

## MOLECULAR MECHANISM OF SLEEP-WAKE REGULATION

Yoshihiro Urade

**Abstract** Prostaglandin (PG) D<sub>2</sub> is the most potent endogenous sleep-promoting substance thus far reported and its sleep-inducing mechanism is the best characterized at the molecular level. PGD<sub>2</sub> is produced by lipocalin-type PGD synthase (L-PGDS) localized in the leptomeninges, choroid plexus, and oligodendrocytes in the brain and is secreted into the cerebrospinal fluid as a sleep hormone. PGD<sub>2</sub> stimulates DP<sub>1</sub> receptors localized in the arachnoid membrane at the basal forebrain to release adenosine as a paracrine sleep-promoting molecule, which activates adenosine A<sub>2A</sub> receptor-expressing neurons located in the basal forebrain. These cells subsequently excite the sleep-active neurons in the ventrolateral preoptic area and concomitantly suppress the tuberomammillary nucleus, a histaminergic arousal center, through GABAergic and galaninergic inhibitory projection, to induce sleep. The administration of a PGD synthase inhibitor (SeCl<sub>4</sub>), DP<sub>1</sub> antagonist (ONO-4127Na) or adenosine A<sub>2</sub> receptor antagonist (caffeine) induces insomnia, indicating that the PGD<sub>2</sub>-adenosine system is crucial for the maintenance of physiological sleep.

Hirosaki Med. J. 61, Supplement : S164—S173, 2010

**Key words:** prostaglandin D<sub>2</sub>; adenosine; ventrolateral preoptic area; tuberomammillary nucleus; histamine

### Introduction

The humoral theory of sleep regulation, the concept that sleep and wakefulness are induced and regulated by a hormone-like chemical substance rather than by a neural network, was initially proposed by Kuniomi Ishimori of Nagoya, Japan, in 1909 and independently and concurrently, by a French neuroscientist, Henri Piéron of Paris, in 1913 (Fig. 1). They took samples of the brain and cerebrospinal fluid (CSF) from sleep-deprived dogs and infused them into the brains of normal dogs. The recipient dogs soon started to sleep. Thus these scientists became the pioneers of sleep research, demonstrating the existence of endogenous sleep-promoting substances. However, the chemical nature of their sleep substance(s) was not identified. During the 20<sup>th</sup> century, numerous investigators have reported more than 30 so-called endogenous sleep- and wake-promoting substances in the brain, CSF, and

urine of animals and humans. Among them, prostaglandin (PG) D<sub>2</sub> is now recognized as the most potent endogenous sleep-promoting substance; and its action mechanism to induce sleep is the best characterized.

### Sleep induction by Prostaglandin (PG) D<sub>2</sub>

PGs are a group of 20-carbon polyunsaturated fatty acids containing a unique 5-carbon ring

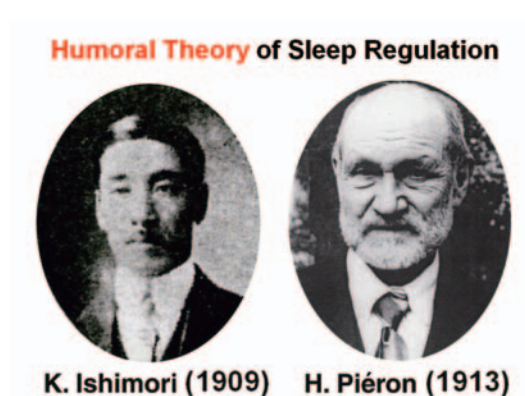


Figure 1 Photos of Dr. Ishimori and Dr. Piéron.

structure. PGs of the 2 series, such as PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub>, PGI<sub>2</sub> (prostacyclin), and thromboxane A<sub>2</sub>, are all produced from arachidonate (C<sub>20:4</sub> fatty acid) via a common intermediate, PGH<sub>2</sub>, which is produced by the action of cyclooxygenase, a target of non-steroidal anti-inflammatory drugs such as aspirin and indomethacin. Each prostanoid is then produced from PGH<sub>2</sub> by its terminal PG synthase, e.g., PGD synthase (PGDS) in the case of PGD<sub>2</sub>. PGD<sub>2</sub> had long been considered as a minor and biologically inactive PG. However, in the 1980's, this prostanoid was found to be the most abundant PG produced in the brains of rats<sup>1</sup> and other mammals including humans<sup>2</sup>, suggesting that PGD<sub>2</sub> may serve some important function in the central nervous system (CNS).

During the search for the neural function of PGD<sub>2</sub>, its sleep-inducing activity was discovered<sup>3</sup>, as evidenced by the fact that the microinjection of nano-molar quantities of PGD<sub>2</sub> into the rat brain increased both non-rapid eye movement (non-REM, NREM) and REM sleep. By using a sleep bioassay system based on the recording of both electroencephalogram and electromyogram during the continuous intracerebroventricular infusion of drugs into freely moving rats, the somnogenic activity of PGD<sub>2</sub> was demonstrated to be dose and time dependent<sup>4</sup>. The PGD<sub>2</sub>-induced sleep was indistinguishable from physiological sleep as judged by several electrophysiological and behavioral criteria. For example, during the PGD<sub>2</sub> infusion, rats were easily aroused by clap sound stimulation; and their sleep was episodic, indicating that PGD<sub>2</sub> does not interfere with the minimum time awake for their survival. Essentially the same sleep induction was demonstrated in the rhesus monkey *Macaca mulatta* during the intracerebroventricular infusion of PGD<sub>2</sub><sup>5</sup>.

The PGD<sub>2</sub> concentration in rat CSF showed a circadian fluctuation that paralleled the sleep-wake cycle<sup>6</sup> and became elevated with

an increase in sleep propensity during sleep deprivation<sup>7</sup>. PGD<sub>2</sub> was reported to be involved in the pathogenesis of mastocytosis, a disorder characterized by episodic and endogenous production of PGD<sub>2</sub> accompanied by deep sleep episodes<sup>8</sup>. PGD<sub>2</sub> concentrations were selectively and time-dependently elevated in the CSF of patients with African sleeping sickness, which is caused by infection with *Trypanosoma*<sup>9</sup>. These findings suggest that PGD<sub>2</sub> induces sleep in humans as well as in rodents and monkeys.

### PGDS and PGD<sub>2</sub> receptor in CNS

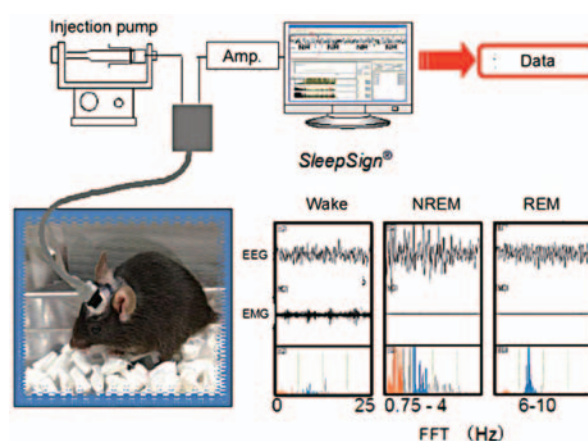
There are two distinct types of PGDS (PGH<sub>2</sub> D-isomerase, EC.5.3.99.2); one is lipocalin-type PGDS (L-PGDS)<sup>10</sup> and the other, hematopoietic PGDS (H-PGDS)<sup>11,12</sup>. We purified L-PGDS and H-PGDS, isolated their cDNAs and chromosomal genes of the human and mouse enzymes, determined their X-ray crystallographic structures, and demonstrated that these two enzymes are quite different from each other in terms of their amino acid sequence, tertiary structure, evolutionary origin, cellular distribution, etc<sup>13,14</sup>. Two distinct subtypes of receptors for PGD<sub>2</sub> have been identified; one is the DP<sub>1</sub> (DP) receptor originally identified as a homolog of other PG receptors<sup>15</sup> and the other is the DP<sub>2</sub> (CRTH2) receptor, identified as a chemoattractant receptor for PGD<sub>2</sub><sup>16</sup>. We developed inhibitors selective for either L-PGDS or H-PGDS (AT-56<sup>17</sup> and HQL-79<sup>18</sup>, respectively). Selective agonists and antagonists for DP<sub>1</sub> or DP<sub>2</sub> are also available. These pharmacological tools are used for various studies including those on sleep.

L-PGDS and the DP<sub>1</sub> receptor are involved in the regulation of physiological sleep, as described later in detail. L-PGDS is localized in the leptomeninges, choroid plexus, and oligodendrocytes in the brain<sup>19, 20</sup>. The DP<sub>1</sub> receptor is highly concentrated in the leptomeninges on the ventral surface of the rostral basal forebrain, whereas other brain areas are almost completely

negative<sup>21</sup>). The DP<sub>1</sub>-enriched leptomeninges was immunohistochemically defined as bilateral wings in the rostral basal forebrain lateral to the optic chiasm in the proximity of the posterior hypothalamus, which contains sleep-wake regulation centers<sup>21</sup>.

L-PGDS is the only member associated with enzyme activity in the lipocalin gene family, which comprises various secretory proteins that bind and transport small hydrophobic substances<sup>22,23</sup>. Having a molecular weight of 26,000 daltons, L-PGDS is composed of 189 and 190 amino acid residues in the mouse and human enzymes, respectively, and is post-translationally modified by the cleavage of an N-terminal hydrophobic signal peptide comprising 24 and 22 amino acid residues in the mouse and human enzymes, respectively, and by N-glycosylation at 2 positions (Asn51 and Asn78) in the mouse and human enzymes. We recently determined the X-ray crystallographic structure of 2 different conformers of L-PGDS, i.e., one with an open and the other with a closed calyx, at 2.1Å resolution<sup>24</sup> and showed that L-PGDS possesses a typical lipocalin-fold,  $\beta$ -barrel structure with a large hydrophobic pocket containing the catalytically essential Cys-65 residue.

L-PGDS is the same protein as  $\beta$ -trace<sup>25,26</sup>, which was originally discovered in 1961 as a major protein of human CSF<sup>27</sup>. The serum L-PGDS/ $\beta$ -trace concentration shows a circadian change with a nocturnal increase, which is suppressed during total sleep deprivation but not affected by deprivation of REM sleep<sup>28</sup>. We recently found that L-PGDS/ $\beta$ -trace binds PGD<sub>2</sub> with a high affinity (Kd = 20 nM; Aritake K. and Y.U., unpublished results), suggesting that PGD<sub>2</sub> may circulate within the CSF as a form bound to L-PGDS/ $\beta$ -trace and be transported to the DP<sub>1</sub> receptor to initiate NREM sleep.



**Figure 2** Sleep bioassay system for freely moving mice. EEG, electroencephalogram. EMG, electromyogram.

### The molecular mechanisms of PGD<sub>2</sub>-induced sleep

We modified the sleep bioassay system to monitor the electroencephalogram and electromyogram of freely moving mice during continuous intracerebroventricular infusion of drugs (Fig. 2), developed SLEEPSIGN software (Kissei Comtec, Nagano, Japan) for automatic scoring of the vigilance states of rats and mice<sup>29</sup>, and used these systems and software to study the molecular mechanism of PGD<sub>2</sub>-induced sleep (Fig. 3).

When PGD<sub>2</sub> was infused into the subarachnoid space of the basal forebrain of wild-type mice, in which DP<sub>1</sub> receptors are remarkably abundant, the extracellular adenosine concentration was increased dose-dependently. The PGD<sub>2</sub>-induced increase in extracellular adenosine was not observed in KO mice for DP<sub>1</sub> receptors, indicating that the increase in adenosine is dependent on the DP<sub>1</sub> receptors<sup>21</sup>.

Adenosine has been proposed to be an endogenous sleep substance, as a number of stable adenosine analogues induce sleep when administered to rats and other experimental animals. When CGS21680, an A<sub>2A</sub> receptor agonist, was infused into the lateral ventricle of

wild-type mice, NREM sleep was induced dose-dependently; whereas the  $A_1$  receptor-selective agonist  $N^6$ -cyclopentyladenosine was totally inactive, indicating that  $A_{2A}$ , but not  $A_1$ , receptors are involved in NREM sleep regulation<sup>30</sup>.

When  $PGD_2$  or the  $A_{2A}$  receptor agonist CGS21680 was infused for 2 h into the  $PGD_2$ -sensitive zone of the subarachnoid space of the basal forebrain, the number of Fos-positive cells was remarkably increased in the leptomeningeal membrane as well as in the ventrolateral preoptic (VLPO) area concomitant with the induction of NREM sleep<sup>31-33</sup>. In contrast, the number of Fos-positive neurons decreased markedly in the tuberomammillary nucleus (TMN) of the posterior hypothalamus. The VLPO is known to send specific inhibitory GABAergic and galaninergic efferents to the TMN the neurons of which contain the ascending histaminergic arousal system<sup>34</sup> (Fig. 3).

The intracellular recording of VLPO neurons in rat brain slices demonstrated the existence of 2 distinct types of VLPO neurons in terms of their responses to serotonin and adenosine. VLPO neurons are inhibited uniformly by 2 arousal neurotransmitters, noradrenaline and acetylcholine, and mostly by an adenosine  $A_1R$  agonist. Serotonin inhibits the type-1 neurons but excites the type-2 neurons; whereas an  $A_{2A}R$  agonist excites postsynaptically the type-2, but not the type-1, neurons. These results suggest that the type-2 neurons are involved in the initiation of sleep and that the type-1 neurons contribute to sleep consolidation, since they are activated only when released from inhibition by arousal systems<sup>35</sup>.

*In vivo* microdialysis experiments revealed that infusion of an adenosine  $A_{2A}R$  agonist, CGS21680, into the basal forebrain inhibited the release of histamine in both the frontal cortex and medial preoptic area in a dose-dependent manner, and increased the GABA release specifically in

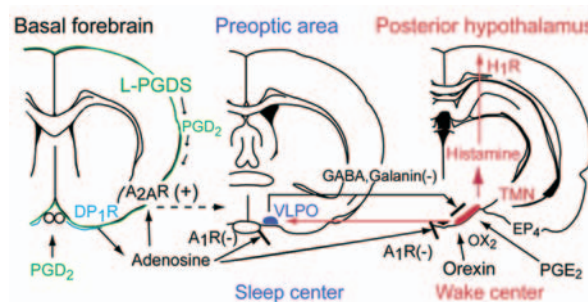


Figure 3 Mechanisms of  $PGD_2$ -induced sleep.

the TMN but not in the frontal cortex<sup>36</sup>. The CGS21680-induced inhibition of histamine release was antagonized by perfusion of the TMN with a  $GABA_A$  antagonist, picrotoxin, suggesting that the  $A_{2A}$  agonist induced sleep by inhibiting the histaminergic system through an increase in GABA release in the TMN. These results support the original idea of the flip-flop mechanism, whereby sleep is promoted by up-regulation of the sleep neurons in the VLPO and at the same time down-regulation of the histaminergic wake neurons in the TMN, as mentioned by Saper and colleagues<sup>37,38</sup>.

### Sleep abnormalities in mice gene-manipulated for L-PGDS, $DP_1$ , and $A_{2A}$ receptors

We generated L-PGDS KO mice with the null mutation by homologous recombination<sup>39</sup> and demonstrated that the KO mice grow normally but show several functional abnormalities in their regulation of sleep<sup>40,41</sup>. The L-PGDS KO mice do not accumulate  $PGD_2$  in their brain during sleep deprivation nor show the NREM sleep rebound after sleep deprivation; whereas the wild-type mice show an increase in the  $PGD_2$  content in their brain during sleep deprivation, which induces the NREM sleep rebound<sup>40,41</sup>. We also generated TG mice<sup>42</sup> that over-expressed the human L-PGDS under the control of the  $\beta$ -actin promoter and serendipitously discovered that these TG mice showed a transient increase



in NREM sleep after their tails had been clipped for DNA sampling used for genetic analysis<sup>40,42</sup>. The noxious stimulation of tail clipping induced a remarkable increase in the PGD<sub>2</sub> content in the brain of the TG mice but not in that of the wild-type ones, although we do not yet understand in detail the mechanism responsible for this increase.

KO mice for various receptors involved in the sleep-wake regulation, such as DP<sub>1</sub>, A<sub>1</sub>, A<sub>2A</sub>, and histamine H<sub>1</sub> receptors, have been already generated and their sleep-wake regulation examined. However, those KO mice showed essentially the same circadian profiles and daily amounts of sleep as wild-type mice, although a minor decrease in the short period of wakefulness was observed in the H<sub>1</sub> KO mice<sup>43</sup>. These phenomena have been explained by genetic compensation, in which the deficiency of one system in a complicated network of the sleep-regulatory system may be effectively compensated by the other systems during embryonic development, because sleep is essential for life. To minimize the effect of functional compensation on the sleep-wake regulation, we used pharmacological tools, such as antagonists for those receptors, to examine the contribution of each system to physiological sleep, as described below. On the other hand, those KO mice showed abnormal behavior in their sleep-wake regulation after some stimulation such as sleep deprivation. Similar to L-PGDS KO mice, DP<sub>1</sub> KO mice did not exhibit NREM sleep rebound after sleep deprivation, indicating that the L-PGDS/PGD<sub>2</sub>/DP<sub>1</sub> receptor system is crucial for the homeostatic regulation of NREM sleep<sup>41</sup>.

### **Contribution of PGD<sub>2</sub> to physiological sleep**

We examined the effect of the DP<sub>1</sub> antagonist ONO-4127Na on the sleep of rats and that of a selective inhibitor of L-PGDS, SeCl<sub>4</sub>, on the sleep

of wild-type and KO mice for PGDS and DP<sub>1</sub> receptors<sup>44</sup>.

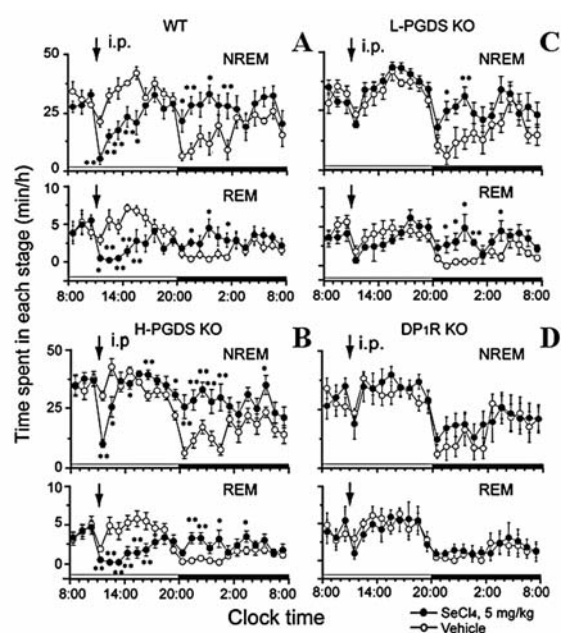
ONO-4127Na is a novel DP<sub>1</sub> antagonist developed by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). This compound exhibited a highly specific binding affinity for DP<sub>1</sub> receptors ( $K_i = 2.5$  nM) and antagonism toward these receptors ( $pA_2 = 9.73$ ). We infused this DP<sub>1</sub> antagonist into the subarachnoid space underlying the rostral basal forebrain of rats during the sleep period. ONO-4127Na infusion had little effect on the sleep-stage distribution at 50 pmol/min but reduced NREM sleep by 23% and 28% and REM sleep by 49% and 63% at 100 and 200 pmol/min, respectively, during perfusion for 6 h and 1 h post-infusion. ONO-4127Na infusion at 200 pmol/min decreased NREM sleep hourly by 30 to 40%, and reduced REM sleep by 60 to 90% commencing about 2 h after the beginning of ONO-4127Na infusion, as compared with the baseline. Although we could not use doses higher than 200 pmol/min due to the low solubility of ONO-4127Na, these results clearly indicate that this DP<sub>1</sub> antagonist dose-dependently reduced NREM and REM sleep, and suggest that the stimulation of DP<sub>1</sub> receptors with endogenous PGD<sub>2</sub> is essential for maintenance of physiological sleep.

Inorganic tetravalent selenium compounds are potent, relatively specific, and reversible inhibitors of PGDS<sup>45</sup>. When we examined the effect of SeCl<sub>4</sub> on the activities of mouse L-PGDS and H-PGDS *in vitro*, SeCl<sub>4</sub> inhibited both L-PGDS and H-PGDS efficiently with IC<sub>50</sub> values of 40 and 90 μM, respectively. We then determined the PGD<sub>2</sub> content in the brain of wild-type mice at 2 h after an intraperitoneal injection of SeCl<sub>4</sub> at a dose of 1.25 to 5 mg/kg body weight during the light period, when mice normally sleep. SeCl<sub>4</sub> given at 1.25 mg/kg had little effect on the PGD<sub>2</sub> content in the brain, but at 2.5 and at 5 mg/kg it reduced the PGD<sub>2</sub> content by 52% and 59%, respectively, without

changing the PGE<sub>2</sub> and PGF<sub>2α</sub> contents. These results indicate that the SeCl<sub>4</sub> administration selectively inhibited the production of PGD<sub>2</sub> in the brain *in vivo* without affecting the production of other PGs<sup>44</sup>.

Next we examined the sleep-wake pattern of wild-type mice before and after an intraperitoneal bolus injection of SeCl<sub>4</sub> at doses of 1.25 to 5 mg/kg at 11:00 during the sleep period. In the case of 1.25 mg/kg SeCl<sub>4</sub>, the sleep-wake pattern was almost identical before and after the injection, similar to the pattern of PGD<sub>2</sub> content in the brain. SeCl<sub>4</sub> at 2.5 mg/kg slightly reduced both NREM and REM sleep for 2 to 3 h after the injection and decreased the cumulative amounts of NREM and REM sleep for 5 h post-injection by 23% and 44%, respectively. At doses of 4 and 5 mg/kg SeCl<sub>4</sub>, both NREM and REM sleep decreased promptly after the injection; and SeCl<sub>4</sub>-injected mice displayed almost complete insomnia within 1 h. The sleep suppression gradually decreased thereafter, lasting about 3 and 5 h after the injection of 4 and 5 mg/kg SeCl<sub>4</sub>, respectively. SeCl<sub>4</sub> given at 4 and 5 mg/kg reduced the 5 h-cumulative amount of NREM sleep during the daytime by 31% and 45%, respectively, and that of REM sleep by 63% and 81%, respectively (Fig. 4A). It also induced a strong rebound-like reaction during the following nighttime to increase NREM and REM sleep. These results revealed that SeCl<sub>4</sub> given during the light period dose dependently inhibited NREM and REM sleep of wild-type mice and increased both during the following dark period.

We then administered SeCl<sub>4</sub> at 5 mg/kg to various KO mice lacking H-PGDS, L-PGDS, both H-/L-PGDSs or DP<sub>1</sub> receptors and compared their sleep-wake cycle with that of the wild-type mice (Fig. 4). In H-PGDS KO mice, SeCl<sub>4</sub> also inhibited both NREM and REM sleep, by 80% and almost completely, respectively, and increased the wake time about 2-fold for 1 h after the injection (Fig. 4B). The hourly amounts



**Figure 4** SeCl<sub>4</sub>-induced insomnia in wild-type (A) and H-PGDS KO mice (B), but not in L-PGDS KO mice (C) or DP<sub>1</sub> KO mice (D)<sup>44</sup>.

of NREM sleep and wakefulness returned to the vehicle-injected level within 2 to 3 h after the administration, but the REM sleep suppression was prolonged for 6 h. H-PGDS KO mice showed strong NREM and REM sleep induction and suppression of wakefulness during the following dark period, similar to the WT mice. Between the wild-type and H-PGDS KO mice, there was no statistically significant difference in the responsiveness of NREM and REM sleep and wakefulness to the SeCl<sub>4</sub> administration, suggesting that H-PGDS was most likely not involved in the SeCl<sub>4</sub>-induced insomnia.

In contrast, when L-PGDS KO mice were examined, SeCl<sub>4</sub> did not change the amounts of NREM and REM sleep or the time spent in wakefulness at all during the daytime (Fig. 4C), clearly indicating the sleep inhibition immediately after the administration of SeCl<sub>4</sub> to be dependent on L-PGDS. When SeCl<sub>4</sub> was administered to H-/L-PGDSs double KO mice or to DP<sub>1</sub> KO mice (Fig. 4D) under the same experimental conditions, neither the inhibition of

sleep immediately after administration of  $\text{SeCl}_4$  nor the delayed increase in sleep during the night was observed, indicating that  $\text{SeCl}_4$  was not toxic to inhibit sleep *per se* but suppressed sleep by inhibiting the endogenous production of  $\text{PGD}_2$ , whose information is transferred by  $\text{DP}_1$  receptors to induce sleep. These results also suggest that sleep in wild-type mice is controlled by  $\text{PGD}_2$  produced by L-PGDS, rather than H-PGDS, and recognized by  $\text{DP}_1$  receptors under physiological conditions.

### Contribution of adenosine to physiological sleep

Complete insomnia was also observed in wild-type mice for 2 to 3 hours after an intraperitoneal injection of caffeine, a non-selective antagonist of adenosine  $A_1$  and  $A_{2A}$  receptors, at a dose of 15 mg/kg, a dose corresponding to an intake of approximately three cups of coffee in humans. In the case of caffeine, there was no disruption of sleep architecture after the 3-h period. We then used KO mice for  $A_1$  or  $A_{2A}$  receptors and their respective wild-type littermates of the inbred C57BL/6 strain obtained from mating of heterozygotes to elucidate which subtype of receptors is involved in caffeine-induced wakefulness/insomnia<sup>46</sup>. The caffeine-induced insomnia was observed in  $A_1$  receptor KO mice to have the same intensity and duration as in wild-type mice. In contrast,  $A_{2A}$  KO mice did not show any change in time spent in wakefulness after the caffeine administration, indicating that the  $A_{2A}$  receptor is crucial in caffeine-induced wakefulness. These results also indicate that the stimulation of  $A_{2A}$  receptors with endogenous adenosine is essential for maintenance of physiological sleep.

Adenosine deaminase, the enzyme that catabolizes adenosine to inosine, is predominantly localized in the TMN in the brain parenchyma, which nucleus is enriched in adenosine  $A_1$  receptors. Therefore, the histaminergic arousal

system is predicted to be actively regulated by adenosine in the TMN. Recently we showed that the bilateral injection of an  $A_1$  receptor agonist,  $\text{N}^6$ -cyclopentyladenosine, into the rat TMN significantly increased the amount of NREM sleep<sup>47</sup>. The bilateral injection of adenosine or of coformycin, an inhibitor of adenosine deaminase, into the rat TMN also increased NREM sleep, which increase was completely abolished by co-administration of a selective  $A_1$ R antagonist, 1,3-dimethyl-8-cyclopentylxanthine. These results indicate that endogenous adenosine in the TMN suppresses the histaminergic system via  $A_1$  receptors to promote NREM sleep.

### Acknowledgements

Studies conducted in our laboratory were supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN); the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO); a Health and Labor Science Research Grant from the Ministry of Health, Labor, and Welfare, Japan; and by research grants from Takeda Science Foundation, Takeda Pharmaceutical Co. Ltd., Ono Pharmaceutical Co. Ltd., and Osaka city.

### References

- 1) Narumiya S, Ogorochi T, Nakao K, Hayaishi O. Prostaglandin  $D_2$  in rat brain, spinal cord and pituitary: Basal level and regional distribution. *Life Sci* 1982;31:2093-103.
- 2) Ogorochi T, Narumiya S, Mizuno N, Yamashita K, Miyazaki H, Hayaishi O. Regional distribution of prostaglandins  $D_2$ ,  $E_2$ , and  $F_{2\alpha}$  and related enzymes in postmortem human brain. *J Neurochem* 1984;43:71-82.
- 3) Ueno R, Ishikawa Y, Nakayama T, Hayaishi O. Prostaglandin  $D_2$  induces sleep when microinjected into the preoptic area of conscious rats. *Biochem Biophys Res Commun* 1982;109:576-82.

- 4) Ueno R, Honda K, Inoue S, Hayaishi O. Prostaglandin D<sub>2</sub>, a cerebral sleep-inducing substance in rats. *Proc Natl Acad Sci USA* 1983;80:1735-7.
- 5) Onoe H, Ueno R, Fujita I, Nishino H, Oomura Y, Hayaishi O. Prostaglandin D<sub>2</sub>, a cerebral sleep-inducing substance in monkeys. *Proc Natl Acad Sci USA* 1988;85:4082-6.
- 6) Pandey HP, Ram A, Matsumura H, Hayaishi O: Concentration of prostaglandin D<sub>2</sub> in cerebrospinal fluid exhibits a circadian alteration in conscious rats. *Biochem Mol Biol Int* 1995;37:431-7.
- 7) Ram A, Pandey HP, Matsumura H, *et al.* CSF levels of prostaglandins, especially the level of prostaglandin D<sub>2</sub>, are correlated with increasing propensity towards sleep in rats. *Brain Res* 1997; 751:81-9.
- 8) Roberts LJ, 2nd, Sweetman BJ, Lewis RA, Austen KF, Oates JA. Increased production of prostaglandin D<sub>2</sub> in patients with systemic mastocytosis. *N Engl J Med* 1980;303:1400-4.
- 9) Pentreath VW, Rees K, Owolabi OA, Philip KA, Doua F. The somnogenic T lymphocyte suppressor prostaglandin D<sub>2</sub> is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. *Trans R Soc Trop Med Hyg* 1990;84:795-9.
- 10) Urade Y, Fujimoto N, Hayaishi O: Purification and characterization of rat brain prostaglandin D synthetase. *J Biol Chem* 1985;260:12410-5.
- 11) Christ-Hazelhof E, Nugteren DH. Purification and characterisation of prostaglandin endoperoxide d-isomerase, a cytoplasmic, glutathione-requiring enzyme. *Biochim Biophys Acta* 1979;572:43-51.
- 12) Urade Y, Fujimoto N, Ujihara M, Hayaishi O. Biochemical and immunological characterization of rat spleen prostaglandin D synthetase. *J Biol Chem* 1987;262:3820-5.
- 13) Urade Y, Eguchi N. Lipocalin-type and hematopoietic prostaglandin D synthases as a novel example of functional convergence. *Prostaglandins Other Lipid Mediat* 2002;68-69:375-82.
- 14) Urade Y, Hayaishi O. Prostaglandin D synthase: Structure and function. *Vitam Horm* 2000;58:89-120.
- 15) Hirata M, Kakizuka A, Aizawa M, Ushikubi F, Narumiya S. Molecular characterization of a mouse prostaglandin d receptor and functional expression of the cloned gene. *Proc Natl Acad Sci USA* 1994;91:11192-6.
- 16) Hirai H, Tanaka K, Yoshie O, *et al.* Prostaglandin D<sub>2</sub> selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor crth2. *J Exp Med* 2001; 193:255-61.
- 17) Irikura D, Aritake K, Nagata N, Maruyama T, Shimamoto S, Urade Y. Biochemical, functional, and pharmacological characterization of AT-56, an orally active and selective inhibitor of lipocalin-type prostaglandin D synthase. *J Biol Chem* 2009; 284:7623-30.
- 18) Aritake K, Kado Y, Inoue T, Miyano M, Urade Y. Structural and functional characterization of HQL-79, an orally selective inhibitor of human hematopoietic prostaglandin D synthase. *J Biol Chem* 2006;281:15277-86.
- 19) Urade Y, Kitahama K, Ohishi H, Kaneko T, Mizuno N, Hayaishi O. Dominant expression of mRNA for prostaglandin D synthase in leptomeninges, choroid plexus, and oligodendrocytes of the adult rat brain. *Proc Natl Acad Sci USA* 1993;90:9070-4.
- 20) Beuckmann Ct, Lazarus M, Gerashchenko D, *et al.* Cellular localization of lipocalin-type prostaglandin D synthase (beta-trace) in the central nervous system of the adult rat. *J Comp Neurol* 2000; 428:62-78.
- 21) Mizoguchi A, Eguchi N, Kimura K, *et al.* Dominant localization of prostaglandin D receptors on arachnoid trabecular cells in mouse basal forebrain and their involvement in the regulation of non-rapid eye movement sleep. *Proc Natl Acad Sci USA* 2001;98:11674-9.
- 22) Urade Y, Hayaishi O: Biochemical, structural, genetic, physiological, and pathophysiological features of lipocalin-type prostaglandin D synthase. *Biochim Biophys Acta* 2000;1482: 259-71.
- 23) Urade Y, Eguchi N, Hayaishi O. Lipocalin-type prostaglandin D synthase as an enzymic lipocalin. Landes Bioscience / Eurekah.com, Georgetown, Texas, USA., (2006).
- 24) Kumasaka T, Aritake K, Ago H *et al.*: Structural



- basis of the catalytic mechanism operating in open-closed conformers of lipocalin type prostaglandin d synthase. *J Biol Chem* 2009;284:22344-52.
- 25) Hoffmann A, Conradt HS, Gross G, Nimtz M, Lottspeich F, Wurster U. Purification and chemical characterization of beta-trace protein from human cerebrospinal fluid: Its identification as prostaglandin d synthase. *J Neurochem* 1993; 61:451-6.
- 26) Watanabe K, Urade Y, Mader M, Murphy C, Hayaishi O. Identification of beta-trace as prostaglandin D synthase. *Biochem Biophys Res Commun* 1994;203:1110-6.
- 27) Clausen J. Proteins in normal cerebrospinal fluid not found in serum. *Proc Soc Exp Biol Med* 1961;107:170-2.
- 28) Jordan W, Tumani H, Cohrs S *et al.*: Prostaglandin D synthase (beta-trace) in healthy human sleep. *Sleep* 2004;27:867-74.
- 29) Kohtoh S, Taguchi Y, Matsumoto N, Wada M, Huang Z, Urade Y. Algorithm for sleep scoring in experimental animals based on fast fourier transform power spectrum analysis of the electroencephalogram. *Sleep Biol Rhythm* 2008; 6:163-71.
- 30) Urade Y, Eguchi N, Qu WM, *et al.* Sleep regulation in adenosine A<sub>2A</sub> receptor-deficient mice. *Neurology* 2003;61(11 Suppl 6):S94-6.
- 31) Scammell T, Gerashchenko D, Urade Y, Onoe H, Saper C, Hayaishi O. Activation of ventrolateral preoptic neurons by the somnogen prostaglandin D<sub>2</sub>. *Proc Natl Acad Sci USA* 1998;95:7754-9.
- 32) Scammell TE, Gerashchenko DY, Mochizuki T, *et al.*: An adenosine A<sub>2A</sub> agonist increases sleep and induces fos in ventrolateral preoptic neurons. *Neuroscience* 2001;107:653-663.
- 33) Satoh S, Matsumura H, Koike N, Tokunaga Y, Maeda T, Hayaishi O. Region-dependent difference in the sleep-promoting potency of an adenosine A<sub>2A</sub> receptor agonist. *Eur J Neurosci* 1999;11: 1587-97.
- 34) Sherin JE, Shiromani PJ, Mccarley RW, Saper CB. Activation of ventrolateral preoptic neurons during sleep. *Science* 1996;271:216-9.
- 35) Gallopin T, Luppi PH, Cauli B, *et al.*: The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A<sub>2A</sub> receptors in the ventrolateral preoptic nucleus. *Neuroscience* 2005;134:1377-90.
- 36) Hong ZY, Huang ZL, Qu WM, Eguchi N, Urade Y, Hayaishi O. An adenosine A<sub>2A</sub> receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats. *J Neurochem* 2005;92:1542-9.
- 37) Saper CB, Chou TC, Scammell TE: The sleep switch: Hypothalamic control of sleep and wakefulness. *Trends Neurosci* 2001;24:726-31.
- 38) Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005;437:1257-63.
- 39) Eguchi N, Minami T, Shirafuji N, *et al.* Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc Natl Acad Sci USA* 1999;96:726-30.
- 40) Eguchi N, Pinzar E, Kuwahata Y *et al.*: *Sleep of transgenic and gene-knockout mice for lipocalin-type prostaglandin D synthase*. In: *Oxygen and Life - Oxygenases, Oxidases and Lipid Mediator* 2002.
- 41) Hayaishi O, Urade Y, Eguchi N, Huang ZL: Genes for prostaglandin D synthase and receptor as well as adenosine A<sub>2A</sub> receptor are involved in the homeostatic regulation of nrem sleep. *Arch Ital Biol* 2004;142:533-9.
- 42) Pinzar E, Kanaoka Y, Inui T, Eguchi N, Urade Y, Hayaishi O: Prostaglandin D synthase gene is involved in the regulation of non-rapid eye movement sleep. *Proc Natl Acad Sci USA* 2000;97: 4903-7.
- 43) Huang ZL, Mochizuki T, Qu WM *et al.*: Altered sleep-wake characteristics and lack of arousal response to H<sub>3</sub> receptor antagonist in histamine H<sub>1</sub> receptor knockout mice. *Proc Natl Acad Sci USA* 2006;103:4687-92.
- 44) Qu WM, Huang ZL, Xu XH, *et al.*: Lipocalin-type prostaglandin D synthase produces prostaglandin D<sub>2</sub> involved in regulation of physiological sleep. *Proc Natl Acad Sci USA* 2006;103:17949-54.
- 45) Islam F, Watanabe Y, Morii H, Hayaishi O.

- Inhibition of rat brain prostaglandin D synthase by inorganic selenocompounds. *Arch Biochem Biophys* 1991;289:161-6.
- 46) Huang ZL, Qu Wm, Eguchi N *et al.*: Adenosine A<sub>2A</sub>, but not A<sub>1</sub>, receptors mediate the arousal effect of caffeine. *Nat Neurosci* 2005;8:858-9.
- 47) Oishi Y, Huang ZL, Fredholm BB, Urade Y, Hayaishi O. Adenosine in the tuberomammillary nucleus inhibits the histaminergic system via A<sub>1</sub> receptors and promotes non-rapid eye movement sleep. *Proc Natl Acad Sci USA* 2008;105:19992-7.