The effects of timolol, epinephrine, and acetazolamide on the rate of flow of aqueous humor through the anterior chamber of awake and sleeping human subjects was studied. Timolol reduced the rate in awake subjects but not sleeping subjects. Epinephrine increased the rate in sleeping subjects to a greater extent than in awake subjects. Acetazolamide reduced the rate of flow in awake subjects or epinephrine-stimulated subjects. Acetazolamide reduced the rate of flow slightly below the basal rate observed during sleep, but the reduction was small and not statistically significant. The authors propose that the diurnal fluctuation of the rate of aqueous humor flow in humans is driven by changes in the concentration of endogenous epinephrine available to the ciliary epithelia. Invest Ophthalmol Vis Sci 26:1315-1319, 1985

Ericson has shown by a suction cup technique that the rate of aqueous humor flow through the anterior chamber undergoes a circadian cycle. His work has been confirmed by fluorophotometric techniques. The rate of flow in the morning hours is almost twice that during sleep. The magnitude of the change which occurs naturally is as great as can be brought about by beta adrenergic blocking drugs or carbonic anhydrase inhibitors.

Pharmacologic agents that alter the rate of aqueous humor formation may not have the same effect at night as they have during the day. Differences in responses of the eye to pharmacologic agents at different times of the circadian cycle may help to identify the local mediator of the day/night cycle of flow. In this article, we report the effects of timolol and epinephrine, applied topically to the eye, and the effects of acetazolamide administered orally. In addition, we report the effects of the supine position and lid closure on the rate of aqueous humor flow.

Materials and Methods

Sixty-three normal human subjects were studied. This pool of subjects was studied in groups of 10 to 20 for individual protocols. An examination of the eye was performed to confirm that no ocular disease was present and that the intraocular pressures of the two eyes were the same (within 3 mmHg). Informed consent was obtained. The volume of the anterior chamber of each subject was measured photogrammetrically.

The rate of flow of aqueous humor was determined during the day (06:00-13:00) and during the night (22:00-05:00). The effects of the following drugs were studied: timolol maleate, epinephrine hydrochloride, and acetazolamide. The effect of acetazolamide and epinephrine in combination was studied. In addition, daytime flow was measured in a group of subjects who on one occasion were ambulatory and on another occasion reclined in the supine position but remained awake. The rate of flow was also measured during the day in both eyes of a group of subjects in whom the upper lid of one eye was kept closed between measurements by means of a short strip of adhesive tape.

The rate of flow of aqueous humor was calculated using the method of Coakes and Brubaker. Sequential studies in each subject were separated by approximately 1 wk to allow the effects of the previously applied drug to decay.

At the beginning of each study, the fluorescence of the unstained ocular structures were measured. Approximately 300 ng of fluorescein was delivered iontophoretically into a 5-mm diameter stromal depot. Tonometry was performed after the iontophoresis by means of the Perkins handheld applanation tonometer. The intraocular pressure recorded at this time is referred to as "P," in the tables. Any excess of fluorescein
was washed out of the conjunctival cul-de-sac with an isotonic solution. Fluorophotometric measurements of the corneal stroma and anterior chamber were made 15 min after iontophoresis to determine the optical spread function of fluorescence. The total mass of fluorescein was measured at 30 min. Fluorescence of the stroma and anterior chamber was measured again at 2 and 7 hr following iontophoresis. The measurements of the concentration in the anterior chamber were corrected for the optical spread function of fluorescence. Tonometry was repeated immediately after the 7-hr reading (referred to as “P2” in the tables).

The daytime segment of the study started with iontophoresis at 06:00 in the morning. The 7-hr reading was taken at 13:00. A small standard breakfast was provided after the 30-min reading. Subjects were instructed to avoid caffeine and other extraneous drugs. Further, they were requested to refrain from additional food intake, heavy exercise, and to avoid direct exposure to bright sunlight. Otherwise, the subjects engaged in their normal daytime activities.

The nighttime segment of the study started with iontophoresis at 22:00 in the evening. The 7-hr reading was taken at 05:00 and ended the procedure. Subjects slept from 22:30 to 05:00, being awakened at 24:00 for the 2-hr measurement. Comfortable, safe sleeping quarters were provided. Subjects were asked to fast and avoid the use of extraneous drugs during the nighttime segment of the study.

It is noteworthy that the measured rate of flow pertains to the 3 hr prior to the final measurement of fluorescence, hr 7. Even though the subject was awakened at 05:00 for the final measurement of the nighttime studies, the time between awakening and measurement of fluorescence is too short for the concentration of fluorescein to change significantly. Thus, the measured flow pertains to flow during sleep. Intraocular pressure, however, was always measured with the subject awake in the sitting position. The pressure can change as a result of the subject’s being awakened and the change of position. We do not consider either of the nighttime measurements of intraocular pressure as representing pressure during sleep.

All drugs were administered from code-labeled containers. The labels were not decoded until all of the studies were complete and the rates of flow had been calculated. The data were then subjected to a statistical analysis.

The t-test for paired samples was used to test statistical significance. A $P$ value of $\leq 0.05$ was considered statistically significant. The variance of the technique employed in this study to measure flow has been estimated from previous studies to be 0.07. A sample size of 10 subjects provides a 90% chance of detecting a 35% reduction of flow. A sample size of 20 provides a 90% chance of detecting a 24% reduction in flow.

**Timolol Maleate**

Nineteen subjects, seven men and 12 women, were studied. Timolol maleate ophthalmic solution (0.25%) and an identically-appearing placebo solution were administered from coded containers. The eye to be treated with the drug was chosen by random assignment. The fellow eye received the placebo. Two drops of drug/placebo were instilled after the 15-min and the 2-hr measurements. Subjects were instructed to be careful to avoid transferring the drug from one eye to the other. A separate tissue was used to blot each eye. Each subject participated in paired daytime and nighttime segments of the study.

**Epinephrine Hydrochloride**

Twenty subjects, eight men and 12 women, were studied. The active drug was epinephrine hydrochloride ophthalmic solution (1.0%), and the placebo was an identically-appearing artificial tear solution. The drug was given topically to one eye of each subject and the placebo was given to the other. Two drops of drug/placebo were instilled after the 15-min and the 2-hr measurements.

This study was conducted simultaneously with the acetazolamide study. Each subject underwent two daytime and two nighttime measurements. The effect of epinephrine alone versus topical placebo was determined by analyzing the data of the daytime and nighttime studies when oral placebo had been administered.

**Acetazolamide**

The same 20 subjects comprised this study group as the epinephrine study (described in previous section) and the combination study (described in next section). Acetazolamide sustained release capsules (500 mg) were administered to each subject. The active capsules were paired with placebo capsules containing lactose. Each capsule was coded. Each subject received a capsule containing acetazolamide on one daytime and nighttime study and a capsule containing lactose on the other daytime and nighttime study. The order was randomized. Each capsule was given orally after the 2-hr measurement.

**Epinephrine and Acetazolamide**

The data for this study was available from the preceding 20 subjects when acetazolamide, rather than oral placebo, had been administered.
Table 1. Study of timolol maleate

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Night*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (µl/min)</td>
<td>2</td>
</tr>
<tr>
<td>Rate of flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Placebo</td>
<td>2.26</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>(0.86)</td>
<td>(3)</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Timolol</td>
<td>1.58</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>(0.49)</td>
<td>(3)</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The conditions under which flow and pressure were measured at night are not comparable. The rate of flow pertains to the state of sleep whereas intraocular pressure was measured before sleep and after awakening the subject.

**Statistically significant differences (P ≤ 0.05) were observed between the following pairs of entries in the table: column 1, row A vs column 1, row B; column 1, row A vs column 4, row A; column 2, row A vs column 3, row A; column 2, row B vs column 3, row B; column 3, row A vs column 3, row B; column 5, row A vs column 6, row A; column 6, row A vs column 6, row B.

**The pretreatment intraocular pressure was 14 mmHg for the placebo-treated and the timolol-treated eyes in this group before both the daytime study and the nighttime study. The intraocular pressure increased to 15 mmHg at the end of the daytime segment of the study in the placebo-treated eyes (P = 0.01). The intraocular pressure fell to 12 mmHg after treatment with timolol in the day (P = 0.0005). The intraocular pressure increased to 16 mmHg by the end of the nighttime segment of the study in the eyes which received placebo drops (P = 0.025). No statistically significant effect of timolol on intraocular pressure at night was observed when comparing the treated eye before treatment to after treatment. However, a significant effect was observed when comparing the timolol-treated eye to placebo-treated eye at 05:00 in the morning after awakening the subject (P = 0.005).

Lid Closure

Fourteen subjects, five men and nine women were studied. The upper lid of one eye was kept closed between measurements with a small strip of adhesive tape. The fellow eye remained open. In half of the subjects the right eye was closed; in the other half, the left eye was closed. In taping the eye, care was taken to position the tape in such a way that direct pressure against the globe was avoided. The tape was applied after the 15-min reading and was removed for 5 min for the 2-hr measurement.

Supine Position

Ten subjects, four men and six women, were studied. Each subject underwent two daytime and one nighttime measurements of the rate of flow. The first measurement established the normal daytime rate while the subject engaged in his customary activities. The second measurement was conducted while each subject reclined in a supine position. This position was disturbed for 5 min for the 2-hr measurement of fluorescein. The subjects were permitted to watch television or to read but were prevented from sleeping or resting with their eyes closed. The third measurement was conducted at night during sleep between 22:00 and 05:00.

Results

Timolol

Table 1 summarizes the results of the timolol experiment. In the daytime, the rate of flow through the anterior chamber was reduced 30% by this β adrenergic blocker (P = 0.0005). The mean rate of aqueous flow during the day in this group of subjects was 2.26 µl/min in the placebo-treated eyes. The timolol-treated eyes had a daytime flow of 1.58 µl/min. At night, the placebo-treated eyes measured 1.61 µl/min, 29% less than the daytime (P = 0.025). Timolol had no statistically significant effect on the rate of flow at night. The rate of flow during the day in the timolol-treated eye was not significantly different from either the timolol-treated eye or the placebo-treated eye at night.

Epinephrine

Table 2 summarizes the results of the epinephrine and acetazolamide experiments. Epinephrine increased the rate of flow by 15% during the day (P = 0.05) and 47% at night (P = 0.005). The daytime rate in the placebo-treated eyes of this group was 2.83 µl/min. In the epinephrine-treated eyes, the mean flow was 3.26 µl/min. At night, the rate was 1.69 µl/min in the placebo-treated eyes and 2.48 µl/min in the epinephrine-treated eyes. The rate of flow in the placebo-treated eyes was 40% lower at night than during the day (P = 0.0005),
**Table 2. Study of epinephrine hydrochloride and acetazolamide**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Night*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of flow (µl/min)</td>
<td>P₁ (mmHg)</td>
<td>P₂ (mmHg)</td>
<td>Rate of flow (µl/min)</td>
<td>P₁ (mmHg)</td>
</tr>
<tr>
<td>A. Topical placebo and oral placebo</td>
<td>2.83 (0.49)</td>
<td>13 (2)</td>
<td>12 (2)</td>
<td>1.69 (0.34)</td>
</tr>
<tr>
<td>B. Epinephrine and oral placebo</td>
<td>3.26 (1.06)</td>
<td>13 (2)</td>
<td>12 (3)</td>
<td>2.48 (0.98)</td>
</tr>
<tr>
<td>C. Acetazolamide and topical placebo</td>
<td>2.27 (0.57)</td>
<td>12 (2)</td>
<td>12 (2)</td>
<td>1.65 (0.36)</td>
</tr>
<tr>
<td>D. Acetazolamide and epinephrine</td>
<td>2.59 (0.50)</td>
<td>12 (2)</td>
<td>12 (2)</td>
<td>2.11 (0.69)</td>
</tr>
</tbody>
</table>

All daytime rates were significantly different ($P < 0.05$) from corresponding nighttime rates. No significant differences in pressure were observed. Statistically significant differences ($P < 0.05$) were observed between the following pairs of entries in the table: column 1, row A vs column 1, row B; column 1, row A vs column 1, row C; column 1, row B vs column 1, row C; column 1, row C vs column 1, row D; column 4, row A vs column 4, row B; column 4, row B vs column 4, row C; column 4, row B vs column 4, row D; column 4, row C vs column 4, row D.

* The conditions under which flow and pressure were measured at night are not comparable. The rate of flow pertains to the state of sleep whereas intraocular pressure was measured before sleep and after awakening the subject.

but only 24% lower at night in the epinephrine-treated eyes ($P = 0.005$).

No statistically significant changes of intraocular pressure were observed in epinephrine-treated eyes.

**Acetazolamide**

The daytime rate of aqueous humor flow was reduced 20% by acetazolamide ($P = 0.005$). The nighttime rate was reduced only 2% by acetazolamide (statistically insignificant). The pretreatment intraocular pressures were not significantly different from the 7-h posttreatment pressures.

**Acetazolamide and Epinephrine**

In the epinephrine-treated eye, acetazolamide was observed to reduce the rate of flow both in the day and at night. The rate during the day was reduced 21% ($P = 0.005$) and at night was reduced 15% ($P = 0.025$) by acetazolamide in the epinephrine-treated eye. In the acetazolamide-treated subject, epinephrine was observed to increase the rate of flow both during the day and at night. The rate during the day was increased 14% ($P = 0.005$) and at night was increased 28% ($P = 0.005$) by epinephrine in the acetazolamide-treated subject. These drugs, singly or in combination, were not observed to affect the intraocular pressure as measured in this protocol (see Materials and Methods).

**Lid Closure**

Table 3 summarizes the results of the lid-closure experiment. Lid closure had no significant effect except to raise intraocular pressure very slightly ($P = 0.01$).

**Table 3. Study of lid closure**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Eye with open lid</th>
<th>Fellow eye with closed lid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of flow (µl/min)</td>
<td>P₁ (mmHg)</td>
<td>P₂ (mmHg)</td>
</tr>
<tr>
<td>Mean</td>
<td>2.60</td>
<td>13</td>
</tr>
<tr>
<td>SD</td>
<td>(0.54)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

* The pressure at the end of the test $P₂$ with the lid open was significantly lower ($P < 0.05$) than the pressure $P₂$ of the fellow eye with the lid closed.
Supine Position

Table 4 summarizes the results of the experiment to test the effect of the supine position. During the day, subjects who were lying in the supine position had rates of flow which were 12% lower than when the same subjects were ambulatory (P = 0.05). At night during sleep, the rate of flow was 1.65 μl/min in the same subjects, significantly lower than the daytime rates during ambulation or at rest in the supine position (P ≤ 0.001).

Discussion

The results of this study reconfirm the studies of Ericson¹ and of Reiss et al.² that the rate of flow of aqueous humor through the anterior chamber of human subjects is higher during the day and lower at night during sleep. This observation cannot be attributed to lid closure, which had no significant effect on the clearance of fluorescein from the anterior chamber. Resting in the supine position partly mimicks the effect of sleep, but neither the body position nor closure of the lid reproduce the effects of sleep.

Timolol, like all beta adrenergic blockers which have been tested, consistently reduced the rate of flow in ambulatory subjects. However, no effect was observed at night. Failure to observe an effect at night could have been due to lack of a drug effect or an undetectably small effect at night. It is possible that the rate of aqueous humor formation cannot be reduced below a threshold level by any but a toxic drug. Alternatively, timolol, which has no intrinsic agonistic effects, could have had no effect because of the absence of endogenous beta adrenergic activity during sleep. The implication of the latter interpretation is that beta adrenergic blockage produces a sleep-like state on the ciliary epithelium.

The effects of epinephrine observed in this study confirm similar studies with this combined alpha and beta adrenergic agonist. This hormone stimulated flow both during the day and during sleep, but a greater stimulation was observed during sleep. The concentration of circulating epinephrine is reduced during sleep and is lower in the supine position than in the upright position.⁸⁹ If endogenous epinephrine stimulates the formation of aqueous humor as does exogenous epinephrine, the greater effect of topical epinephrine at night can be explained. Alternatively, the effect of epinephrine during the day could be less than at night because the rate of formation is already stimulated by other endogenous mediators during the day and is near its maximum rate.

Acetazolamide had the expected effects during the day and in the epinephrine-stimulated eye. Curiously, we were unable to demonstrate any effect of this carbonic anhydrase inhibitor during sleep in the unstimulated eye. It is possible that the rate of formation cannot be reduced below a basal level. Alternatively, at a lower rate of aqueous humor formation, lower demands for conversion of CO₂ to carbonic acid might permit the uncatalyzed rate to be sufficient to meet the metabolic needs of the ciliary epithelium.

The results of this study and others lead us to propose the following hypothesis: the rate of aqueous humor formation is stimulated by circulating endogenous epinephrine and the effect of this hormone is blocked by beta adrenergic blockers. This hypothesis can account for the circadian pattern of aqueous humor flow, for the inhibitory effects of beta adrenergic blockers during the day, and for the lack of their effect at night.

Key words: rate of aqueous humor flow, timolol, acetazolamide, sleep, epinephrine

Table 4. Study of supine position

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rate of flow (μl/min)</th>
<th>Pressure P1 (mmHg)</th>
<th>Pressure P2 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day: awake</td>
<td>Mean: 3.03 (0.68)</td>
<td>13 (4)</td>
<td>14 (3)</td>
</tr>
<tr>
<td>Night: awake</td>
<td>Mean: 2.66 (0.60)</td>
<td>13 (3)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>Night: asleep</td>
<td>Mean: 1.65 (0.25)</td>
<td>14 (2)</td>
<td>13 (4)</td>
</tr>
</tbody>
</table>

The rate of flow under each set of conditions was significantly different from the other two rates of flow (P < 0.05). No significant differences in pressure were observed.

References