The Role of Adenosine in the Regulation of Sleep

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Abstract: This paper presents an overview of the current knowledge about the role of adenosine in the sleep-wake regulation with a focus on adenosine in the central nervous system, regulation of adenosine levels, adenosine receptors, and manipulations of the adenosine system by the use of pharmacological and molecular biological tools. The endogenous somnogen prostaglandin (PG) D_2 increases the extracellular level of adenosine under the subarachnoid space of the basal forebrain and promotes physiological sleep. Adenosine is neither stored nor released as a classical neurotransmitter and is thought to be formed inside cells or on their surface, mostly by breakdown of adenine nucleotides. The extracellular concentration of adenosine increases in the cortex and basal forebrain during prolonged wakefulness and decreases during the sleep recovery period. Therefore, adenosine is proposed to act as a homeostatic regulator of sleep and to be a link between the humoral and neural mechanisms of sleep-wake regulation. Both the adenosine A_1 receptor (A_1R) and $A_{2A}R$ are involved in sleep induction. The $A_{2A}R$ plays a predominant role in the somnogenic effects of PGD_2. By use of gene-manipulated mice, the arousal effect of caffeine was shown to be dependent on the $A_{2A}R$. On the other hand, inhibition of wake-promoting neurons via the A_1R also mediates the sleep-inducing effects of adenosine, whereas activation of A_1R in the lateral preoptic area induces wakefulness, suggesting that A_1R regulates the sleep-wake cycle in a site-dependent manner. The potential therapeutic applications of agonists and antagonists of these receptors in sleep disorders are briefly discussed.

Keywords: Adenosine, knockout mice, prostaglandin D_2, receptor, sleep, wakefulness.

1. INTRODUCTION

The drive to sleep begins with the onset of wakefulness and dissipates slowly with the progression of sleep. Endogenous sleep factors acting on specific neurons in the brain are hypothesized to regulate the waxing and waning of the sleep drive [1]. Two such sleep factors are adenosine, a naturally occurring purine nucleoside present in all cells [2], and prostaglandin (PG) D_2, an eicosanoid acting as a tissue or local hormone [3], both of which are released as neuromodulators in the brain. Here, we stress that we are discussing some areas of high general complexity: (a) adenosine is a key signaling molecule for PGD_2-induced sleep; (b) the metabolism of adenosine, which is ubiquitously present in all cells; (c) key roles of the A_{2A} receptor ($A_{2A}R$) in sleep induction; (d) activation of adenosine A_1R regulates the sleep-wake cycle in a region or site-dependent manner. This paper briefly reviews these points, with particular emphasis on the recent progress made by the use of biochemical and pharmacological tools and gene-manipulated mice.

2. ADENOSINE IS A KEY SIGNALING MOLECULE FOR PGD_2-INDUCED SLEEP

Identification of the mechanism by which endogenous PGD_2 promotes sleep (Fig. **1**) led to the discovery that adenosine acts as a mediator of PGD_2-induced sleep [4]. PGD_2 was found to be one of the most potent somnogens involved in physiological sleep [5]. It is the most abundant PG produced in the brains of rats [6] and other mammals including humans [7]. The PGD_2 concentration in rat cerebrospinal fluid (CSF) shows a circadian fluctuation that parallels the sleep-wake cycle [8] and becomes elevated with an increase in sleep propensity during sleep deprivation [9], suggesting that PGD_2 may play some important function in the CNS. During the search to uncover the neural function of PGD_2, its sleep-inducing activity was discovered [10], in which microinjection of nano-molar quantities of PGD_2 in the rat brain increased both non-rapid eye movement (non-REM, NREM) and REM sleep [11]. Essentially the same sleep induction was demonstrated in the rhesus monkey (*Macaca mulatta)* during the i.c.v. infusion of PGD_2 [12]. The PGD_2-induced sleep was significant with as little as picomolar quantities per minute and indistinguishable from physiological sleep as judged by several electrophysiological and behavioral criteria.

There are two distinct types of PGD synthase (PGDS) for the production of PGD_2 in the CNS: one is lipocalin-type PGDS (L-PGDS), dominantly expressed in the arachnoid membrane, choroid plexus, and oligodendrocytes [13-14]; and the other, hematopoietic PGDS (H-PGDS), localized in microglia [15]. PGD_2 produced by either of these enzymes is secreted into the CSF, and its level there exhibits a circadian alteration in freely moving rats [8]. L-PGDS is identical to beta-trace, a major protein in human CSF. Also, the serum L-PGDS/β-trace concentration shows a circadian change

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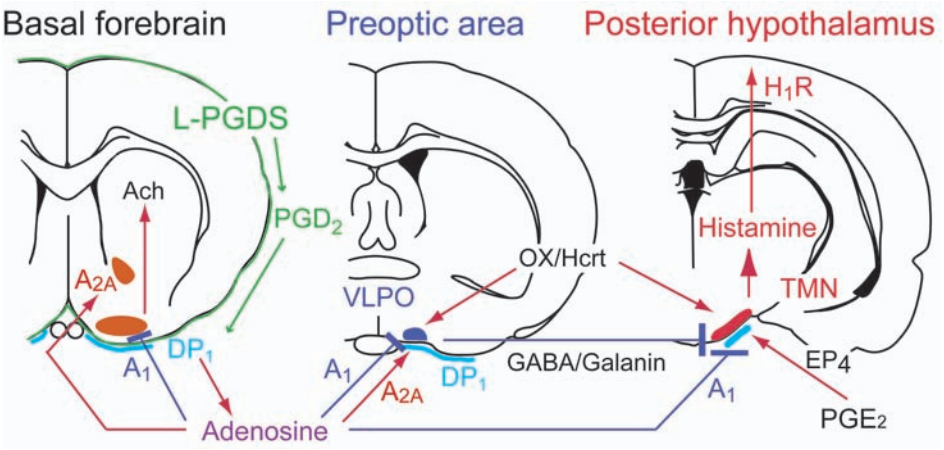

Fig. (1). Molecular mechanism of sleep–wake regulation. The endogenous somnogen PGD_2 (green) is produced by L-PGDS, circulates within the CSF, stimulates DP_1R (light blue) on the ventral surface from the basal forebrain to the hypothalamus, and increases the level of extracellular adenosine. Adenosine (purple) diffuses into the brain parenchyma as the secondary somnogen, inhibits arousal neurons in the basal forebrain (orange area) and TMN (red area) via A_1R (blue lines), and activates sleep–active VLPO neurons (blue area) via $A_{2A}R$ (red arrows) to induce sleep. The flip–flop switch of sleep–wakefulness regulation between the VLPO and TMN is stabilized by their OX/Hcrt-mediated activation and adenosine A_1R-mediated suppression. Ach: Acetylcholine; EP_4: Prostaglandin E_2 receptor, subtype EP_4; H_1R: Histamine H_1 receptor; Hcrt: Hypocretin; L-PGDS: Lipocalin-type prostaglandin D synthase; Ox: Orexin; TMN: Tuberomammillary nucleus; VLPO: Ventrolateral preoptic area. Adapted and modified from Urade and Hayaishi [5] with permission.

with a nocturnal increase, which is suppressed during total sleep deprivation but not affected by deprivation of REM sleep in humans [16]. Furthermore, when rats are administered inorganic tetravalent selenium (Se^{4+}) compounds such as selenium tetrachloride ($SeCl_4$), which are specific and reversible inhibitors of PGDS [17], sleep is inhibited time- and dose-dependently and reversibly [18-19]. These findings indicate that PGD_2/PGDS plays an important role in sleep induction.

To clarify the involvement of PGD_2 in regulating physiological sleep, we examined the effect of inhibition of PGD_2 synthesis by $SeCl_4$ on the sleep of wild-type (WT) mice and knockout (KO) mice for PGDS and PGD_2 receptor (DP_1R), as well as the effect of a DP_1R antagonist, ONO-4127Na, on the sleep of rats. The i.p. injection of $SeCl_4$ into WT mice decreased the PGD_2 content in the brain without affecting the amounts of PGE_2 and $PGF_{2\alpha}$. It inhibited sleep dose-dependently to induce almost complete insomnia after the administration during the light period when mice normally sleep. The $SeCl_4$-induced insomnia was observed in H-PGDS KO mice but not at all in L-PGDS KO, H- and L-PGDS double KO or DP_1R KO mice. Furthermore, the DP_1R antagonist ONO-4127Na reduced the sleep of rats during infusion into the subarachnoid space under the rostral basal forebrain [20]. These results clearly show that the L-PGDS/PGD_2/DP_1R system plays a pivotal role in the regulation of physiological sleep.

When PGD_2 was infused to the subarachnoid space of the basal forebrain of WT mice, in which DP_1Rs are remarkably abundant, the extracellular adenosine concentration was increased dose-dependently, although the mechanism linking PGD_2 and adenosine accumulation remains unknown. The PGD_2-induced increase in extracellular adenosine was observed in WT mice, but not in KO mice of DP_1Rs (Fig. **2**) [13]. Furthermore, the somnogenic effect of PGD_2 was blocked by the intraperitoneal injection of the $A_{2A}R$-specific antagonist KF 17837, indicating that the adenosine increase was dependent on the activation of DP_1R and that endogenous adenosine acting at $A_{2A}Rs$ may be a mediator of the PGD_2-induced sleep (Fig. **1**).

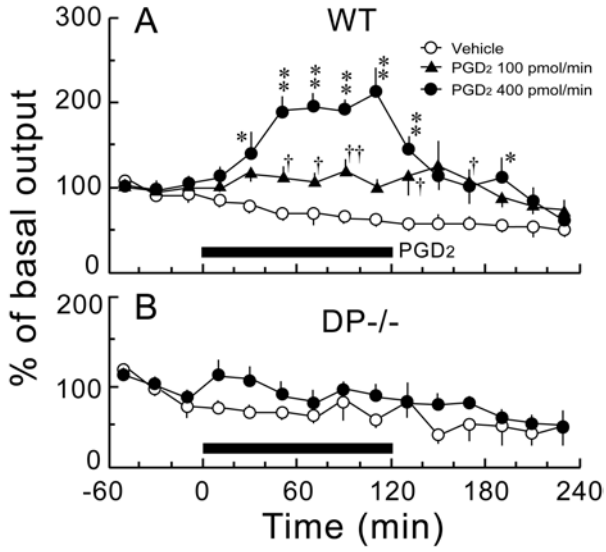

Fig. (2). Effect of PGD_2 perfusion on the extracellular adenosine level in the subarachnoid space below the rostral basal forebrain of WT and $DP^{-/-}$ mice. Vehicle or PGD_2 was perfused for 2 h (black bar) into the subarachnoid space below the rostral basal forebrain of WT (***A***) and $DP^{-/-}$ (***B***) mice under anesthesia. The basal level of adenosine during 1 h before the PGD_2 perfusion was 0.46 ± 0.04 pmol/20 µl in WT mice and 0.24 ± 0.04 pmol/20 µl in $DP^{-/-}$ mice. The data are expressed as a percentage of the baseline value (mean ± SEM, n = 5–8). *, †, $P < 0.05$; **, ††, $P < 0.01$, compared with the vehicle group. Adapted from Mizoguchi *et al.* [13] with modification and permission.

3. FORMATION, METABOLISM, AND TRANSPORT OF ADENOSINE IN THE CNS

Adenosine is a nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) via a β-N_9-glycosidic bond for its chemical structure, see Fig. (**3**). It is formed inside cells or on their surface and is mainly formed by the breakdown of intra- or extracellular adenine nucleotides [21]. Inside the cell, adenosine can be formed from adenosine triphosphate (ATP) by intracellular 5'-nucleotidase and then transported outside the cell via bidirectional nucleoside transporters, or it can be generated outside the cell by the metabolism of released nucleotides by ecto-nucleotidases. Two intracellular 5'-nucleotidase enzymes have been cloned, with cytosolic nucleotidase (cN)-I breaking down AMP to adenosine, and cN-II breaking down inosine 5'-monophosphate and guanosine monophosphate to inosine and guanosine, respectively [22]. There is a family of ecto-nucleotidases that can generate adenosine from ATP, but ecto-5'-nucleotidase is the major enzyme responsible for this under physiological conditions [23]. Adenosine can also arise from cyclic adenosine 3', 5'-monophosphate (cAMP), either generated inside the cell by a G-protein-coupled receptor with subsequent conversion to AMP by phosphodiesterase, or following the efflux of cAMP by a probenecid-sensitive transporter and metabolism by ecto-enzymes [24-25]. However, the conversion of cAMP to AMP by phosphodiesterase is slow, and activation of adenosine receptors following increased availability of cAMP is limited [26]. A further source of adenosine inside the cell is hydrolysis of S-adenosyl homocysteine (SAH) [27], but this pathway is not important in the brain [28].

While ATP may function as a neurotransmitter in some brain areas [29], adenosine is neither stored nor released as a classical neurotransmitter since it does not accumulate in synaptic vesicles, but is released from the cytoplasm into the extracellular space through a nucleoside transporter. Intra- and extracellular adenosine concentrations are kept in equilibrium by means of equilibrating transporters, which mediate adenosine reuptake, the direction of the transport being dependent upon the concentration gradient on both sides of the membrane [30]. These equilibrating transporters are abundant in most cells, including astrocytes [31].

Extracellular adenosine appears through two major mechanisms: (1) the release of adenosine as such through nucleoside transporters [32] after an increase in the intracellular levels of adenosine; and (2) the extracellular formation of adenosine through the ectonucleotidase pathway on the release of adenine nucleotides, especially ATP.

The clearance of adenosine depends on nucleoside transporters and three kinds of enzymes, i.e., adenosine kinase (AK), adenosine deaminase (ADA), and S-adenosyl-homocysteine hydrolase (SAHH). The clearance of extracellular adenosine mostly occurs through the non-concentrating nucleoside transporters, and ecto-ADA deaminates adenosine to inosine [33]. The main intracellular metabolic pathways of adenosine are the formation of AMP by AK and the irreversible breakdown to inosine by ADA. Neurons appears

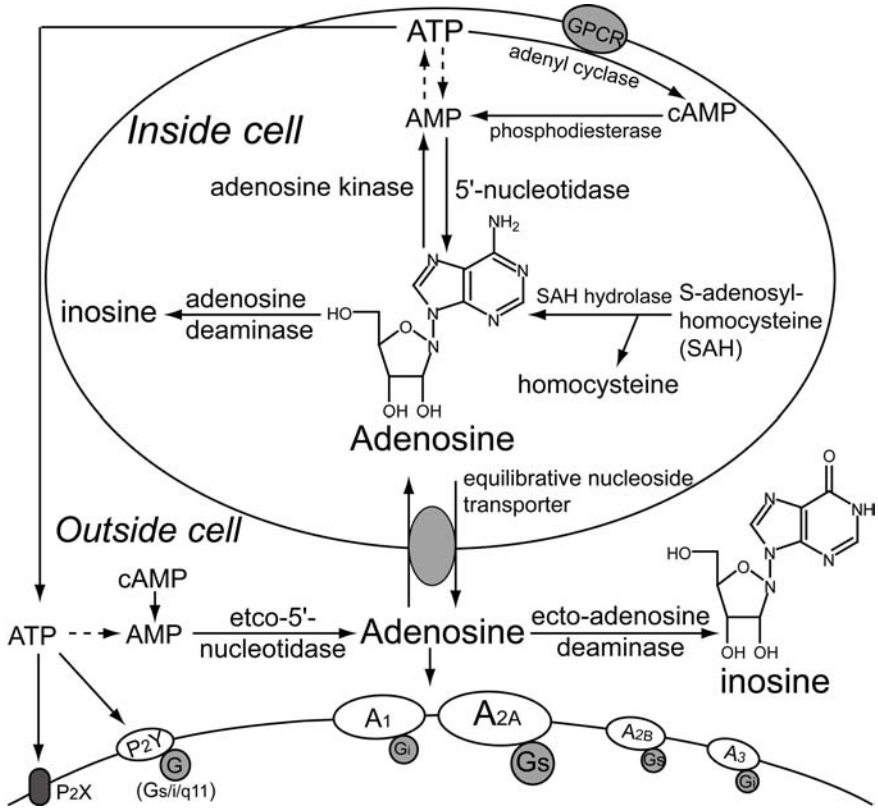

Fig. (3). Intracellular and extracellular pathways for the formation and metabolism of adenosine. Inside the cell, adenosine is formed from ATP, cAMP or SAH, while outside the cell it arises from equilibrating nucleoside transporter-mediated release or metabolism from ATP or cAMP. Adapted from Sawynok and Liu [97] with modification and permission.

to be enriched in AK, whereas ADA appears to be more abundant in astrocytes [33] and histaminergic neurons in the tuberomammillary nuclei (TMN) [34-35]. A third enzyme, SAHH, converts adenosine to S-adenosyl-homocysteine in cardiomyocytes, yet may be less important in the CNS [33].

4. INCREASE IN THE EXTRACELLULAR ADENOSINE LEVEL PROMOTES SLEEP

In 1954, Feldberg and Sherwood [36] showed that intraventricular injection of micromole quantities of adenosine into cats caused a state resembling natural sleep of 30-min duration. Subsequent pharmacological studies from several laboratories demonstrated that adenosine and its receptor agonists promoted, but antagonists such as caffeine, inhibited both NREM and REM sleep (for reviews, see refs. [3, 37-38]).

Under physiological conditions, the actions of highly active ADA, adenosine transport or AK are important particularly when large amounts of adenosine have to be cleared. Thus, inhibition of adenosine metabolism by blocking AK with ABT-702 [39] and ADA with (deoxy) coformycin [35, 40-41], or microdialysis perfusion with an adenosine transport inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBTI) in the cholinergic basal forebrain [42], increases extracellular adenosine levels, prolongs sleep and increases slow wave activity (SWA) in the EEG.

These pharmacological data in rats are consistent with genetic findings in mice showing that a genomic region encoding genes which contribute to the regulation of extracellular adenosine levels modifies the rate at which the need for NREM sleep accumulates during wakefulness [43]. In humans, a genetic variant of ADA, which is associated with the reduced metabolism of adenosine to inosine, has been reported to specifically enhance deep sleep and SWA during sleep, suggesting that genetic variability in the adenosine system contributes to the inter-individual variability in brain electrical activity during sleep and wakefulness [44].

Sleep deprivation is a useful way to provide a physiological challenge to promote sleep homeostasis. Elevated sleep propensity after prolonged wakefulness is counteracted not only by prolonged sleep duration, but also by enhanced sleep intensity [45], such as EEG SWA and spindle frequency activity (power within 11–15 Hz). Sleep deprivation increases local adenosine levels in the basal forebrain, cortex and hippocampus in rats and cats during prolonged wakefulness. Enhanced sleep intensity and adenosine levels decline during recovery sleep [42, 46-47]. Because changes in the adenosine level appear to be more pronounced in the basal forebrain than in other cerebral regions [48], the local increase in the extracellular level of adenosine in the basal forebrain was proposed to provide a signal for the homeostatic regulation of NREM sleep (see refs.[37, 49] for reviews). Recent evidence indicates that, during sleep deprivation, nitric oxide production in the basal forebrain acts to induce sleep through adenosine production [50].

The source of extracellular adenosine from neurons and glia for sleep regulation in the CNS still remains in debate. Sleep is regulated by a homeostatic process that has long been thought to involve adenosine originating from neurons [51]. Astrocytes release ATP and glutamate via many pathways including exocytosis [52-53] and regulate extracellular adenosine by releasing ATP. Conditional astrocyte-selective expression of the SNARE domain of the protein synaptobrevin II (dnSNARE) prevents both tonic and activity- dependent extracellular accumulation of adenosine [54]. Halassa *et al.* [55] showed that the dnSNARE transgenic mice lost their compensatory response to sleep deprivation, such as enhancement of SWA and an increase in sleep amount that occurs in WT mice, suggesting the importance of the glia-dependent accumulation of adenosine for sleep drive and homeostasis.

5. PREDOMINANT ROLES OF A_{2A}R IN SLEEP REGULATION BY ADENOSINE

There are four adenosine receptor subtypes, all of which are G-protein-coupled receptors (GPCR): A_1 and A_3 are primarily coupled to the Gi family of G proteins; whereas A_{2A} and A_{2B} are mostly coupled to Gs, like G proteins [33]. Stimulation of A_1Rs inhibits adenylate cyclase through activation of Gi proteins, activates phospholipase C (PLC), opens several types of K^+ channels, and inactivates Q-, P- and N-type Ca^{2+} channels [33, 56]. On the other hand, activation of the A_{2A}R subtype increases adenylate cyclase activity through activation of Gs or Golf (in the striatum) proteins, induces the formation of inositol phosphates, and activates protein kinase C [33, 56]. Accumulated findings indicate that both A_1 and A_{2A}R are involved in sleep induction, with A_{2A}R being more important in sleep induction by adenosine. The A_{2B}R is expressed widely, but generally at very low levels; whereas the A_3R is expressed at intermediate levels in the hippocampus and cerebellum [57]. Little is known about the functional significance of A_{2B}R and A_3R in sleep. The major properties of adenosine receptors are shown in Table **1**.

Evidence accumulated in recent years indicates that the A_{2A}R plays a key role in the effects of adenosine on sleep. The A_{2A}Rs are present at high concentration in the CNS, mainly in the striatum, nucleus accumbens, and olfactory bulb [58]. In rats, selective A_{2A}R agonists such as CGS21680 administered to the subarachnoid space adjacent to the basal forebrain and lateral preoptic area reliably induce NREM sleep, whereas infusion of A_1R agonists produces weak and variable effects [59-62]. When infused into the medial pontine reticular formation in rats, CGS 21680 was 10-fold more potent than the A_1R agonist N^6-cyclohexyl-adenosine, in inducing REM sleep [63]. Sleep induced by the A_{2A}R agonist CGS 21680 is followed by a strong rebound of wakefulness after the cessation of CGS 21680 infusion [64]. The major site responsible for A_{2A}R-mediated sleep is apparently located in or near the rostral basal forebrain, as shown by the sleep-promoting effect and c-Fos expression after local infusion of CGS 21680 there [65]. Furthermore, CGS 21680–induced sleep is almost completely abolished in A_{2A}R knockout mice, confirming the specificity of CGS 21680 for A_{2A}Rs [62].

A possible anatomical pathway for A_{2A}R-mediated PGD_2-induced sleep was mapped by detecting Fos-positive neurons [61, 65]. When PGD_2 or the A_{2A}R agonist

Table 1. Roles of Adenosine Receptors in Sleep-Wake Regulation

Receptor	A_1R	$A_{2A}R$	$A_{2B}R$	A_3R
Expression High	Cortex, cerebellum, hippocampus	Caudate putamen, nucleus accumbens, tuberculum olfactorium, olfactory bulb		
Intermediate	Other brain regions		Median eminence	Cerebellum (human?), hippocampus (human?)
Low		Rest of brain		most of brain (rat, mouse)
G protein	Gi, Go	Gs, Golf	Gs, Gq	Gi
Chromosomal location	chr 1q32.1	chr 22g11.2	chr 17p11.2-12	chr 1p21-13
Selective agonist	CPA, CCPA, CHA	CGS 21680, HE-NECA, CV-1808, CV-1674, ATL146e	None	Cl-IB-MECA
Selective antagonist	DPCPX 8-cyclopentyltheophylline, WRC0571	SCH 58261 moderately: ZM 241385, KF 17387, CSC	MRS1754, enprofylline, 1-butyl-8-[4-(4-benzyl) (piperazino-2-oxyethoxy) phenyl] xanthine	MRS1220, MRE3008-F20, MRS1191; MRS1523
Roles in animal sleep	Activation of A_1R in basal forebrain[89], TMN [35], and lateral hypothalamus [78,94] induces sleep; whereas activation of A_1R in the lateral preoptic area promotes wakefulness [59]	$A_{2A}R$ agonists administered to the brain induce a dramatic increase in sleep [4,60,65,67]; arousal effect of caffeine is seen in A_1R KO, but not in $A_{2A}R$ KO mice [70]	None reported	None reported
Roles in human sleep	Prolonged wakefulness induces A_1R up-regulation [85]	Variations in $A_{2A}R$ gene contribute to individual sensitivity to caffeine effects on sleep [95]	None reported	None reported

From Fredholm *et al.* [33] and Fredholm [96] with modification. Abbreviations: CHA, N6-cyclohexyl-adenosine; CCPA, 2-chloro-N6-cyclopentyladenosine; CGS 21680, 2-p-(2-carboxyethyl) phenethylamino-5'-N-ethylcarboxamido adenosine hydrochloride; HE-NECA , 2-hexynyl- 5'-(N-ethylcarboxamido) adenosine; CV-1808, 2-phenylaminoadenosine; CV 1674, 2-(4-methoxyphenyl) adenosine; ATL-146e, 4-{3-[6-Amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester; IB-MECA, 1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9H-purine-9-yl]-N-methyl-D-ribofuranuronamide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; WRC-0571, 8-(N-Methylisopropyl) amino -N-(5'-endohydroxy-endonorbornyl)-9-methyladenine; SCH-58261, 5-Amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo(4,3-e)-1,2,4-triazolo(1,5-c)pyrimidine; ZM 241385, 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]t riazin-5-ylamino]ethyl)phenol; KF 17387, 1,3-dipropyl-8-[3,4-dimethoxystyryl]-7-methylxanthine; CSC, (8-(3-Chlorostyryl) caffeine; MRS 1754, N-(4-Cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)phenoxy]acetamide; Enprofylline, 3-n-propylxanthine; MRS-1191, 3-ethyl - 5-benzyl-2-methyl-6-phenyl- 4-phenylethynyl-1,4-(+)-dihydropridine-3,5-dicarboxylate; MRS-1220, 9-chloro-2-(2-furyl)-5-phenylacetylamino-[1,2,4]-triazolo[1,5-c]quinazoline; MRS 1523, 6-Ethyl-5-(ethylsulfanylcarbonyl)-2-phenyl-4-propylpyridine-3-carboxylic acid propyl ester; MRE 3008F20, 5N-(4-methoxyphenylcarbamoyl) amino-8-propyl-2-(2-furyl) pyrazolo [4,3-e] -1,2,4- triazolo[1,5-c]pyrimidine.

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, which increase was concomitant with the induction of NREM sleep [61, 65]. In contrast, the number of Fos-positive neurons decreased markedly in the 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 [66].

Inhibition of the histaminergic system promotes sleep. *In vivo* microdialysis experiments revealed that infusion of an adenosine $A_{2A}R$ agonist, CGS21680, into the subarachnoid space of 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 the TMN but not in the frontal cortex [67]. The CGS21680-induced inhibition of histamine release was antagonized by perfusion of the TMN with a $GABA_A$ antagonist, picrotoxin, suggesting that the $A_{2A}R$ 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 [68].

The local application of CGS21680 increases c-fos expression in the VLPO [61], but $A_{2A}R$ seems to be undetectable or very weakly expressed in this brain region. Direct activation of sleep-promoting VLPO neurons via postsynaptic stimulation of $A_{2A}R$ was demonstrated in VLPO slices [69]. The intracellular recording of VLPO neurons in rat

brain slices demonstrated the existence of two distinct types of VLPO neurons in terms of their responses to serotonin and adenosine. VLPO neurons were inhibited uniformly by two arousal neurotransmitters, noradrenaline and acetylcholine, and mostly by an adenosine A_1R agonist. Serotonin inhibited the type-1 neurons but excited the type-2 neurons. An $A_{2A}R$ agonist excited 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 type-1 neurons are activated only when released from inhibition by arousal systems [69]. Furthermore, the administration of CGS21680 to the rostral basal forebrain produced predominant expression of Fos within the shell of the nucleus accumbens and the medial portion of the olfactory tubercle. The microdialysis perfusion of CGS21680 into the shell of the nucleus accumbens also exhibited a sleep-promoting effect [65]. Scammell *et al.* [61] suggested that activation of adenosine $A_{2A}Rs$ in cells of the leptomeninges or nucleus accumbens would increase the activity of the VLPO. These VLPO neurons may then coordinate the inhibition of multiple wake-promoting regions, resulting in sleep.

Acting opposite to adenosine, caffeine promotes wakefulness. Caffeine binds to A_1R and $A_{2A}R$ with similar high affinities and acts as an antagonist for both receptor subtypes [58]. We used gene-manipulated mice to demonstrate that caffeine promoted wakefulness in WT and A_1R KO mice but not at all in $A_{2A}R$ KO mice (Fig. **4**), indicating that the arousal effect of caffeine was due to blockade of the $A_{2A}R$, not the A_1R [70]. Caffeine may also reduce the hypnotic effects of alcohol via $A_{2A}R$ [71]. These results strongly suggest a predominant role of $A_{2A}R$ in sleep regulation.

$A_{2A}Rs$ are abundantly expressed in the caudate–putamen, nucleus accumbens, and the tuberculum olfactorium, in which dopamine D_2Rs are co-localized [72]. Recently we demonstrated that the D_2R is important for the maintenance of wakefulness [73-75], whereas activation of $A_{2A}R$ induces potent sleep. Opposing effects of $A_{2A}R$ and D_2R have also been shown at the levels of neurotransmitter release, receptor binding, and gene expression [76-77]. Therefore, $A_{2A}R$ and D_2R are involved in sleep–wake regulation in a different and coordinate manner.

6. A_1R-MEDIATED EFFECTS ON SLEEP-WAKE CYCLES ARE BRAIN REGION DEPENDENT

The A_1Rs are widely expressed in the brain cortex, thalamus, hippocampus, and basal ganglia, as well as in the lateral hypothalamus and TMN [35, 57, 78]. Because of the widespread distribution of cerebral A_1Rs and the inhibition of excitatory neurotransmission following presynaptic A_1R activation, it has been generally assumed that adenosine affects sleep primarily via the A_1R. Several findings from pharmacological studies are compatible with this assumption. For example, i.p. or i.c.v. administration of the A_1R-selective agonist N^6-cyclopentyladenosine (CPA) to rats increases NREM sleep, suppresses REM sleep, and induces changes in the NREM sleep EEG similar to those brought about by prolonged wakefulness [79-80]. Moreover, microdialysis perfusion of the rat basal forebrain with A_1R antisense oligonucleotides reduces NREM sleep and increases wakefulness [81]. *In vitro* electrophysiological studies demonstrated that adenosine had a post-synaptic inhibitory effect via A_1R on basal forebrain neurons, as well as neurons in the cholinergic laterodorsal tegmental nuclei [82-83]. Christie *et*

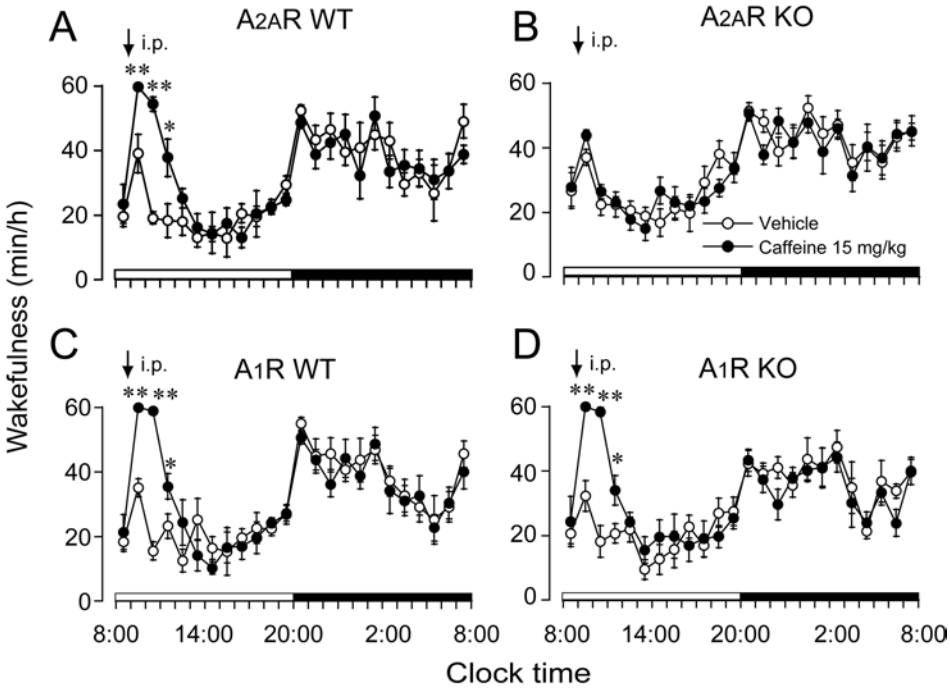

Fig. (4). Time-course of changes in wakefulness after caffeine 15 mg/kg treatment of $A_{2A}R$ WT (**A**), A_{2A} R KO (**B**), A_1R WT (**C**), and A_1R KO (**D**) mice. Each circle represents the hourly mean ± SEM (n =5-7). The arrows indicate the injection time (9 a.m.). *, $P < 0.05$; **, $P < 0.01$, significantly different from the vehicle, by the paired *t*-test. Adapted from Huang *et al.* [70] with modification and permission.

al. suggested that sleep loss induces elevations of basal forebrain adenosine acting via A_1R, leading to increased sleepiness and impaired vigilance [84]. During the rat Psychomotor Vigilance Task performance, response latencies and performance lapses of rats increased significantly after adenosine was dialyzed in the basal forebrain of rats when compared with baseline (no dialysis) or vehicle dialysis sessions. The codialysis of A_1R antagonist, 8-cyclopentyltheophylline with adenosine completely blocked the effects produced by adenosine alone, resulting in performance equivalent to that of the vehicle sessions. These reports suggest that the somnogenic effect of adenosine is mediated by A_1R in the cholinergic region of the basal forebrain. In addition, both in humans [85] and in rats [86] A_1R binding was increased after prolonged wakefulness/sleep deprivation, presumptively due to increases in adenosine, as demonstrated for the basal forebrain [87].

Although local administration of adenosine or an A_1R agonist into the basal forebrain results in an increase in NREM sleep, infusion of the A_1R agonist CPA into the lateral ventricle of mice does not alter the amounts of NREM and REM sleep [62], suggesting that activation of A_1R in other brain regions may cause wakefulness. Methippara *et al.* [59] studied the effects on sleep of an adenosine transport inhibitor, NBTI, and A_1R agonists/antagonists by microdialyzing them into the lateral preoptic area. The results showed that A_1R stimulation or inhibition of adenosine transport by NBTI induced wakefulness. Furthermore, the homeostatic facet of sleep–wake regulation is unaltered in animals lacking A_1Rs [88]. These observations suggest that adenosine-mediated effects on sleep-wake cycles are site and receptor dependent and that A_1Rs may not be absolutely necessary for sleep homeostasis.

Blanco-Centurion *et al.* [89] reported that adenosine levels in the basal forebrain did not increase after 6 h of prolonged wakefulness in rats with 95% of their basal forebrain cholinergic neurons lesioned. The lesioned rats had an intact sleep drive after 6 and 12 h of prolonged wakefulness. In the absence of cholinergic neurons in the basal forebrain, another selective A_1R agonist, N^6-cyclohexyladenosine, effectively induced sleep after administration to the basal forebrain. Thus, neither the activity of cholinergic neurons nor the accumulation of adenosine in the basal forebrain during wakefulness is necessary for the sleep drive. However, Kalinchuk *et al.* [90] have found that lesions of the cholinergic basal forebrain neurons abolish both the increase of adenosine levels and homeostatic sleep drive. These findings leave open the possibility raised by both studies that A_1R on noncholinergic neurons could affect sleep and that the hypnogenic response to adenosine may not be specific to the basal forebrain [91]. To understand the roles of noncholinergic and cholinergic basal forebrain neurons in spontaneous sleep–waking and EEG, and in homeostatic sleep regulation, Kaur *et al.* [92] lesioned noncholinergic basal forebrain neurons with ibotenate, and cholinergic neurons with 192-IgG saporin, and found that the noncholinergic basal forebrain neurons promoted cortical activation by inhibiting delta waves, that the cholinergic basal forebrain neurons played a nonexclusive role in promoting wakefulness, and that both types of basal forebrain neurons played important roles in the increases in NREM sleep and EEG delta power after sleep loss.

Inhibition of the adenosine system via A_1R in the orexin neuronal regions [93] and histaminergic TMN [35] are also reported to promote NREM sleep. Thakkar *et al.* [78] reported that 30% of the orexin-containing neurons were immunoreactive with A_1R antibody. Perfusion with the A_1R agonist CPA significantly suppressed the sleep-wake discharge activity of perifornical-lateral hypothalamic neurons and suppressed arousal [94]. Recently, we found that adenosine A_1R is also abundant in the TMN. Bilateral injection into the rat TMN of the A_1R agonist CPA significantly increased the amount of NREM sleep. The bilateral injection of adenosine or an inhibitor of ADA, coformycin, into the rat TMN also increased NREM sleep (Fig. **5**), which was completely abolished by co-administration of 1,3-dimethyl-8-cyclopenthylxanthine, a selective A_1R antagonist [35]. These results indicate that endogenous adenosine in the TMN suppresses the histaminergic system via A_1Rs to promote NREM sleep.

7. POTENTIAL APPLICATION OF ADENOSINE RECEPTOR AGONISTS TO SLEEP DISORDERS

Although many hypnotic and antihypnotic drugs are on the market, most of these compounds exhibit a variety of undesirable side effects. Recent progress in our understanding of the molecular mechanism underlying sleep-wake regulation by adenosine described in this paper may provide a valid and useful approach for the development of drugs for more rational treatment of sleep disorders based upon the pharmacological use of enzyme inhibitors and receptor-specific therapies. The concept of using adenosine receptor agonists as modulators for sleep disorders is intriguing, however, in practice this would be dependent on brain-selective receptor activation [56]. It must be emphasized that much more work is necessary to elucidate the detailed mechanisms of sleep-wake regulation in terms of these mediators.

CONCLUSIONS

The roles of adenosine receptors in sleep-wake regulation are summarized in Table **1**. The most potent endogenous sleep-promoting factor, adenosine, accumulates in the brain during wakefulness and induces physiological sleep. Among adenosine receptors responsible for sleep induction, the role of $A_{2A}R$ is predominant in sleep regulation since the selective $A_{2A}R$ agonist CGS21680 administered to the subarachnoid space adjacent to the basal forebrain and lateral preoptic area reliably induces a dramatic increase in NREM sleep, whereas the infusion of A_1R agonists produces weak and variable effects [4, 59, 61-62, 67]. A_1Rs contribute to sleep induction in a region-dependent manner but may not be absolutely necessary for sleep homeostasis. At the present time little is known about how and where adenosine originates under physiological conditions or after sleep deprivation.

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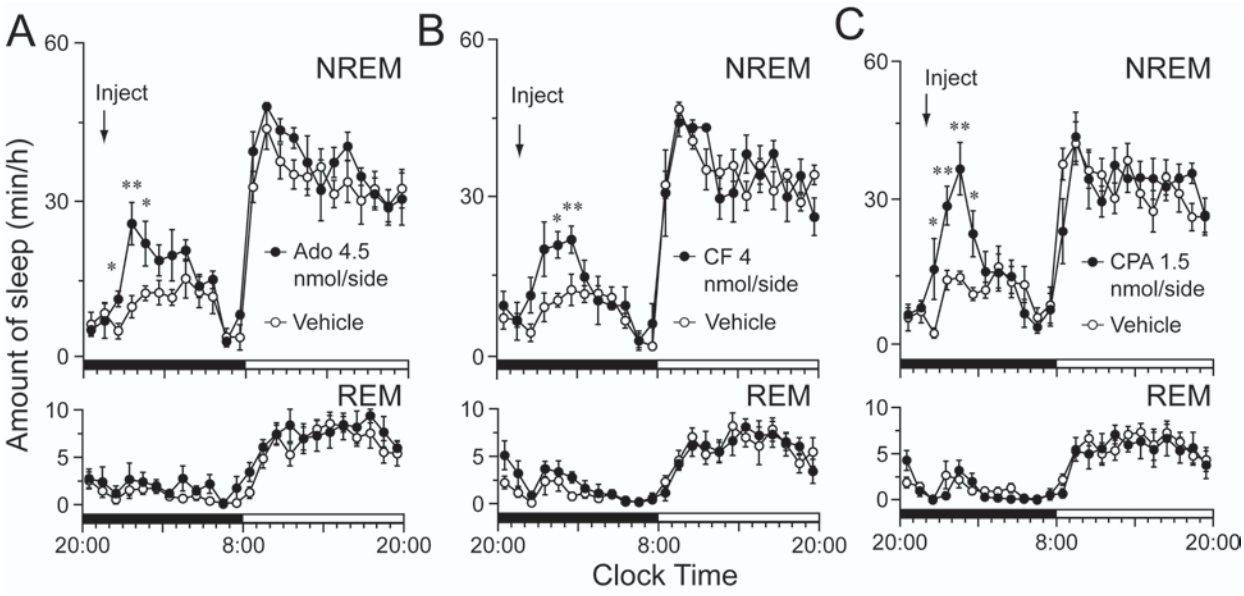

Fig. (5). Time courses of NREM and REM sleep in rats administered adenosine (Ado) at 4.5 nmol/side (**A**) , ADA inhibitor coformycin (CF) at 4 nmol/side (**B**) or A_1R agonist CPA at 1.5 nmol/side (**C**). Values are means ± SEM (n=5–8). *, $P<0.05$; **, $P<0.01$, significantly different from the vehicle injection. Adapted from Oishi *et al.* [35] with modification and permission.

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ABBREVIATIONS

R	=	Receptor
ADA	=	Adenosine deaminase
AK	=	Adenosine kinase
ATP	=	Adenosine triphosphate
cAMP	=	Cyclic adenosine 3', 5'-monophosphate
cN	=	Cytosolic nucleotidase
CPA	=	N6-cyclopentyladenosine
CSF	=	Cerebrospinal fluid
dnSNARE	=	The SNARE domain of the protein synaptobrevin II
DP_1R	=	PGD_2 receptor
GPCR	=	G-protein-coupled receptors
H_1R	=	Histamine H_1 receptor
H-PGDS	=	Hematopoietic PGDS
KO	=	Knock-out
L-PGDS	=	Lipocalin-type PGDS
NBTI	=	S-(4-nitrobenzyl)-6-thioinosine
NREM	=	Non-rapid eye movement
PLC	=	Phospholipase C
PG	=	Prostaglandin
PGDS	=	PGD synthase
REM	=	Rapid eye movement
SAHH	=	S-adenosyl-homocysteine hydrolase
Se^{4+}	=	Tetravalent selenium
$SeCl_4$	=	Selenium tetrachloride
SWA	=	Slow wave activity
TMN	=	Tuberomammillary nucleus
VLPO	=	Ventrolateral preoptic area
WT	=	Wild type

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