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Aqueous Humor Flow in Sleeping Humans Is Unaffected by Norepinephrine Infusion

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PURPOSE. Intravenous administration of the catecholamine epinephrine is known to have a stimulatory effect on aqueous humor flow in sleeping human subjects, an effect that is augmented by plasma corticosteroids. This study was performed to determine whether the closely related catecholamine norepinephrine has a similar effect on aqueous humor flow.

METHODS. Twenty normal subjects were studied. Aqueous flow was measured by fluorophotometry. At night during sleep, norepinephrine or placebo was infused intravenously (IV) between midnight and 6 AM. The rate of aqueous flow during the norepinephrine infusion was compared with the rate of flow during placebo infusion, with each subject serving as his/her own control. The urinary excretions of epinephrine and norepinephrine were measured at the end of each infusion period.

RESULTS. The norepinephrine infusion caused an 8% increase in systolic blood pressure ($P < 0.001$), a 15% increase in diastolic blood pressure ($P < 0.001$), and a 9% decrease in heart rate ($P = 0.003$) compared with the placebo. The rate of aqueous humor flow during sleep from 12 AM to 6 AM was unchanged by norepinephrine. The rate was 1.27 ± 0.31 μl/min (mean ± SD) during IV infusion of placebo and 1.30 ± 0.27 μl/min during infusion of norepinephrine ($P = 0.63$).

CONCLUSIONS. An infusion of norepinephrine during sleep that causes measurable changes in cardiovascular parameters has no measurable effects on the rate of aqueous humor flow. The lack of a measurable effect of a norepinephrine infusion contrasts to the stimulatory effect of an epinephrine infusion. (*Invest Ophthalmol Vis Sci.* 1998; 39:1759-1762)

There are numerous reasons to hypothesize that circulating norepinephrine is a stimulator of aqueous humor formation. It has been shown that the closely related catecholamine epinephrine stimulates the flow of aqueous humor when received intravenously (IV) to sleeping human subjects.[1]

Norepinephrine has been tested in some studies. In one study, topical norepinephrine increased aqueous flow in humans, but this effect did not reach statistical significance.[2] Studies in monkey eyes in which norepinephrine was perfused through the anterior chamber showed a slightly higher rate of aqueous flow, but this effect did not reach statistical significance.[3] Studies of catecholamine concentrations in rabbit aqueous humor showed a much higher concentration of norepinephrine than epinephrine.[4]

If norepinephrine were an endogenous stimulator of aqueous humor formation, its action could account for the observations in several published studies. For example, patients without adrenal glands who lack plasma epinephrine maintain a rhythm of aqueous humor flow,[5] suggesting that some other cyclic hormone such as norepinephrine is active. As another example, during sleep deprivation at night when plasma epinephrine is extremely low, but when plasma norepinephrine is higher, aqueous humor flow is higher than it is during sleep when both catecholamines are low.[6] Thus, the flow of aqueous humor correlates better with the combined plasma concentrations of epinephrine and norepinephrine than with the concentration of either catecholamine alone.[6]

The concentration of norepinephrine in plasma is six times the concentration of epinephrine. Its binding affinity is 30% that of epinephrine in experiments with pindolol competition assays in membrane preparations from the human iris-ciliary body.[7] Norepinephrine has one eighth the potency of epinephrine as an activator of adenylate cyclase in human ciliary processes.[8] Thus, theoretically, there is a sufficient concentration of norepinephrine in the plasma during active hours to suggest that it might be able to stimulate receptors in the ciliary body.

The hypothesis that plasma norepinephrine is a stimulator of aqueous humor flow was tested in this study by comparing

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the effect of IV norepinephrine with placebo on the rate of aqueous flow in sleeping human subjects.

METHODS

Selection of Subjects

Twenty healthy human volunteers (10 men and 10 women) between the ages of 21 and 36 years were studied. An ophthalmic examination was carried out and included corrected visual acuity, slit lamp examination, undilated funduscopy, and tonometry. Exclusion criteria included any ocular disease, small palpebral fissures, photophobia, the use of contact lenses within 24 hours before the study, chronic systemic disease or any acute illness (namely, respiratory tract infection), drug use (except oral contraceptives), pregnancy, or participation in a trial of any investigational drug within 30 days.

The protocol was approved by the Institutional Review Board of the Mayo Clinic. Written informed consent according to US federal guidelines was obtained from all subjects. All subjects were treated in accordance with the tenets of the Declaration of Helsinki.

Kinetic studies of norepinephrine in reclining persons during the day have shown that the rate of release of norepinephrine into plasma is 1.87 ± 0.08 μg/min per m^2.[9] Upright posture increases this rate to 3.25 μg/min per m^2,[9] a difference of 1.38 μg/min per m^2. To mimic the norepinephrine release rate of active persons, norepinephrine must be infused at a rate of 1.38 μg/min per m^2 of body surface area. For a person of average build, an infusion rate of 0.03 μg/kg per minute would achieve this goal.

The plasma concentration of norepinephrine in resting supine subjects is 200 pg/ml to 400 pg/ml. If the plasma clearance of norepinephrine is 3 l/min, the additional infusion would be expected to raise the plasma concentration by 700 pg/ml, making a total of 900 pg/ml to 1100 pg/ml (endogenous plus exogenous norepinephrine). This concentration is similar to what might be observed in an active person during the day.

This infusion protocol was tested in a preliminary group of 20 healthy human volunteers from whom plasma samples were withdrawn during placebo and during norepinephrine infusion. The plasma concentration was 259 ± 75 pg/ml (mean ± SD) during placebo infusion at night during sleep and 1027 ± 247 pg/ml during infusion of norepinephrine at 0.03 μg/kg per minute. This preliminary experiment confirmed the theoretical calculations.

The solution for infusion was prepared from norepinephrine bitartrate at a concentration of 4 μg/ml in 5% dextrose. The placebo was 5% dextrose. The solutions were infused at a rate of 0.45 ml/h per kilogram body weight, which is equivalent to 0.03 μg/kg per minute. The norepinephrine and the placebo solutions were labeled by subject number and by the date to be infused. The solutions appeared identical, and their content remained masked until the acquisition of the data was complete. Each subject was studied on two separate nights, at least 7 days apart. Norepinephrine was infused from midnight until 6 AM on one occasion, and placebo was infused on the second occasion. The order of the two infusions was masked from the examiners and the subjects. Half of the subjects, chosen by random assignment, received the placebo on the first night, and the other half received the placebo on the second night.

Procedures

The subjects were admitted to the General Clinical Research Center in St. Mary's Hospital (Rochester, MN). An 8-hour urine collection was started for measurement of catecholamines at 8 AM. At 4 PM, the urine was collected and fluorescein eye drops were instilled for measuring aqueous flow. A nighttime 8-hour urine collection was started at 10 PM. Thereafter, subjects retired to bed. An IV catheter was placed in one arm for the infusion of norepinephrine or placebo. A wrist Actigraph (Ambulatory Monitoring, Ardsly, NY) was placed on one wrist to measure the efficiency of sleep. The sleep efficiency index was defined as the percentage of 1-minute intervals during which no motion of the wrist was measured by the accelerometers of the Actigraph.

Just before midnight the subjects awakened, walked approximately 20 m to a scanning fluorophotometer, and underwent a fluorophotometric scan of both eyes before returning to sleep 5 minutes later. At midnight, the infusion of norepinephrine or placebo commenced using the IV catheter. The infusion was controlled by a digitally programmable infusion pump set at 0.45 ml/h per kilogram body weight. The heart rate was continuously monitored telemetrically. Blood pressure was recorded by an automatic sphygmomanometer every 15 minutes. At 2 AM, 4 AM, and 6 AM the subject was awakened and fluorophotometric scans of both eyes were performed. At 6 AM the nighttime urine collection was terminated and subjects were dismissed. The residual of the IV solution was frozen and stored. The concentration of norepinephrine was measured in each of these containers to verify that the randomization code was correct and that degradation of norepinephrine had not occurred.

All data were collected and stored in a computer for statistical analysis. At this time, the code was broken. Urine samples were analyzed for catecholamines. The data were analyzed and compared statistically with a Student's *t*-test for paired samples. A sample size of 20 has the power to detect a 34% change in aqueous flow during sleep ($\alpha = 0.05$, $\beta = 0.95$).

RESULTS

The daytime rate of urinary excretion of epinephrine and norepinephrine, when the subjects were active and when no IV infusion was given, was not significantly different for the 20 subjects on the day before their placebo infusion compared with the day before their norepinephrine infusion (Table 1).

The nighttime rate of urinary excretion of epinephrine when placebo was infused was 1.27 ± 1.89 μl/8 h, and when norepinephrine was infused, it was 0.43 ± 0.24 μg/8 h ($P = 0.06$). The nighttime rate of urinary excretion of norepinephrine when placebo was infused was 8.3 ± 2.3 μg/8 h, and when norepinephrine was infused, it was 40.6 ± 11.0 μg/8 h ($P < 0.001$).

The rate of excretion of epinephrine was faster during each day than during the night (2.61 ± 1.20 μg/8 h placebo day versus 1.27 ± 1.89 μg/8 h placebo night, $P = 0.011$; 2.57 ± 1.20 μg/8 h norepinephrine day versus 0.43 ± 0.24 μg/8 h norepinephrine night, $P < 0.001$). The rate of excretion of norepinephrine was faster during the placebo day (15.5 ± 6.4 μg/8 h) compared with the placebo night (8.3 ± 2.3 μg/8 h); $P < 0.001$. However, norepinephrine was excreted much

TABLE 1. Monitored Variables

Conditions	Placebo Run	Norepinephrine Run	% Difference	*P* Value
Daytime, 8 AM to 4 PM				
Urine epinephrine (μg/8 h)	2.61 ± 1.20	2.57 ± 1.20	−1	0.94
Urine norepinephrine (μg/8 h)	15.5 ± 6.4	13.7 ± 4.1	−12	0.20
Nighttime, 10 PM to 6 AM				
Urine epinephrine (μg/8 h)	1.27 ± 1.89	0.43 ± 0.24	−65	0.06
Urine norepinephrine (μg/8 h)	8.3 ± 2.3	40.6 ± 11.0	388	<0.001
Nighttime, 12 AM to 6 AM				
Heart rate (bpm)	58 ± 10	53 ± 9	−9	0.003
Systolic blood pressure (mm Hg)	117 ± 9	126 ± 9	8	<0.001
Diastolic blood pressure (mm Hg)	62 ± 6	71 ± 6	15	<0.001
Sleep efficiency index (%)*	83 ± 4	82 ± 3	−1	0.57

bpm, beats per minute. Values are means ± SD; $n = 20$.

* Sleep efficiency index is the percentage of 1-minute intervals during which no motion was detected by the accelerometers of the wrist Actigraph from midnight to 6 AM.

faster during norepinephrine infusion at night (40.6 ± 11.0 μg/8 h) than during the day when no infusion was given (13.7 ± 4.1 μg/8 h); $P < 0.001$.

Compared with the placebo infusion, the norepinephrine infusion caused an 8% increase in systolic blood pressure ($P < 0.001$), a 15% increase in diastolic blood pressure ($P < 0.001$), and a 9% decrease in heart rate ($P = 0.003$) (Table 2). No difference was seen in the sleep efficiency index during norepinephrine infusion, 82% ± 3%, compared with placebo infusion, 83% ± 4% ($P = 0.57$).

Aqueous flow was measured during three 2-hour intervals during sleep: from midnight to 2 AM; from 2 AM to 4 AM; and from 4 AM to 6 AM (Table 2). On the night when placebo was infused, the rate was 1.12 ± 0.36 μl/min during the early period, 1.27 ± 0.30 during the middle period, and 1.42 ± 0.31 during the late period. The flow was 27% faster during the late period of sleep compared with the early period ($P < 0.001$). On the night when norepinephrine was infused, the same pattern was observed. The rate during the early period was 1.16 ± 0.33 μl/min, during the middle period it was 1.28 ± 0.28, and during the later period it was 1.45 ± 0.28. The rate during the late period was 25% greater than during the early period ($P < 0.001$).

When aqueous flows during the same periods of the placebo and norepinephrine infusion were compared statistically, no significant differences were observed and the actual measured differences were very small.

DISCUSSION

The rate of infusion of norepinephrine necessary to exceed daytime concentrations in resting, supine subjects (200 pg/ml to 400 pg/ml) was estimated on the basis of calculations from previous studies[9] and was confirmed in a preliminary group of 20 persons of similar age under identical conditions. The infusion raised plasma norepinephrine to 1027 ± 247 pg/ml, exactly the level that had been expected from pharmacokinetic theory. This infusion also produced changes in the cardiovascular parameters that were measured. The primary effect of the increased concentration of norepinephrine was to raise systolic blood pressure by 8% and diastolic blood pressure by 15%. A reflex effect was to lower the cardiac pulse rate by approximately 9%. These changes demonstrated that the elevated plasma norepinephrine was able to reach active receptors.

The experiment was carried out to determine whether norepinephrine also would have an effect on the rate of aqueous humor formation, as has been found for the catecholamine epinephrine. However, no such response was observed. If the norepinephrine infusion used in this experiment were equally as effective as the epinephrine infusion used in Kacere's experiment,[1] our sample size would have been of sufficient size to make it very likely that the effect would have been discovered.

The finding that urine epinephrine excretion was reduced during the norepinephrine infusion was unexpected. This effect could have partly offset the putative stimulatory effect of norepinephrine on aqueous flow. The likelihood that this sit-

TABLE 2. Aqueous Humor Flow

Time	Placebo	Norepinephrine	% Difference	*P* Value
12 AM to 2 AM	1.12 ± 0.36*	1.16 ± 0.33†	4	0.57
2 AM to 4 AM	1.27 ± 0.30	1.28 ± 0.28	1	0.84
4 AM to 6 AM	1.42 ± 0.31*	1.45 ± 0.28†	2	0.65
12 AM to 6 AM	1.27 ± 0.31	1.30 ± 0.27	2	0.63

Values are means ± SD, expressed in microliters per minute; $n = 20$.

* Percentage difference between earliest and latest flow measurements was 27%, $P < 0.001$.

† Percentage difference between earliest and latest flow measurements was 25%, $P < 0.001$.

uation could have masked a norepinephrine effect can be estimated from the data of Kacere et al.[1] They found a 27% increase in aqueous flow that was caused by the epinephrine infusion. The epinephrine infusion caused a 6000% increase in urinary epinephrine in their experiment. In our experiment, the norepinephrine infusion was accompanied by a 65% decrease in urinary epinephrine secretion, a decrease that was not quite statistically significant ($P = 0.06$). It seems unlikely that the lowering of epinephrine in association with norepinephrine infusion would have offset any putative clinically significant effect of norepinephrine. This point of view also is supported by the data in humans who have undergone adrenalectomy[5] whose epinephrine excretion was unmeasurable but whose average aqueous humor flow from midnight to 6 AM during sleep was 1.37 ± 0.44 μl/min, very close to the 1.27 ± 0.31 μl/min and 1.30 ± 27 μl/min rates observed during placebo and norepinephrine infusions of this experiment.

It is unclear why norepinephrine failed to stimulate aqueous humor flow as expected. One possibility, among many, is that plasma norepinephrine is cleared by uptake or degradation before it can reach relevant receptors such as those associated with autonomic nerve terminals in the ciliary body. Another possibility is that norepinephrine may interact primarily with α-adrenergic receptors that mediate lessor effects on aqueous humor formation than β-adrenergic receptors with which epinephrine can also interact.[10] A third possibility is that norepinephrine has an effect, but that it is too small to be detected reliably in a sample composed of 20 subjects. The experiment does not shed any light on why norepinephrine was not observed to have an effect.

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