Aqueous Flow in Humans After Adrenalectomy

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Purpose. This study was performed to determine if the circadian rhythm of aqueous humor formation and the aqueous humor suppressing effect of β-adrenergic antagonists can occur in the absence of adrenally derived epinephrine.

Methods. Twenty-one human subjects who had undergone bilateral adrenalectomy were studied during a 28-hour period. The study was divided into four time periods as follows: morning 1 (8 AM to noon), afternoon (noon to 4 PM), night (midnight to 6 AM), and morning 2 (8 AM to noon). At 6:45 AM before the morning 2 measurements, one drop of 0.5% timolol was applied to one eye and one drop of placebo (artificial tears) was applied to the other eye. Topical fluorescein and a scanning fluorophotometer were used to measure the rate of aqueous humor flow. Twenty normal controls were studied in a similar fashion but did not undergo the morning 2 measurement.

Results. In the subjects lacking adrenals, the daytime rates of aqueous flow were 3.17 ± 0.78 μl/min (mean ± SD) and 3.16 ± 0.67 μl/min for the morning 1 and afternoon periods, respectively. The rates in daytime periods were not significantly different from each other ($P = 0.699$). The rate of aqueous flow for the night period was 1.37 ± 0.37 μl/min, a 57% reduction from both morning 1 and afternoon periods ($P < 0.001$). The morning, afternoon, and night rates of flow in normal controls were not significantly different from the rates in subjects lacking adrenals. For the morning 2 period, the aqueous flow was 2.74 ± 0.54 μl/min for the placebo-treated eye and 1.77 ± 0.38 μl/min for the timolol-treated eye. The rate of aqueous flow was reduced (35%) in the timolol-treated eye when compared to the fellow placebo-treated eye ($P < 0.001$). The timolol-treated eye also showed a 26% reduction in intraocular pressure when compared to the fellow placebo-treated eye ($P < 0.001$).

Conclusion. The study demonstrates that both the circadian rhythm of aqueous flow and the daytime response to timolol persist in the absence of the adrenal glands. Invest Ophthalmol Vis Sci. 1994;35:3325–3331.

During sleep, the human eye produces aqueous humor at a rate less than half that of the daytime rate.[1–4] 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,[5–7] β-adrenergic antagonists,[8–10] and selective α_2-adrenergic agonists.[11–14] This rhythm of aqueous flow must be controlled hormonally or neuronally by a poorly understood mechanism. One possible explanation is 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 evidence suggests epinephrine, a hormone of the adrenal medulla, deserves further investigation. The rate of secretion of epinephrine by the adrenal gland is higher during daytime activity, when aqueous formation is highest, and low during sleep, when aqueous formation is low.[15–17] Three decades ago, Linnér,[18] using a suction cup method of aqueous flow measurement developed by Rosengren,[19–21] measured the circadian rhythm of aqueous flow in nine persons with adrenal insufficiency who were on replacement steroid therapy. In contrast to Ericson,[2] who had observed a 70% suppression of aqueous formation during sleep in normal subjects, Linnér ob-

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served only an 18% suppression in his subjects with adrenal insufficiency. Linnér concluded that the hormones of the adrenal gland must play an important role in the circadian rhythm of aqueous flow. A number of studies have shown that various catecholamines, including epinephrine, that possess β-adrenergic activity can stimulate aqueous humor flow when administered systemically[22] or topically to the eye.[6,23–28] A study by Topper and Brubaker[6] suggests that topically administered epinephrine increased the rate of flow by 15% during the day and 47% at night. A recent study by Kacere[22] suggests that intravenously administered epinephrine stimulates the rate of aqueous flow in sleeping subjects.

It is known that β-adrenergic antagonists suppress aqueous flow in humans during the day,[5–10,29–31] but this effect has not been observed in sleeping subjects.[6,7,32] This observation is consistent with the idea that β-adrenergic antagonists block a hormone that stimulates aqueous formation during the day but is low or absent at night. Topper and Brubaker[6] hypothesized that this hormone could be epinephrine.

We decided to test the idea that the circadian rhythm of aqueous flow is driven principally by the circadian rhythm of epinephrine synthesis and release by the adrenal gland. We did so by repeating Linnér's experiment with currently available techniques of measuring aqueous flow. We measured the circadian rhythm of aqueous flow and daytime response to timolol in patients lacking adrenal glands.

MATERIALS AND METHODS

Selection of Subjects

Twenty-one human subjects who had undergone bilateral adrenalectomy were studied during a 28-hour period. These subjects were recruited from patients of the endocrinology department at the Mayo Clinic under the supervision of one of us (WFY). All subjects required corticosteroid replacement therapy (14 subjects were taking prednisone, and 7 subjects were taking hydrocortisone.) Their standard daily regimens mimicked a circadian cycle; a full dose was taken in the morning and a half dose was taken in the evening (prednisone: 5 mg morning, 2.5 mg evening; hydrocortisone: 20 mg morning and 10 mg evening). On the study day, subjects were asked to double their evening dose to equal their morning dose. This change was made to eliminate any confounding effects of a circadian variation in corticosteroid action. All but one subject took 0.1 mg of fludrocortisone (Florinef, Bristol-Myers Squibb, Princeton, NJ) daily for mineralocorticoid replacement therapy. This regimen was not altered.

Subjects ranged in age from 22 to 63 years (mean age, 43 years). All subjects underwent a screening examination that included medical and ophthalmic history, visual acuity measurement, slit-lamp examination, applanation tonometry, and ophthalmoscopy. Subjects were excluded if they had asthma, previous eye surgery, chronic eye disease, or asymmetric intraocular pressures (difference >3 mm Hg), or if they were taking medications known to affect aqueous flow, including β-adrenergic antagonists or agonists, α_2-adrenergic agonists, or carbonic anhydrase inhibitors. One subject, who was otherwise eligible, was taking β-adrenergic antagonist eye drops. His aqueous flow results had to be excluded because of the well-known effect of this class of drug on aqueous flow.

Twenty normal human subjects without historic or physical evidence of ocular or systemic disease were studied during a 22-hour period and served as controls. These control subjects ranged in age from 22 to 45 years (mean age, 31 years).

The study was approved by the Institutional Review Board of the Mayo Clinic. Written informed consent was obtained from all subjects in accordance with federal guidelines for investigation of human subjects. The study met the recommendation of the Declaration of Helsinki.

Materials

A scanning ocular fluorophotometer was used to measure the concentration of fluorescein in the anterior chamber and cornea.[33] This measurement is referred to as a scan. The autofluorescence of the anterior chamber and cornea were subtracted from these values to give the amount of fluorescence due to fluorescein alone. The rate of aqueous flow was calculated from the rate of clearance of fluorescein from the anterior chamber minus a small adjustment (0.25 μl/min) for diffusional loss of fluorescein from the system.[34] The volume of the anterior chamber, used in the calculation of flow, was determined by a photogrammetric method.[35]

Intraocular pressure was measured with a Goldmann tonometer employing Fluress (Barnes Hind, Sunnyvale, CA). Each intraocular pressure measurement was made two or three times in each eye, beginning in the right eye and alternating between eyes. The intraocular pressure was recorded as the mean of the measurements for each eye.

Sterile dropper bottles containing either timolol 0.5% (Timoptic, Merck Sharp & Dohme, West Point, PA) or a placebo (Hypo Tears, IOLAB, Claremont, CA) were prepared by the pharmacy of the Rochester Methodist Hospital. These bottles were randomized to the right and left eyes and labeled as follows: subject #1, right eye; subject #1, left eye; subject #2, right eye, and so on. The investigators did not know the contents of the bottles until all the data were recorded and stored on a computer spread sheet. At that point,

the code was broken, and the contents of each container were confirmed by absorption spectrometry at 200 and 340 nm.

Measurements of catecholamines in plasma (obtained from venipuncture) and urine were made in the clinical laboratories of the Mayo Clinic using high-pressure liquid chromatography.

Procedures

On a day when no fluorescein had been administered, each subject underwent a measurement of the autofluorescence of the anterior chamber and cornea. At the same sitting, the volume of both anterior chambers was measured by photogrammetry.

In the evening before the study day, the subjects were admitted to the General Clinical Research Center of the Mayo Clinic and were provided with quiet, comfortable rooms not far from the fluorophotometer. At 10 PM the subjects went to sleep. At 2 AM, a nurse or investigator awakened the subjects and instilled 2 to 5 drops of 2% fluorescein in each eye (younger subjects received more drops, older subjects fewer.) Subjects then returned to sleep. The remaining portion of the study was divided into four time periods as follows: morning 1 (8 AM to noon), afternoon (noon to 4 PM), night (midnight to 6 AM), and morning 2 (8 AM to noon).

At 8 AM, fluorophotometric measurements began. Subjects' lids were cleaned, and scans of both eyes were performed. After scanning, subjects without adrenals voided to commence a 24-hour urine collection for catecholamines (norepinephrine, epinephrine, and dopamine). Scans were repeated at 10 AM, noon, 2 PM, and 4 PM After the 4 PM scans, intraocular pressures were measured and fluorescein was reinstilled, as previously described, in preparation for the remainder of the study (night and morning 2 periods). Subjects kept their eyes closed for 30 minutes after fluorescein instillation, after which their lids were carefully cleansed of excess dye.

At 10 PM, both eyes were rescanned to determine if the concentration of fluorescein was adequate for the remainder of the study. Subjects then retired to sleep. At midnight, 3 AM, and 6 AM, subjects were awakened and walked a short distance from their beds to undergo repeat scans, a procedure that would keep them awake for only 5 minutes.

After the 6 AM scans, subjects walked around the research center for 10 minutes to stimulate any potential release of epinephrine, and venipuncture was carried out in the standing position to measure plasma catecholamines. (At this point, the control subjects had completed their study and were dismissed.) At 6:45 AM, the investigator placed one drop of 0.5% timolol in a subject's eye and one drop of placebo (artificial tears) in the other eye. A separate tissue was used to blot each eye. The placebo and timolol drops were administered in a double-masked, randomized manner. In half the subjects, the right eye received the drug; in the other half, the left eye received the drug.

At 8 AM, the 24-hour urine collection was completed, and fluorophotometry scans were resumed. Additional scans were made at 9 AM, 10 AM, 11 AM, and noon. After the last scan, intraocular pressures were again measured, and the study was complete.

The twenty control subjects also followed the same protocol for the morning 1, afternoon, and night periods to compare circadian rhythm and plasma catecholamine analysis to the subjects who had undergone adrenalectomy. Control subjects did not undergo 24-hour urine collections or measurements with timolol.

Statistics

For comparisons within the adrenalectomy group or the control group, statistical significance was determined using the two-sided Student's *t*-test for paired samples. For comparisons between the adrenalectomy group and the control group, a two-sided *t*-test for unpaired samples was used. To determine the circadian rhythm of aqueous flow, the data for the two eyes of each subject were averaged, and the average was treated as a single datum for the morning 1, afternoon, and night periods. The rates of aqueous flow during the three periods were then compared. To determine the effect of timolol on aqueous flow, the mean flows for the drug-treated eye and the fellow placebo-treated eye during the morning 2 period were compared. In addition, the mean flows for the drug-treated eye and the same eye for the morning 1 period were compared. A P value of less than 0.05 was considered significant. The method of measuring flow as described in this paper has a coefficient of variation of approximately 16%. Our sample size of 20 has a 95% probability of detecting a 15% difference in flow.

RESULTS

The study time was divided into four time periods as follows: morning 1 (8 AM to noon), afternoon (noon to 4 PM), night (midnight to 6 AM), and morning 2 (8 AM to noon).

Aqueous flows for the 20 subjects who underwent adrenalectomy and for the 20 control subjects were observed to follow the normal circadian rhythm found by other investigators[1-4] (see Table 1). For the 20 adrenalectomy subjects, the daytime rates of aqueous flow were 3.17 ± 0.78 μl/min (mean $\pm$ SD) and 3.16 ± 0.67 μl/min for the morning 1 and afternoon periods, respectively. The rates for daytime periods were not significantly different from each other ($P = 0.699$). The rate of aqueous flow for the night period was 1.37 ± 0.37 μl/min, significantly slower than both the morn-

ing 1 ($P < 0.001$) and afternoon ($P < 0.001$) periods. These subjects demonstrated a 57% nighttime reduction in flow from the morning 1 period, which corresponds to the 59% reduction observed in our controls, the 57% reduction observed by Koskela in normals,[4] and the 48% reduction observed by Reiss in normals[3] (Table 1).

For the 20 control subjects, the mean flow was 3.33 ± 0.70 μl/min for the morning 1 period, 3.05 ± 0.61 μl/min for the afternoon period (an 8% reduction from the morning 1 period, $P < 0.001$), and 1.37 ± 0.30 μl/min for the night period (a 59% reduction from the morning 1 period, $P < 0.001$). Although a significant difference was found between morning and afternoon rates of aqueous flow for the control group but not the adrenalectomy group, the two groups were not significantly different at any of the first three time periods: morning 1, $P = 0.495$; afternoon, $P = 0.581$; and night, $P = 0.985$. In addition, the morning 1 to night difference between the two groups was not significant ($P = 0.487$).

Table 2 summarizes the results of the morning 2 timolol experiment. The rate of aqueous flow was 2.74 ± 0.54 μl/min for the placebo-treated eyes and 1.77 ± 0.38 μl/min for the timolol-treated eyes. The rate of aqueous flow was reduced 35% by this β-adrenergic antagonist when compared to the fellow placebo-treated eye for this period ($P < 0.001$). In addition, the rate of flow during the morning 2 period in the timolol-treated eye was reduced 46% from the same eye (untreated) during the morning 1 period ($P < 0.001$). The rate of aqueous flow during the morning 2 period in the placebo-treated eye was 11% slower than the same eye (untreated) during the morning 1 period ($P = 0.035$). The decreased flow observed in the placebo-treated eyes may have been a contralaterally mediated effect of timolol, or it may have been the result of an inadvertent transfer of a small quantity of timolol from one eye to the other if the subject blotted or rubbed an eye at any time during the morning 2 experiment. This small effect of timolol on the untreated eye has been observed by other investigators.[5,8]

The pretreatment intraocular pressures were not significantly different for the placebo-treated and timolol-treated eyes when measured at 4 PM in the afternoon before the drops were given ($P = 0.429$). However, a 26% reduction in intraocular pressure was observed in the timolol-treated eye compared to the fellow placebo-treated at noon the next day, 5 hours after the drops were given ($P < 0.001$) (see Table 3).

Table 4 summarizes the results of the plasma catecholamine analysis for the controls and subjects who underwent adrenalectomy. As expected, epinephrine secretion concentration for the adrenalectomy group was negligible at 1.3 ± 2.7 pg/ml. Plasma analysis showed that 16 of the subjects who underwent adrenalectomy had 0.0 pg/ml of circulating epinephrine, and in five there were trace amounts of <10 pg/ml after walking 10 minutes and having a standing blood draw taken. All control subjects showed plasma epinephrine levels greater than zero after the standing blood draw. Plasma epinephrine concentration for the control group was 24.1 ± 11.3 pg/ml, significantly greater than for the adrenalectomy group ($P < 0.001$). No statistically significant differences were found between the two groups for plasma concentration of norepinephrine ($P = 0.609$) or dopamine ($P = 0.169$). Analysis of the 24-hour urine samples showed that 18 of the subjects who underwent adrenalectomy had 0.0 μg/24 hours of epinephrine, and the remaining three reported trace amounts of <2.0 μg/24 hours. The normal 24-hour secretion is 10 μg (range, 0 to 20 μg).

DISCUSSION

The results of this study reconfirm previous studies 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 rhythm was observed in normal control subjects and in sub-

TABLE 1. Aqueous Flow: Diurnal Variation

	Aqueous Flow, μL/min (Mean ± SD)			
	Morning 1, 8 am–Noon	*Afternoon Noon–4 pm*	*Night, Midnight–6 am*	***Morning 1–Night Difference***
Adrenalectomy subjects ($n = 20$)	3.17 ± 0.78	3.16 ± 0.67 $P = 0.699$	1.37 ± 0.37 $P < 0.001$	↓57%
Control subjects ($n = 20$)	3.33 ± 0.70	3.05 ± 0.61 $P < 0.001$	1.37 ± 0.30 $P < 0.001$	↓59%
Data frm Koskela[4] ($n = 20$)	3.02 ± 0.69	2.67 ± 0.68 $P < 0.001$	1.31 ± 0.36 $P < 0.001$	↓57%
Data from Reiss[3] ($n = 19$)	3.10 ± 0.60	—	1.60 ± 0.50 $P < 0.001$	↓48%

Probability values are comparisons to Morning 1 (8 am–noon) flow rates.

TABLE 2. Aqueous Flow of 20 Adrenalectomy Subjects: Effects of β-Adrenergic Suppression in Timolol-Treated and Placebo-Treated Eyes

	Aqueous Flow, μL/min (Mean ± SD)				
	Untreated Eyes			*Treated Eyes*	
	Morning 1, 8 am–Noon	*Afternoon, Noon–4 pm*	*Night, Midnight–6 am*	*Morning 2 8 am–Noon*	*% Change From Morning 1*
Placebo eyes (n = 20)	3.07 ± 0.76	3.09 ± 0.66 P = 0.41	1.37 ± 0.44 P < 0.001	2.74 ± 0.54 P = 0.035	↓11%
Timolol eyes (n = 20)	3.27 ± 0.87	3.23 ± 0.73 P = 0.75	1.41 ± 0.40 P < 0.001	1.77 ± 0.38 P < 0.001	↓46%
Sig* (placebo versus timolol)	ns (P = 0.065)	ns (P = 0.10)	ns (P = 0.917)	P < 0.001	

* Sig = Statistically significant, $P < 0.05$.
Probability values in first two rows are comparisons to the same eye during the Morning 1 period.

jects after adrenalectomy. The amplitude of the rhythm in persons after adrenalectomy was indistinguishable from that of normals even though the persons who underwent adrenalectomy were shown to lack evidence of circulating epinephrine. Our results differed from Linner's.[18] In that study, subjects had a variety of conditions, the method was less specific for the measurement of flow, and the experiment lacked controls. Nevertheless, in view of the results of published studies cited in the introduction, our results were somewhat surprising.

Of a number of hormones that have been tested in humans for their ability to stimulate or suppress aqueous flow as evidenced by the rate of clearance of topically applied fluorescein, epinephrine stands out as the most active. The recent study by Kacere and coworkers[22] has confirmed that circulating epinephrine can measurably stimulate aqueous flow, an effect that is blocked by topical timolol.[36] Yet the current results show that the circadian rhythm and the daytime response to a β-adrenergic antagonist can occur in the absence of circulating epinephrine.

Epinephrine is not the only catecholamine secreted more rapidly during the day than during sleep. Åkerstedt and Levi[15] and Åkerstedt and Fröberg[16] have shown that norepinephrine is secreted at a much lower rate during inactivity at night. This reduction is not a circadian rhythm, as is the case with epinephrine; norepinephrine secretion continues at daytime rates in subjects who remain active at night.[16]

Reiss[3] has shown that the rhythm of aqueous flow persists in subjects who remain active during sleeping hours. The rate of aqueous flow during sleep deprivation was midway between the normal daytime rate and the normal rate during sleep. Under these conditions, epinephrine concentration in plasma should be low, but norepinephrine concentrations should be normal. The results of Reiss' experiment are consistent with the idea that both catecholamines can act on the ciliary processes.

Wax and Molinoff[37] have observed that the ability of norepinephrine to inhibit the binding of pindolol to membrane preparations of human iris–ciliary body is only three times weaker than that of epinephrine. Nathanson[38] has shown that norepinephrine is eight times weaker as an activator of adenylate cyclase prepared from human ciliary processes. These data, coupled with the fact that plasma norepinephrine in normal subjects is nearly 20 times greater than epinephrine concentration, suggest that norepinephrine might play a significant role in the rhythm of aqueous flow. It is also possible that the chronic absence of epinephrine in persons lacking adrenals may lead to increased sensitivity of the ciliary processes to norepinephrine.

This study does not resolve the question of epinephrine's role in the circadian rhythm of aqueous flow in normal persons. It does show, however, that the rate of aqueous flow can be normal during waking hours, and it can be normal during sleep in the absence of circulating epinephrine. It would now be in-

TABLE 3. Intraocular Pressure: Timolol-treated Versus Placebo-treated Eyes

	Intraocular Pressure, mm Hg (Mean ± SD)	
	Untreated Eyes Day 1, 4 pm	*Treated Eyes Morning 2, noon*
Placebo eyes (n = 19)	13.9 ± 1.3	13.9 ± 1.3
Timolol eyes (n = 19)	14.0 ± 1.2	10.3 ± 1.7
% Difference	0%	↓26%
Sig* (placebo versus timolol)	ns (P = 0.429)	P < 0.001

* Sig = Statistically significant, $P < 0.05$.

TABLE 4. Plasma Catecholamine Results: Adrenalectomy Subjects and Control Subjects

	Plasma Concentration pg/mL (Mean ± SD)		
	Epinephrine	*Norepinephrine*	*Dopamine*
Adrenalectomy subjects (n = 20)	1.3 ± 2.7	470.6 ± 188.2	8.5 ± 6.1
Control subjects (n = 20)	24.1 ± 11.3	437.5 ± 217.1	15.4 ± 20.5
Sig*	$P < 0.001$	$P = 0.609$	$P = 0.169$

* Statistical significance was determined using a two-sided t-test for unpaired samples between the adrenalectomy group and control group. $P < 0.05$ was considered significant.

teresting to determine if persons who suffer from dysautonomia and lack the postural stimulus to norepinephrine secretion or persons with norepinephrine secreting tumors such as pheochromocytoma have an abnormal rhythm of aqueous flow. It would also be interesting to compare the concentrations of epinephrine and norepinephrine and the rates of flow during sleep deprivation. Such studies would help clarify the relative roles of these two catecholamines in this system.

Key Words

adrenal gland, adrenalectomy, aqueous humor, human eye, circadian rhythm

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