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Rapid communication

Prostaglandin E_2 produced by microsomal prostaglandin E synthase-1 regulates the onset and the maintenance of wakefulness

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ABSTRACT

This study examined the effect of prostaglandin E_2 (PGE_2) produced by microsomal prostaglandin E synthase-1 (mPGES-1) on circadian rhythm. Using wild-type mice (WT) and mPGES-1 knockout mice (*mPGES-1*$^{-/-}$), I recorded and automatically analyzed the natural behavior of mice in home cages for 24 h and measured brain levels of PGE_2. The switch to wakefulness was not smooth, and sleepiness and the total duration of sleep were significantly longer in the *mPGES-1*$^{-/-}$ mice. Moreover, the basal concentration of PGE_2 was significantly lower in the *mPGES-1*$^{-/-}$ mice. These findings suggest that PGE_2 produced by mPGES-1 regulates the onset of wakefulness and the maintenance of circadian rhythm.

1. Introduction

Prostaglandin E_2 (PGE_2) has been identified as a modulator of wakefulness in the brain because microinjection of PGE_2 into the preoptic area or infusion of PGE_2 into the third cerebral ventricle of rats reduced the duration of slow-wave and paradoxical sleep, respectively (Matsumura et al., 1988, 1989). In addition, the treatment of narcoleptic dogs with PGE_2 induced a dose-dependent reduction in canine cataplexy (Nishino et al., 1989). Recently, PGE_2 was shown to induce wakefulness through activation of the histaminergic system via EP4 receptors at the tuberomammillary nucleus (TMN) in posterior hypothalamus (Huang et al., 2003, 2007).

However, microsomal prostaglandin E synthase-1 (mPGES-1) is known as an inducible PGE_2 synthesis enzyme, with a role in inflammation. In the brain, PGE_2 produced by inducible mPGES-1 regulates febrile responses (Yamagata et al., 2001) or neuronal loss elicited by kainic acid (KA) (Takemiya et al., 2010, 2011), whereas the role of basal mPGES-1 is not clear. In this study, I aimed to show the effect of PGE_2 produced by basic mPGES-1 on circadian rhythms of sleep/wakefulness.

For this study, I used the automated behavior recognition software HomeCageScan, which can detect detailed behavioral changes and is suitable for the observation of circadian rhythm in mice.

2. Materials and methods

2.1. Mice

Three wild-type (C57BL/6J; WT) and three *mPGES-1* knockout mice (*mPGES-1*$^{-/-}$) aged 10–14 weeks were used for behavior recording, and six WT and eight *mPGES-1*$^{-/-}$ were used for measurement of PGE_2 concentration. In addition, *mPGES-1*$^{-/-}$ were provided by Prof. S. Akira and Dr. S. Uematsu (Osaka University) and have been described previously (Uematsu et al., 2002). Food and water were provided ad libitum, and the mice were singly housed for the period of the study. The room was maintained at 24 ± 2 °C with a standard 12 h light/dark cycle. The cages and bedding were changed once a week, and the last change occurred 48 h before the initiation of each experiment. The experimental protocols were approved by the Animal Care and Use Committees of Tokyo Women's Medical University.

2.2. Behavior recording and analysis

A digital video camera was mounted perpendicular to the home cages, and mouse behavior was recorded as video data for 24 h. The video data were analyzed by HomeCageScan software (Clever Sys., Inc., Reston, VA, USA) using a Dell Dimension 490 computer. During recording, the mice were housed in standard cages. The circadian rhythm of sleep/wakefulness and the total duration of sleep were compared between the WT and *mPGES-1*$^{-/-}$ groups.

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2.3. PGE_2 enzyme immunoassay

Brain PGE_2 was measured in six WT and eight *mPGES-1*$^{-/-}$ under basal conditions. The mice were decapitated under deep anesthesia, and their brains were quickly removed during the window from 3 p.m. to 5 p.m. and frozen in liquid nitrogen. The PGE_2 concentration was determined using a spectrophotometer after incubation with a tracer and the anti-PGE_2 monoclonal antibody in a microplate according to the manufacturer's instructions (Cayman Chemical). The PGE_2 enzyme immunoassay was performed as previously described (Takemiya et al., 2003).

2.4. Statistics

Data are presented as the means ± s.e.m. The statistical analysis was performed using Student's *t*-test and one-way ANOVA. *P* values less than 0.05 were considered significant.

3. Results

3.1. Circadian rhythm and the total duration of sleep

Total sleeping time per each 60-min period is shown in Fig. 1. The figure depicts circadian rhythm during the 24-h observation period. The WT mice displayed clear circadian rhythms for 24 h, with definite wakening periods in the nighttime and sleeping periods during the daytime (Fig. 1A and B). Meanwhile, the onset of wakefulness was not smooth at the initial phase of the nighttime in *mPGES-1*$^{-/-}$ (Fig. 1C and D). The *mPGES-1*$^{-/-}$ mice also slept during the daytime same as the WT mice; thus, the total duration of sleep in the *mPGES-1*$^{-/-}$ mice (11.8 ± 0.7 h) was significantly longer than that in the WT mice (7.2 ± 1.4 h) by ANOVA (Fig. 2A). There was a significant difference in the duration of nighttime sleep, but not in that of daytime sleep by ANOVA (Fig. 2B and C)

3.2. PGE_2 levels

The basal PGE_2 concentrations of WT and *mPGES-1*$^{-/-}$ were as follows: 19.1 ± 1.9 ng/g wet tissue in WT, 10.1 ± 0.7 ng/g wet tissue in *mPGES-1*$^{-/-}$. There was a significant difference in PGE_2 levels between the two groups (Fig. 2D). This indicates that mPGES-1 is related to baseline synthesis of PGE_2.

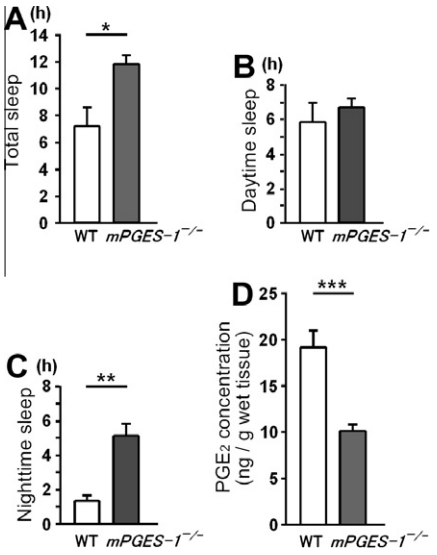

Fig. 2. Total sleep time over 24 h and PGE_2 concentration. (**A**) There is a significant difference between the WT and *mPGES-1*$^{-/-}$ ($n = 3$, each) groups in terms of total sleep time during the 24-h period. $^{*}p < 0.05$. (B and C) There is a significant difference in the duration of nighttime sleep (C), but not in that of daytime sleep (B). $^{**}p < 0.01$. (D) There is a significant difference in the concentration of brain PGE_2 between the two groups; WT and *mPGES-1*$^{-/-}$ ($n = 6$–8). $^{***}p < 0.0005$.

4. Discussion

In this study, I compared the circadian rhythm of sleep/wakefulness between WT and *mPGES-1*$^{-/-}$ mice. The results showed that the switch to wakefulness was not smooth at the initial phase of the nighttime in the *mPGES-1*$^{-/-}$ mice, showing sleepiness continuously throughout the 24-h period (Fig. 1C and D). The total duration of sleep was also increased because the duration of nighttime sleep, but not daytime sleep, was increased in this group (Fig. 2A–C). In addition, PGE_2 is produced by mPGES-1 not only after stimuli but also at baseline (Fig. 2D). These results indicate that PGE_2 produced by mPGES-1 regulates the onset of wakefulness and the maintenance of circadian rhythm.

Baseline levels of PGE_2 have been shown to regulate wakefulness in the brain (Matsumura et al., 1988, 1989; Nishino et al., 1989). The crucial mechanism is activation of the histaminergic

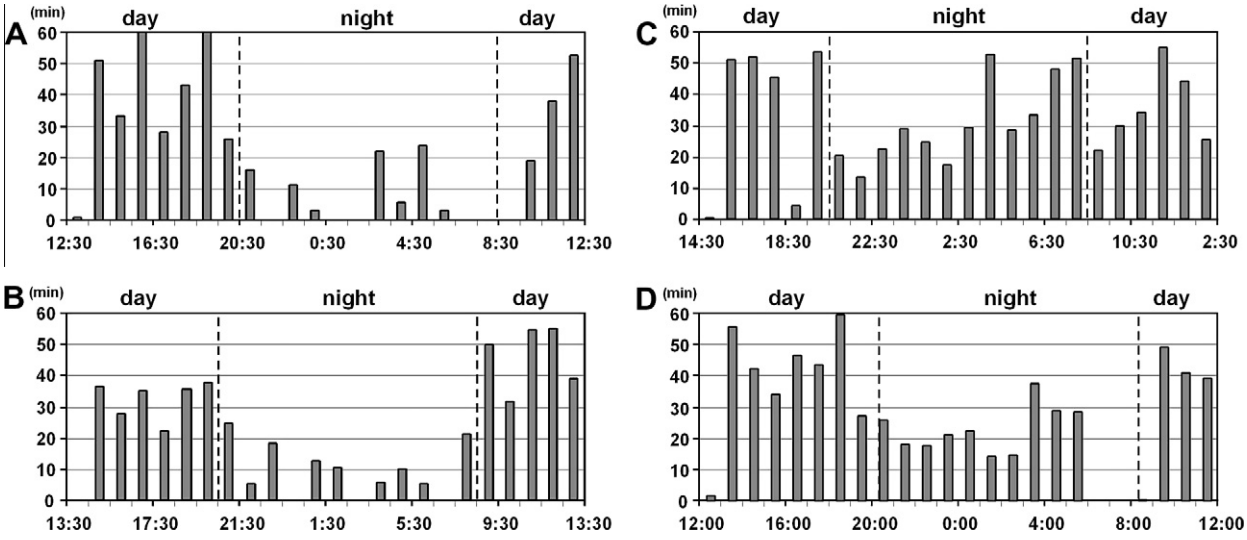

Fig. 1. 24-h Circadian rhythm of WT and *mPGES-1*$^{-/-}$ mice in home cages. (**A** and **B**) Total sleep time per each 60-min period over 24 h in two WT mice. There are clear circadian rhythms during the 24-h, with a definite wakening period in the nighttime and sleeping period in the daytime. (**C** and **D**) Total sleep time per each 60 min over 24 h in two *mPGES-1*$^{-/-}$ mice. Two graphs show sleepiness in the nighttime. The switch to wakefulness was not smooth.

system via EP4 receptors at the TMN in posterior hypothalamus (Huang et al., 2003, 2007). Basic PGE_2 is thought to be synthesized by constitutive PGE_2 enzymes such as cytosolic PGES or mPGES-2. On the contrary, inducible PGE_2 is known to be produced by mPGES-1 in the brain and affects the febrile response and KA-induced seizures (Takemiya et al., 2010, 2011; Yamagata et al., 2001). We did not detect basal mPGES-1 in primary hippocampal slice cultures or in vivo experiments in our previous immunohistochemistry study (Takemiya et al., 2010, 2011); therefore, I thought that mPGES-1 was not related to the production of basic PGE_2. However, the level of PGE_2 in the brains of the *mPGES-1*$^{-/-}$ mice was significantly lower than that in the WT mice (Fig. 2D), suggesting that mPGES-1 also regulates the production of basal PGE_2. In addition, low basal levels of *mPGES-1* mRNA were detected in the brain (Yamagata et al., 2001), and we observed basal mPGES-1 levels in cultured primary endothelial cells (Takemiya et al., 2010, 2011). Thus, PGE_2 might be produced by brain endothelial mPGES-1 under baseline conditions.

Tonic *mPGES-1* mRNA expression is also known to be enriched in the testis and kidney (Yamagata et al., 2001). In addition, because PGE_2 regulates circadian gene expression in peripheral tissues, PGE_2 might act as a clock-resetting agent in peripheral tissues in vivo (Tsuchiya et al., 2005). These reports indicate the possible effect of peripheral mPGES-1 on circadian rhythm.

To research the circadian rhythms of mice, observation of home cage behaviors is most suitable because it provides an opportunity to investigate naturally occurring behavior. Because the study was performed in the mouse home environment, there are no confounding factors associated with human interference, experimental protocol, or behavior changes associated with moving the mice to another testing apparatus; there is only basic, natural observation of normal laboratory animal behavior. In this study, I analyzed mouse behavior in the home cage using the automated behavior recognition software 'HomeCageScan'; thus, I was able to demonstrate the effect of mPGES-1 on circadian rhythms of sleep/wakefulness. When *mPGES-1*$^{-/-}$ mice were used for the cage-exchange stress experiment, the motor activity of the WT and *mPGES-1*$^{-/-}$ mice was similar, with no significant difference between the two groups (Saha et al., 2005). The results presented here demonstrate, for the first time, that PGE_2 produced by mPGES-1 regulates the onset and maintenance of wakefulness, as determined by the observation of home-cage behaviors.

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References

Huang, Z.L., Sato, Y., Mochizuki, T., Okada, T., Qu, W.M., Yamatodani, A., Urade, Y., Hayaishi, O., 2003. Prostaglandin E2 activates the histaminergic system via the EP4 receptor to induce wakefulness in rats. J. Neurosci. 23, 5975–5983.

Huang, Z.L., Urade, Y., Hayaishi, O., 2007. Prostaglandins and adenosine in the regulation of sleep and wakefulness. Curr. Opin. Pharmacol. 7, 33–38.

Matsumura, H., Goh, Y., Ueno, R., Sakai, T., Hayaishi, O., 1988. Awaking effect of PGE2 microinjected into the preoptic area of rats. Brain Res. 444, 265–272.

Matsumura, H., Honda, K., Goh, Y., Ueno, R., Sakai, T., Inoue, S., Hayaishi, O., 1989. Awaking effect of prostaglandin E2 in freely moving rats. Brain Res. 481, 242–249.

Nishino, S., Mignot, E., Fruhstorfer, B., Dement, W.C., Hayaishi, O., 1989. Prostaglandin E2 and its methyl ester reduce cataplexy in canine narcolepsy. Proc. Natl. Acad. Sci. USA 86, 2483–2487.

Saha, S., Engstrom, L., Mackerlova, L., Jakobsson, P.J., Blomqvist, A., 2005. Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1100–R1107.

Takemiya, T., Suzuki, K., Sugiura, H., Yasuda, S., Yamagata, K., Kawakami, Y., Maru, E., 2003. Inducible brain COX-2 facilitates the recurrence of hippocampal seizures in mouse rapid kindling. Prostaglandins Other Lipid Mediat. 71, 205–216.

Takemiya, T., Matsumura, K., Sugiura, H., Maehara, M., Yasuda, S., Uematsu, S., Akira, S., Yamagata, K., 2010. Endothelial microsomal prostaglandin E synthase-1 exacerbates neuronal loss induced by kainate. J. Neurosci. Res. 88, 381–390.

Takemiya, T., Matsumura, K., Sugiura, H., Yasuda, S., Uematsu, S., Akira, S., Yamagata, K., 2011. Endothelial microsomal prostaglandin E synthase-1 facilitates neurotoxicity by elevating astrocytic Ca^{2+} levels. Neurochem. Int. 58, 489–496.

Tsuchiya, Y., Minami, I., Kadotani, H., Nishida, E., 2005. Resetting of peripheral circadian clock by prostaglandin E2. EMBO Rep. 6, 256–261.

Uematsu, S., Matsumoto, M., Takeda, K., Akira, S., 2002. Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. J. Immunol. 168, 5811–5816.

Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugiura, H., Cao, C., Watanabe, Y., Kobayashi, S., 2001. Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. J. Neurosci. 21, 2669–2677.