statistical significance was tested using the student t-test for paired samples; differences were considered significant if \(P < 0.05\).

**Results**

**\(\alpha_2\)-Adrenergic Receptors**

Because we observed little or no contralateral effect of 0.3% YOH or RWL on IOP (data not shown), contralateral eyes were used as controls. Topical YOH or RWL produced small statistically significant de-
RWL increased aqueous NE during both the light and dark phases of the circadian cycle but did not have a statistically significant effect on aqueous cAMP in either light or dark (Table 2). Although the absolute increase of aqueous NE after RWL was considerably larger during the dark, the percent increase was approximately the same during light and dark, 62% and 78%, respectively. Aqueous NE and cAMP concentrations observed in contralateral eyes were comparable to those we reported earlier.12

α₁-Adrenergic Receptors

Unilateral application of BNZ at lights off (12:00 CT) produced statistically significant, dose-dependent, unilateral decreases of IOP from 0.01–0.3% drug concentrations (Fig. 2). When applied at lights on (00:00 CT), 0.3% BNZ produced a smaller maximum decrease of IOP (2.5 versus 6.5 mmHg, Fig. 3). In this experiment, there was evidence of a small contralateral response that was not seen in the dose–response experiment. Bilateral CGX or DX abolished the response of IOP to 0.3% BNZ (Fig. 4). Unilateral application of 0.1% BNZ during the dark produced statistically significant, unilateral decreases of aqueous flow measured by the intravitreal depot method at 13:00 and 15:00 CT (−0.40 ± 0.13 [−13%] and −0.52 ± 0.17 μl/min [−18%], respectively (Fig. 5). However, when aqueous flow was measured by the corneal depot method, BNZ did not significantly change flow (Table 1). During the dark phase of the circadian cycle, 0.3% BNZ produced a small decrease in pupillary diameter relative to contralateral control eyes (Fig. 6).

β-Adrenergic Receptors

We had shown earlier that 0.1% TIM applied during the dark produced a small decrease of aqueous flow measured by the intravitreal depot method.11 Because we obtained conflicting results with the two methods used for measuring aqueous flow after BNZ treatment, we measured flow by the corneal depot

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Table 1. Effect of RWL, BNZ, and TIM on aqueous humor flow measured by the corneal depot method

<table>
<thead>
<tr>
<th>Circadian phase</th>
<th>n</th>
<th>Treated</th>
<th>Contralateral</th>
<th>Crossover control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWL (0.3%)</td>
<td>10</td>
<td>3.99 ± 0.18</td>
<td>4.21 ± 0.26</td>
<td>—</td>
</tr>
<tr>
<td>RWL (0.3%)</td>
<td>10</td>
<td>4.02 ± 0.10</td>
<td>3.99 ± 0.13</td>
<td>—</td>
</tr>
<tr>
<td>BNZ (0.1%)</td>
<td>11</td>
<td>3.09 ± 0.13</td>
<td>2.94 ± 0.15</td>
<td>—</td>
</tr>
<tr>
<td>TIM (0.1%)</td>
<td>12</td>
<td>2.77 ± 0.12*</td>
<td>2.87 ± 0.11*</td>
<td>3.25 ± 0.14</td>
</tr>
</tbody>
</table>

*Significantly different from crossover control, P < 0.05.

For dark phase flow measurements, rauwolscine (RWL; 0.3%) or bunazosin (BNZ; 0.1%) were applied unilaterally at 12:00 and 15:00 circadian time (CT); corneal and anterior chamber fluorescence were measured at 12:30, 14:00, 15:30, and 17:00 CT. Timolol (TIM; 0.1%) was applied unilaterally at 11:00, 12:00, and 13:00 CT using a crossover protocol; corneal and anterior chamber fluorescence were measured at 12:30, 13:30, 14:30, 15:30, and 16:30 CT. For light phase flow measurements, RWL (0.3%) was applied unilaterally at 00:00 and 03:00 CT; corneal and anterior chamber fluorescence were measured at 00:30, 02:00, 03:30, and 05:00 CT.
Table 2. Effect of RWL on aqueous norepinephrine (NE) and cyclic adenosine monophosphate (AMP) during the light (03:00 circadian time) and dark (15:00 CT)

<table>
<thead>
<tr>
<th></th>
<th>Treated eye</th>
<th>Contralateral eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>[NE]aq (ng/ml aqueous humor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03:00 CT</td>
<td>2.1 ± 0.5*</td>
<td>1.3 ± 0.2 (n = 9)</td>
</tr>
<tr>
<td>15:00 CT</td>
<td>9.8 ± 1.9*</td>
<td>5.5 ± 0.7 (n = 7)</td>
</tr>
<tr>
<td>[Cyclic AMP]aq (pmol/ml aqueous humor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03:00 CT</td>
<td>14.7 ± 4.0</td>
<td>9.6 ± 3.8 (n = 7)</td>
</tr>
<tr>
<td>15:00 CT</td>
<td>22.2 ± 4.1</td>
<td>19.0 ± 3.5 (n = 11)</td>
</tr>
</tbody>
</table>

* Significantly different from contralateral control eyes, P < 0.05.

Rabbits were treated unilaterally with 0.3% rauwolscine (RWL) 3 hr and 1 hr before being killed at 03:00 or 15:00 CT for paracentesis.

method after treatment with TIM during the dark. Unilateral application of 0.1% TIM produced significant decreases of aqueous flow during the dark in both treated (−0.47 ± 0.16 μl/min, −14%) and contralateral eyes (−0.38 ± 0.13 μl/min, −12%, Table 1).

Discussion

α2-Adrenergic Receptors

Blockade of prejunctional α2-adrenergic receptors potentiated release of NE from sympathetic nerves in rabbits iris-ciliary body in vitro by decreasing α2-adrenergic receptor-mediated feedback inhibition of NE release from nerve endings. Postjunctional α2-adrenergic receptors inhibited adenylate cyclase activity and cAMP production by rabbit ciliary processes; therefore, blockade of these receptors increases adenylate cyclase activity and cAMP production. We believe that increased release of NE from sympathetic nerve endings during the dark stimulates β-adrenergic receptors to increase IOP by int-
creasing aqueous flow and stimulates \( \alpha_1 \)-adrenergic receptors to increase IOP by a mechanism that is not yet clear. Therefore, we might expect that blockade of \( \alpha_2 \)-adrenergic receptors would increase IOP and aqueous flow during the dark by increasing sympathetic input to ocular \( \alpha_1 \) and \( \beta \)-adrenergic receptors and by enhancing the response of \( \beta \)-adrenergic receptors to sympathetic input. Because CGX and DX increase IOP and aqueous flow during the light phase, it is possible that sympathetic input to the eye has two different effects on IOP and flow during light and dark. Alpha2-adrenergic agonists are known to decrease IOP in rabbits. Perhaps stimulation of \( \alpha_2 \)-adrenergic receptors decreases (or maintains lowered) IOP and aqueous flow during the light phase of the circadian cycle. If this were correct, we also might expect that blockade of \( \alpha_2 \)-adrenergic receptors would increase IOP and aqueous flow during the light.

We found topical YOH or RWL produced small, statistically significant decreases of IOP during both light and dark; there was little difference between the responses to \( \alpha_2 \)-adrenergic blockade during the light or dark. Others showed that a higher concentration (1%) of either YOH or RWL decreased IOP in rabbits by approximately 3 mmHg. In another study, no blockade by RWL (0.1% and 1.0%) was found of the IOP increase from 10:00 to 14:00 CT. However, at no time or dose did YOH or RWL increase IOP; nor did RWL increase aqueous flow during light or dark. Although the data from each of the aqueous flow experiments in Table 1 were from different groups of animals and we noticed substantial variation of flow from one group of animals to another, it was surprising that flow was so high in the animals treated with RWL during the light phase. Aqueous flow measurements from our laboratory (intravitreal depot method) and from another laboratory (corneal depot method) and measurement of aqueous flare by others showed that aqueous flow in the light phase should be substantially less than flow in the dark phase. We have no satisfactory explanation for the high flow we observed in RWL-treated animals.

Liu et al concluded that \( \alpha_2 \)-adrenergic receptors do not participate in control of the dark phase increases of IOP and aqueous flow, but this is not an appealing conclusion because \( \alpha_2 \)-agonists are known to reduce IOP in rabbits. In addition, apraclonidine blocked the dark phase increases of IOP and aqueous flow in rabbits maintained in alternating 12-hr light-dark cycles. Furthermore, apraclonidine decreased aqueous NE at 14:00 CT, and RWL increased aqueous NE during both the light and dark (Table 2). Therefore, it would seem that ocular prejunctional \( \alpha_2 \)-adrenergic receptors are functional during both phases of the circadian cycle. Because we did not observe a significant increase of aqueous cAMP after topical RWL, we cannot conclude similarly for postjunctional \( \alpha_2 \)-adrenergic receptors. We and others have argued that increases of IOP and aqueous flow observed during the dark phase of the circadian cycle in rabbits result from increased sympathetic input to the eye during the dark. This conclusion was supported in part by observations that aqueous NE increased during the dark and that increased aqueous NE reflects ocular sympathetic activity. However, our results with RWL show that increased aqueous NE is not accompanied necessarily by increased aqueous flow and IOP. We have no satisfactory explanation for our observations after blockade of \( \alpha_2 \)-adrenergic receptors. The role of \( \alpha_2 \)-adrenergic...
adrenergic receptors in control of the circadian rhythm of IOP remains unclear.

$\alpha_1$-Adrenergic Receptors

Topical application of BNZ produced a marked decrease of IOP during the dark phase of the circadian cycle and a smaller decrease during the light phase. The effect of BNZ on IOP during both light and dark required intact sympathetic innervation to the eye. These data are consistent with earlier data showing that prazosin (PRZ), another $\alpha_1$-adrenergic antagonist, decreased IOP in rabbits and that CGX partially blocked the effect of this drug on IOP. Our data also support earlier results demonstrating that PRZ blocked the increase of IOP between 10:00 and 14:00 CT in rabbits maintained in alternating 12-hr light–dark cycles. In addition, BNZ decreased aqueous flow measured by the intravitreal depot method. These data support the earlier observation that PRZ decreased aqueous flow (estimated by measuring posterior chamber ascorbic acid levels). Furthermore, phenylephrine, an $\alpha_1$-adrenergic agonist, can increase aqueous flow, and this increase was blocked by intravenous administration of phenoxybenzamine. Based on these findings, it would appear that $\alpha_1$-adrenergic receptors participate in the regulation of the circadian rhythms of IOP and aqueous flow and that stimulation of these receptors by NE during the dark phase of the circadian cycle increases IOP and flow in concert with stimulation of $\beta$-adrenergic receptors. However, Sugiuara and Araie showed that although BNZ appeared to decrease aqueous flow measured by the intravitreal depot method, it had no effect on flow measured by the corneal depot method. These authors interpreted their data to mean that BNZ decreased IOP without changing the aqueous flow rate, but it increased bulk fluid movement from the vitreous to the aqueous. This increased the rate of movement of fluorescein isothiocyanate-conjugated dextran from the vitreous to the aqueous, increased the anterior chamber concentration of this marker, and therefore, gave the appearance of decreased aqueous flow. When we measured aqueous flow by the corneal depot method during the dark in rabbits entrained to alternating 12-hr light–dark cycles, we observed no significant change of flow after topical application of BNZ. Similar results were obtained in rabbits maintained in a similar environment in another study; PRZ did not block the increase of aqueous flow measured by the corneal depot method between 08:00–10:00 and 12:00–14:00 CT. It would appear, therefore, that $\alpha_1$-adrenergic receptor blockade blunts the dark phase increase of IOP in rabbits by a mechanism that is independent of decreased aqueous flow. What might this mechanism be?

Recently, it was reported that BNZ had no effect on pupillary diameter in rabbits nor did it block the mydriasis produced by NE (0.1%) or phenylephrine (0.1%). This led to concern on our part that BNZ may not be an effective antagonist of ocular $\alpha_1$-adrenergic receptors. We found that BNZ could reduce the pupillary diameter during the dark, showing that this drug was able to block ocular $\alpha_1$-adrenergic receptors.

It was suggested that part of the dark-phase IOP increase during the circadian cycle in rabbits results from decreased outflow facility, that decreased facility is mediated by increased NE stimulation of $\alpha_1$-adrenergic receptors in the dark, and therefore, PRZ blunted the dark-phase IOP increase by blocking the NE stimulation of the $\alpha_1$-adrenergic receptors that control outflow facility. Although this mechanism is not supported by data showing that NE increased the outflow facility in rabbits and that PRZ had no effect on facility in rabbits as estimated by tonography, it is possible that the adrenergic mechanisms that control outflow facility change during the circadian cycle in such a way that stimulation of $\alpha_1$-adrenergic receptors during the dark decreases outflow facility. It is unlikely that the IOP decrease we observed after BNZ and the blockade by PRZ of the dark-phase IOP increase resulted from decreased episcleral venous pressure; others could not detect a change in episcleral venous pressure in rabbits after PRZ. Another possible explanation of the results with BNZ and PRZ is that blockade of ocular $\alpha_1$-adrenergic receptors increased uveoscleral outflow. Because BNZ does not change the rate of aqueous flow, this would reduce the rate of outflow through the pressure-dependent trabecular pathway and decrease IOP. However, uveoscleral outflow is thought to be unimportant in rabbits. Furthermore, NE had no effect on uveoscleral outflow in monkeys, and epinephrine increased uveoscleral outflow in monkeys and humans. $\alpha_1$-Adrenergic receptors appear to play a role in control of the circadian rhythm of IOP by a yet to be identified mechanism that depends on sympathetic innervation to the eye but is independent of aqueous flow.

$\beta$-Adrenergic Receptors

We found earlier that bilateral topical application of TIM produced a small decrease of IOP during the dark, no change in IOP during the light, had no effect on IOP during light or dark in rabbits after bilateral CGX, and produced a small decrease of aqueous flow (measured by the intravitreal depot method) during
the dark but not during the light. We confirmed the dark-phase decrease of aqueous flow after TIM using the corneal depot method (Table 1). In another study, unilateral application of TIM (0.1% and 1.0%) did not block the increase of IOP from 10:00–14:00 CT relative to contralateral control eyes. However, topical application of TIM is known to have a contralateral effect on IOP in rabbits. Furthermore, we presented evidence in this report for a contralateral effect of topical TIM on aqueous flow in rabbits (Table 1) and also showed that unilateral application of 0.1% or 1.0% TIM at 12:00 or 15:00 CT decreased IOP in contralateral eyes of rabbits entrained to the alternating 12-hr light–dark cycle described (unpublished data). Therefore, it is unlikely that partial blockade by TIM of the increase in IOP from 10:00–14:00 CT would have been detected.

Stimulation of β-adrenergic receptors by NE released from ocular sympathetic nerves may produce part of the dark-phase increases of the circadian rhythms of IOP and aqueous flow in rabbits. Humans have a daily rhythm of aqueous flow that is roughly 180° out of phase with the rhythms of IOP and flow in rabbits; flow is high during the day and low at night. Studies implicate β-adrenergic receptors in the regulation of the daily rhythm of aqueous flow in humans. However, based on the observation that patients with unilateral Horner’s syndrome have a normal rhythm of aqueous flow in the Horner’s eye and the normal eye, it was concluded that systemic epinephrine stimulates β-adrenergic receptors to increase flow during the day in humans. Although catecholamines are crucial for the regulation of the rhythm of aqueous flow in humans and the rhythms of IOP and flow in rabbits, the agonist and its origin may differ in these two species. In humans, the agonist appears to be epinephrine from the adrenal medulla. In rabbits, the agonist appears to be NE from the ocular sympathetic nerves.

Key words: circadian, α-adrenergic receptors, intraocular pressure, aqueous flow, rabbit

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