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Direct effect of light on 24-hour variation of aqueous humor protein concentration in Sprague-Dawley rats

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Abstract

Sprague-Dawley rats 10-12 weeks of age were entrained to a standard light-dark cycle with lights turned on at 6 am and off at 6 pm. Variations of 24-hour aqueous humor protein concentration were determined. Samples were taken every 4 hours ($N = 10-14$) under the standard light-dark condition at 8 pm, midnight, 4 am, 8 am, noon, and 4 pm. Under an acute constant dark condition, when lights were not turned on at 6 am, samples were collected at 8 am, noon, 4 pm, and 8 pm. Aqueous humor protein concentrations under the standard light-dark condition were found in the range of 0.305 ± 0.115 mg/ml (mean $\pm$ SD, $N = 10$) at midnight to 1.505 ± 0.342 mg/ml ($N = 14$) at noon. The 3 light-phase protein concentrations were each higher than the 3 dark-phase concentrations. Aqueous humor protein concentrations at 8 am, noon, and 4 pm under the acute constant dark condition were each higher than the concentrations at 8 pm (after both 2 hours and 26 hours in the dark), midnight, and 4 am, demonstrating an endogenously driven 24-hour pattern. At 8 am, noon, and 4 pm, protein concentrations were 56-147% higher when exposed to light. Intraocular pressure (IOP) was monitored using telemetry in separate groups of light-dark entrained rats under the standard light-dark condition and the acute constant dark condition. The 24-hour IOP pattern was inverse to the 24-hour pattern of aqueous humor protein concentration under the standard light-dark condition, and this IOP pattern was not altered by the acute constant dark condition. In conclusion, an endogenously driven 24-hour variation of aqueous humor protein concentration occurred in Sprague-Dawley rats with higher concentrations during the light-phase than the dark-phase. This endogenous pattern of protein concentration was accentuated by a direct effect of light, which was unrelated to the 24-hour pattern of IOP.

Keywords

aqueous humor; circadian rhythm; intraocular pressure; light; protein; rat

INTRODUCTION

There are considerable interests in the aqueous humor protein level associated with ocular diseases such as uveitis and glaucoma (Freddo, 1993; Ladas et al., 2005). Total protein level in aqueous humor is regulated by the permeability of blood-aqueous barrier and the movement of proteins in and out of adjacent tissues (Bill, 1986; Stastna et al., 2007). It is known that total

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aqueous humor protein concentration in normal physiological conditions can vary significantly through time. Circadian (24-hour) changes of aqueous humor protein concentration have been observed in humans (Anjou, 1961a; Oshika et al., 1988; McLaren et al., 1990), rabbits (Anjou, 1961c; McLaren et al., 1990; Takahashi et al., 1995; Liu, 2002), and mice (Zhou and Liu, 2006). Previous experiments showed that the circadian rhythms of aqueous humor protein concentration in rabbits and mice were driven endogenously and were not a direct response to light exposure since these rhythms persisted under an acute 24-hour constant dark condition (Anjou, 1961b; Liu et al., 1998; Liu, 2002; Zhou and Liu, 2006).

The circadian variation in aqueous humor protein concentration is believed to be partially due to the variation in aqueous humor formation, assuming newly formed aqueous humor contains little protein (Anjou, 1961a). The circadian rhythm of aqueous humor formation is also a major determinant for the circadian rhythm of intraocular pressure (IOP) in rabbits (Smith and Gregory, 1989; Liu et al., 1991). Thus, circadian rhythms of aqueous humor protein concentration and IOP in rabbits are off phase with each other by approximately 12 hours (Liu et al., 1996; Liu et al., 1998; Liu, 2002). A similar correlation in phase timings of these two circadian rhythms occurs in mice (Aihara et al., 2003; Zhou and Liu, 2006; Li and Liu, 2008). There was no report in other animal species on the correlation of circadian rhythms in aqueous humor protein and IOP.

The widely used Sprague-Dawley rat has a daytime aqueous humor protein concentration of approximately 1.00 mg/ml (Stjernschantz et al., 1973); higher than the 0.12 - 0.52 mg/ml aqueous humor protein concentrations in all other species studied including humans (Wurster et al., 1982; Tripathi et al., 1989), cattle (Wurster et al., 1982), horses (Wurster et al., 1982), sheep (Wurster et al., 1982), dogs (Wurster et al., 1982; Krohne et al., 1995), cats (Bucu et al., 1978; Wurster et al., 1982), rabbits (Wurster et al., 1982; Liu, 2002), and mice (Zhou and Liu, 2006). This high concentration in rats may reflect a direct effect of light exposure on aqueous humor protein level. In the present study, 24-hour variation of aqueous humor protein concentration in the Sprague-Dawley rat was determined and the effect of light exposure on the aqueous humor protein concentration during the accustomed light-phase was examined. Since the light effect on aqueous humor protein concentration may be due to an effect on aqueous humor formation, the 24-hour IOP pattern was examined in conscious and freely moving rats with and without light exposure during the accustomed light-phase.

MATERIALS AND METHODS

This study followed the guidelines of the Institute for Laboratory Animal Research and was approved by the Institutional Animal Care and Use Committee. Sprague-Dawley rats 10-12 weeks of age (200 - 300 g) were obtained from the Charles River Laboratories (Hollister, CA). Rats were entrained to a standard light-dark cycle with fluorescent lights (200-300 lux) turned on at 6 am and off at 6 pm for at least two weeks prior to the experiment. Food and water were freely available and the housing temperature was maintained at 22°C.

Samples of aqueous humor in light-dark entrained rats were collected under one of two conditions: the standard light-dark condition and the acute constant dark condition. Under the standard light-dark condition, aqueous humor samples were collected from 10-14 rats every four hours (8 pm, midnight, 4 am, 8 am, noon, and 4 pm). Under the acute constant dark condition (lights not turned on at 6 am the day of experiment), samples were collected at 8 am, noon, 4 pm, and 8 pm from 10 rats for each time point (after rats had 14-26 hours in constant dark). To collect the samples, rats were euthanized with CO₂. A dim photo-safe red light (<5 lux) was used to assist this procedure under a dark environment. Immediately following euthanasia, each rat eye was washed with 0.9% saline and dried using soft filter paper. The central cornea was punctured shallowly using a 30G needle, avoiding damage to the iris and

compromise of the blood-aqueous barrier. Aqueous humor exited passively and 15-20 μ l gathered on the corneal surface. Using a microtubing connected to a peristaltic pump (Zhou and Liu, 2006), this aqueous humor sample was carefully removed for protein assay.

Total protein concentration in the aqueous humor sample was immediately determined in a microplate (Bradford, 1976; Zhou and Liu, 2006). Samples of 4 μ l from each eye were assayed in duplicate. Dilution of aqueous humor sample by 2-4 folds was taken when needed to bring the protein concentration within the linear range of 0.8 mg/ml. The averages of protein concentrations in the right and left eyes were used for data analysis. Caution was taken to affirm the integrity of blood-aqueous barrier during sample collection. A previous study in Sprague-Dawley rats showed that the average protein concentration of rapidly formed aqueous humor after paracentesis (secondary aqueous humor) was 3.39 mg/ml; a 239% increase over the average protein concentration of primary aqueous humor sample (Stjernschantz et al., 1973). In the present study, any aqueous humor sample with a protein concentration higher than 1.00 mg/ml and a right-left difference of more than 100% was assumed to have a compromised blood-aqueous barrier during sample collection. Two pairs of right-left samples showing such questionable collections were not included in data analyses.

In a separate group of Sprague-Dawley rats, IOP was monitored using telemetry (Schnell et al., 1996; Li and Liu, 2008). A battery-powered pressure transmitter (Model PA-C20; Data Sciences International, St. Paul, MN) was implanted on the upper back under anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine). A midline incision was made to the dorsal neck and a subcutaneous pocket was created at the same height as the eyeball to house the transmitter. A pressure catheter was routed subcutaneously to the temporal eyelid and the catheter tip was inserted 4 mm into the vitreous chamber, passing the pupillary midpoint. The distal pressure catheter was stabilized on the parietal bone (Li and Liu, 2008). The postoperative rat was allowed to recover in an individual cage under the standard light-dark condition. Food and water sources were placed on the cage floor to minimize the pressure artifact in the recording system related to postural changes (Li and Liu, 2008). The overhead steel grid was removed from the cage to prevent pressure artifacts due to climbing.

Pressure data collection began 24-48 hours postoperatively in 11 rats under the standard light-dark condition and continued for 6-14 days until the pressure recording system failed (Li and Liu, 2008). Data was collected at 120 Hz for two minutes every five minutes and the average pressure for each hour was calculated. For 6 of these 11 postoperative rats, after a consistent 24-hour IOP pattern had been established, data was collected under an acute constant dark condition by replacing one 12-hour light-phase with darkness. Control experiments for monitoring pressure artifacts in the recording system under the standard light-dark condition were performed in 5 other rats for 4-10 days. These 5 rats underwent the same procedure of implanting a pressure transmitter and routing the pressure catheter. However, the pressure catheter tip was positioned outside the eyeball and not inserted into the vitreous chamber.

For statistical analyses of aqueous humor protein concentration, ANOVA and post-hoc Bonferroni test were used to detect any differences across the 6 time points within 24 hours under the standard light-dark condition. A similar statistical procedure was used to analyze data collected from the 7 time points when the rats were in the dark for 2, 6, 10, 14, 18, 22, and 26 hours. Student's t-test was used to compare the aqueous humor protein concentrations at 8 am, noon, and 4 pm with light exposure (under the standard light-dark condition) to the concentrations at the same clock time points under the acute constant dark condition. To determine the IOP change pattern under the standard light-dark condition, a paired t-test was used to compare the light-phase and the dark-phase IOP averages (Li and Liu, 2008). Under the 24-hour constant dark condition, the IOP average during the period of 6 am-6 pm (light-phase) was compared to the IOP average during the period of 6 pm-6 am (dark-phase). To

evaluate the direct light effect on IOP, a paired t-test was used to compare the IOP differences between the periods of 6 am-6 pm and 6 pm-6 am in the same group of 6 postoperative rats housed under the constant dark condition and under the standard light-dark condition. $P < 0.05$ was considered statistically significant.

RESULTS

Variation of 24-hour aqueous humor protein concentration appeared in Sprague-Dawley rats under the standard light-dark condition (Fig. 1). Aqueous humor protein concentrations ranged from a minimum of 0.305 ± 0.115 mg/ml (mean $\pm$ SD, $N = 10$) at midnight to a maximum of 1.505 ± 0.342 mg/ml ($N = 14$) at noon. One-way ANOVA and post-hoc Bonferroni test showed that the aqueous humor protein concentration at each light-phase time point (8 am, noon, or 4 pm) was statistically higher than ($P < 0.05$) the 3 dark-phase concentrations (8 pm, midnight, and 4 am). Protein concentration at noon was statistically higher than the concentration at 4 pm ($P < 0.05$). No significant differences were found between any two concentrations among the 3 dark-phase concentrations.

Under the constant dark condition, aqueous humor protein concentration remained relatively unchanged across the time points of 8 am, noon, and 4 pm (Fig. 1). For these 3 clock time points, aqueous humor protein concentration was significantly higher (56-147%) with light exposure (under the standard light-dark condition) than without light exposure (under the acute constant dark condition) ($P < 0.001$, Student's t-test). For the aqueous humor protein samples collected at the 7 time points under dark environment, protein concentrations at 8 am, noon, and 4 pm were each statistically higher than the concentrations at 8 pm (either having 2 hours or 26 hours in the dark), midnight, and 4 am ($P < 0.05$). There were no significant differences between any 2 concentrations among the time points of 8 am, noon, and 4 pm, nor were there differences among the concentrations at 8 pm, midnight, and 4 am. Aqueous humor protein concentrations in the rats that had remained in the dark for 2 hours and 26 hours, both sampled at 8 PM, showed no statistical difference.

Variation of 24-hour IOP appeared under the standard light-dark condition with the peak IOP in the early dark-phase (Fig. 2). The 24-hour IOP pattern was inverse to the 24-hour pattern of aqueous humor protein concentration. Average IOP was 23.5 ± 10.3 mm Hg during the dark-phase and 18.1 ± 9.2 mm Hg ($N = 11$) during the light-phase. The dark-light IOP difference of 5.3 ± 3.9 mm Hg was statistically significant ($P < 0.01$, paired t-test). For the 6 rats housed one day under an acute constant dark condition, the 24-hour IOP pattern was similar to the general 24-hour IOP pattern under the standard light-dark condition (Fig. 2). The IOP decrease of 6.3 ± 4.0 mm Hg from the 6 pm-6 am period (22.1 ± 7.7 mm Hg) to the 6 am-6 pm period (15.8 ± 8.7 mm Hg) was statistically significant ($P < 0.05$). This IOP decrease was not statistically different from the dark-light IOP decrease of 7.1 ± 4.4 mm Hg when the same 6 rats were housed under the standard light-dark condition ($P = 0.492$, paired t-test). Power calculations indicated that 6 rats provided a 0.82 power to detect a difference of 3.5 mm Hg IOP (half the magnitude of the 7.1 mm Hg dark-light IOP difference) when accepting the type I error of 0.05.

Comparing to the 24-hour IOP profile, the pressure variation in the control experiments was minimal (Fig. 2). The pressure difference of 0.5 ± 0.6 mm Hg between the dark-phase (0.3 ± 2.7 mm Hg) and the light-phase (-0.2 ± 3.2 mm Hg) was not significant ($P = 0.165$, $N = 5$), indicating that the overall pressure artifacts registered in the pressure transmitter were not significant.

DISCUSSION

Aqueous humor protein concentration varied around the clock in these albino Sprague-Dawley rats under the standard light-dark condition. A significant rise of protein concentration occurred during the transition from the dark-phase to the light-phase (between 4 am and 8 am). A significant fall of protein concentration occurred during the transition from the light-phase to the dark-phase (between 4 pm and 8 pm). Under a constant dark condition, the persistence, although diminished in magnitude, of the rise and fall pattern of aqueous humor protein concentration indicated that this rhythm had an endogenous oscillator. The presence of an endogenous oscillator for 24-hour pattern of aqueous humor protein concentration in rats agreed with previous observations in both pigmented and albino rabbits (Anjou, 1961b; Liu et al., 1998) and pigmented C57BL/6J mice (Zhou and Liu, 2006). Previous studies also showed that light exposure had no direct effect on the endogenously driven 24-hour aqueous humor protein concentration in rabbits and mice (Anjou, 1961b; Liu, 2002; Zhou and Liu, 2006). However, the endogenously driven aqueous humor protein concentration in Sprague-Dawley rats is superimposed by a direct effect of light on aqueous humor protein concentration. This light effect occurred during the accustomed light-phase, which was different from the photo-entrainment of circadian oscillator occurring during the accustomed dark-phase. Whether or not this observation of direct light effect is applicable to pigmented rat strains needs to be verified.

It has not been reported in the literature that regular light exposure can directly increase aqueous humor protein concentration. The elevated aqueous humor protein concentration found upon light exposure in the present study could be related to light responses in adjacent ocular tissues or associated cellular repair mechanisms. Among the four major groups of aqueous humor proteins classified by their functions, one protein group is related to antioxidant protection (Stastna et al., 2007). It is known that retinal susceptibility to light damage in albino rats is not constant throughout 24 hours (Duncan and O'Steen, 1985; White and Fisher, 1987) with susceptibility peaking in the dark period (Organisciak et al., 2000; Vaughan et al., 2002). Multiple proteins were shown to play a role in mitigating such light damage (LaVail et al., 1992). The present study suggests that the degree of susceptibility to light damage in tissues adjacent to the aqueous humor may also be contingent upon time-dependent and light-induced appearance of certain proteins in aqueous humor.

Telemetric 24-hour IOP pattern in conscious and freely moving Sprague-Dawley rats was comparable to 24-hour IOP patterns observed using periodic IOP measurements in female albino Lewis rats (Krishna et al., 1995) and male Brown Norway rats (Moore et al., 1996), demonstrating a higher IOP during the dark-phase than the light-phase. Results from the present study also showed a similar 24-hour IOP pattern under the acute constant dark condition as under the standard light-dark condition, indicating light exposure did not have a significant, direct effect on the endogenous 24-hour IOP pattern in rats. In rabbits and mice, change patterns of 24-hour IOP also remained the same under both the standard light-dark condition and the acute constant dark condition (Liu et al., 1996; Li and Liu, 2008).

The permeability of the blood-aqueous barrier to protein can remain unchanged over a 24-hour period (Oshika et al., 1993), and the aqueous humor protein concentration is related to a higher formation rate of protein-free aqueous humor (Anjou, 1961a). Since other parameters of aqueous humor dynamics including the outflow resistance do not constitute a barrier to protein movement, no change other than the parameter of aqueous humor formation can affect the protein concentration in aqueous humor. If the direct light effect on aqueous humor protein concentration (an elevation) was due to a slowdown of aqueous humor formation, IOP would probably be reduced as well in the present study. The average protein concentration during the light-phase (8 am, noon, and 4 pm) was 1.300 mg/ml with light exposure and 0.622 mg/ml

without light exposure. This doubling of protein concentration upon light exposure could occur if there were a 50% reduction in the aqueous humor formation rate. A 50% parallel decrease in IOP would be expected, assuming no other change in aqueous humor dynamics. The relatively small IOP difference during the period of 6 am to 6 pm with and without light exposure in the present study did not support a role of aqueous humor formation in the direct light effect on aqueous humor protein concentration.

One may propose that the elevated aqueous humor protein concentration caused a decrease of outflow facility and an increase of IOP (Sit et al., 1997) to counterbalance a direct light effect on IOP. This possibility was not supported by the fact that a lower IOP occurred at the time with a higher aqueous humor protein concentration in the rats housed under the constant dark condition. The present study supports the view that 24-hour variation of aqueous humor protein concentration can be modulated by the formation of aqueous humor as well as by a direct protein entry into aqueous humor (Murray and Bartels, 1993). When exposed to light, a direct protein entry into aqueous humor may occur in rats at the anterior part of the iris as in rabbits (Freddo et al., 1990) and monkeys (Barsotti et al., 1992).

In summary, the present study established the 24-hour pattern of aqueous humor protein concentration in Sprague-Dawley rats. The basic 24-hour change pattern of aqueous humor protein is driven endogenously. Light exposure during the accustomed light-phase causes additional increase in aqueous humor protein, but does not affect IOP. This direct light effect on the aqueous humor protein concentration has not been observed in other species. It may explain why daytime aqueous humor protein concentration in Sprague-Dawley rats is significantly higher than aqueous humor protein concentrations in all other species studied. Whether or not this unique light effect on aqueous humor protein level plays a role in ocular physiology or any pathological condition in Sprague-Dawley rats will be interesting to explore.

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Figure 1.

Time and light-dependent variations in 24-hour aqueous humor protein concentrations in Sprague-Dawley rats. Error bars represent standard deviations ($N = 10-14$). Rats were entrained under 12-hour light (6 am to 6 pm) and 12-hour dark cycle. Black boxes arrayed below the light-phase represent acute constant dark condition during the light-phase. * These 8 pm samples were collected after 26 hours in the dark.

Figure 2. Time-dependent variation in intraocular pressure (IOP) in conscious and freely moving Sprague-Dawley rats. Error bars represent standard error of the mean. Rats were entrained under 12-hour light (6 am to 6 pm) and 12-hour dark cycle. Control experiment monitored pressure artifacts in the telemetric recording system.