Investigative Ophthalmology & Visual Science, Vol. 32, No. 9, August 1991

The Nocturnal Suppression of Aqueous Humor Flow in Humans is Not Blocked by Bright Light

Timo Koskela* and Richard F. Brubaker†

Normal human subjects were studied hourly by fluorophotometry to measure the normal circadian rhythm of aqueous humor flow. On a separate day, the subjects slept for approximately 1 hr after lunch; this short nap was not found to have any effect on aqueous flow. On a separate night, the subjects slept under a bright light of 2500 lux; the light was not found to have any effect on flow in the sleeping subjects. Because bright light blocks melatonin release in humans, nocturnal suppression of aqueous flow in humans may not be driven by systemic melatonin release. Invest Ophthalmol Vis Sci 32:2504–2506, 1991

It is well established that humans have a reduced rate of aqueous humor flow at night during sleep.[1,2] It is not known, however, what factors mediate this phenomenon. One hypothesis is that melatonin, released during sleep into the general circulation from the paired gland, could reach the eye and suppress aqueous formation.[3]

In animals, melatonin release can be suppressed easily by ambient light. In humans, suppression can also be accomplished, but only with bright light.[4] If melatonin is the major hormonal mediator of the nocturnal suppression of aqueous humor flow in humans, then bright light might suppress this phenomenon in sleeping subjects. We also would expect that a short period of sleep, even in the dark, during normal waking hours—too short to alter the circadian rhythm of melatonin release—would not produce the same suppression of flow observed during nocturnal sleep in the dark. We did an experiment to test the melatonin hypothesis by comparing aqueous flow during nocturnal sleep in the dark and during nocturnal sleep under bright lights. We also measured aqueous flow during a short period of sleep in a dark room during the day.

Methods

To measure the effect of sleep and light on aqueous humor flow, four different studies were done with 20 healthy subjects (12 men and 8 women; mean age, 28 ± 4 yr). Before the study, all subjects were examined for visual acuity and underwent slit-lamp examination, Goldmann applanation tonometry, and direct ophthalmoscopy. We excluded those whose intraocular pressures varied by more than 3 mm Hg between their eyes. The subjects were asked not to consume alcoholic beverages and to avoid prolonged exposure to sunlight and vigorous exercise during the study. A careful history was taken to exclude a sleep disorder.

In both eyes of each subject, the flow of aqueous humor was measured by observing the rate of disappearance of a fluorescent tracer (fluorescein) from the cornea and anterior chamber. The flow rates were calculated by observing the rate of clearance of fluorescein from the anterior segment.[5,6]

Fluorophotometry was done as follows, Each subject was asked to instill fluorescein 2% into each eye 6 hr before the time when the fluorophotometric examination was to begin. Each subject instilled the dye in each eye four times, with instillations spaced 5 min apart. In preparation for the daytime study, the subjects returned to sleep for the remainder of the night after the fluorescein instillation. The subjects reported to the test area at 8:00 AM. During sleep portions of the study, the length and quality of sleep was monitored with a wrist Actigraph (Ambulatory Monitoring Inc., Ardsley, NY) that registers sleeping time by monitoring the movement of the subject's arm.[7]

During the first daytime study, hourly fluorescence measures were made between 8:00 AM and 4:00 PM. Between measurements, the subjects were allowed to resume their normal daily activities.

During the second daytime study, a fluorophotometric measurement was made at 12:00 noon and then at 1:00 PM to get the value for aqueous flow between these times. After the 1:00 PM scan, the subjects went to sleep in a dark room for 60–90 min. A

From the *Department of Ophthalmology, University of Umeå, Umeå, Sweden, and the †Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota.

Supported in part by National Institutes of Health grant EY 02037, Research to Prevent Blindness, Inc., New York, New York, and the Mayo Foundation, Rochester, Minnesota.

Submitted for publication: January 16, 1991; accepted April 9, 1991.

Reprint requests: Richard F. Brubaker, MD, Department of Ophthalmology, Mayo Foundation, 200 First Street SW, Rochester, MN 55905.

measurement was made immediately after the subjects awakened. Only one of the subjects was unable to sleep, and the data of this subject were not used in the analysis.

During the first night, hourly flows were measured between 10:00 PM and 6:00 AM. The subjects slept in a dark quiet room, and they were encouraged to sleep as much as possible between the measurements, approximately 5 min/hr. During the second night, between 10:00 PM and 12:00 midnight, the subjects faced a bright fluorescent light (luminance, over 2500 lux at 3 feet distance; Sylvania F40D, 24429, GTE Products Division, Danvers, MA), and hourly fluorescein measurements were done. Exposure to bright light (2500 lux) for 2 hr suppresses nocturnal melatonin secretion in humans.[4,8] After the 12:00 midnight scan, the subjects slept under the bright light. Sleep was again monitored with the Actigraph. The subjects were undisturbed until 6:00 AM when a measurement of fluorescence was made. The last measurement was done at 7:00 AM. The subjects were awake during the last hour of this study.

Analysis of Data

Flows always were calculated as the average flow of the two eyes of each subject. Daytime flow was calculated as the average flow between 8:00 AM and 4:00 PM. Nighttime flows were calculated as the average flows between 12:00 midnight and 6:00 AM. Prenap flows were calculated between 12:00 noon and 1:00 PM. Nap flows were calculated between 1:00 PM and the time of awakening, usually 1 hr later.

Three subjects did not complete all aspects of the study satisfactorily. One of these had erratic measurements of corneal fluorescence on the first night and was observed to have heavy crusts of fluorescein-stained material on the lid margins and lashes that evening. A second subject was unable to sleep during the daytime sleep period. A third subject was not able to start the last nighttime study because of corneal irritation, presumably from repeated instillations of fluorescein. These three data points are missing from the analysis, and comparable paired data points were not considered.

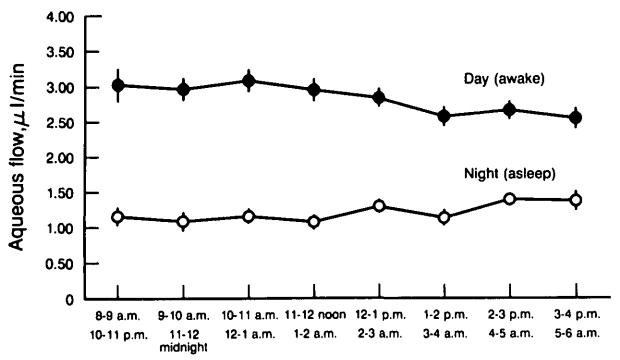

Fig. 1. Rate of aqueous flow measured hourly during the day and at night during sleep to show the normal circadian rhythm in humans.

We analyzed the data statistically with the student t-test for paired samples. The coefficient of variation of the flow measurement when one eye was compared with the same eye or the fellow eye was approximately 15%. We tested whether light inhibits the normal nocturnal suppression of aqueous flow or not. If a two-sided test of significance is used with a significance level of 0.05, a sample size of 20 subjects gives odds of 0.95 that we could detect inhibition of approximately one third of the normal nocturnal suppression.

Results

On the first day, when hourly determinations were made of aqueous flows, the highest rates were observed in the morning between 8:00 AM and 12 noon (3.02 ± 0.60 µl/min). In the afternoon between 12:00 noon and 4:00 PM, the rate slowed to 2.67 ± 0.53 µl/min ($P < 0.0001$), an 11% change (Fig. 1). Measurements were not made between 4:00 PM and 10:00 PM because fluorescein was reinstilled after the 4:00 PM measurement, and a 6-hr delay was necessary to allow fluorescein to become uniformly distributed in the cornea. The hourly flows during sleep from 10:00 PM until 6:00 AM were much lower than the daytime flows, averaging 1.31 ± 0.36 µl/min. Recordings from the unit Actigraph confirmed that all subjects slept during all these intervals, except when they were aroused for the measurements (approximately 5 min/hr). Flow was slowest during early sleep as can be seen in Figure 1. The flow between 10 PM and 2 AM was 1.13 ± 0.41, and between 2 AM and 6 AM, it was 1.31 ± 0.32 ($P = 0.003$).

During the second day, flow was measured from 12 noon to 1:00 PM. Then the subject was placed in a dark room and went to sleep for 60–90 min. The flow before sleep was 2.44 ± 0.88 µl/min. During the afternoon nap, it was 2.06 ± 0.87, a drop of 16% ($P < 0.0001$). The comparable figures on the day without a nap were 2.96 ± 0.68 from 12 noon to 1:00 PM and 2.64 ± 0.63, a drop of 11% ($P = 0.0005$). There was no statistically significant difference between the noon/afternoon fall in flow on the 2 days ($P = 0.70$). Thus, we were unable to detect any significant effect of a short period of sleep per se for 1 hr during the day.

On the second night, from 10 PM to 12:00 midnight while the subject was kept awake and faced a bright extended light source, the flow was 1.37 ± 0.52 µl/min, somewhat more than on the first night when the subject slept in a dark room 1, 1.12 ± 0.51 ($P = 0.03$). Between the hours of 12:00 midnight and 6:00 AM when the subject was allowed to sleep undisturbed (confirmed by Actigraph) under the bright

Table 1.

	Rate of flow, μl min⁻¹ (mean ± SD)
First day (awake)	
8:00 AM–12:00 noon	3.02 ± 0.69
12:00 noon–4:00 PM	2.67 ± 0.53
12:00 noon–1:00 PM	2.96 ± 0.68
1:00 PM–2:00 PM	2.64 ± 0.63
First night (asleep, awakened hourly, dark)	
10 PM–12:00 midnight	1.12 ± 0.51
10 PM–2:00 AM	1.13 ± 0.41
2:00 AM–6:00 AM	1.31 ± 0.32
12:00 midnight–6:00 AM	1.31 ± 0.36*
Second day	
12:00 noon–1:00 PM (awake)	2.44 ± 0.88
1:00 PM–2:30 (asleep)	2.06 ± 0.87
Second night (bright light)	
10:00 PM–12:00 midnight (awake)	1.37 ± 0.52
12:00 midnight–6:00 AM	1.00 ± 0.44*

* Calculation based on fluorescence measurement made only at midnight and 6:00 AM.

light, the flow was 1.00 ± 0.44, less than the rate on the initial night during the comparable time, 1.31 ± 0.36 ($P = 0.018$). The average rate of flow on the "dark night" and the "light night" during the subject's normal sleeping hours (10:00 PM to 6:00 AM) was almost exactly the same, 1.17 ± 0.43 versus 1.31 ± 0.47 ($P = 0.16$). These data are summarized in Table 1.

Discussion

The study confirms previous studies showing the circadian rhythm of aqueous flow in human subjects to be highest in the morning, somewhat lower in the afternoon, and lowest during sleep. A short period of sleep during daylight hours caused no statistically significant effect. An effect of sleep could have been missed either because the flow was decreasing anyway or because the period of measurement was too short to detect an effect. However, forcing subjects to stay awake for 2 hr during the time when they would normally sleep was still accompanied by a lower rate of flow, 1.37 ± 0.52 compared with 3.02 ± 0.69 in the morning ($P < 0.0001$) and 2.67 ± 0.53 in the afternoon ($P < 0.0001$). As previously shown by Reiss et al,[9] subjects deprived of sleep all night have a lower rate of flow than during daylight hours but higher than when actually asleep during night hours. As in this previous study, our subjects had a lower rate of flow at night during sleep (10 PM to midnight) than when they were awake at the same hours. Thus, it appears that both sleep and the time of day in the circadian cycle are factors that influence aqueous production.

Light per se seemed to have no effect on flow. If melatonin release were suppressed by the light, then melatonin seems unlikely to be the major hormone mediating the nocturnal suppression of aqueous humor flow. Although this view is supported by the recent study of Heinrich et al[10] (who showed that a large dose of melatonin given to human subjects during the day has no effect on the rate of aqueous humor flow), melatonin was not measured in this experiment, and any conclusion regarding its role can only be inferred.

It is appealing to hypothesize that the circadian rhythm of aqueous flow, which occurs both in rabbits[11] and in humans,[1] is driven by a single hormone. Like other studies, our experiment does not answer the questions: which hormone drives this rhythm and how does it reach and interact with ocular tissues?

Key words: aqueous flow, circadian rhythm, light, melatonin, human eye

References

1. Ericsson LA: Twenty-four hourly variations in the flow of aqueous humor. Acta Ophthalmol (Copenh) 36:381, 1958.
2. Reiss GR, Lee DA, Topper JE, and Brubaker RF: Aqueous humor flow during sleep. Invest Ophthalmol Vis Sci 25:776, 1984.
3. Samples JR, Krause G, and Lewy AJ: Melatonin lowers intraocular pressure. Curr Eye Res 7:649, 1988.
4. Lewy AJ, Wehr AT, and Goodwin K: Light suppresses melatonin secretion in humans. Science 210:1267, 1980.
5. Jones RF and Maurice DM: New methods of measuring the rate of aqueous flow in man with fluorescein. Exp Eye Res 5:208, 1966.
6. Brubaker RF: Clinical evaluation of the circulation of aqueous humor. *In* Clinical Ophthalmology, Vol 3, Duane TD and Jaeger EA, editors. Philadelphia, Harper & Row, 1986, pp. 1–11.
7. Mullaney DJ, Kripke DF, and Messin S: Wrist-actigraphic estimation of sleep time. Sleep 3:83, 1980.
8. Terman M: On the question of mechanism in phototherapy for seasonal affective disorder: Considerations of clinical efficacy and epidemiology. Journal Biological Rhythms 3:155, 1988.
9. Reiss FR, Lee DA, Topper JE, and Brubaker RF: Aqueous humor flow during sleep. Invest Ophthalmol Vis Sci 25:776, 1984.
10. Heinrich SR and Brubaker RF: The effect of melatonin on aqueous humor flow in humans during the day. ARVO Abstracts. Invest Ophthalmol Vis Sci 31(Suppl):234, 1990.
11. Smith SD and Gregory DS: A circadian rhythm of aqueous flow underlies the circadian rhythm of IOP in NZW rabbits. Invest Ophthalmol Vis Sci 30:775, 1989.