The Effect of Topical Timolol on Epinephrine-Stimulated Aqueous Humor Flow in Sleeping Humans

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Purpose. It has been shown that intravenously administered epinephrine can increase the rate of aqueous humor flow in sleeping humans. This experiment was conducted to determine if this stimulatory effect can be blocked by the beta-adrenergic antagonist timolol.

Methods. Twenty normal human subjects were studied for one sleep cycle at night. Epinephrine was infused intravenously at a rate of 1 μg/min. One eye received a single drop of 0.25% timolol, and the fellow eye received a placebo. Aqueous humor flow was measured by the rate of disappearance of fluorescein from the eye.

Results. The rate of aqueous humor flow was 14% lower in the timolol-treated eye than in the placebo-treated eye during 6 hours of sleep. This difference was interpreted as being due to blockage of part of epinephrine's effect on aqueous humor flow by topical timolol.

Conclusion. It was concluded that the effect of systemically administered epinephrine on aqueous humor flow is at least partly mediated locally on the eye and that some portion of timolol's well-known effect on daytime aqueous humor flow could be due to inhibition of the ocular effects of epinephrine. Invest Ophthalmol Vis Sci. 1994;35:554-559

There is accumulating evidence that epinephrine, a hormone of the adrenal medulla, is a stimulator of aqueous humor formation in humans. An early study by Friedenwald and Buschke1 showed that intravenous epinephrine stimulated the rate of aqueous humor formation in rabbits. Subsequent work with humans has shown that topical epinephrine2-7 as well as intravenously administered epinephrine8 raises the rate of flow of aqueous humor, especially in sleeping humans.

It is not known whether the effect of intravenously administered epinephrine observed by Kacere and coworkers8 on aqueous humor flow in sleeping humans was due to a systemic effect of the hormone or whether it was due to a local effect on the eye. Topical epinephrine's effect on aqueous humor flow may be related to its ability to stimulate beta-adrenergic receptors because beta-selective adrenergic drugs have the same effect.9,10 Thus, to determine if systemically administered epinephrine acts on the eye or elsewhere, it would seem logical to determine experimentally if its effect can be blocked by a locally applied beta-adrenergic antagonist as has been shown to occur after topical application of timolol in the terbutaline-stimulated monkey eye.11 This article describes such an experiment.

METHODS

Subject Selection

Twenty young healthy volunteers with normal eyes were studied. To be eligible, subjects were required to be free of any medications except oral contraceptives, have normal sleep patterns, and have no history of eye disease, eye surgery, hypersensitivity to fluorescein, or any significant chronic disease. To ensure suitability, all potential subjects underwent a preliminary examination to determine eligibility. The examination consisted of a general medical history, an ocular history, measurement of visual acuity, a slit-lamp examination, tonometry, and undilated funduscopy. Subjects that exhibited photophobia with the slit-lamp examination, had narrow palpebral fissures, or had an intraocular

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Submit for publication: April 8, 1993; revised June 7, 1993; accepted July 2, 1993.
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pressure difference greater than 3 mm Hg were excluded. Twenty-three persons were screened and three were excluded: one person with asthma, one with Hepatitis B virus antibodies, and one with questionable ability to sleep in the Clinical Research Center. Contact lens wear was not an exclusion criterion, and seven of twenty wore contacts. However, contact lens use was discontinued for 48 hours before the study to allow the epithelium of the cornea to attain its maximum luster. The research followed the tenets of the Declaration of Helsinki, informed consent was obtained after the nature and possible consequences of the study were explained to the subjects, and the research was approved by the Mayo Foundation Institutional Review Board.

MATERIALS

Epinephrine was prepared by the pharmacy staff of the Rochester Methodist Hospital. A 1 ml ampule of intravenous epinephrine (1 mg/ml; Elkins-Sinn, Inc., Cherry Hill, NJ) was diluted in 100 ml normal saline to form a final concentration of 10 μg/ml. These preparations were protected from light to ensure their stability up to the time of infusion.

Sterile eyedrop bottles were prepared by the same pharmacy containing either timolol 0.25% (Timoptic, Merck Sharp & Dohme, West Point, PA) or a placebo (Tears Naturale, Alcon Laboratories, Fort Worth, TX). These were randomized to the right and left eyes and labeled subject 1, right eye; subject 1, left eye; subject 2, right eye; subject 1, left eye; subject 2, right eye, and so forth. The investigators did not know the contents of the bottles until all the data were recorded and stored in a computer spreadsheet program. At that point, the code was broken, and the contents of the eyedrop bottles confirmed by absorption spectrometry at 200 and 340 nm.

Fluorophotometer measurements of subjects were made with the instrument described by McLaren and Brubaker.12 Measurements of catecholamines in plasma and urine were made in the clinical laboratories of the Mayo Clinic.

PROCEDURES

On a day when no fluorescein had been administered, the autofluorescence of the cornea of each subject was measured. At the same sitting, the volume of both anterior chambers was measured by photogammetry.13 Each subject subsequently reported at 4:00 PM to the outpatient eye clinic. Intraocular pressure was measured with Goldmann tonometry employing Flores (Barnes Hind, Sunnyvale, CA). After tonometry, fluorescein sodium 2% (Iolab Corp., Claremont, CA) was administered every 5 minutes to each eye for a total of five instillations per eye. Each subject rested with his eyes closed between drops and for 30 minutes after the last instillation. The lids were carefully cleansed of excess fluorescein and the subject was dismissed and told to report to the Clinical Research Center of St. Mary's Hospital at 9:00 PM that evening.

At 9:00 PM, the subject was checked into the Clinical Research Center. Baseline blood pressure and pulse rates were recorded. A history and brief physical examination were carried out. The concentration of fluorescein in the cornea was measured to ensure that it was greater than 250 ng/ml. (Three subjects had to be rescheduled, one because of an upper respiratory infection and a resting heart rate of greater than 100 beats per minute and two because of inadequate concentrations of fluorescein in the cornea.)

Each subject was assigned quiet, safe, and private sleeping quarters. Adhesive electrodes for a 3-lead electrocardiogram were attached to the chest. An intravenous line was placed into a forearm vein and connected to an infusion apparatus. Normal saline 0.9% was infused at a constant rate of 30 ml/hr. A similar line was placed in the opposite arm to permit withdrawal of blood without waking the subject.

At 11:00 PM the timolol/placebo eyedrops were instilled into the assigned eye according to the labeled containers. Facial tissues, a separate one for each eye, were used for blotting the tears after drop instillation. The subject was asked not to touch either eye thereafter to reduce the chance that fluid from the timolol-treated eye might contaminate the placebo-treated eye. The subject was then allowed to retire to sleep.

At midnight, before waking the subject, the pulse was recorded from the cardiographic tracing and a blood sample was drawn. Blood pressure was then taken after gently waking but not moving the subject. The subject was then asked to get up and void, beginning a 6-hour urine collection for measurement of catecholamines. The subject then walked approximately 50 feet to the fluorophotometer where the concentrations of fluorescein in the stroma and anterior chamber of both eyes were measured. In approximately 5 minutes the subject returned to bed, and the epinephrine infusion was begun. The infusion rate was given by a constant rate pump at 6 ml/hr/70 kg body weight. The infusion rate and concentration of epinephrine resulted in an epinephrine infusion rate of 14 ng/kg body weight/min, identical to the rate infused by Kacere.9 This rate is approximately three times the basal rate of endogenous epinephrine secretion of supine 19 to 28-year-old subjects measured between 7 AM and 10 AM.14 This rate had been chosen to ensure a blood level somewhat higher than that produced by the basal rate but not so high as to interfere with sleep or produce symptoms.

Pulse measurement, blood sampling, blood pressure measurement, and fluorophotometric measure-
ments were repeated at 3:00 AM and at 6:00 PM. After the 6:00 AM series of measurements, the subject voided completely into a container of 23 ml acetic acid. At this point, the experiment was complete; the intravenous lines and the electrodes were removed, and the subject was interviewed to determine the subjective quality of sleep. The subject was then dismissed.

As a precaution for the subject’s safety, the rate of the epinephrine infusion was decreased if the pulse exceeded the baseline measurement by 30% or the blood pressure exceeded the baseline measurement by 20%. This situation occurred with one subject at the 3:00 AM measurement when the blood pressure was measured to be 20% higher than the baseline. The epinephrine infusion rate was reduced by 50%, and the study was continued at the lower infusion rate. This one subject noted lightheadedness at the 3:00 AM examination, but no other effects possibly attributable to the epinephrine infusion were noted by any of the subjects.

CALCULATIONS AND STATISTICS

The rate of clearance of fluorescein from the anterior chamber was calculated from the disappearance of fluorescein in the combined cornea and anterior chamber (AM) and from the average concentration of fluorescein in the anterior chamber (Ca) during the time interval between measurements (t).

\[
\text{Clearance} = \frac{\text{AM}}{(\text{Ca} \cdot t)}
\]

The rate of flow of aqueous humor was regarded as being equal to the clearance, less the diffusional clearance of fluorescein in the normal eye, approximately 0.25 μl/min.\textsuperscript{15}

The purpose of the study was to determine if timolol reduces the rate of aqueous humor flow in the epinephrine-stimulated eye compared to the placebo-treated eye during sleep. Because the eyes are paired, we have carried out a one-sided Student’s t test for paired samples. A probability of 0.05 was regarded as statistically significant. The same statistical test was used to detect differences in blood pressure or pulse rate caused by the epinephrine infusion compared to the preinfusion baselines. The coefficient of variation of aqueous humor flow measured at night during sleep from 12 midnight to 6 AM during epinephrine infusion can be estimated from Kacere’s data\textsuperscript{a} as being 31%. Thus a sample size of 20 subjects should permit a 95% chance of detecting at least a 24% suppression of aqueous humor flow by timolol in the treated eye compared to the untreated eye.

RESULTS

The rate of aqueous humor flow in the placebo-treated eyes of this group of 20 subjects during sleep from midnight to 3 AM was \(1.40 \pm 0.45\) μl/min (mean ± sd) and from 3 AM to 6 AM was \(1.68 \pm 0.51\) μl/min. The rates in the timolol-treated eyes for the comparable times were \(1.25 \pm 0.47\) μl/min and \(1.42 \pm 0.36\) μl/min. These rates were lower by 11% \((P = 0.065)\) and 16% \((P = 0.003)\), respectively (Table 1). The average flow for the entire midnight to 6 AM period for placebo-treated eyes was \(1.54 \pm 0.46\) μl/min and for the timolol-treated eyes was \(1.33 \pm 0.40\) μl/min, a difference of 14% \((P = 0.01)\).

The heart rate at midnight just before starting the epinephrine infusion was 60.7 ± 6.1 beats per minute. At 3 a.m. the rate was 61.8 ± 9.0 beats per minute and at 6 AM 62.5 ± 8.7 beats per minute, increases of 2% and 3% over preinfusion rates that were not statistically significant \((P = 0.29, P = 0.19)\).

Systolic blood pressure at midnight just before starting the infusion was 112 ± 12 mm Hg. At 3 AM systolic blood pressure was 111 ± 10 mm Hg and at 6 AM was 111 ± 10 mm Hg. These tiny differences (<1%) were not significant. Diastolic blood pressures were slightly lower during the infusion compared to before infusion, but not significantly so (Table 2).

The concentrations of epinephrine, norepinephrine, and dopamine at midnight (before epinephrine infusion) and at 3 AM and 6 AM (during epinephrine infusion) are given in Table 3. As expected, the plasma concentration of epinephrine was much higher during the infusion. The concentration of dopamine was steady whereas the concentration of norepinephrine gradually fell (Table 3). The amount of epinephrine

**TABLE 1.** Aqueous Humor Flow μl/min (n = 20)

<table>
<thead>
<tr>
<th></th>
<th>Midnight to 3 AM</th>
<th>3 AM to 6 AM</th>
<th>Midnight to 6 AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Timolol</td>
<td>Placebo</td>
</tr>
<tr>
<td>Mean</td>
<td>1.40</td>
<td>1.25</td>
<td>1.68</td>
</tr>
<tr>
<td>SD</td>
<td>0.45</td>
<td>0.47</td>
<td>0.51</td>
</tr>
<tr>
<td>% Difference</td>
<td>↓11%</td>
<td>↓16%</td>
<td>↓14%</td>
</tr>
<tr>
<td>P</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Topical Timolol and Intravenous Epinephrine

TABLE 2. Pulse and Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Pulse (bpm)</th>
<th>Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Midnight</td>
<td>3 AM</td>
</tr>
<tr>
<td>Mean</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

recovered in the urine during the 6-hour infusion was 24.5 ± 7.0 µg. This amount is 7% of the amount of epinephrine infused over the same period of time. (Kacere and coworkers\textsuperscript{\textregistered} infused epinephrine at the same rate and recovered 18.5 ± 2.5 µg during the 6-hour urine collection. The same subjects, during infusion of a saline placebo, were found to have much less epinephrine in the 6-hour specimen, 0.3 ± 0.3 µg.)

DISCUSSION

In a recent study, Kacere and coworkers\textsuperscript{8} demonstrated that infusion of epinephrine at a rate of 1 µg/min\textsuperscript{*} in sleeping humans increased the rate of aqueous humor formation 27% compared to a placebo infusion. It was not possible to determine from their experiment if the effect of the infusion was a local effect on the eye or if it was mediated by a more indirect route.

The current study was not designed to determine the effect of intravenous epinephrine but rather the effect of topical timolol. However, the flow in the placebo-treated eyes of this study (1.54 µl/min) was higher than the flow in the saline infused eyes in Kacere’s study (1.13 µl/min) and comparable to the epinephrine-infused eye in Kacere’s study (1.44 µl/min). In addition, a small suppressing effect of timolol in comparison to placebo was observed. The difference between the timolol-treated eye and the placebo-treated eye was greatest between 3 AM and 6 AM.

The difference between the stimulating effect of epinephrine in the Kacere study (0.31 µl/min) and the suppressing effect of timolol on the epinephrine-stimulated eye in the current study (0.21 µl/min) is not statistically significant but hints that topically applied timolol did not completely block the effect of systemically administered epinephrine. Alternatively, topical timolol in one eye may have suppressed aqueous humor flow to a small degree in the placebo-treated eye as well as suppressing the flow to a greater degree in the timolol-treated eye. A systemic effect would also explain why no significant effect of epinephrine was observed on heart rate in this study while Kacere observed a 10% increase in heart rate. In studies of the effect on daytime aqueous humor flow of unilaterally instilled topical timolol on the untreated eye, it appears that the drug causes somewhere near a 10% suppression of flow in the untreated eye. If this 10% were added to the observed 14% difference in flows between the two eyes during the midnight to 6 AM period, the overall suppressing effect of timolol, 24%, is nearly the same as the stimulating effect of epinephrine observed by Kacere.

The suppressing effect of timolol on aqueous humor flow in sleeping humans has not been demonstrated previously though the effect has been sought in at least three previous experiments. Topper and Brubaker,\textsuperscript{3} using an iontophoretic method of applying fluorescein, found an insignificantly higher rate of flow in the timolol-treated eye compared to the placebo-treated eye of sleeping subjects. Brubaker and coworkers\textsuperscript{16} and McCannel and coworkers,\textsuperscript{17} using the same techniques of measuring aqueous flow as used in this study, also found an insignificantly higher rate of flow in the timolol-treated eye of sleeping subjects (Table 4). It was concluded that timolol has no measurable effect on aqueous humor flow in sleeping subjects because of the lack of some hormone present during

TABLE 3. Plasma Catecholamines (pg/ml)

<table>
<thead>
<tr>
<th>Epinephrine</th>
<th>Norepinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midnight</td>
<td>3 AM</td>
<td>6 AM</td>
</tr>
<tr>
<td>Mean</td>
<td>13.3</td>
<td>374</td>
</tr>
<tr>
<td>SD</td>
<td>7.0</td>
<td>142</td>
</tr>
</tbody>
</table>

* In the previous paper, the rate of infusion was correctly stated as $14 \times 10^{-8}$ g/kg/min but incorrectly stated as 1 µg/hr. The correct rate was 1 µg/min.
TABLE 4. Effect of Timolol on Aqueous Flow in Sleeping Subjects

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Methods</th>
<th>Flow, µl/min</th>
<th>Placebo</th>
<th>Timolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topper⁴</td>
<td>1985</td>
<td>Fluorescein iontophoresis, nomogram measure 10 PM, MN, 5 AM</td>
<td>1.61 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brubaker¹⁵</td>
<td>1987</td>
<td>Fluorescein drops, AM/C₉·t</td>
<td>1.2 ± 0.12</td>
<td>1.5 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td></td>
<td>Measure 10 PM and 2 AM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 AM and 6 AM</td>
<td>1.5 ± 0.09</td>
<td>1.6 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>McCannel¹⁶</td>
<td>1992</td>
<td>Fluorescein drops, AM/C₉·t</td>
<td>1.08 ± 0.59</td>
<td>1.13 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>n = 18</td>
<td></td>
<td>Measure MN and 3 AM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study</td>
<td></td>
<td>Fluorescein drops, AM/C₉·t</td>
<td>1.40 ± 0.45</td>
<td>1.25 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td>Measure MN and 3 AM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(epinephrine</td>
<td></td>
<td>3 AM and 6 AM</td>
<td>1.68 ± 0.51</td>
<td>1.42 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>stimulated)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

daytime activities. Epinephrine was speculated to be one such hormone.

The rate of infusion of epinephrine in this study and in Kacere’s study was chosen to mimic daytime production rates of epinephrine by the adrenal gland to determine if epinephrine alone could account for the normal circadian rhythm of aqueous humor flow that was originally discovered by Ericson.¹⁸,¹⁹ The rate of aqueous humor flow in these experiments did not approach the rates observed during daytime hours in active subjects. To produce daytime rates, the epinephrine infusion would have had to more than double the rate of aqueous flow of the sleeping eye, yet only a 27% increase was actually seen. Either the rate of infusion of epinephrine was insufficient in comparison to the rate of release by the adrenal glands or a steady infusion does not mimic the natural pulsatile release of epinephrine. Alternatively, epinephrine secretion alone may be insufficient to account for the entire or even the major portion of the circadian rhythm of aqueous humor flow. Also, the suppression of aqueous humor flow by timolol in this experiment was considerably less than the suppression observed when timolol is applied to humans during waking hours. The difference between the large daytime effect of timolol in suppressing aqueous humor flow and its small effect in this experiment can be interpreted as evidence that timolol’s major effect on daytime flow depends on antagonism of hormones or neurotransmitters other than epinephrine.

An interesting experiment would be to measure the circadian rhythm of aqueous humor flow and the daytime response to timolol of persons lacking any source of adrenal secretion. This experiment was performed many years ago by Linnér employing the suction cup method of measuring aqueous humor flow.²⁰ Interestingly, in contrast to the results of Ericson who originally concluded from suction cup studies that aqueous formation is suppressed almost 70% during sleep, Linnér observed only an 18% suppression of flow at night in such patients. Linnér’s experiment should be repeated with currently available techniques of measuring aqueous humor flow.

Key Words
aqueous flow, fluorophotometry, human eye, timolol, epinephrine, circadian rhythm

References


