Intravenous Epinephrine Stimulates Aqueous Formation in the Human Eye

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An experiment was done to determine if intravenously administered epinephrine would stimulate the rate of aqueous humor flow in sleeping human subjects. Twenty normal volunteers participated in a double-masked randomized placebo-controlled study. The rate of aqueous humor flow was measured by the rate of clearance of topically applied fluorescein. Measurements were made on two separate nights while the subjects slept, once during a placebo infusion and again during an intravenous infusion of epinephrine (rate, 1 µg/hr; a rate calculated to be threefold the basal adrenal secretion in supine sedentary human subjects). Compared with placebo, epinephrine infusion had no effect on the blood pressure of sleeping subjects, increased the heart rate by 10%, and increased aqueous flow by 27%. Systolic blood pressure was 112 ± 9 mmHg (mean ± standard deviation) during placebo infusion and 114 ± 13 during epinephrine infusion. The heart rate was 60 ± 10 beats/min during placebo and 67 ± 14 during epinephrine infusion (P = 0.011). Aqueous flow was 1.11 ± 0.51 µl/min during placebo and 1.42 ± 0.52 during epinephrine infusion (P = 0.016). It was concluded that adrenal medullary secretion can stimulate aqueous formation and may explain in part the circadian rhythm of aqueous flow. Invest Ophthalmol Vis Sci 33:2861-2865, 1992

It has been established that the rate of flow of aqueous humor in the human eye decreases during sleep to a rate that is less than one half the daytime rate.1-3 The effect of sleep on aqueous flow is as great as the effects of several classes of therapeutic agents used for the treatment of glaucoma, including carbonic-anhydrase inhibitors,4-6 β-adrenergic antagonists,7-9 and α-2 selective adrenergic agonists.10-13 The biologic mechanism that causes the observed day-to-night difference in aqueous flow is unknown. One explanation might be that an endogenous hormone that increases during the day stimulates aqueous formation. Alternatively, a hormone that rises during sleep might inhibit aqueous formation and be the driving force behind this circadian rhythm of aqueous flow. Previous studies of several hormones have not produced credible evidence that either one of these mechanisms explains this rhythm.14-16

Epinephrine is a hormone that deserves further investigation. The rate of secretion of epinephrine is higher during daytime activity when aqueous formation is high.17 It also has been shown that various catecholamines that possess β-adrenergic activity can stimulate aqueous humor flow when applied topically to the eye.5,18-23 In addition, β-adrenergic antagonists suppress aqueous flow in humans during the day.4-9,24-26 However, during sleep, no such effect is observed.4-6,27 Therefore, β-adrenergic agonists completely eliminate the circadian rhythm of aqueous flow; a somewhat dampened circadian rhythm of flow persists after administration of carbonic-anhydrase inhibitors or α-2 selective agonists.6

Indirect evidence that the diurnal rhythm of flow is mediated by a circulating hormone rather than the sympathetic nervous system comes from studies of unilateral terminal-neuron Horner's syndrome. These patients have a normal day-to-night cycle of aqueous flow28 and respond normally to β-adrenergic antagonists.29 These observations suggest that a complement of adrenergic nerves to the eye is necessary for the rhythm of flow to persist.

There is evidence (from investigations in anesthetized animals) that catecholamines administered intravenously can stimulate aqueous humor formation. In one study, intravenously administered epinephrine stimulated aqueous humor formation in anesthetized rabbits.30 In anesthetized monkeys, catecholamines with β-adrenergic activity stimulated aqueous formation.31,32

Could epinephrine released by the adrenal medulla be a hormonal regulator of aqueous formation and
account for the day-to-night difference in aqueous humor formation in humans? To help answer this question, we might infuse epinephrine intravenously at night at a rate that mimicked the daytime secretion of this hormone. We would expect the infusion to blunt the nadir of aqueous flow that occurs normally during sleep. We did such an experiment, and the results suggest that endogenous epinephrine release may be a significant determinant of the rate of aqueous formation.

Materials and Methods

Twenty normal human volunteers (15 men and 5 women) were studied. An eye history and examination of each subject was done (consisting of best-corrected visual acuity, confrontation visual field, external examination, indirect ophthalmoscopy, tonometry, and slit-lamp examination). Subjects were excluded from the study for any of the following reasons: ocular disease, systemic disease requiring chronic treatment, drug use except oral contraceptives, abnormal sleep patterns, inability to undergo tonometry or fluorophotometry satisfactorily, drug hypersensitivity (especially to fluorescein), or participation in a trial of any investigational drug within 30 days. After Mayo Institutional Review Board approval no. 103-N-91, written informed consent according to federal guidelines was obtained from all subjects. Preliminary tests included photogrammetry to measure anterior chamber volumes and fluorophotometric measurement of autofluorescence of each cornea. Results of blood tests for pregnancy were negative in all women 2-3 days before beginning the study.

Epinephrine kinetics studies showed that, during the hours of 7 AM-10 AM, supine 19-28-yr-old subjects produced $279 \times 10^{-9}$ g/min of epinephrine. In a 70-kg person, this equals approximately $4 \times 10^{-9}$ g/kg/min. In light of these data, we elected to infuse 14 $\times 10^{-9}$ g/kg/min of epinephrine from 12 midnight to 6 AM to ensure a blood level somewhat higher than the basal rate but not so high that it would interfere with sleep or produce symptoms that would unmask the study.

The experimental solutions were prepared and randomized by the research personnel of the Rochester Methodist Hospital. The active solution of epinephrine was prepared from 1-ml ampules of epinephrine 1:1000 (1 mg/ml) diluted in 100 ml of 0.9% sterile saline; the placebo solution was 0.9% saline. Both epinephrine and placebo containers were numbered 1-20, representing subject numbers. One half of the subject numbers were chosen by random to receive the epinephrine containers, labeled "Study #1." The placebo containers for these subjects were labeled "Study #2." The contents were otherwise identical. The remaining ten subject containers for placebo were labeled "Study #1" and for epinephrine, "Study #2." The contents remained unknown to any of the investigators until the entire study was completed, and all the data had been entered into a computer spreadsheet.

The study consisted of the following sequence for each subject. The subject instilled one drop (0.03 ml) of 2% fluorescein into each eye five times at 5-min intervals beginning at 2 AM the first morning of the study. At 8 AM, 12 noon, and 4 PM, the subject reported to Saint Marys Clinical Research Center (CRC) where scanning fluorophotometric measurements were made of the cornea and the anterior chamber of each eye (a "scan"). After the 4 PM scan, the subject instilled 2% fluorescein in each eye as was done previously. The subject then was released and instructed to return at 9 PM for the nighttime portion of the study. After the subject was readmitted to the CRC, an intravenous catheter was placed in their midforearm veins in each arm. Normal saline was infused at 30 ml/hr to keep the lines open. Later, one catheter was used for blood withdrawal to measure epinephrine levels, and the other was used to infuse the experimental solution (epinephrine or placebo). Fluorophotometric measurements were made again at 10 PM. Electrocardiographic leads were placed on the subject's chest to monitor their heart rate. At 12 midnight, the infusion of the experimental solution marked Study #1 was begun. A calibrated Travenol infusion pump (Baxter Health Care Corp., Deerfield, IL), set at 6 $\mu$g/hr, delivered 1 $\mu$g/hr from 12 midnight to 6 AM. For a 70-kg subject, this rate was $14 \times 10^{-9}$ g/kg/min.

At 12 midnight, 3 AM, and 6 AM, the following procedures were done. While the subject was still asleep or lying still, 10 ml of blood was drawn for catecholamine levels, and their heart rate and blood pressure were measured. Subjects then got out of bed, walked approximately 30 feet to the fluorophotometer, and underwent scans of both eyes. They then returned to their beds and went back to sleep. These procedures took 10-15 min. Sleep was monitored by a wrist actigraph (Ambulatory Monitoring Inc., Ardsly, NY) that detects and records movement.

A 6-hr urine specimen was collected for catecholamines from midnight to 6 AM. After the protocol was completed, the intravenous catheters were removed, and the remaining experimental solution was retained for analysis after the code had been broken. This step was taken to ensure that no clerical error had been committed and that the epinephrine had not been degraded between the time of its preparation and infusion. The subjects then were dismissed from the CRC.
This complete sequence was repeated at least 1 wk later; at this time, the container marked Study #2 was infused. Neither the subject nor the examiner were able to determine on which of the two nights the epinephrine solution had been infused. All data were collected and stored in a spreadsheet for statistical analysis. Then the code was broken, the contents of the containers confirmed by analysis, and the data stratified by placebo versus epinephrine.

The data were evaluated using student t-tests for paired samples. A single-sided P value < 0.025 was considered significant. Flow was calculated from the clearance of fluorescein by subtracting an assumed normal rate of diffusional loss (0.25 μl/min). The coefficient of variation of the difference of measurements in a single eye (measured on two occasions) was determined in a previous group of normal humans to be 21%. Based on this figure, we calculated that a sample size of 20 subjects has a 90% chance of detecting a 15% stimulation caused by epinephrine.

### Results

The rates of aqueous humor flow under the various conditions are summarized in Table 1. The flow during the daytime from 8:00 AM to 4:00 PM was 2.72 ± 0.66 μl/min (mean ± standard deviation) for the subjects before receiving the nighttime epinephrine infusion and 2.83 ± 0.58 μl/min before receiving placebo. These rates of flow were normal compared with a large group of normal subjects (age range, 5-82 yr), where a flow of 2.75 ± 0.63 μl/min was found. The rate of flow during the night during placebo infusion from 12:00 midnight to 6:00 AM was 1.13 ± 0.42 μl/min. This rate was normal compared with a previous group of 67 subjects (age range, 21-65 yr) tested under similar conditions with the same fluorometric protocol (mean, 1.18 ± 0.41 μl/min). The rate of flow during the epinephrine infusion was 1.44 ± 0.45 μl/min; this was 27% higher compared with the placebo infusion (P = 0.016, one sided t-test).

The epinephrine infusion had a small but measurable effect on heart rate but no measurable effect on blood pressure. The heart rate at 3:00 AM during the placebo infusion was 60.4 ± 10.3 beats/min, but during the epinephrine infusion, it was 66.7 ± 13.6 beats/min, an increase of 10% (P = 0.011). The mean blood pressure at 3:00 AM during the placebo infusion was 85.9 ± 9.3 mmHg, and during the epinephrine infusion, it was 85.1 ± 7.1 mmHg. This difference was not significant (P = 0.32).

Several precautions (taken to ensure that the infusions were correct) confirmed that they were. The mean plasma concentration of epinephrine at 3:00 AM during the placebo infusion was 13 ± 30.6 pg/ml, and during the epinephrine infusion, it was 195 ± 79.8 pg/ml. The urinary epinephrine during the 12:00 midnight to 6:00 AM collection on the night of the placebo infusion was 0.33 ± 0.29 μg, and on the night of the epinephrine infusion, it was 18.5 ± 2.5 μg. Finally, the actual concentration in the epinephrine infusion bags, which were frozen and measured several weeks later, was 84% of the initial concentration.

The recorded wrist motion was studied for systematic differences between the nights of placebo and epinephrine infusion. The tracings indicated that subjects slept well on both nights; this agreed with their subjective reports to the examiner at the end of each session.

Two subjects reported feeling lightheaded at the time of the 3:00 AM scan on the night they received the epinephrine infusion. This feeling had disappeared in both subjects by the time of the 6:00 AM scan. No other subject reported any side effects. No subject was able to determine whether placebo or epinephrine had been administered.

### Discussion

To our knowledge, this study is the first demonstration that systemically administered epinephrine can stimulate aqueous flow in the human eye. However,
previous evidence made such a possibility seem likely. First, the secretion of epinephrine by the adrenal gland is faster during times of increased human activity; aqueous humor flow also is greater during these times. This finding alone could be mere coincidence because many other hormones behave similarly. Second, aqueous humor formation is sensitive to substances that alter the rate of synthesis of the intracellular signaling compound cyclic adenosine monophosphate.40-48 This signaling system is known to be coupled to surface receptors in many cells that are sensitive to β-adrenergic agonists, including epinephrine.59-62 The direction of the effect of β-adrenergic stimulation on aqueous humor formation in some animals and under certain conditions is inhibitory.53 In other systems, it is stimulating,18 but many experiments have linked the two. Third, it is known that β-adrenergic antagonists can suppress aqueous flow whether administered systemically64 or topically to the eye.5 This effect does not depend on complete sympathetic innervation of the eye because it occurs in patients with the clinical and pharmacologic signs of terminal-neuron Horner’s syndrome.29 However, this effect depends on a state of wakefulness; timolol, for example, has no effect on aqueous flow in sleeping humans.4,6-27 Thus, it appears that some hormone not derived from sympathetic nerves to the eye must be present for β-adrenergic blockers to have an effect on aqueous flow, a hormone that is present during the day but not at night.

The foregoing discussion suggests that adrenally secreted epinephrine could be the hormone that drives the diurnal cycle of aqueous flow and might explain the well-known effect of β-adrenergic blockers on flow and intraocular pressure. However, is the natural secretory rate of this hormone sufficient to have any measurable effect on the eye? This question was addressed in our study, and the results showed that the normal adrenal gland can affect aqueous flow. In this study, we infused epinephrine intravenously at a rate that increased the pulse rate slightly but did not raise the blood pressure of sleeping subjects measurably. However, aqueous flow was increased almost 30%, a significant change. Considering that the infusion rate in this experiment was less than the adrenal release rate of active humans, it seems credible that aqueous flow could be stimulated sufficiently by this hormone to reach the rate observed during daytime activity.

This experiment did not provide evidence for the site or the mechanism of action of systemically released epinephrine on aqueous flow. The site could be the eye or distant structures. Other hormones or physiologic functions that, in turn, affect the eye could be involved. These questions must be answered by additional experiments. Others32 found in anesthetized monkeys that terbutaline-stimulated aqueous flow was inhibited by topically applied timolol. Their experiment suggests that the epinephrine effect we measured was a direct ocular effect, but a similar experiment must be conducted in sleeping humans before this question can be answered.

Key words: epinephrine, circadian rhythm, aqueous humor flow, human eye, intravenous

References

17. Cryer PE: Physiology and pathophysiology of the human sym-