

Effect of Chronic Epinephrine on Aqueous Humor Flow During the Day and During Sleep in Normal Healthy Subjects

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Epinephrine 2% drops were administered to one eye and a placebo to the fellow eye twice a day for 2 weeks in 18 normal human volunteers. The circadian rhythm of aqueous humor flow was measured by fluorophotometry. Epinephrine (compared with placebo) had no measurable effect on the rate of aqueous flow or the circadian rhythm of aqueous humor flow. These results suggest either that topical application of epinephrine cannot achieve a sustained effect on the mechanism of aqueous formation compared with endogenous epinephrine or that circulating epinephrine is not the sole or principal hormonal messenger that mediates the circadian rhythm of aqueous flow in humans. An experiment in which epinephrine was administered systemically might clarify this ambiguity. Invest Ophthalmol Vis Sci 32:2507-2510, 1991

It has been observed that the rate of aqueous humor flow in humans undergoes a circadian rhythm characterized by a higher flow during waking hours and a lower flow during sleep.^{1,2} This rhythm is obliterated by the beta-adrenergic blocker timolol.³ One hypothesis that would explain this observation is that the circadian rhythm of flow is driven by endogenous epinephrine, a hormone known to vary with the time of day.^{4,5} A corollary of this hypothesis is that timolol blocks epinephrine's action on the tissues of the ciliary body. If this hypothesis were correct, we might expect acute doses of topical epinephrine to stimulate aqueous flow to a greater extent during sleep than during the day. This action has been observed, not only for epinephrine,³ but also for the catecholamines isoproterenol⁶ and terbutaline.⁷ If a high and steady concentration of epinephrine could be maintained in the ciliary body by topical application, the endogenous fluctuations of epinephrine might not be seen in comparison with its total concentration in ocular tissues. Thus, high concentrations (if steady) might dampen or obliterate the diurnal cycle of aqueous flow.

One experiment answers part of this question.⁸ These investigators administered 2% epinephrine

drops twice a day for 1 week to one eye of 14 human subjects and observed that the treated eye had a rate of aqueous flow at the end of the treatment that was greater than the untreated eye. Their measurements were made during the day but not at night. In this experiment, we attempted to test the hypothesis further by giving 2% epinephrine drops twice a day for 2 weeks to one eye of a group of normal human subjects and comparing the amplitude of the day-night difference in flow of the treated eye to the untreated eye.

Materials and Methods

Eighteen normal subjects (nine women and nine men) were studied. An eye examination was done on each subject, consisting of best-corrected visual acuity, external examination, confrontation visual field, slit-lamp examination, undilated fundus examination, and applanation tonometry. Written informed consent was obtained. The volume of the anterior chamber of each subject was measured photogrammetrically.⁹ The study consisted of three separate 24-hr fluorophotometric determinations of aqueous humor flow: pretreatment, after 24 hr of treatment, and after 2 weeks of treatment.

The night before the fluorophotometric determination, each subject instilled four drops of 2% fluorescein at 3:00 AM so that stromal fluorescence was relatively uniform. Fluorescence was measured using a two-dimensional scanning fluorophotometer.¹⁰

Flow was measured during the day (9:00 AM to 5:00 PM) and during the night (10:00 PM to 7:00

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AM). For the daytime segment of the study, the subjects were asked to abstain from caffeine, heavy exertion, and overeating to avoid unnecessary variations in aqueous flow. Otherwise, the subjects engaged in normal daytime activity. For the nighttime study, the subjects were provided comfortable sleeping quarters. The measurements were timed so that the subjects could sleep uninterrupted between midnight and 6:00 AM.

The first 24-hr study measured each subject's pretreatment flow and was the basis for calculating the statistical power of the sample size to detect a reduction of the diurnal variation of flow. Each subject then received a commercially prepared solution of 2% epinephrine hydrochloride (Epifrin; Allergan, Irvine, CA) to one eye and nothing to the fellow eye. The eye receiving the drug was chosen randomly. One drop of the drug was administered to the treated eye at 8:00 AM and another at 8:00 PM for a 24-hr period. Another fluorophotometric determination of aqueous humor flow was done to determine the effect of epinephrine after 1 day of treatment. The subject continued the epinephrine twice daily for a minimum of 14 days (mean treatment period, 15 days). The subject then underwent the third and final 24-hr fluorophotometric determination of aqueous humor flow.

Intraocular pressure was not measured to avoid any alteration in the clarity or permeability of the corneal epithelium, both of which were critical to accurate measurements of flow by this technique.

In determining the effect of the drug, the difference between the daytime and nighttime flow (the diurnal variation of flow) of the treated eye of each subject was compared with the untreated fellow eye of the same subject. To test statistical significance, the student t-test for paired samples was used, and a *P* value less than 0.05 was considered significant. To determine the statistical power of this experiment of 18 subjects, we calculated the normalized standard deviation of the difference of the diurnal variation of flow (*V*) between the two eyes of each subject during the 24-hr pretreatment test when neither eye was treated:

$$\sqrt{\frac{\sum_{i=1}^n (V_{OD} - V_{OS})^2}{n-1}} / \bar{V}$$

where \bar{V} is the mean diurnal variation of flow of the subjects. This statistic had a calculated value of 0.32. The statistical power was calculated from a standard table.¹¹ Table 1 summarizes the statistical power of our sample:

Table 1. Statistical power

<i>Reduction of diurnal variation caused by treatment</i>	<i>Odds of detection ($\alpha = 0.05$)</i>
27%	95%
24%	90%
21%	80%
13%	50%

Results

Tables 2 and 3 are summaries of the treatment effects on flow in this experiment. The rate of flow before treatment in the eyes assigned to be treated was $2.88 \pm 0.59 \mu\text{l}/\text{min}$ (mean \pm standard deviation) in the morning from 09:00–11:00 AM, 2.64 ± 0.44 in the afternoon from 11:00 AM to 4:00 PM, and 1.19 ± 0.34 during sleep midnight to 06:00 AM. In the control eyes, the rates were 2.91 ± 0.60 in the morning, 2.60 ± 0.38 in the afternoon, and 1.22 ± 0.28 during sleep. These rates are normal, and the normal circadian rhythm was observed.

The difference between the morning flow and the flow at night (the diurnal variation) in the eyes to be treated was 1.69 ± 0.63 and in the fellow eyes was 1.68 ± 0.71 . The difference between the diurnal variations of the fellow eyes before treatment was 0.01 ± 0.51 .

When the measurement was repeated, beginning 24 hr after the initiation of epinephrine drops, the flow in the treated eye in the morning was 2.55 ± 0.78 ; in the afternoon, 2.38 ± 0.56 ; and during sleep, 1.44 ± 0.54 . In the control eye, the flow in the morning was 2.58 ± 0.75 ; in the afternoon, 2.40 ± 0.51 ; and during sleep, 1.28 ± 0.31 . The diurnal variation was 1.11 ± 0.82 in the treated eyes after 24 hr of treatment and 1.30 ± 0.90 in the control eyes at the same time. Although it appears that epinephrine suppressed the diurnal swing, this difference was not statistically significant ($P = 0.10$).

After at least 14 days of treatment, the flows for the treated eye in the morning were 2.61 ± 0.73 ; in the afternoon, 2.49 ± 0.53 ; and during sleep, 1.32 ± 0.36 . In the control eye, the values in the morning were 2.41 ± 0.75 ; in the afternoon, 2.36 ± 0.53 ; and during sleep, 1.24 ± 0.25 . The diurnal variation was 1.29 ± 0.79 in the treated eyes and 1.17 ± 0.72 in the control eyes. This difference was not statistically significant ($P = 0.36$).

In both the treated and the control eyes, there appeared to be a lessening of the diurnal variation over time. This phenomenon was observed in the treated eyes at 24 hr and in the control eyes at 14 days ($P = 0.02$). The reason for this observation is not apparent. It seems unlikely that this observed trend could

Table 2. Treatment effects on flow

	Baseline			24 Hours			14 Days		
	AM (9-11)	PM (11-4)	Night (12-6)	AM (9-11)	PM (11-4)	Night (12-6)	AM (9-11)	PM (11-4)	Night (12-6)
Flow, $\mu\text{l min}^{-1}$, mean \pm SD									
Treated eye (T)	2.88 \pm 0.59	2.64 \pm 0.44	1.19 \pm 0.34	2.55 \pm 0.78	2.38 \pm 0.56	1.44 \pm 0.54	2.61 \pm 0.73	2.49 \pm 0.53	1.32 \pm 0.36
Control eye (C)	2.91 \pm 0.60	2.60 \pm 0.38	1.22 \pm 0.28	2.58 \pm 0.75	2.40 \pm 0.51	1.28 \pm 0.31	2.41 \pm 0.75	2.36 \pm 0.53	1.24 \pm 0.25
Statistics (<i>P</i> values)									
C vs. T	0.79	0.57	0.41	0.82	0.84	0.15	0.10	0.16	0.26
Baseline vs. treatment period									
Treated				0.10	0.06	0.07	0.13	0.18	0.13
Control				0.09	0.11	0.60	0.03	0.12	0.81

have been due to a local effect of epinephrine because it was observed in the treated and in the untreated eye.

Discussion

In this experiment, we observed (both at the 24-hr measurement and at the 14-day measurement) that the direction of the difference between the nighttime flows of the treated eye and the untreated eye was the same as observed in studies of the acute effects of catecholamines on flow in sleeping subjects, namely, a stimulation of aqueous flow.^{3,6,7} However, in this group of subjects the difference was not statistically significant. As can be seen from Table 1, the probability of finding a clinically significant effect, if it were present, was good.

The effect of chronic treatment that would be expected on the basis of the hypothesis leading to the study was not observed. That is, we expected to see a reduction in the day-night difference in flow in the treated eye but no reduction in the untreated eye. We observed a reduction in the day-night difference in both eyes at both treatment intervals. In the case of the treated eye, this difference was significant at 24 hr; in the case of the control eye, it was significant at 14 days. Thus, this experiment does not support the hypothesis.

There are at least three reasons that could explain the experiment's failure to support the hypothesis. First, and most obvious, is that the hypothesis is incor-

rect, ie, epinephrine is not the sole or major hormonal mediator of the circadian rhythm of aqueous flow in humans. Second, topically applied epinephrine, which reaches the outflow apparatus in sufficient concentration to enhance outflow facility, might not reach the ciliary epithelium in the posterior chamber in a high enough concentration for long enough to mimic the effects of epinephrine released endogenously by the adrenal glands. Third, the systemic absorption of topically applied 2% epinephrine may have affected both eyes, rendering the comparison between the "treated eye" and the "untreated eye" meaningless. The last two possibilities deserve further comment.

It is well known that topically applied epinephrine is able to lower intraocular pressure in some eyes by enhancing the outflow of aqueous humor. A system of trabecular cells was developed in vitro in which hydraulic conductivity could be measured.¹² In this system, epinephrine at a concentration of 10^{-5} M was able to increase the conductivity of these cells.¹³ It is known that epinephrine has difficulty penetrating the cornea and is metabolized rapidly in ocular tissues.¹⁴⁻¹⁹ Despite these difficulties, it is likely that the concentrations used clinically (10,000-fold the concentrations necessary to enhance conductivity) can reach the trabeculum and produce the desired effects on outflow. However, the concentrations that reach the ciliary epithelium must be much lower.

If the concentration difference between the posterior and the anterior chamber is as large as the difference between the tears and the anterior chamber, then epinephrine placed in the tear film that produces a concentration of 10^{-5} M in the trabeculum would produce a concentration of only 10^{-9} M in the posterior chamber. This concentration is only twice the plasma concentration in resting humans²⁰ and may be barely sufficient to stimulate aqueous formation to a measurable degree.

Because the resting plasma concentration of epinephrine is approximately 5.5×10^{-10} M,²⁰ 200 mil-

Table 3. Diurnal variation (AM minus night)

	Baseline	24 Hours	14 Days
Flow, $\mu\text{l min}^{-1}$, mean \pm SD			
Treated (T)	1.69 \pm 0.63	1.11 \pm 0.82	1.29 \pm 0.79
Control (C)	1.68 \pm 0.71	1.30 \pm 0.90	1.17 \pm 0.72
Statistics (<i>P</i>)			
T vs. C	0.94	0.25	0.36
Baseline vs. treatment period			
Treated		0.02	0.46
Control		0.13	0.02

lion times less than the concentration in 2% epinephrine eye drops (0.11 M), we must consider the possibility that topical administration in one eye can have systemically mediated effects on the fellow eye. The entry of epinephrine into the blood from the ocular depot would be rapid at first, but it would diminish exponentially. An estimate of the kinetics can be made from the tear volume and the rate of tear turnover. The volume of the tear pool is approximately 7 μl .²¹ The coefficient of basal tear turnover is approximately 6 hr^{-1} .¹⁹ If we assume that a volume of undiluted 2% epinephrine equal to the tear volume is absorbed completely into the blood stream with an absorption rate coefficient of 6 hr^{-1} , the rate of entry of epinephrine into the blood would fall below the basal rate of epinephrine production by the adrenal gland within 40 min of drop instillation. If a lower estimate of bioavailability of the drop is assumed, say 10%, the rate of absorption from the ocular depot will fall below the basal rate in 15 min. The rate of entry is the important parameter since the half-life of epinephrine in the plasma of humans is 1.2 min.²⁰ In other words, administration of this concentration of eye drops is likely to produce systemic elevations of epinephrine but only for a short period relative to the duration of the experiment. Also, the total amount of epinephrine that will be absorbed through the ocular route, estimated in this case to be from 14–140 μg , is less than the resting adrenal gland will release over the 12-hr dosage interval, estimated to be 200 μg .²⁰ What is not known is the temporal relationship between any stimulus by epinephrine in the ciliary body and the duration of any response. Thus, a systemic effect, although unlikely, cannot be excluded.

In summary, our results neither support nor refute the hypothesis that adrenally released epinephrine plays a role in the circadian cycle of aqueous humor flow in humans. An experiment that might give a definitive answer would be to infuse epinephrine intravenously into sleeping subjects at a rate mimicking the rate of release of epinephrine from the adrenal medulla in active subjects. At present a satisfactory explanation for the mechanisms of this cycle in humans is elusive.

Key words: epinephrine, aqueous humor flow, chronic treatment, fluorophotometry, diurnal cycle

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