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Nocturnal Light Exposure Alters Hepatic *Pai-1* Expression by Stimulating the Adrenal Pathway in C3H Mice

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Abstract: Recent studies have suggested the possibility that nocturnal light exposure affects many biological processes in rodents, especially the circadian rhythm, an endogenous oscillation of approximately 24 h. However, there is still insufficient information about the physiological effects of nocturnal light exposure. In this study, we examined the changes in gene expression and serum levels of plasminogen activator inhibitor-1 (PAI-1), a major component of the fibrinolytic system that shows typical circadian rhythmicity, in C3H/He mice. Zeitgeber time (ZT) was assessed with reference to the onset of light period (ZT0). Exposure to fluorescent light (70 lux) for 1 h in the dark period (ZT14) caused a significant increase in hepatic *Pai-1* gene expression at ZT16. Serum PAI-1 levels also tended to increase, albeit not significantly. Expression levels of the typical clock genes *Bmal1*, *Clock*, and *Per1* were significantly increased at ZT21, ZT16, and ZT18, respectively. Exposure to nocturnal light significantly increased plasma adrenalin levels. The effects of nocturnal light exposure on *Pai-1* expression disappeared in adrenalectomized mice, although the changes in clock genes were still apparent. In conclusion, our results suggest that nocturnal light exposure, even for 1 h, alters hepatic *Pai-1* gene expression by stimulating the adrenal pathway. Adrenalin secreted from the adrenal gland may be an important signaling mediator of the change in *Pai-1* expression in response to nocturnal light exposure.
Key words: adrenal pathway, light exposure, liver, mice, plasminogen activator inhibitor-1

Introduction

Recent studies have suggested that many biological processes in mammals, including the absorption and metabolism of ingested compounds, display an endogenous oscillation of approximately 24 h (circadian rhythm) [32]. This implies that the biological effects of the ingested compounds may vary according to the time of ingestion [2, 13, 15, 27, 28]. Therefore, it is important to determine when the target compounds should be administrated as well as their amounts. When conducting experiments using nocturnal rodents, researchers often need to perform some tasks, such as nutrient/drug administration, in a dark environment. However light is a dominant stimulus for entraining mammalian circadian rhythms [12, 30] and exposure to nocturnal light might

disturb many biological processes. Therefore, the fluorescent lights should not be turned on and the procedures are instead conducted under a dim red light [15, 19, 32] because rodents, including mice, are largely insensitive to red light (wavelength >650 nm) [7, 16]. Despite these practices, there is limited information about the effects of nocturnal light exposure on biological processes displaying a circadian rhythm.

The plasminogen activator (PA) system plays an important role in vascular homeostasis and constitutes a critical response mechanism to cardiovascular injury, such as myocardial infarction. Plasminogen activator inhibitor-1 (PAI-1) is one of the major components of the PA system [22]. Because the half-life of plasma PAI-1 is relatively short, circulating PAI-1 concentrations are regulated at the gene expression level [33], and the *Pai-1* gene is highly expressed in peripheral tissues, such as the liver, adipose tissue, heart, and kidney [20, 25, 26]. Blood PAI-1 levels show a typical circadian oscillation, reaching peak levels just before the active period in both humans and rodents [3, 23, 24]. Its gene expression profile also seems to display a typical circadian oscillation [25]. These findings led us to hypothesize that nocturnal light exposure might affect the gene expression and circulating levels of PAI-1, and consequently disturb the PA system.

In the present study, we examined the effects of nocturnal light exposure on hepatic *Pai-1* gene expression and serum PAI-1 levels in mice. We also analyzed the changes in expression levels of typical clock genes, such as circadian locomotor output cycles kaput (*Clock*) in the liver. The primary reason focusing on the liver is that this peripheral organ is the major site of PAI-1 synthesis in response to physiological signals, such as glucocorticoids and catecholamines [9]. We also measured serum glucocorticoid levels, the rodent analog of corticosterone, and plasma catecholamines (adrenalin and noradrenalin). Because glucocorticoids and catecholamines are secreted from the adrenal grand, we also examined the effects of adrenalectomy (ADX) on the changes in hepatic *Pai-1* gene expression in response to nocturnal light exposure. We used C3H/He mice in this study because this strain displays a high-amplitude melatonin rhythm that modifies circadian rhythms by altering the phase of the biological clock, whereas melatonin is undetectable in other commonly used mouse strains, such as BALB/c and DBA/2 mice [18, 21].

Materials and Methods

Animals

Male C3H/He mice (4 weeks old) were purchased from Japan SLC (Shizuoka, Japan), and five mice were housed per cage (338 × 225 × 140 mm). The mice had free access to a certified control diet (MF, Oriental Yeast Co., Tokyo, Japan) and tap water in the animal-care room that was controlled at $23 \pm 1°C$ and 60% humidity under a 12-h light/12-h dark cycle. High color rendering fluorescent lamps (20 W, FL20S·N-EDL, Toshiba Lighting & Technology Co., Kanagawa, Japan) were used as the light source for the light period and nocturnal light exposure. The lights were installed on the ceiling and had a luminance of 70 lux on the floor. To ensure all cages were exposed to a uniform light intensity, each cage was put in a foam plastic box and placed on the floor. In this study, a dim red lamp (1 lux) without spectral radiance of <650 nm was turned on all day, including the light period, to allow the researchers to work efficiently during the dark period without affecting the mice [7, 16]. Additional 4-week-old male C3H/He (Japan SLC) underwent ADX or sham surgery (sham). To prevent the expected loss of sodium, which occurs because of ADX, the ADX mice were given 0.9% sodium chloride in their drinking water throughout the experimental period. All experimental procedures were carried out in strict accordance with the recommendations of the American Association for Laboratory Animal Science. The protocol was approved by Animal Experiments Ethics Committee of the University of Shizuoka. In this study, ether was used as an anesthesia to minimize stress responses during handling and injections, because Croft *et al.* reported that the stress response was evident within 5 min after saline injections [8]. To protect against the effects of ether, the dissections were carried out in a dissecting room with sufficient ventilation. The dissectors used equipment, such as a mask, glasses, gloves, and dissecting robe, after adhering to practices that minimized risks associated with the explosiveness of ether. All efforts were made to minimize suffering.

Light exposure and sample collection

After 4 weeks of acclimatization, mice were exposed to light under two protocols, as follows.

Protocol-I. Sixty normal mice were randomly divided into two groups. One group was exposed to nocturnal light with a combination of 70 lux from a white fluores-

cent lamp for 1 h starting at Zeitgeber time (ZT) 14, where ZT0 represents the time when the light was turned on at the start of the light period. Therefore, the light period was from ZT0 to ZT12 and the dark period was from ZT12 to ZT24. The mice were decapitated after being anesthetized with ether. Trunk blood was collected into Capiject tubes (Terumo Medical Corporation, Somerset, NJ, USA) and EDTA-coated plasma tubes before light exposure at ZT14 and after light exposure at ZT15, 16, 18, 21, and 24 (n=5 at each time point). Serum and plasma fractions were obtained by centrifugation (3,500 × g, 90 s), and stored at −80°C until analysis. The liver was removed immediately after blood collection, cut into approximately 5-mm cubes, immersed in RNA*later*® (Ambion Inc., Austin, TX, USA) overnight at 4°C, and then stored at −20°C until RNA extraction.

Protocol-II. ADX and sham mice (n=10 each) were randomly divided into two groups. One group was exposed to light as in protocol -I for 1 h starting at ZT14, while the other group was not exposed to light. Serum fractions and liver were collected at ZT16 as described in protocol-I.

Determination of serum PAI-1, adrenalin, noradrenalin, and serum corticosterone levels

Total serum PAI-1 was measured in a multi-analyte profile using the Luminex-200 system with the Milliplex™ MAP kit (Mouse Adipokine Panel, Millipore Co., Billerica, MA, USA) according to the manufacturer's instructions. Plasma adrenalin and noradrenalin levels were analyzed by high performance liquid chromatography with a coulometric array system according to our previously described method [31]. Serum corticosterone was determined using an enzyme immunoassay kit (Enzo Biochem Inc., Farmingdale, NY, USA).

RNA extraction

Total RNA was extracted from each liver sample using the QuickGene RNA tissue kit S II (RT-S2) with Quick-Gene-Mini80 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturer's instructions. The ratio of the optical densities of the RNA samples measured at 260 and 280 nm was used to evaluate nucleic acid purity, and the total RNA concentrations were determined by measuring absorbance at 260 nm. The extracted RNA (400 ng) was reverse transcribed in a final volume of 10 μl using the PrimeScript® RT reagent kit (RR037A, Takara Bio Inc., Shiga, Japan)

according to the manufacturer's instructions.

Quantitative RT-PCR

cDNA solution (0.5 μl) was added to 19.5 μl of the PCR mixture containing TaqMan Gene Expression Master Mix (10 μl, Applied Biosystems, Foster City, CA, USA), DNase/RNase-free water (7.5 μl), house-keeping gene solution (glyceraldehyde-3-phosphate dehydrogenase; *Gapdh*, 1 μl), and individual target primer (1 μl). The following primers from Applied Biosystems, were used: brain and muscle aryl hydrocarbon receptor nuclear translocator-like (*Bmal1*), assay ID, Mm00500226_ m1; *Clock*, Mm_00455950_m1; period (*Per*) 1, Mm_00501813_m1; *Per2*, Mm_00478113_m1; cryptochrome (*Cry1*), Mm_00514392_m1; and *Pai-1*, Mm_00435860_m1. Quantitative RT-PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems).The relative expression level of the target gene was calculated using *Gapdh* as a calibrator.

Statistical analysis

Statistical analyses were performed using StatView software for Windows (Version 5.0, SAS Institute, Cary, NC, USA). Time-dependent data analysis were analyzed by repeated measures analysis of variance followed by Dunnett's test for between group comparisons. The control and light-exposed groups were compared using Student's t-test. The results were considered significant if the possibility of error was <5%.

Results

Effects of nocturnal light exposure in normal C3H/He mice

Before starting this study, we confirmed that the hepatic *Pai-1* gene displayed a circadian oscillation in C3H/He mice under a 12-h light/12-h dark cycle (Supplemental Fig. 1). *Pai-1* gene expression exhibited a typical diurnal rhythmicity, in which the peak expression preceded the middle of the light period and then started to decrease with patterns similar to those reported by other research groups [25]. We next evaluated the effects of nocturnal light exposure on hepatic *Pai-1* expression and serum PAI-1 levels. *Pai-1* gene expression did not change during the 1-h light exposure starting at ZT14 relative to the control group without nocturnal light exposure (Fig. 1A). After turning off the light at ZT15, *Pai-1* expression was significantly increased at ZT16 ($F_{1.5}$=4.470, P=0.038).
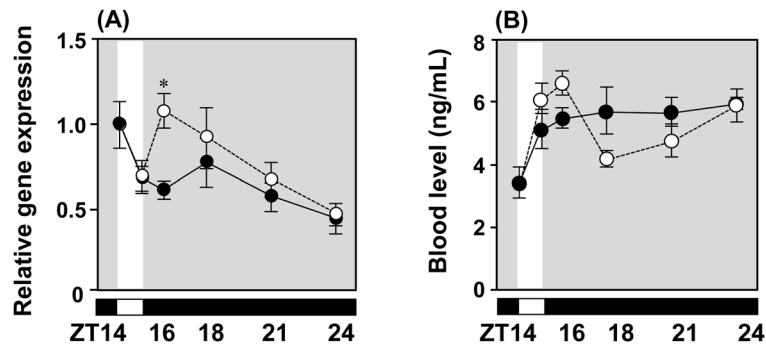
Fig. 1. Effects of nocturnal light exposure on hepatic *Pai-1* gene expression and serum PAI-1 levels. After acclimatization, C3H/He mice were exposed to light (70 lux) for 1 h starting at ZT14. Liver and serum samples were collected just before light exposure at ZT14, and just after light exposure at ZT15, 16, 18, 21, and 24 to determine hepatic *Pai-1* gene expression (A) and serum PAI-1 level (B). Open circles and filled circles represent the nocturnal light exposure group and the control group, respectively. Values are presented as the mean ± SE (n=5). *$P<0.05$ *vs* the control group at each time point (Dunnett's test).
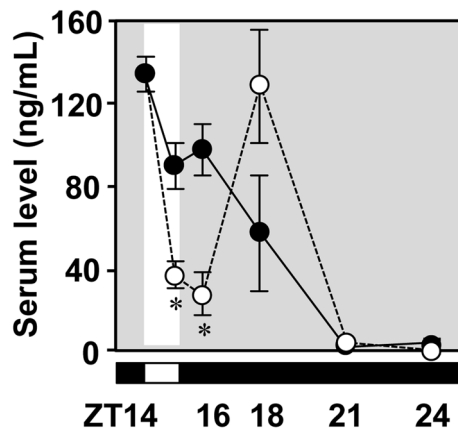

Fig. 2. Effects of nocturnal light exposure on serum corticosterone levels. After acclimatization, C3H/He mice were exposed to light (70 lux) for 1 h starting at ZT14. Serum samples were collected just before light exposure at ZT14 and just after light exposure at ZT15, 16, 18, 21 and 24 to measure corticosterone levels. Open circles and filled circles represent the nocturnal light exposure group and the control group, respectively. Values are presented as the mean ± SE (n=5). *$P<0.05$ *vs* the control group at each time point (Dunnett's test).

The total serum PAI-1 levels tended to increase until ZT16, although the change was not statistically significant (Fig. 1B). Serum corticosterone levels decreased significantly after the light exposure (ZT14), which continued until ZT16, but then dramatically increased (Fig. 2).

The representative clock genes analyzed in this study were unaffected during nocturnal light exposure for 1 h from ZT14, but the expression levels of *Bmal1*, *Clock*, and *Per1* were significantly increased at ZT21 ($F_{1.5}=19.869$, $P=0.013$), ZT16 ($F_{1.5}=8.490$, $P=0.011$), and ZT18 ($F_{1.5}=2.492$, $P=0.027$), respectively (Figs. 3A–C). Nocturnal light exposure did not affect *Per2* and *Cry1* mRNA levels (Figs. 3D–E).

Effects of nocturnal light exposure on plasma adrenalin and noradrenalin levels in normal mice

In normal mice, the plasma adrenalin levels were significantly increased at ZT16 after 1 h of light exposure starting at ZT14 (Fig. 4). Nocturnal light exposure also increased noradrenalin levels, although not significantly.

Effects of nocturnal light exposure in ADX mice

The serum corticosterone levels were below the detection limit in ADX mice (data not shown). ADX and sham mice were exposed to 1 h of light starting at ZT14. Nocturnal light exposure significantly increased the hepatic expression levels of *Clock* and *Pai-1* in sham mice compared with control mice at ZT16 ($P=0.007$ and $P=0.037$, respectively) (Fig. 5). However, the effects of nocturnal light exposure on *Pai-1* expression disappeared in ADX mice, although *Clock* expression remained significantly increased ($P=0.027$).
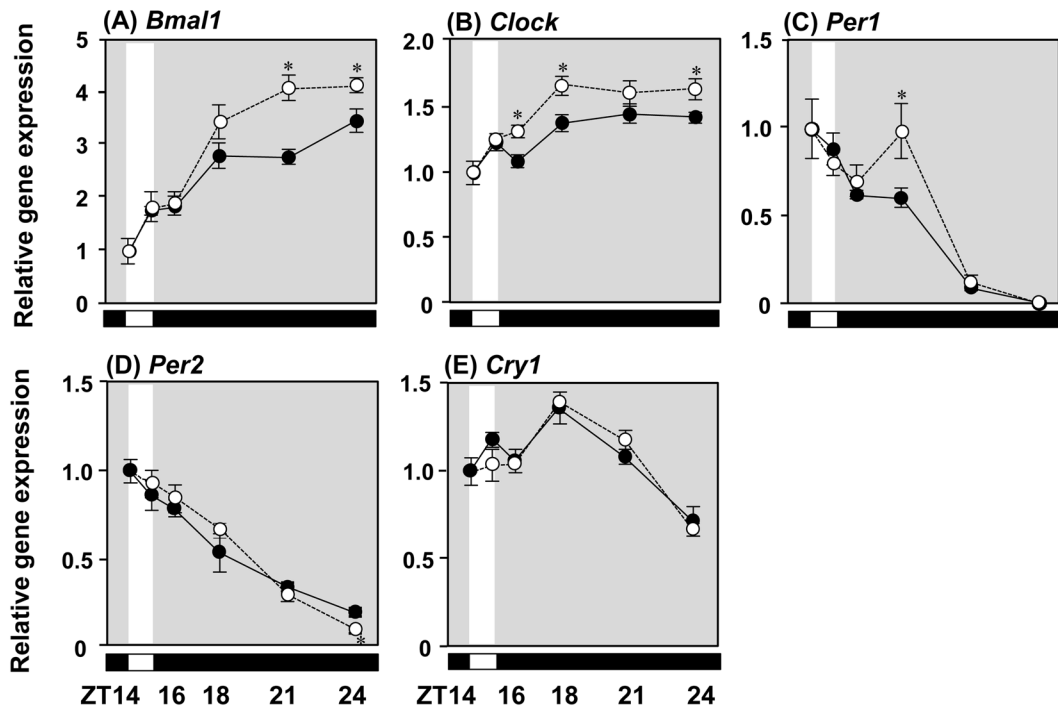
Fig. 3. Effects of nocturnal light exposure on hepatic expression of clock genes. After acclimatization, C3H/He mice were exposed to light (70 lux) for 1 h starting at ZT14. Liver samples were collected just before light exposure at ZT14 and just after light exposure at ZT15, 16, 18, 21, and 24 to determine the expression levels of the major clock genes *Bmal1* (A), *Clock* (B), *Per1* (C), *Per2* (D), and *Cry1* (E). Open circles and filled circles represent the nocturnal light exposure group and the control group, respectively. Values are presented as the mean ± SE (n=5). *P<0.05 *vs* the control group at each time point (Dunnett's test).
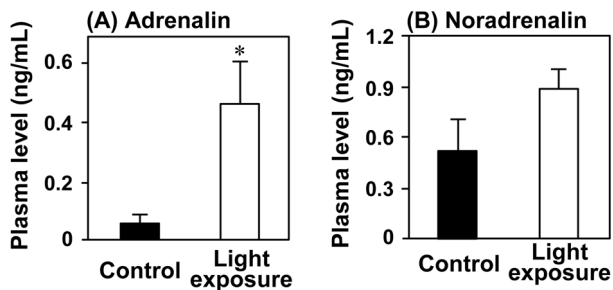

Fig. 4. Effects of nocturnal light exposure on plasma adrenalin and noradrenalin levels. After acclimatization, C3H/He mice were exposed to light (70 lux) for 1 h starting at ZT14. Plasma samples were collected 1 h after exposure (ZT16). Values are presented as the mean ± SE (n=5) in the nocturnal light exposure group relative to the control group. *P<0.05 *vs* the control group (Student's *t* -test).

Discussion

In this study, we demonstrated that the liver receives a signal related to nocturnal light exposure that induces hepatic *Pai-1* mRNA expression in C3H mice (Fig. 1A),

indicating that nocturnal light exposure, even if only for 1 h, may stimulate hepatic *Pai-1* expression in mice. Our findings also suggest that the serum PAI-1 levels were increased after nocturnal light exposure, although the increase was not significant (Fig. 1B). However, this change was considered not to be induced by the hepatic *Pai-1* expression, because increasing of serum PAI-1 levels was more rapidly appeared than that of hepatic *Pai-1* expression. Although further studies are needed, the other origins might be existed for increasing serum PAI-1 after light exposure, for example affecting of *Pai-1* gene expressions in another peripheral tissues such as adipose, and platelet activation [4, 10, 20, 25, 26]. The stimulation of serum PAI-1 was small after single exposure of nocturnal light, but there is remaining possibility to induce significantly changes when repeated exposure of light. Additionally, the effects of the lighting environment should be considered on the future study because the timing, illuminance level, and spectral distribution of the light were reported to affect the expression levels of some genes displaying circadian rhythms
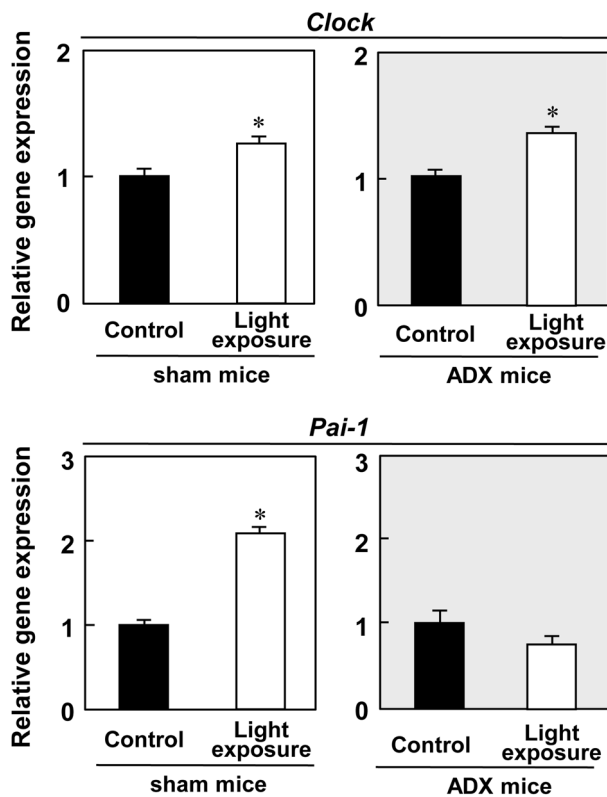
Fig. 5. Effects of adrenalectomy (ADX) on hepatic gene expression following nocturnal light exposure. After acclimatization, ADX or sham C3H/He mice were exposed to light (70 lux) for 1 h starting at ZT14. Liver samples were collected at ZT16 to determine the gene expression levels of *Clock* and *Pai-1* by quantitative RT-PCR. Values are presented as the mean ± SE (n=5) in the nocturnal light exposure group relative to the control group. *$P<0.05$ *vs* the control group (Student's *t*-test).

[1].

The circadian rhythmic oscillation of the *Pai-1* gene in peripheral organs is probably regulated by the molecular circadian clockwork [23]. The hepatic expression levels of the clock genes *Bmal1*, *Clock,* and *Per1* were increased after nocturnal light exposure (Fig. 3). However, the increase in *Pai-1* expression in the liver occurred at the same time as that of *Clock* but earlier than that of *Bmal1*. These findings indicate that light regulates hepatic *Pai-1* gene expression through a pathway that is independent of the clock genes.

Corticosterone, which is secreted from the adrenal gland, was candidate stimulus to increase *Pai-1* gene expression [9]. Some research groups reported that light exposure increases blood levels of corticosterone in rodents [14, 29]. Interestingly, we found that nocturnal light exposure at ZT14 for 1 h significantly decreased the serum corticosterone level at ZT16 (Fig. 2). We also found that this decrement appeared within 5 min after the light exposure (data not shown). Buijs *et al.* reported that the immediate decrease in plasma corticosterone by the light exposure was only observed at the beginning of the dark period (ZT14), but not at other time points (ZT2 and ZT20) [6]. Light did not inhibit corticosterone secretion from the adrenal cortex at ZT20, which indicates that light plays an inhibitory role on the adrenal cortex, but only at the start of the dark phase [6]. Our observations obtained in this study strongly agree with the results of the study by Buijs *et al.*, and suggest that corticosterone did not mediate the increase in *Pai-1* gene expression after nocturnal light exposure.

Jiang *et al.* investigated the effects of restraint stress on plasma PAI-1 levels using C57BL/6 mice [17]. They found that 30 min of stress exposure dramatically increased plasma PAI-1 levels, and concomitantly elevated catecholamine levels (adrenalin plus noradrenalin). Cell culture and animal experiments revealed that adrenalin and noradrenalin induce *Pai-1* mRNA levels in cardiac blood vessel cells and cardiomyocytes, as well as in the brain and heart [5, 34]. The physiological responses to stress are initiated by activation of the sympatho-adrenomedullary system, resulting in the release of catecholamines. In this study, we found that plasma adrenalin levels were significantly increased at ZT16 in mice exposed to light (Fig. 4). We also evaluated the effects of ADX on hepatic *Pai-1* expression induced by nocturnal light exposure for 1 h from ZT14. The hepatic gene expression levels of *Clock* and *Pai-1* were significantly greater at ZT16 (1 h of nocturnal light) than at ZT14 in sham mice (Fig. 5). This increase in *Pai-1* expression disappeared in ADX mice, although the expression of *Clock* was still affected by nocturnal light exposure. These data suggest that nocturnal light exposure alters hepatic *Pai-1* expression by stimulating the sympatho-adrenomedullary pathway. Interestingly, biological responses to exogenous stimulus have been reported to be different among the mouse strains [11]. Hence, the experiment using the other strains than C3H should be conducted in the future study.

In conclusion, our study has shown that nocturnal light exposure for 1 h starting at ZT14 alters hepatic gene expression of *Pai-1*, as well as the clock genes *Bmal1*, *Clock*, and *Per1* in C3H/He mice. Light exposure also significantly increased the plasma adrenalin levels. The

effects of light exposure on *Pai-1* expression had disappeared in ADX mice, although the clock genes were still modified. These observations suggest that nocturnal light affects hepatic *Pai-1* gene expression by stimulating the adrenal pathway, and adrenalin secreted from the adrenal gland might be a signaling mediator for these effects of nocturnal light. Our results also imply that the lighting environment should be considered when conducting animal experiments to evaluate the biological effects, at least in the fibrinolytic system, of target compounds in mice.

References

1. Abe, H., Honma, S., Namihira, M., Tanahashi, Y., Ikeda, M., and Honma, K. 1998. Circadian rhythm and light responsiveness of BMAL1 expression, a partner of mammalian clock gene Clock, in the suprachiasmatic nucleus of rats. *Neurosci. Lett.* 258: 93–96. [Medline] [CrossRef]

2. Boughattas, N.A., Lévi, F., Fournier, C., Lemaigre, G., Roulon, A., Hecquet, B., Mathé, G., and Reinberg, A. 1989. Circadian rhythm in toxicities and tissue uptake of 1,2-diamminocyclohexane(trans-1)oxalatoplatinum(II) in mice. *Cancer Res.* 49: 3362–3368. [Medline]

3. Bridges, A.B., McLaren, M., Scott, N.A., Pringle, T.H., McNeill, G.P., and Belch, J.J. 1993. Circadian variation of tissue plasminogen activator and its inhibitor, von Willebrand factor antigen, and prostacyclin stimulating factor in men with ischaemic heart disease. *Br. Heart J.* 69: 121–124. [Medline] [CrossRef]

4. Brogren, H., Wallmark, K., Deinum, J., Karlsson, L., and Jern, S. 2011. Platelets retain high levels of active plasminogen activator inhibitor 1. *PLoS ONE* 6: e26762. [Medline] [CrossRef]

5. Brown, N.J., Bradford, J., Wang, Z., Lea, W., Ma, L., Ma, J., Vaughan, D.E., and Fogo, A.B. 2007. Modulation of angiotensin II and norepinephrine-induced plasminogen activator inhibitor-1 expression by AT1a receptor deficiency. *Kidney Int.* 72: 72–81. [Medline] [CrossRef]

6. Buijs, R.M., Wortel, J., Van Heerikhuize, J.J., Feenstra, M.G., Ter Horst, G.J., Romijn, H.J., and Kalsbeek, A. 1999. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur. J. Neurosci.* 11: 1535–1544. [Medline] [CrossRef]

7. Conway, B.R. 2007. Color vision: mice see hue too. *Curr. Biol.* 17: R457–R460. [Medline] [CrossRef]

8. Croft, A.P., O'Callaghan, M.J., Shaw, S.G., Connolly, G., Jacquot, C., and Little, H.J. 2008. Effects of minor laboratory procedures, adrenalectomy, social defeat or acute alcohol on regional brain concentrations of corticosterone. *Brain Res.* 1238: 12–22. [Medline] [CrossRef]

9. Dimova, E.Y. and Kietzmann, T. 2008. Metabolic, hormonal and environmental regulation of plasminogen activator inhibitor-1 (PAI-1) expression: lessons from the liver. *Thromb. Haemost.* 100: 992–1006. [Medline]

10. Erickson, L.A., Ginsberg, M.H., and Loskutoff, D.J. 1984. Detection and partial characterization of an inhibitor of plasminogen activator in human platelets. *J. Clin. Invest.* 74: 1465–1472. [Medline] [CrossRef]

11. Gatien, M.L., Hotchkiss, A.K., Neigh, G.N., Dhabhar, F.S., and Nelson, R.J. 2004. Immune and stress responses in C57BL/6 and C3H/HeN mouse strains following photoperiod manipulation. *Neuroendocrinol. Lett.* 25: 267–272. [Medline]

12. Hirota, T. and Fukada, Y. 2004. Resetting mechanism of central and peripheral circadian clocks in mammals. *Zoolog. Sci.* 21: 359–368. [Medline] [CrossRef]

13. Houghton, S.G., Iqbal, C.W., Duenes, J.A., Fatima, J., Kasparek, M.S., and Sarr, M.G. 2008. Coordinated, diurnal hexose transporter expression in rat small bowel: implications for small bowel resection. *Surgery* 143: 79–93. [Medline] [CrossRef]

14. Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., Tsujimoto, G., and Okamura, H. 2005. Light activates the adrenal gland: timing of gene expression and glucocorticoid release. *Cell Metab.* 2: 297–307. [Medline] [CrossRef]

15. Itoh, K., Masumori, S., Nakajima, M., Hayashi, M., Sakakibara, H., and Shimoi, K. 2012. Differences in micronucleus induction in peripheral blood reticulocytes of mice exposed to N-ethyl-N-nitrosourea at light and dark dosing times. *J. Toxicol. Sci.* 37: 427–430. [Medline] [CrossRef]

16. Jacobs, G.H., Williams, G.A., Cahill, H., and Nathans, J. 2007. Emergence of novel color vision in mice engineered to express a human cone photopigment. *Science* 315: 1723–1725. [Medline] [CrossRef]

17. Jiang, Q., Gingles, N.A., Olivier, M.A., Miles, L.A., and Parmer, R.J. 2011. The anti-fibrinolytic SERPIN, plasminogen activator inhibitor 1 (PAI-1), is targeted to and released from catecholamine storage vesicles. *Blood* 117: 7155–7163. [Medline] [CrossRef]

18. Kennaway, D.J., Voultsios, A., Varcoe, T.J., and Moyer, R.W.

2002. Melatonin in mice: rhythms, response to light, adrenergic stimulation, and metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282: R358–R365. [Medline]

19. Kumazaki, M., Ando, H., Ushijima, K., Maekawa, T., Motosugi, Y., Takada, M., Tateishi, M., and Fujimura, A. 2011. Influence of dosing time on the efficacy and safety of finasteride in rats. *J. Pharmacol. Exp. Ther.* 338: 718–723. [Medline] [CrossRef]

20. Maemura, K., de la Monte, S.M., Chin, M.T., Layne, M.D., Hsieh, C.M., Yet, S.F., Perrella, M.A., and Lee, M.E. 2000. CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. *J. Biol. Chem.* 275: 36847–36851. [Medline] [CrossRef]

21. Masana, M.I., Benloucif, S., and Dubocovich, M.L. 2000. Circadian rhythm of mt1 melatonin receptor expression in the suprachiasmatic nucleus of the C3H/HeN mouse. *J. Pineal Res.* 28: 185–192. [Medline] [CrossRef]

22. Nicholl, S.M., Roztocil, E., and Davies, M.G. 2006. Plasminogen activator system and vascular disease. *Curr. Vasc. Pharmacol.* 4: 101–116. [Medline] [CrossRef]

23. Oishi, K. 2009. Plasminogen activator inhibitor-1 and the circadian clock in metabolic disorders. *Clin. Exp. Hypertens.* 31: 208–219. [Medline] [CrossRef]

24. Oishi, K., Ohkura, N., Amagai, N., and Ishida, N. 2005. Involvement of circadian clock gene Clock in diabetes-induced circadian augmentation of plasminogen activator inhibitor-1 (PAI-1) expression in the mouse heart. *FEBS Lett.* 579: 3555–3559. [Medline] [CrossRef]

25. Oishi, K., Ohkura, N., Wakabayashi, M., Shirai, H., Sato, K., Matsuda, J., Atsumi, G., and Ishida, N. 2006. CLOCK is involved in obesity-induced disordered fibrinolysis in ob/ob mice by regulating PAI-1 gene expression. *J. Thromb. Haemost.* 4: 1774–1780. [Medline] [CrossRef]

26. Oishi, K., Uchida, D., Ohkura, N., and Horie, S. 2010. PPARα deficiency augments a ketogenic diet-induced circadian PAI-1 expression possibly through PPARγ activation in the liver. *Biochem. Biophys. Res. Commun.* 401: 313–318. [Medline] [CrossRef]

27. Pan, X., Terada, T., Okuda, M., and Inui, K. 2003. Altered diurnal rhythm of intestinal peptide transporter by fasting and its effects on the pharmacokinetics of ceftibuten. *J. Pharmacol. Exp. Ther.* 307: 626–632. [Medline] [CrossRef]

28. Pleschka, K., Heinrich, A., Witte, K., and Lemmer, B. 1996. Diurnal and seasonal changes in sympathetic signal transduction in cardiac ventricles of European hamsters. *Am. J. Physiol.* 270: R304–R309. [Medline]

29. Rahman, S.A., Kollara, A., Brown, T.J., and Casper, R.F. 2008. Selectively filtering short wavelengths attenuates the disruptive effects of nocturnal light on endocrine and molecular circadian phase markers in rats. *Endocrinology* 149: 6125–6135. [Medline] [CrossRef]

30. Reppert, S.M. and Weaver, D.R. 2002. Coordination of circadian timing in mammals. *Nature* 418: 935–941. [Medline] [CrossRef]

31. Sakakibara, H., Izawa, Y., Nakajima, J., Seo, S., Tamaki, T., Kawai, Y., and Terao, J. 2008. Effects of polyphenol rich herbal medicine, *Ginkgo biloba* extracts on neurotransmitter levels in rat brain. pp. 429–434. *In*: *Functional Food and Health* (Shibamoto T, Kanazawa K, Shahidi F, and Ho CT eds.), Oxford University Press, Oxford.

32. Tsurusaki, T., Sakakibara, H., Aoshima, Y., Yamazaki, S., Sakono, M., and Shimoi, K. 2013. Diurnal rhythmicity in biological processes involved in bioavailability of functional food factors. *J. Clin. Biochem. Nutr.* 52: 208–214. [Medline] [CrossRef]

33. Vaughan, D.E., Declerck, P.J., Van Houtte, E., De Mol, M., and Collen, D. 1990. Studies of recombinant plasminogen activator inhibitor-1 in rabbits. Pharmacokinetics and evidence for reactivation of latent plasminogen activator inhibitor-1 in vivo. *Circ. Res.* 67: 1281–1286. [Medline] [CrossRef]

34. Venugopal, B., Sharon, R., Abramovitz, R., Khasin, A., and Miskin, R. 2001. Plasminogen activator inhibitor-1 in cardiovascular cells: rapid induction after injecting mice with kainate or adrenergic agents. *Cardiovasc. Res.* 49: 476–483. [Medline] [CrossRef]