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Circadian variation of mouse aqueous humor protein

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Purpose: The first aim was to document the 24 h pattern of total protein concentration in aqueous humor of the C57BL/6J mouse strain under various environmental light-dark conditions. The second aim was to determine the diurnal/nocturnal change of aqueous humor protein concentration in the CBA/CaJ mouse strain reported to show a unique absence of diurnal/nocturnal elevation of intraocular pressure (IOP).

Methods: Mice of both strains were entrained to a daily 12 h diurnal/light (6 AM to 6 PM) and 12 h nocturnal/dark cycle. Total protein concentrations of aqueous humor were determined using specimens collected from C57BL/6J mice every 4 h under standard light-dark, acute constant dark, and acute constant light conditions. Aqueous humor protein concentrations in CBA/CaJ mice were determined at three diurnal times and three nocturnal times.

Results: The C57BL/6J mouse strain showed a higher aqueous humor protein concentration during the diurnal period than during the nocturnal period under standard light-dark conditions. This pattern persisted under acute constant dark and was modulated under acute constant light. The CBA/CaJ mouse strain showed a similar diurnal/nocturnal decrease of total aqueous humor protein concentration.

Conclusions: Endogenous circadian variation of aqueous humor protein concentration occurs in the C57BL/6J mouse strain. The nocturnal concentration is significantly lower than the diurnal concentration. A similar diurnal/nocturnal decrease of aqueous humor protein concentration occurs in the CBA/CaJ mouse strain, indicating that this change of aqueous humor protein is unrelated to the diurnal/nocturnal pattern of IOP.

Aqueous humor contains a small amount of protein with a concentration roughly about 1/500 of the plasma protein level [1]. The level of total aqueous humor protein represents the overall balance of circulating plasma proteins across the blood-aqueous barrier and protein metabolism in the adjacent anterior segment tissues of iris, ciliary processes, corneal endothelium, lens epithelium, and trabecular meshwork [1-3]. Previous studies have shown that total protein concentration in aqueous humor is not constant around the clock in rabbits and humans [4-11]. A diurnal/nocturnal decrease of total aqueous humor protein occurs in rabbits [4,6,8,10,11], and an opposite change pattern occurs in humans [5,7,9]. Plasma protein concentration remains constant around the clock in humans [9]. A time-dependent variation of protein concentration in aqueous humor may reflect parallel variations of physiological activities in anterior segment tissues. For example, there is a negative correlation between the 24 h variation of aqueous humor protein and the 24 h variation of aqueous humor flow [5,8,9], or intraocular pressure (IOP) [6,7,10].

Due to the increasing use of mouse models in studying molecular mechanisms of ocular physiology and pathology, around the clock changes of total protein concentration in the mouse aqueous humor can be informative. We are unaware of any report of daily variation of total aqueous humor protein concentration in mice. Therefore, we set out to document the 24 h pattern of aqueous humor protein concentration in the commonly used C57BL/6J mouse strain under various environmental light-dark conditions. We also examined aqueous humor protein concentrations in the CBA/CaJ mouse strain during the diurnal period and during the nocturnal period. Unlike the C57BL/6J and many other mouse strains, the CBA/CaJ mouse is the only pigmented strain that lacks a diurnal/nocturnal elevation of IOP due to a high IOP during the diurnal period [12,13].

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METHODS

All procedures in this study were approved by our Institutional Animal Care and Use Committee, and followed the guidelines of the Institute for Laboratory Animal Research. We obtained 170 C57BL/6J and 60 CBA/CaJ young adult mice (8-12 weeks of age, about 25 g) from the Jackson Laboratory (Bar Harbor, ME). All mice were entrained to a daily 12 h diurnal/light (6 AM to 6 PM) and 12 h nocturnal/dark cycle for at least two weeks before the experiments. Food and water were freely available and the housing air temperature was constant at 21 °C.

Sixty light-dark entrained C57BL/6J mice were used to establish the 24 h pattern of aqueous humor protein under standard light-dark conditions. At each of six times: 8 AM, noon, 4 PM, 8 PM, midnight, and 4 AM, 10 mice were sacrificed using CO_2 exposure. In the dark period, a dim photo-safe red light (<5 lux) was used to assist the procedure. A euthanized mouse was laid on its side with its right eye facing up. The ocular surface was thoroughly washed with sterile saline and tapped dry with filter paper. Under a dissecting microscope, the eye was proptosed and the central cornea was carefully punctured with a 30 gauge needle. Aqueous humor exited in-

stantly through the incision and 2-4 μl of aqueous humor gathered on the corneal surface. Using a peristaltic pump (Gilson minipuls 2 model; Middleton, WI) and clean PVC manifold microtubing, aqueous humor on the corneal surface was transferred to a clean culture dish. Aqueous humor specimens were collected in a similar manner from the left eye. Samples from both eyes were mixed and 4 μl mixture was pipetted into a 96 well microplate for the determination of protein concentration. Sample collecting from one mouse usually took a few minutes. In order to meet the time restriction, only two to three mice were processed within a window of 20 min around each clock time during one day of experiment.

Quantification of protein was performed immediately following sample collection to avoid sample freezing that may affect the accuracy of protein concentration. Protein concentrations were determined in microplates using the Bradford method [14]. Mean protein concentration at each clock time was calculated from samples of 10 mice. The variations in the protein concentrations among the six clock times were evaluated using one-way ANOVA and post-hoc Bonferroni test for all 15 possible comparisons. A $p<0.05$ was considered statistically significant.

Like many circadian rhythms of biological activities, 24 h change of aqueous humor protein can be driven endogenously. Alternatively, 24 h change of aqueous humor protein concentration may be a spontaneous response to environmental light. An endogenously driven biological rhythm should persist under a constant dark condition, also called a free run. We investigated whether or not the 24 h change pattern of aqueous humor protein in the C57BL/6J mouse persists in an acute constant dark condition. Thirty light-dark entrained mice were put under an acute constant dark condition on the day of experiment after disabling the 6 AM lights-on switch in the mouse housing. Aqueous humor specimens were collected from two to three mice at 8 AM, noon, and 4 PM from both eyes, and the protein concentrations determined immediately as described previously. For each clock time, 10 mice were used on different days of the experiment under acute constant dark. Mean aqueous humor protein concentrations under acute constant dark were statistically compared with the values obtained under the standard light-dark condition at each time of 8 AM, noon, and 4 PM using the Student's t-test.

It is known that, in the light-dark entrained mouse and other species, light exposure during the regular dark period can modulate the endogenous circadian rhythm [15]. Sixty light-dark entrained C57BL/6J mice were used to study the influence of acute constant light on the 24 h pattern of aqueous humor protein. On the days of experiment, lights in the mouse housing were not turned off at 6 PM, and mice were under acute constant light. Samples of aqueous humor were collected starting at 8 PM (2 h into the acute constant light condition) and at 4 h intervals of midnight, 4 AM, 8 AM, noon, and 4 PM. Aqueous humor protein concentrations were determined immediately. Ten mice were used for each clock time. The mean aqueous humor protein concentrations at the six clock times under the acute constant light condition were compared with the values obtained under the standard light-dark condition using the Student's t-test.

A major aqueous humor protein is albumin [1]. A previous study in rabbits showed that the 24 h change pattern of albumin in aqueous humor is similar to the 24 h change pattern of total protein concentration in aqueous humor [11]. In the present study, aqueous humor samples of light-dark entrained C57BL/6J mice were collected to determine the albumin concentration in the early light period (8 AM) and early dark period (8 PM). Samples were collected from 10 mice at each time and assayed immediately using an ELISA kit specifically designed for mouse serum albumin (Alpha Diagnostic International, San Antonio, TX). The mean albumin concentrations were compared between 8 AM and 8 PM using the Student's t-test.

Recent studies showed that IOP in the mouse is not constant around the clock [12,13,16]. IOP in the early nocturnal period (1 to 3 h after lights-off) was higher than IOP during the diurnal period (hours were not specified) in all pigmented mouse strains studied, except the CBA/CaJ strain [12,13]. In the present study, we compared total aqueous humor protein in the CBA/CaJ mouse strain at three nocturnal times (8 PM, midnight, and 4 AM) and at three diurnal times (8 AM, noon, and 4 PM). Sixty light-dark entrained CBA/CaJ mice were used; 10 mice at each time. Aqueous humor samples were collected, and the protein concentrations were determined as described previously. One-way ANOVA and post-hoc Bonferroni tests were used to compare aqueous humor protein concentrations in this mouse strain among the six clock times. In addition, aqueous humor protein concentrations were compared between the C57BL/6J and CBA/CaJ strains at each of the six clock times using the Student's t-test.

RESULTS

Figure 1 presents the 24 h change patterns of total aqueous humor protein concentration in the C57BL/6J mouse strain under various environmental light-dark conditions. Under the standard 12 h:12 h light-dark condition, total aqueous humor protein concentrations at 8 AM and 4 PM were statistically higher than the concentrations at 8 PM ($p<0.01$) and at the other two nocturnal times of midnight and 4 AM ($p<0.05$). Protein concentration at noon was significantly higher than the concentration at 8 PM ($p<0.05$). There was no difference in the aqueous humor protein concentrations among the three diurnal times, and there was no difference among the three nocturnal times. The average aqueous humor protein concentration at 8 PM was 41% lower than the concentration at 8 AM.

Under acute constant dark, the diurnal pattern of aqueous humor protein persisted as under standard light-dark. The total protein concentrations at 8 AM and 4 PM under acute constant dark showed no difference from those at the same times under regular light. Total protein concentration at noon under the acute dark condition was significantly higher than that under the regular light condition. Under 24 h constant dark, aqueous humor protein concentrations at 8 AM, noon, and 4

PM were all statistically higher than the concentrations at 8 PM, midnight, and 4 AM. There was no difference among the three nocturnal times or among the three diurnal times.

Under acute constant light, the 24 h variation of aqueous humor protein concentration in the C57BL/6J mouse strain was modulated. A significant attenuation of aqueous humor protein concentration first occurred at midnight, 6 h after the onset of acute constant light condition. The aqueous humor protein concentrations during the acute constant light condition afterward were consistently lower than the values under the standard light-dark condition. Although the nocturnal to diurnal increase of total aqueous humor protein concentration still occurred, the rate of change was slower (Figure 1). The average magnitude of increase between 4 AM and 8 AM was smaller under the acute constant light condition (0.048 mg/ml) compared to the standard light-dark condition (0.169 mg/ml).

Aqueous humor concentration of albumin in the C57BL/6J mouse strain at 8 PM, 0.013±0.003 mg/ml (mean±SD), was significantly lower (48%) than the concentration at 8 AM, 0.025±0.007 mg/ml ($p<0.05$). In the CBA/CaJ mouse strain, the aqueous humor protein concentrations at 8 PM, midnight, and 4 AM were significantly less ($p<0.01$) than the concentrations at 8 AM, noon, and 4 PM (Figure 2). There was no difference among the concentrations at the three diurnal times or among the three nocturnal times. The aqueous humor protein concentration in the CBA/CaJ mouse strain was not statistically different from the concentration in the C57BL/6J mouse strain at each time.

DISCUSSION

The present study showed for the first time a significant 24 h variation of total aqueous humor protein in mice. In the light-dark entrained C57BL/6J mouse strain, the total aqueous humor protein concentration was significantly higher during the diurnal/light period than during the nocturnal/dark period. The acute constant dark condition had little influence on the nocturnal:diurnal elevation of total protein concentration in aqueous humor. Since the 24 h variation of mouse aqueous humor protein concentration persisted in the constant dark condition, this change pattern was driven endogenously. The acute constant light environment modulated the 24 h variation of aqueous humor protein concentration observed under the standard light-dark condition. This is consistent with the notion that a new environmental light period affects an endogenous circadian rhythm.

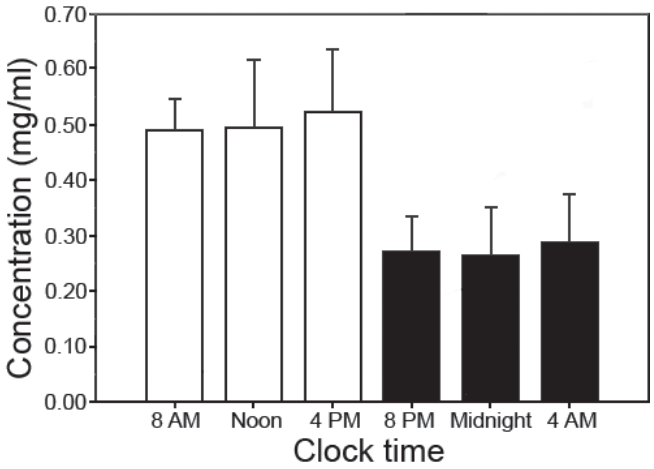

Figure 2. The 24 h pattern of aqueous humor protein concentration in the CBA/CaJ mice under 12 h diurnal/light (6 AM to 6 PM) and 12 h nocturnal/dark cycle. The error bars represent standard deviations (n=10).

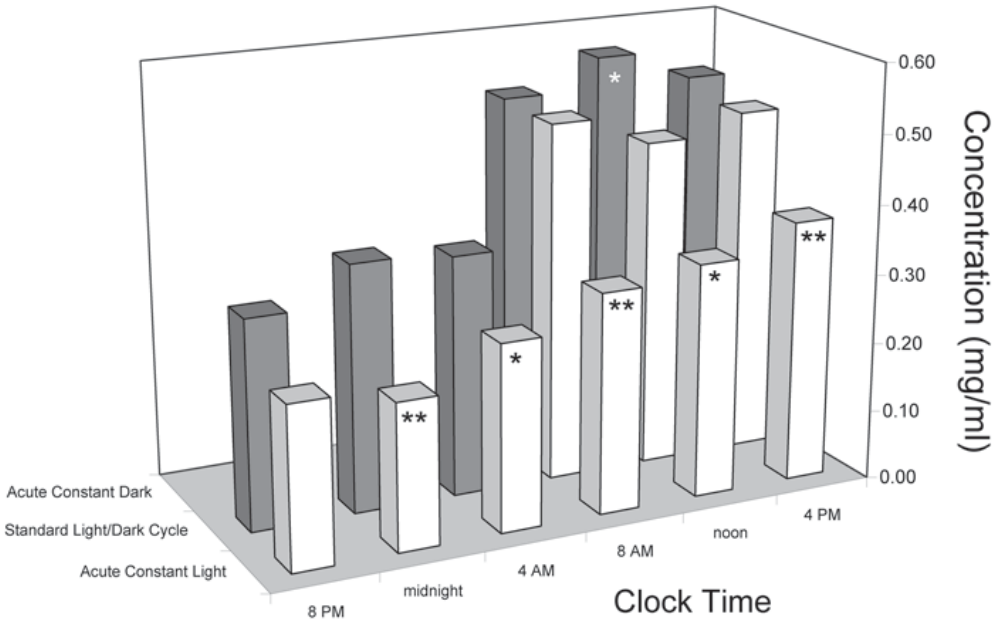

Figure 1. The 24 h patterns of aqueous humor protein concentration in C57BL/6J mice under acute constant light, standard light-dark, and acute constant dark conditions. Mice were entrained to daily 12 h diurnal/light (6 AM to 6 PM) and 12 h nocturnal/dark cycle prior to the experiments. Each column represents the mean of 10 mice. The single asterisk indicates a $p<0.05$ and the double asterisk indicates a $p<0.01$ for the comparison to the value at the same time under the standard light-dark condition (Student's t-test).

The 24 h change pattern of total aqueous humor protein in mice is similar to the pattern observed in rabbits [4,6,8,10,11] and opposite from that in humans [5,7,9]. It is interesting that, for all three species, the higher aqueous humor protein concentrations appear when biological activities are low and the eyes may be closed for sleep. Mice and laboratory rabbits are nocturnally active and humans are diurnally active. Previous studies in rabbits and humans suggested that a high aqueous humor protein concentration is related to a slow aqueous humor flow rate or low IOP [4-10].

It was reported that the inbred CBA/CaJ mouse strain has a unique absence of a diurnal/nocturnal IOP elevation because of a high IOP level during the diurnal period [12]. We assumed that a slightly different photoperiod used for housing mice in the present study (12 h:12 h light-dark cycle) from the previous study [12] (14 h light/10 h dark cycle) had no effect on the diurnal/nocturnal IOP pattern. In Swiss white mice, the diurnal/nocturnal IOP elevations under the two photoperiods were similar [12,16]. If the total protein concentration in aqueous humor were directly related to an IOP change, the diurnal/nocturnal change pattern of aqueous humor protein in the CBA/CaJ mouse strain would be different from the C57BL/6J mouse strain. Our data showed that the change pattern of aqueous humor protein concentration from any diurnal time (8 AM, noon, or 4 PM) to any nocturnal time (8 PM, midnight, or 4 AM) in the CBA/CaJ mouse was not different from the C57BL/6J mouse. There was also no difference of aqueous humor protein concentration at each time between the two mouse strains.

Mechanisms responsible for attenuating the diurnal/nocturnal elevation of IOP in the CBA/CaJ mouse strain played little role in the diurnal/nocturnal change pattern of aqueous humor protein concentration. This observation raises the possibility that the 24 h variation of aqueous humor protein concentration is unrelated to the 24 h variation of IOP or aqueous humor flow in other mouse strains. Mechanisms regulating the circadian variation of mouse aqueous humor protein may be different from the regulatory mechanisms in rabbits and humans [4-10]. In the latter two species, blood-aqueous barrier permeability to plasma protein was regarded to be fairly constant [5,8,9]. Thus, 24 h variation of aqueous humor protein concentration was assumed due to the variation of relatively protein-free aqueous humor flow.

The aqueous humor albumin concentration in the C57BL/6J mice showed a decrease from 8 AM to 8 PM, similar to the change pattern of aqueous humor protein concentration. This is consistent with the observation in light-dark entrained rabbits that 24 h aqueous humor albumin concentration followed the 24 h pattern of aqueous humor protein concentration [11]. Albumin serves as an important carrier for enzymes, fatty acids, and hormones in systemic and local circulations. Circulatory albumins use aqueous humor as the passage to reach nonvascular tissues in anterior segment tissues, such as the lens [17,18]. Since aqueous humor albumin concentration in mice is higher during the diurnal/light period than during the nocturnal/dark period, physiological activities related to albumin in the anterior segment tissues are probably high during the diurnal/light period. In light-dark entrained rabbits, there are also circadian rhythms of prostaglandins, transforming growth factor-β_2, and basic fibroblast growth factors in aqueous humor [19,20]. Circadian rhythms of these extracellular signaling molecules may also exist in the mouse aqueous humor. Examination of the 24 h variations of aqueous humor protein and its components in mouse models of anterior segment physiology and pathology may be useful for understanding the related molecular mechanisms.

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