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Involvement of adenosine A_{2A} receptor in sleep promotion

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Abstract

We examined the sleep-modulatory effects of four adenosine agonists, namely, (1) 2-(4-(2-carboxyethyl)phenylethylamino)adenosine-5′-*N*-ethylcarboxamideadenosine (CGS21680), a highly selective adenosine A_{2A} receptor agonist; (2) 2-(4-(2-(2-aminoethylamino-carbonyl)ethyl)phenylethylamino)-5′-*N*-ethylcarboxamidoadenosine (APEC), a selective adenosine A_{2A} receptor agonist; (3) 5′-*N*-ethyl-carboxamidoadenosine (NECA), a nonselective adenosine A_1/A_2 receptor agonist, and (4) N^6-cyclopentyladenosine (CPA), a selective adenosine A_1 receptor agonist. Each agonist was administered in the subarachnoid space underlying the rostral basal forebrain of rats through chronically implanted cannulae at the rate of 0.02, 0.2, 2.0, 12.0, or 20.0 pmol/min over a 6-h period starting from 2300 h, which period is the active phase of the animals. CGS21680, APEC, and NECA produced significant increases in the total amounts of non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep after at least one dose within the range of administration rates. CPA did not produce any significant increase in the total amount of either type of sleep at any of the above administration rates, but instead suppressed REM sleep at the administration rates of 12.0 and 20.0 pmol/min. These results indicate that the activities of adenosine A_{2A} receptors are crucially involved in the promotion of sleep.

Keywords: Adenosine A_{2A} receptor; Non-rapid-eye-movement (NREM) sleep; Rapid-eye-movement (REM) sleep; Brain temperature; (Rat)

1. Introduction

A series of papers from this and other laboratories during the past 15 yr showed prostaglandin D_2 to induce physiological sleep in mice, rats, monkeys, and probably in humans (Hayaishi, 1997). The site of action of prostaglandin D_2 for the promotion of sleep was located on the ventral surface of the rostral basal forebrain in rats, and administration of prostaglandin D_2 in any other part of the brain was hardly effective (Matsumura et al., 1994). More recently, we found that CGS21680, a selective adenosine A_{2A} receptor agonist, also promoted sleep when it was infused into or near the prostaglandin D_2-sensitive zone, namely, the subarachnoid space underlying the rostral basal forebrain. The sleep profile induced by CGS21680 was clearly different from that induced by N^6-cyclohexyladenosine (CHA), a selective adenosine A_1 receptor agonist. The sleep-promoting effect of the former was attenuated by pretreatment with (*E*)-1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine (KF17837), a selective adenosine A_2 receptor antagonist. In the light of these results, we suggested that the activation of adenosine A_{2A} receptors was involved in the promotion of sleep (Satoh et al., 1996).

Several lines of experimental evidence indicate that adenosine is involved in the regulation of sleep–wake phenomena (Haulicã et al., 1973; Radulovacki et al., 1983a; Rainne et al., 1994; Portas et al., 1997). To date, the receptors for adenosine have been pharmacologically and structurally classified into A_1, A_{2A}, A_{2B}, and A_3 (reviewed by Fredholm et al., 1994). A role for the adenosine A_1 receptor in the promotion of sleep has been proposed, based on the findings that the intraperitoneal injection of approximately 0.3–9 μmol/kg of N^6-(L-phenylisopropyl)adenosine (L-PIA), CHA, or CPA, all selective adenosine A_1 receptor agonists, and the direct administration of approximately 0.3–30 nmol of CPA into the lateral ventricle, promoted deep slow wave sleep in rats (Radulovacki et al., 1982, 1983b; Ticho and Radulovacki, 1991; Benington et al., 1995; Schwierin et al., 1996). On

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the other hand, the intraperitoneal injection of selective adenosine A_{2A} receptor agonists, such as CGS21680 (5.6–560 nmol/kg) and 2-hexynyl-5′-*N*-ethylcarboxamide-adenosine (2HE-NECA; 77–770 nmol/kg) did not promote sleep in rats (Monti et al., 1995; Bertorelli et al., 1996).

In order to further clarify the involvement of adenosine A_{2A} receptors in sleep promotion, we compared the sleep-modulatory potencies of four adenosine agonists that have different spectra of affinity and selectivity for the adenosine receptor subtypes. We now confirmed and extended results of our previous study on the effect of CGS21680. We also examined the effects of three other adenosine agonists, namely, APEC, NECA, and CPA. The K_i (nM) values of these agonists for adenosine A_1, A_{2A}, and A_3 receptors and the EC_{50} (nM) values of these agonists for the adenosine A_{2B} receptor are listed in Table 1 (Hide et al., 1991; Rivkees and Reppert, 1992; Kim et al., 1994; Daly and Jacobson, 1995). CGS21680 is a highly selective adenosine A_{2A} receptor agonist, whereas APEC is also a selective adenosine A_{2A} receptor agonist, but with less selectivity for this receptor subtype than CGS21680. NECA is a nonselective adenosine A_1/A_2 receptor agonist. In contrast, CPA is a selective adenosine A_1 receptor agonist. All these compounds are water soluble, which property is essential for our sleep research that involves a continuous infusion method, since a solvent like dimethyl sulfoxide (DMSO) itself often changes the sleep–wake pattern (personal observation).

As the sleep-promoting effect of CGS21680 was initially found in a nocturnal experiment (Satoh et al., 1996), we also examined the sleep-modulatory effect of CGS21680 by administering it over a 6-h period during the rest phase of the animal, i.e., the counter-period of the nocturnal experimental period, to determine whether the effect of the compound is influenced by the timing of its administration.

The results of the present study together with those of our previous study clearly indicate that the adenosine A_{2A} receptor is involved in sleep promotion. This effect of adenosine A_{2A} receptor agonist is observed during the night but not during the day.

Table 1
Receptor selectivity of the adenosine agonists

Agonist	Class	K_i (nM)			EC_{50} (nM)
		A_1	A_{2A}	A_3	A_{2B}
CGS21680	A_{2A}	2600[ad]	15[ad]	584[ad]	nearly inactive[bc]
APEC	A_{2A}	400[a]	5.7[a]	50[a]	nearly inactive[c]
NECA	A_1/A_2	6.3[d]	10[d]	110[d]	1900[b]
CPA	A_1	0.6[ad]	462[ad]	240[ad]	66000[b]

[a] Taken from Kim et al. (1994).
[b] Taken from Rivkees and Reppert (1992).
[c] Taken from Hide et al. (1991).
[d] Taken from Jacobson et al. (1993).

2. Materials and methods

2.1. Animals

Adult male rats of the Sprague–Dawley strain, weighing 300–350 g, were supplied from Japan SLC ($n = 162$). They were maintained for 7–10 days before the surgical operation under constant environmental conditions of controlled relative humidity ($60 \pm 5\%$) and room temperature (25 ± 0.5°C) on a 12-h light/12-h dark schedule (lights on at 0800 h), which were the same environmental conditions as those of the experimental chambers. They were permitted free access to food and water.

2.2. Surgery

Under deep pentobarbital anesthesia (50 mg/kg body weight, i.p.), each rat underwent a surgical operation for the implantation of electrodes to record the electroencephalogram (EEG) and electromyogram (EMG) and of a thermistor probe to monitor brain temperature. In some cases, implantations of electrodes to record the electrooculogram (EOG) were performed in addition. For delivery of agonists, paired stainless-steel cannulae were inserted according to the stereotaxic coordinates from the brain atlas of Paxinos and Watson (1986): AP + 1.5 mm, ML + 1.7 mm, $D - 8.3$ mm from the brain surface with an angle of 10° towards the midline from the parasagittal plane. Thus, the tips of the cannulae were situated in the subarachnoid space underlying the rostral basal forebrain.

2.3. Experimental protocol

The protocol adopted in this study was essentially the same as that described before (Matsumura et al., 1994; Satoh et al., 1996); After a period of 7–10 days for recovery from surgery, the rats were set inside the experimental chamber, the interior of which was maintained under the same conditions as described above. Continuous infusion of saline through the infusion cannulae was commenced, and this infusion lasted until the end of the experimental session except for the period when the test compound was infused. The infusion speed of vehicle and test solution was set at 0.2 μl/min. After a period of 4 days for acclimation to the experimental milieu, continuous recordings of EEG, EMG and brain temperature were started at 2000 or 0800 h. In some cases, continuous recordings of EOG were also additionally performed. The subsequent two 24-h periods were designated as baseline day and experimental day, respectively. Each rat received only one experimental infusion according to the following protocols:

CGS21680, APEC, NECA and CPA were each dissolved in saline to make 0.05, 0.5, 5.0, 30 and 50 μM solutions. The bilateral infusion of each test solution re-

sulted in the total administration rate of 0.02, 0.2, 2.0, 12.0 and 20.0 pmol/min, respectively. The baseline day was started at 2000 h. The continuous infusion of saline was replaced by that of a particular test solution from 2300 h to 0500 h on the experimental day. For the diurnal infusion experiments, CGS21680 was dissolved in the saline solution to make 0.5, 5.0 and 30 μM solutions (corresponding to 0.2, 2.0, and 12.0 pmol/min, respectively, for the total rate of the bilateral infusion). The baseline day was started at 0800 h. The continuous infusion of saline was replaced by that of the CGS21680 solution between 1100 and 1700 h on the experimental day.

After the end of each experimental session, 2% methylene blue solution was continuously administered for 3 h at the infusion rate of 0.2 μl/min. Then, the rats were deeply anesthetized with pentobarbital sodium and perfused transcardially with saline solution, followed by 0.1 M phosphate buffer/4% paraformaldehyde. The location of cannula tips was identified from blue spots on the ventral surface of rostral basal forebrain. In some cases, the brains were postfixed and then transferred to 0.1 M phosphate buffer/20% sucrose until equilibration. The brains were cut coronally at a thickness of 50 μM with a freezing microtome. The location of cannula tips was also identified from the cannula traces within the section.

2.4. Analytical procedure

The recordings of EEG and EMG from each animal were scored as non-rapid-eye-movement (NREM) sleep, rapid-eye-movement (REM) sleep, or wakefulness by visual inspection according to the criteria described earlier (Matsumura et al., 1994). Thus, light slow wave sleep and deep slow wave sleep were combined and treated as NREM sleep. An episode of vigilance state lasting less than 15 s was not treated as an independent episode but was included in the preceding episode. The values obtained from experimental administration were compared with corresponding values from the baseline recordings (control), and statistical analysis was done with a paired *t*-test. The changes in sleep periods induced by different doses of a given adenosine receptor agonist, were compared by means of one-way analysis of variance (One-way ANOVA). *P* values were corrected for multiple comparisons according to Scheffe's *F* post-hoc procedure. To compare the changes in sleep periods induced by CGS21680 with those induced by the other adenosine receptor agonists at the respective infusion rates, we used an One-way ANOVA. *P* values were corrected according to Dunnett's post-hoc procedure for comparing a control to all other means. $P < 0.05$ was considered significant.

2.5. Chemicals

Agonists 2-(4-(2-carboxyethyl)phenylethylamino)-adenosine-5′-*N*-ethylcarboxamideadenosine (CGS21680), 5′-*N*-ethylcarboxamidoadenosine (NECA), and N^6-cyclopentyladenosine (CPA) were purchased from Research Biochemicals (Natick, MA). The agonist, 2-(4-(2-(2-aminoethylaminocarbonyl)ethyl)phenylethylamino)-5′-*N*-ethylcarboxamidoadenosine (APEC), was provided by Research Biochemicals International as part of the Chemical Synthesis Program of the National Institute of Mental Health. (Contract N01MH3003)

3. Results

3.1. Dose–response studies

The means and standard error values for the total amounts of NREM sleep and REM sleep that occurred during the 6-h control periods (open circles and black bars) and experimental periods (closed circles and black bars) are summarized in Fig. 1 for the four adenosine receptor agonists given at different infusion rates. During the 6-h control period, the rats of each group showed nearly identical periods of sleep, about 100 min for NREM sleep and about 15 min for REM sleep.

Firstly, we evaluated the changes in sleep that occurred at different infusion rates of an adenosine receptor agonist. ANOVA revealed a significant variance in NREM sleep (CGS21680: $F(4,34) = 23.69$, $P < 0.01$; APEC: $F(4,25) = 19.51$, $P < 0.01$; NECA: $F(3,24) = 4.65$, $P < 0.05$; CPA: $F(4,29) = 3.24$, $P < 0.05$) and in REM sleep (CGS21680: $F(4,34) = 6.10$, $P < 0.01$; APEC: $F(4,25) = 12.11$, $P < 0.01$; NECA: $F(3,24) = 9.73$, $P < 0.01$; CPA: $F(4,29) = 3.99$, $P < 0.05$).

CGS21680 and APEC, selective adenosine A_{2A} receptor agonists, showed dose-dependent increases in NREM sleep within the dose range of 0.02–2.0 pmol/min. The increases in NREM sleep that occurred at the infusion rate of 2.0 pmol/min from the respective baseline values were 97.5 ± 4.4 min (a 99.5 ± 6.9% increase; paired *t*-test, $P < 0.001$) for CGS21680 and 116.4 ± 8.8 min (a 121.8 ± 14.3% increase; paired *t*-test, $P < 0.001$) for APEC, amounts which were significantly larger than those recorded at the infusion rate of 0.2 pmol/min (Scheffe's *F*, $P < 0.01$). The NREM sleep-promoting effect of CGS21680 reached its plateau at the administration rate of 2.0 pmol/min and was not further modified at the rate of 12.0 or 20.0 pmol/min. The NREM sleep-promoting effect of APEC was attenuated in a dose-dependent manner above 2.0 pmol/min, and this attenuation reached statistical significance at the infusion rate of 20.0 pmol/min (Scheffe's *F*, $P < 0.01$).

NECA, a nonselective A_1/A_2 adenosine receptor agonist, also produced an increasing tendency to NREM sleep within the dose range of 0.02–2.0 pmol/min. The increase in NREM sleep from the baseline value at the infusion rate of 2.0 pmol/min was 67.1 ± 16.3 min (a 74.5 ± 17.9% increase; paired *t*-test, $P < 0.001$); however, this amount

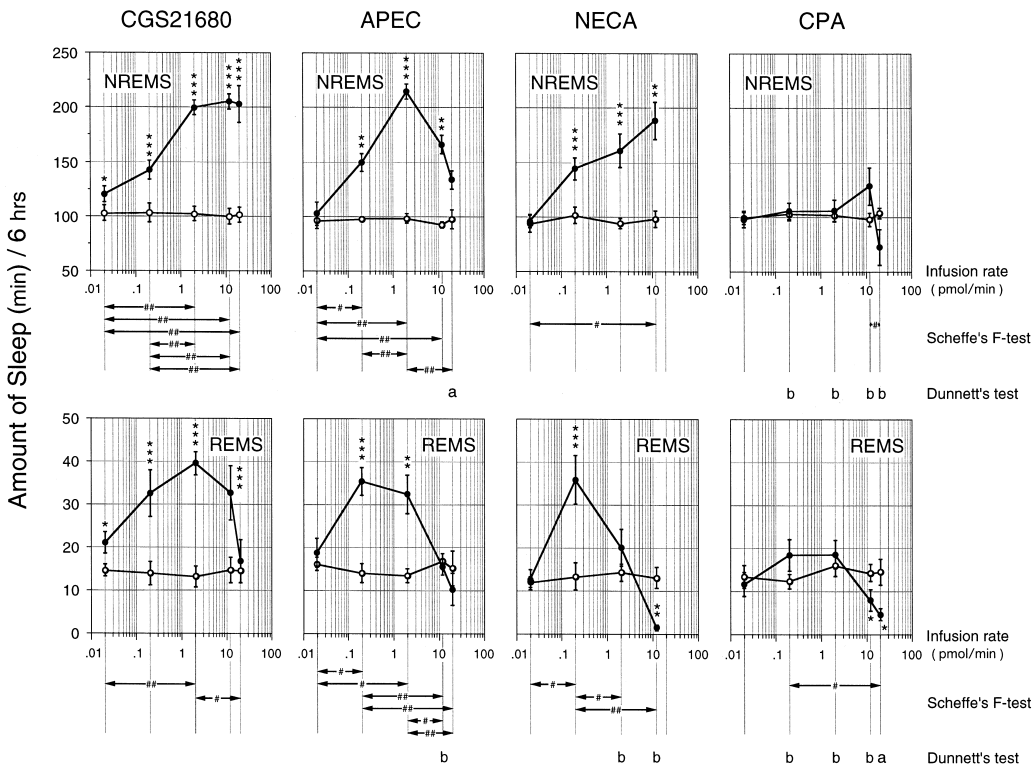

Fig. 1. Effects of adenosine agonists on sleep. CGS21680, APEC, NECA, and CPA were separately and continuously infused into the subarachnoid space underlying the rostral basal forebrain over a 6-h period during the active phase of rats, at administration rates of 0.02, 0.2, 2.0, 12.0, or 20.0 pmol/min. The total amounts (mean ± S.E.M.) of NREM sleep and REM sleep during the 6-h control period (open circles and black bars) or the experimental period (closed circles and black bars) at the respective administration rates are shown. $n = 5$–11 per each group; $*$, $P < 0.05$. $**$, $P < 0.01$. $***$, $P < 0.001$, vs. control by paired *t*-test. The changes in sleep induced by an adenosine agonist given at different infusion rates were compared with each other. Significance of differences was determined by one-way analysis of variance (One-way ANOVA) followed by Scheffe's *F*-test. Bars with arrowheads on both sides show the significance of differences ($^{\#}$, $P < 0.05$. $^{\#\#}$, $P < 0.01$) between the changes in sleep at the infusion rates indicated. The changes in sleep induced by CGS21680 at the various infusion rates were compared with those induced by APEC, NECA, and CPA at the respective rates. Significance of difference—(a) $P < 0.05$; (b) $P < 0.01$—was determined by One-way ANOVA followed by Dunnett's test.

was not significantly different from that found at the infusion rate of 0.2 pmol/min (Scheffe's F, $P = 0.745$). At the infusion rate of 12.0 pmol/min, NECA produced unusual synchronization of the EEG. NREM sleep episodes with a duration longer than 600 s often appeared in the latter half of the administration period, whereas the normal duration of an NREM sleep episode of a rat was within 450 s under our experimental conditions (data not shown).

As for REM sleep, CGS21680 and APEC showed a bell-shaped, dose–response relationship. The most effective dosage for the respective agonists and the increase in response to it were as follows: 2.0 pmol/min, 26.3 ± 1.4 min (a 305 ± 77.0% increase; paired *t*-test, $P < 0.001$) for CGS21680; 0.2 pmol/min, 21.3 ± 3.3 min for APEC (a 177.3 ± 32.8% increase; paired *t*-test, $P < 0.001$). These increases were significantly larger than those found at the infusion rate of 0.02 (Scheffe's F; $P < 0.01$ for CGS21680, $P < 0.05$ for APEC) and 20.0 pmol/min (Scheffe's F; $P < 0.01$ for CGS21680 and APEC).

NECA also showed this bell-shaped, dose–response relationship. The greatest increase appeared at the infusion rate of 0.2 pmol/min, which was 22.5 ± 3.1 min (a 206.7 ± 40.1% increase; paired *t*-test, $P < 0.001$). This increase was significantly greater than increases at the infusion rates of 0.02, 2.0 (Scheffe's F, $P < 0.05$), and 12.0 pmol/min (Scheffe's F, $P < 0.01$).

In contrast to these agonists, CPA, a selective adenosine A_1 receptor agonist, caused negligible changes in the amount of NREM sleep or REM sleep within the dose range of 0.02–2.0 pmol/min. Further increases in dosage did not produce a consistent response in NREM sleep, but did cause a significant decrease in REM sleep from the baseline values (paired *t*-test, $P < 0.05$).

Secondly, we compared the changes in sleep induced by the four adenosine agonists at the respective infusion rates. ANOVA revealed a significant variance in NREM sleep (0.2 pmol/min: $F(3,23) = 7.34$, $P < 0.01$; 2.0 pmol/min: $F(3,31) = 17.67$, $P < 0.01$; 12.0 pmol/min: $F(3,23) = 5.30$, $P < 0.01$; 20.0 pmol/min: $F(2,15) = 15.41$, $P < 0.01$) and in REM sleep (0.2 pmol/min: $F(3,23) = 3.69$, $P < 0.05$ pmol/min; 2.0 pmol/min: $F(3,31) = 11.52$, $P < 0.01$; 12.0 pmol/min: $F(3,23) = 12.52$, $P < 0.01$; 20.0 pmol/min: $F(2,15) = 3.87$, $P < 0.05$).

The changes in NREM sleep induced by CGS21680 were significantly different from those induced by CPA at the infusion rates of 0.2, 2.0, 12.0 and 20.0 pmol/min

(Dunnett two-tailed, $P < 0.01$), and the changes induced by the former were also significantly different from that induced by APEC at the infusion rate of 20.0 pmol/min (Dunnett two-tailed, $P < 0.05$). The changes in REM sleep induced by CGS21680 were significantly different from those caused by CPA at the infusion rates of 0.2, 2.0, 12.0 and 20.0 pmol/min (Dunnett two-tailed, $P < 0.01$ for 0.2, 2.0, 12.0 pmol/min, $P < 0.05$ for 20.0 pmol/min), and from those caused by NECA at the infusion rate of 2.0 and 12.0 pmol/min (Dunnett two-tailed, $P < 0.01$). At the infusion rate of 12.0 pmol/min, a significant difference was also seen in the changes between CGS21680 and APEC (Dunnett two-tailed, $P < 0.01$).

3.2. Profiles of the changes in sleep and brain temperature induced by the adenosine agonists

The 24-h profiles of sleep and of brain temperature at the administration rate of 2.0 pmol/min of the adenosine agonists are shown in Fig. 2A and B, respectively. At this administration rate, CGS21680 produced increases in NREM sleep and REM sleep, both with a maximal magnitude.

CGS21680 and APEC produced the increases in NREM sleep from the first hour of their administration, and increases in REM sleep from the second or third hour. These increases in NREM sleep and REM sleep lasted throughout the administration period. In the case of NECA, a lag period of 2 h preceded the beginning of the increase in NREM sleep, and the increase in REM sleep occurred during the last 2 h of the administration period. In contrast, CPA appeared to cause a biphasic response composed of an initial decrease and a subsequent increase in the profile of NREM sleep, whereas it caused solely an increase in REM sleep during the last 2 h of the administration period. A further increase in the dose of CPA also produced a biphasic response in NREM sleep, but a continuous decrease in REM sleep from the first hour of its administration (data not shown).

CGS21680 and APEC caused a gradual decrease of the profile of brain temperature from the second hour of the administration period, which decrease lasted during the remainder of the infusion period. NECA also caused a drop in temperature but only after a lag period of 4 h. CPA caused only marginal changes in the brain temperature except for the fifth hour of the administration period, at which time the temperature decreased slightly.

3.3. Effect of diurnal treatment with CGS21680 on sleep

Diurnal infusion of CGS21680 also caused a dose-dependent increasing tendency in NREM sleep, but a dose-dependent decreasing tendency in REM sleep (Fig. 3). At the infusion rate of 0.2, 2.0 and 12.0 pmol/min, the increases in NREM sleep from their respective baseline values were 6.7 ± 8.1 min (paired t-test, n.s.), 15.4 ± 5.4

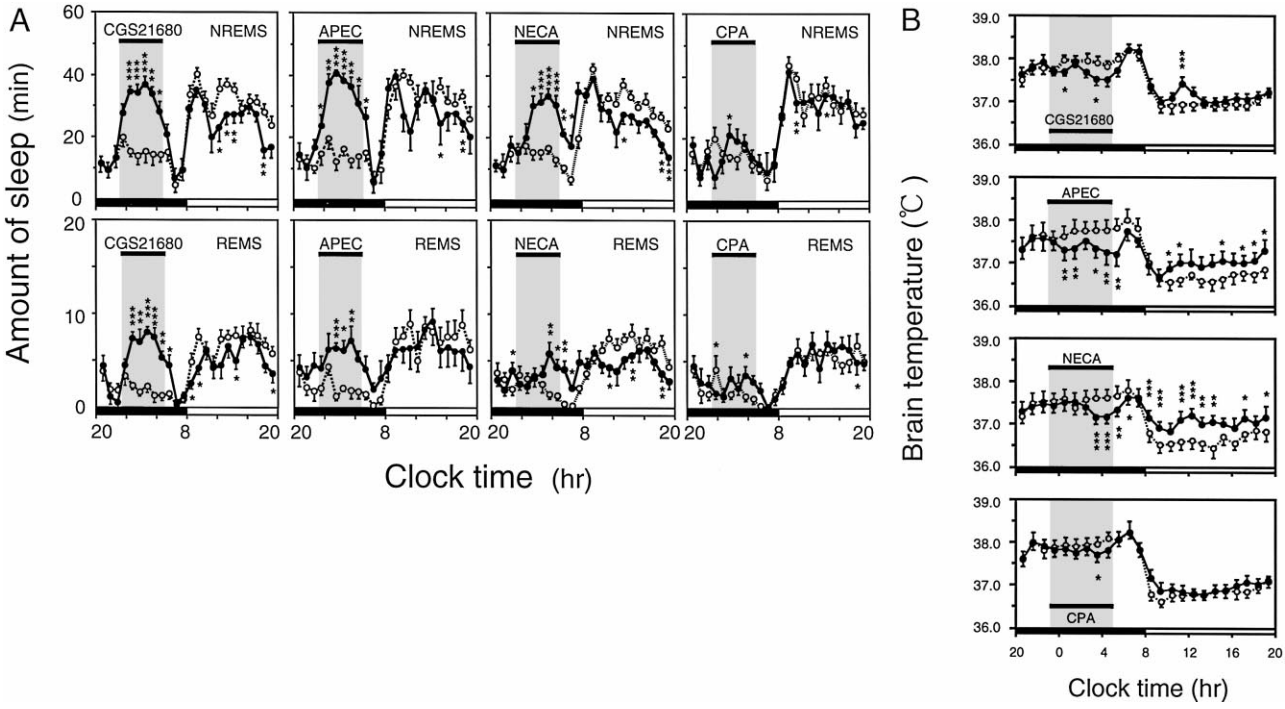

Fig. 2. Twenty-four-hour profiles of sleep (A) and brain temperature (B) on the control day (open circles) and the experimental day (closed circles). CGS21680, APEC, NECA, and CPA were separately administered into the subarachnoid space underlying the rostral basal forebrain bilaterally at the total infusion rate of 2.0 pmol/min during the middle part (2300 h–0500 h) of the active period of the animals. $n = 7–11$ per group; $*$, $P < 0.05$. $**$, $P < 0.01$. $***$, $P < 0.001$, vs. control by paired t-test.

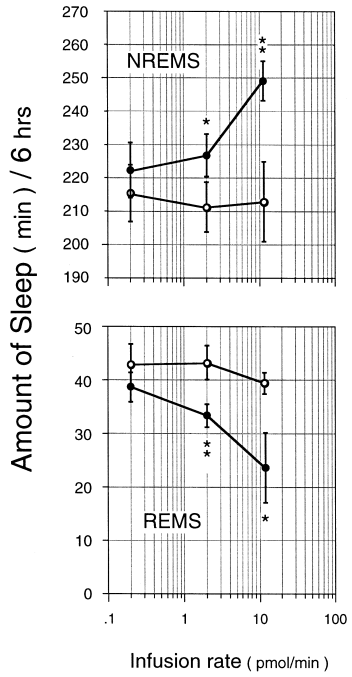

Fig. 3. Effect of diurnal administration of CGS21680 on sleep. CGS21680 was continuously infused into the subarachnoid space underlying the rostral basal forebrain bilaterally at the total infusion rate of 0.2, 2.0, or 12 pmol/min over a 6-h period (1100 h–1700 h) during the rest period of the rat. The total amounts (mean ± S.E.M.) of NREM sleep and REM sleep during the 6-h control (open circles and black bars) and experimental (closed circles and black bars) periods are shown. $n = 7–13$ per each group; *, $P < 0.05$. **, $P < 0.01$, vs. control by paired *t*-test.

min (paired *t*-test, $P < 0.05$), and 33.2 ± 5.7 min (paired *t*-test, $P < 0.01$), respectively; whereas the decreases in REM sleep were 4.2 ± 2.5 min (paired *t*-test, n.s.), 9.8 ± 2.7 min (paired *t*-test, $P < 0.01$) and 15.7 ± 6.3 min (paired *t*-test, $P < 0.05$), respectively. ANOVA revealed a significant variance among the changes in NREM sleep ($F(2,16) = 3.65$, $P < 0.05$), but not in REM sleep ($F(2,16) = 1.21$, $P = 0.32$). However, no significant difference was found between any pairs of the changes in NREM sleep as evaluated in Scheffe's *F*-test.

4. Discussion

The data from the present study, demonstrating that adenosine A_{2A} receptor agonists promoted sleep when administered directly into the rostral basal forebrain during the night, i.e., the active phase of the rats, are consistent with our previous findings. These data further support our hypothesis that the activity of adenosine A_{2A} receptors is involved in the promotion of sleep.

4.1. Administration of adenosine agonists to ventral and rostral part of forebrain

The differences between the marked increases in NREM sleep and REM sleep induced by CGS21680, and the negligible changes in sleep induced by CPA clearly indicate that activation of adenosine A_{2A} receptors is involved in the promotion of sleep. In addition, the increases in NREM sleep and REM sleep induced by APEC and NECA at the relatively low dosages appeared to be due to the activity of adenosine A_{2A} receptors. Attenuation of sleep-promoting potency at relatively high dosages of APEC, as well as the lag period before the start of the increases in sleep and unusual pattern of EEG during the treatment with NECA, might be explained by the additional activation of adenosine A_1 and A_3 receptors. The activation of adenosine A_1 receptors in particular may have produced initially a subtracting and, subsequently, an additive effect on the A_{2A}-mediated increases in NREM sleep, since CPA, an adenosine A_1 receptor agonist, produced a biphasic response composed of an initial decrease and a subsequent increase in NREM sleep when it was administered in the subarachnoid space of the rostral basal forebrain in this study. Activation of adenosine A_3 receptors might suppress sleep, because it was reported that histamine, which promotes wakefulness, is released from mast cells in response to the administration of 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide (2-Cl-IB-MECA), a selective adenosine A_3 receptor agonist (Van Schaick et al., 1996).

Recently, it was reported that 8-(3-chlorostyryl)caffeine (CSC) and 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-c]-1,2,4,-triazolo-[1,5-c]-pyrimidine (SCH58261), both selective adenosine A_{2A} receptor antagonists, increase locomotion activity and, thus, wakefulness (Bertorelli et al., 1996; Jacobson et al., 1993). These reports suggest that a certain part of physiological sleep is produced through the activity of adenosine A_{2A} receptors.

On the other hand, CPA, an adenosine A_1 receptor agonist, did not produce a consistent increase in NREM sleep and, instead, suppressed REM sleep in the present study. These effects were similar to those of CHA on sleep that were found in our previous study and to the results obtained following the intraperitoneal injection of CPA (1.0 mg/kg body weight) in rats (Schwierin et al., 1996). However, it was reported that the intraperitoneal and intracerebroventricular administration of CPA, and the microinjection of CPA into preoptic area increase the slow-wave activity in NREM sleep (Ticho and Radulovacki, 1991; Benington et al., 1995; Schwierin et al., 1996). Since we did not carry out a spectral analysis of EEG, it is difficult to know whether our treatment with CPA increased the slow-wave activity in NREM sleep or not. Furthermore, it is also unclear whether our experimental site was mainly responsible for the adenosine A_1 receptor-mediated promotion of slow-wave activity in NREM sleep or not.

4.2. Effects of adenosine agonists on brain temperature

It is well known that brain temperature decreases during the natural sleep period (reviewed by McGinty and Szymu-

siak, 1990). Similar findings were obtained in the present study. The decrease in the brain temperature induced by CGS21680, APEC, and NECA started along with or after the beginning of the increase in NREM sleep, and did not precede the change in NREM sleep. In these cases, the magnitude of the changes in brain temperature remained within the range of natural circadian variation. The continuous infusion of CPA produced a small decrease in brain temperature only at the fifth hour of administration, around which period a small increase in NREM sleep appeared. Other groups also showed that the microinjection of CPA (0.5 nmol) or NECA (1.0 nmol) into the preoptic area produced an approximately 0.5–1.0°C reduction in brain temperature and an increase in deep slow wave sleep (Ticho and Radulovacki, 1991). Therefore, in contrast to some cytokines that produce sleep and fever (reviewed by Kruger and Majde, 1994), an adenosine receptor agonist could produce the natural relation between sleep and brain temperature if an appropriate amount was directly administered to the brain.

4.3. Effect of day time treatment with CGS21680 on sleep

There were several reports indicating a difference between the response to the day time and to the night time treatment with sleep-promoting substances. Diurnal administration of sleep substances, such as delta-sleep-inducing-peptide (DSIP), prostaglandin D_2, and uridine caused almost no changes in sleep parameters (Inoué et al., 1984). Furthermore, triazolam, one of the benzodiazepines, failed to show sleep-promoting efficacy in rats when injected in the middle of their rest phase (Edger et al., 1991). Thus, it is likely that the effectiveness of exogenously administered CGS21680 and other substances is affected by intrinsic circadian factors. The total sleep period during the diurnal treatment with CGS21680 was around 260 min, and was almost consistent at the various infusion rates used in this study. This phenomenon well resembles the upper limit that total sleep does not exceed in spite of prior sleep deprivation and was named the 'ceiling effect' (Mistlberger et al., 1983). Furthermore, this might be one reason why the other research groups did not observe an increase in sleep following the intraperitoneal injection of A_{2A} adenosine agonists during the day period (Monti et al., 1995; Bertorelli et al., 1996).

4.4. For further study of a sleep-generating structure located in the rostral basal forebrain

Early studies demonstrating that chemical or electrical lesioning of a particular brain region caused the loss of sleep, whereas electrical stimulation of this region produced synchronization of the EEG, indicated that some sleep-generating structure is located in the rostral part of the brain, such as the anterior hypothalamus, preoptic area, and basal forebrain (reviewed by Jones, 1994). However, the pharmacological and anatomical characteristics of this sleep-promoting structure have not yet been clearly elucidated.

In the present study, the tip of each infusion cannula was situated in the subarachnoid space underlying the ventral and medial part of the shell of the accumbens nucleus. In this area and in its vicinity, a dense distribution of adenosine A_{2A} receptors was demonstrated by use of ligand binding autoradiography using [^{3}H]CGS21680 and in situ hybridization with antisense RNA for the adenosine A_{2A} receptor. Adenosine A_{2A} receptors are known to be localized in GABA (γ-aminobutyric acid)-ergic neurons that coexpress enkephalin (reviewed by Ongini and Fredholm, 1996). These neurons might be responsible for the sleep-promoting effect of adenosine A_{2A} receptor agonists.

Several brain regions have been proposed to be involved in the generation of sleep. The ventral surface of the rostral basal forebrain is considered to be the site of action of prostaglandin D_2 for sleep-promotion in rats (Matsumura et al., 1994). The cholinergic neurons of the basal forebrain and the laterodorsal tegmentum are considered to be involved in the adenosine-induced sleep in cats (Rainne et al., 1994; Portas et al., 1997). Some neurons in the medial preoptic area respond to local warming and prolong the period of slow wave sleep (McGinty and Szymusiak, 1990). The average number of FOS-immunoreactive cells per ventrolateral preoptic area (VLPO) sector increased with increasing percent total sleep time for spontaneously behaving rats (Sherin et al., 1996). The microinjection of muscimol, a $GABA_A$ receptor agonist, into the ventrolateral region of the periaqueductal gray and adjacent tegmentum increases the period of REM sleep. (Sastre et al., 1996). The cholinoceptive desynchronized sleep induction zone is localized within the dorsal tegmentum of the pons (Vanni-Mercier et al., 1989; Yamamoto et al., 1990). In contrast, the posterior hypothalamus, especially the histaminergic neurons in the tuberomamillary nucleus, participates in the regulation of wakefulness (Lin et al., 1989). The putative adenosine A_{2A} receptor mediating, the sleep-promoting system would relate directly or indirectly to the activities of these structures. Particularly, the increase in REM sleep might be explained by a disinhibition of mesopontine REM-on neurons, as mentioned by Portas et al. (1997).

For clarification of this issue, it will be necessary to decide the site of action of an adenosine A_{2A} agonist for sleep promotion. To this end, anatomical studies with tracers will be helpful. CGS21680 should serve as a useful tool for further study of the sleep-generating structures located in the rostral and ventral part of the forebrain and their relation to other established sleep-generating structures.

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