Ocular Adrenergic Nerves Contribute to Control of the Circadian Rhythm of Aqueous Flow in Rabbits

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Aqueous flow was measured fluorophotometrically in New Zealand white rabbits after unilateral decentralization of the cervical ganglion or cervical ganglionectomy to determine the role of ocular adrenergic input in regulating the circadian rhythm of aqueous flow. Both surgical procedures decreased the rate of aqueous flow during the dark phase when flow is high. During the light phase when flow is low, cervical ganglionectomy increased aqueous flow; decentralization may have increased flow also, but the increases were not statistically significant. Aqueous flow was also measured in normal rabbits after topical application of timolol during the light or dark to determine whether β-adrenergic receptors play a role in controlling the circadian rhythm of flow. Timolol produced a small reduction of the rate of aqueous flow when applied topically during the dark but not during the light. These results suggest that part of the increase of aqueous flow during the dark phase is produced by adrenergic input to the ciliary processes and that β-adrenergic receptors mediate part of this increase. Invest Ophthalmol Vis Sci 32:523-528, 1991

Rabbits exposed to alternating 12-hr periods of light and dark (12L:12D) have daily rhythms of intraocular pressure (IOP) and aqueous flow; IOP and flow are highest during the dark phase and lowest during the light phase of the circadian cycle.1-11 Because the phase of both rhythms is determined by the phase of the light-dark cycle7-9 and both rhythms persist in constant dark,6,7,9 they are both circadian rhythms—that is, they are governed by an endogenous "clock." Adrenergic control of the rhythm of IOP is suggested by earlier studies showing that cervical ganglionectomy (CGX) or decentralization of the cervical ganglion (DX) reduce the dark phase increase of IOP but have little effect on IOP during the light phase.7,10,12 Timolol produces a small decrease of IOP during the dark, but not during the light, and does not reduce IOP during either light or dark in rabbits previously subjected to bilateral CGX.12 Therefore, adrenergic control of the circadian rhythm of IOP is mediated, in part, by β-adrenergic receptors; however, it is likely that other receptors play important roles in producing the rhythm of IOP in rabbits.

The mechanisms which control the circadian rhythm of aqueous flow in rabbits are unknown. Because of our earlier observations on the role of adrenergic mechanisms in controlling the rhythm of IOP and recent ultrastructural studies of rabbit iridal processes which demonstrated the presence of adrenergic nerve fibers within the ciliary epithelium,13,14 we explored the role of adrenergic mechanisms in control of the circadian rhythm of aqueous flow in rabbits. We report here the results of measurements of aqueous flow after unilateral DX or CGX, and of the rate of aqueous flow after topical timolol, during the light and dark phases of the circadian cycle in New Zealand white (NZW) rabbits entrained to 12L:12D.

Materials and Methods

Animals and Surgery

All experimental procedures employing animals adhered to the ARVO Resolution on the Use of Animals in Research. Male NZW rabbits weighing 2-3 kg underwent unilateral CGX (eight right and five left) or unilateral DX (nine right and nine left).7,10 The animals were allowed to recover in 12L:12D. CGX was confirmed 2-3 weeks after surgery by treating both eyes with hydroxyamphetamine (Pare- drine 1%; SmithKline French, Philadelphia, PA); the irises on the CGX side did not respond.15 DX was confirmed 2-3 weeks after surgery by treating both eyes with hydroxyamphetamine, then 2-7 days later with cocaine (5%; Mallinckrodt, St. Louis, MO); the irises on the DX side responded to hydroxyamphetamine but not to cocaine.15

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Aqueous Flow Measurements

The rate of aqueous flow was estimated using the technique of Johnson and Maurice as previously described with the following changes: (1) fluorescein isothiocyanate-conjugated (FITC) dextran was delivered by intravitreal injection of 15 μl of a 10% solution (molecular weight, 148,000; Sigma, St. Louis, MO); (2) the rabbits were killed with T-61 Euthanasia Solution (Hoechst-Roussel Agri-Vet, Somerville, NJ) or Beuthanasia-D (Schering, Kenilworth, NJ) immediately after the final measurement of the concentration of FITC dextran in the anterior chamber; and (3) FITC dextran was extracted from the vitreous by gently swirling the vitreous in phosphate-buffered saline, pH 7.2, for at least 24 hr. Fluorescence was measured with a scanning fluorophotometer (Fluorotron Master; Coherent, Palo Alto, CA).

Effect of DX or CGX on Aqueous Flow

Flow estimates were done 4–8 weeks after CGX or DX in both eyes of each animal every 3 hr over one 24-hr cycle beginning 1.5 hr after “lights on,” ie, 01:30 circadian time (CT). Lights on is defined as 00:00 CT. Flow measurements were done on two animals per 24-hr cycle. Dark-phase aqueous flow measurements were done by the light of a Bright Lab, Jr. (Delta 1, Dallas, TX) red light. Mean daily flow is the mean for each animal of all eight flow rate estimates over the 24-hr circadian cycle. The range of flow was the difference between the maximum and minimum aqueous flow rate. Mean daily flow varies from animal to animal which are unrelated to the circadian change of flow (and has no effect on the range of flow). Absolute flow rates are presented in Tables 1 and 2.

Effect of Timolol on Aqueous Flow

Rabbits were entrained to 12L:12D for at least 4 weeks before use in this experiment. One half of the rabbits received 50 μl of timolol 0.1% (50 μg of free base) in saline to both eyes 5–7 days after intravitreal injection of FITC dextran; 50 μl of saline was applied to both eyes of the rest of the group. Either 3 or 4 days later, the animals previously treated with saline received timolol, and those previously treated with timolol received saline. Timolol was applied three times: at 0, +2, and +4 hr. The concentration of FITC dextran in the anterior chamber was measured in both eyes at −1, +1, +3, +5, and +7 hr. Flow measurements were done on two animals daily.

Data and Statistical Analysis

All data are expressed as the mean ± the standard error of the mean where n is the number of animals. Statistical significance was tested using the student t-test (two-tailed) for paired samples and by analysis of variance.

Results

The flow data from contralateral control eyes, summarized in Figure 1 and Tables 1 and 2, confirmed
earlier findings that the rate of aqueous flow was higher during the dark than during the light phase in rabbits entrained to a 12L:12D cycle and that minimum and maximum flow are at 01:30 and 13:30 CT, respectively. The mean range of flow in control eyes of DX and CGX animals (Fig. 1) was 1.00 ± 0.11 and 0.96 ± 0.12 \( \mu l \text{ min}^{-1} \), respectively.

Both surgical procedures blunted the circadian rhythm of aqueous flow; the range of flow in DX and CGX eyes was 0.75 ± 0.11 and 0.64 ± 0.11 \( \mu l \text{ min}^{-1} \) or 75% and 67% of the mean range in contralateral control eyes, respectively. However, the effects of the two procedures on the rhythm of flow differed. DX resulted in statistically significant decreases of aqueous flow rates at 13:30 and 19:30 CT (Fig. 1 and Table 1); CGX resulted in statistically significant decreases in aqueous flow rates at 13:30 through 19:30 CT and, in addition, produced statistically significant increases of aqueous flow from 04:30 through 10:30 CT (Fig. 1 and Table 2). Analysis of variance comparing all four mean flows in eyes on the side of the surgery with all four flows in contralateral control eyes in both light and dark phases confirmed these conclusions. Both DX and CGX produced a significant decrease in flow (\( P < 0.05 \)) during the dark; on the other hand, only CGX produced a statistically significant change of flow (\( P = 0.01 \)) during the light phase.

The effects of timolol on the rate of aqueous flow during the light and dark phases are contrasted in Figure 2 and Table 3. Timolol was applied at three times (02:00, 04:00, and 06:00 or 12:00, 14:00, and 16:00 CT) to ensure that any reduction of flow would be sustained long enough to be detected. Timolol (0.1%) produced a small, statistically significant decrease of the rate of aqueous flow during the dark phase. When applied during the light phase, the same dose of timolol had no effect on flow.

**Discussion**

The aqueous flow rate in right and left eyes of normal rabbits is the same over the entire circadian cycle, therefore, the differences in flow rates reported here between ipsilateral and contralateral control eyes after unilateral DX and CGX result specifically from the effects of the operations. The changes in aqueous flow produced by DX and CGX are small; decreases during the dark phase after DX or CGX and increases during the light phase after CGX are about 10% of flow in control eyes (Tables 1 and 2). Changes in flow produced by DX and CGX are small even when compared with the range of flow in contralateral control eyes. At the time of maximum flow (13:30 CT) DX and CGX produced decreases of 0.29 and 0.24 \( \mu l \text{ min}^{-1} \) or 30% and 24%, respectively, of the mean range of flow in contralateral control eyes. Nevertheless, these data suggest that (1) intact adrenergic innervation to the eye is required for complete expression of the circadian rhythm of aqueous flow and (2) circadian information from the central nervous system is required for the expression of the circadian rhythm of aqueous flow.
Carried to the anterior segment by postganglionic preganglionic fibers of the sympathetic chain and is adrenergic nerves.

Receptors in the ciliary processes. The results of our flow during the dark phase producing increased aqueous flow consistent with increased adrenergic activity during these times when applied to rabbits after bilateral CGX. Since timolol is thought to reduce IOP by reducing flow in other species and has recently been shown to reduce flow in rabbits, these data are consistent with increased adrenergic activity during the dark phase producing increased aqueous flow mediated in part by stimulation of \( \beta \)-adrenergic receptors in the ciliary processes. The results of our flow measurements after DX or CGX during the dark and after timolol treatment are consistent with this idea. Depriving the eye of adrenergic input reduced flow during the dark phase, and blockade of \( \alpha \)-adrenergic receptors by topical application of timolol reduced flow during the dark but not during the light phase of the circadian cycle. An alternative explanation for the timolol data is that timolol appears more effective at reducing aqueous flow in the dark than in the light simply because a small fractional decrease of elevated dark-phase flow produced a statistically significant absolute decrease of flow; whereas a similar fractional decrease of the lower flow observed in the light phase did not. If this interpretation is correct, then all agents capable of reducing flow in rabbits would be expected to be more effective during the dark than during the light phase of the circadian cycle.

Although only CGX produced statistically significant increases of flow during the light phase, the data in Figure 1 and Table 1 suggest that DX may have increased aqueous flow also, but not enough for the increases to be statistically significant. Therefore, the difference between the effects of DX and CGX on aqueous flow during the light phase may be quantitative rather than qualitative. We have as yet no plausible explanation for our observation that CGX, and perhaps DX, increases flow during the light phase.

The dose of timolol we used (0.1% three times in both eyes, 0.3 mg total) produced a small decrease (less than 10%) of aqueous flow during the dark phase. Earlier experiments showed that 0.1% (0.1 mg total) timolol was about as effective in reducing IOP in rabbits when applied at

<table>
<thead>
<tr>
<th>Time</th>
<th>CGX</th>
<th>Control</th>
<th>Δ*</th>
<th>( P )</th>
<th>% Change**</th>
</tr>
</thead>
<tbody>
<tr>
<td>01:30</td>
<td>1.64 ± 0.12</td>
<td>1.54 ± 0.12</td>
<td>+0.10 ± 0.05</td>
<td>NS</td>
<td>+7.5</td>
</tr>
<tr>
<td>04:30</td>
<td>1.87 ± 0.16</td>
<td>1.74 ± 0.16</td>
<td>+0.13 ± 0.06</td>
<td>&lt;0.05</td>
<td>+12.9</td>
</tr>
<tr>
<td>07:30</td>
<td>2.02 ± 0.17</td>
<td>1.82 ± 0.16</td>
<td>+0.22 ± 0.06</td>
<td>&lt;0.01</td>
<td>+10.4</td>
</tr>
<tr>
<td>10:30</td>
<td>2.16 ± 0.19</td>
<td>2.40 ± 0.22</td>
<td>-0.24 ± 0.09</td>
<td>&lt;0.05</td>
<td>-10.0</td>
</tr>
<tr>
<td>13:30</td>
<td>2.30 ± 0.20</td>
<td>2.77 ± 0.21</td>
<td>-0.16 ± 0.07</td>
<td>&lt;0.05</td>
<td>-11.8</td>
</tr>
<tr>
<td>16:30</td>
<td>2.20 ± 0.15</td>
<td>2.20 ± 0.17</td>
<td>-0.10 ± 0.05</td>
<td>NS</td>
<td>-8.0</td>
</tr>
<tr>
<td>22:30</td>
<td>1.78 ± 0.12</td>
<td>1.89 ± 0.12</td>
<td>NS</td>
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</tr>
</tbody>
</table>

CGX (n = 13). Relative to flow in contralateral control eyes.

Table 2. Effect of CGX on aqueous flow

Table 3. Effect of 0.1% timolol on aqueous flow in rabbits during light and dark phases

<table>
<thead>
<tr>
<th>CT</th>
<th>Treated</th>
<th>Control</th>
<th>Δ*</th>
<th>( P )</th>
<th>% Change**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>01:00</td>
<td>1.18 ± 0.05</td>
<td>1.17 ± 0.09</td>
<td>+0.01 ± 0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>03:00</td>
<td>1.13 ± 0.05</td>
<td>1.11 ± 0.08</td>
<td>+0.02 ± 0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>05:00</td>
<td>1.16 ± 0.05</td>
<td>1.20 ± 0.08</td>
<td>-0.04 ± 0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>07:00</td>
<td>1.25 ± 0.05</td>
<td>1.29 ± 0.07</td>
<td>-0.03 ± 0.06</td>
<td>NS</td>
<td></td>
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<tr>
<td>09:00</td>
<td>1.37 ± 0.07</td>
<td>1.41 ± 0.09</td>
<td>-0.03 ± 0.07</td>
<td>NS</td>
<td></td>
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<tr>
<td>Dark phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>2.25 ± 0.17</td>
<td>2.46 ± 0.16</td>
<td>+0.06 ± 0.07</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>3.40 ± 0.25</td>
<td>3.52 ± 0.26</td>
<td>-0.12 ± 0.05</td>
<td>&lt;0.05</td>
<td>-3.5</td>
</tr>
<tr>
<td>15:00</td>
<td>3.10 ± 0.21</td>
<td>3.39 ± 0.25</td>
<td>-0.28 ± 0.10</td>
<td>&lt;0.025</td>
<td>-8.3</td>
</tr>
<tr>
<td>17:00</td>
<td>2.61 ± 0.20</td>
<td>2.77 ± 0.21</td>
<td>-0.15 ± 0.08</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td>2.19 ± 0.16</td>
<td>2.36 ± 0.18</td>
<td>-0.16 ± 0.06</td>
<td>&lt;0.025</td>
<td>-7.0</td>
</tr>
</tbody>
</table>

* Treated-control; **relative to control flow. NZW rabbits were treated with timolol (0.5%, bilateral) at 02:00, 04:00, and 06:00 CT during the light (n = 12) and at 12:00, 14:00, and 16:00 CT during the dark (n = 14). The concentration of FITC-dextran in the anterior chamber was measured at the times indicated. On day 1, half of the animals were treated with timolol; the other half were treated with saline. On days 3 or 4, control animals were treated with timolol and animals previously treated with timolol became controls.
12:00 or 15:00 CT. \textsuperscript{12} Timolol is thought to have no effect on outflow facility in humans.\textsuperscript{27-29} We have assumed the same is true for rabbits and that changes in IOP produced by timolol directly reflect changes in the rate of aqueous flow. Therefore, a higher dose of timolol is unlikely to have reduced aqueous flow significantly more than what we observed with 0.1%. Although the absolute decreases in flow produced by timolol in the dark were comparable to those produced by DX and CGX during the dark phase, timolol produced smaller fractional decreases in flow relative to controls. (Since flow measurements after timolol treatment were done on two different groups of rabbits during the light and dark phases, it is not possible to compare the dark-phase decrease with the range of flow in the same animals, as was possible for DX and CGX animals.) Therefore, timolol treatment appears to decrease flow during the dark phase less than either DX or CGX, and it is unlikely that \( \beta \)-adrenergic receptors are solely responsible for the dark-phase increase of flow in rabbits which requires adrenergic input. It is likely that \( \alpha \)-adrenergic or other receptors play important roles in the dark-phase increase of aqueous flow.

Humans have a daily rhythm of aqueous flow which is approximately 180\(^\circ\) out of phase with the rhythm of flow in rabbits.\textsuperscript{11,13-19,30-39} The temporal change in sensitivity of the rate of aqueous flow to timolol in rabbits we reported is analogous to that reported by Topper and Brubaker\textsuperscript{35} who showed that flow was reduced by timolol in humans during the day (when flow is high) but not during the night (when flow is low). Timolol produces a greater decrease in the rate of aqueous flow in humans than either DX or CGX, and it is unlikely that \( \beta \)-adrenergic receptors are solely responsible for the increase of flow in rabbits which requires adrenergic input. It is likely that \( \alpha \)-adrenergic or other receptors play a more important role in control of aqueous flow in humans than in rabbits.

Key words: aqueous flow, adrenergic innervation, \( \beta \)-adrenergic, circadian, rabbit

References


