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An adenosine A_{2A} receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats

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Abstract

The adenosine A_{2A} receptor (A_{2A}R) has been demonstrated to play a crucial role in the regulation of the sleep process. However, the molecular mechanism of the A_{2A}R-mediated sleep remains to be elucidated. Here we used electroencephalogram and electromyogram recordings coupled with *in vivo* microdialysis to investigate the effects of an A_{2A}R agonist, CGS21680, on sleep and on the release of histamine and GABA in the brain. In freely moving rats, CGS21680 applied to the subarachnoid space underlying the rostral basal forebrain significantly promoted sleep and inhibited histamine release in the frontal cortex. The histamine release was negatively correlated with the amount of non-rapid eye movement sleep ($r = -0.652$). In urethane-anesthetized rats, CGS21680 inhibited histamine release in both the frontal cortex and medial pre-optic area in a dose-dependent manner, and increased GABA release specifically in the histaminergic tuberomammillary nucleus but not in the frontal cortex. Moreover, the CGS21680-induced inhibition of histamine release was antagonized by perfusion of the tuberomammillary nucleus with a $GABA_A$ antagonist, picrotoxin. These results suggest that the A_{2A}R agonist induced sleep by inhibiting the histaminergic system through increasing GABA release in the tuberomammillary nucleus.

Keywords: adenosine A_{2A} receptor agonist, γ-aminobutyric acid, histamine, microdialysis, sleep, tuberomammillary nucleus.

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Histaminergic output from the tuberomammillary nucleus (TMN) is considered to play a crucial role in mediating arousal (Monti 1993; Lin 2000; Haas and Panula 2003). Under physiological conditions, the extracellular histamine levels in the pre-optic/anterior hypothalamus areas of cats are the highest during waking, lower during non-rapid eye movement (non-REM) sleep, and the lowest during REM sleep (Strecker *et al*. 2002). In freely moving rats, histamine release in the frontal cortex (FrCx) exhibits a circadian variation and is positively correlated with wakefulness (Chu *et al*. 2004). Enhancement of histaminergic neuron activity produces arousal, as we previously reported based on experiments using either orexin or EP4 agonist (Huang *et al*. 2001, 2003; Hayaishi and Huang 2004). In contrast, sleep is promoted by decreasing histaminergic transmission through pharmacological blockade of central histaminergic receptors (Nicholson *et al*. 1985; Tasaka *et al*. 1989; Lin *et al*. 1990), inhibition of histidine decarboxylase, a key enzyme for histamine biosynthesis (Kiyono *et al*. 1985; Itowi *et al*. 1991), and hyperpolarization of the TMN with GABAergic agonists in cats (Lin *et al*. 1989) and rats (Nelson *et al*. 2002). Furthermore, histidine decarboxylase

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Abbreviations used: A_{2A}R, adenosine A_{2A} receptor; CGS21680, 2-p-(2-carboxyethyl)phenylethylamino-5′-*N*-ethylcarboxyamidoadenosine; EEG, electroencephalogram; EMG, electromyogram; FrCx, frontal cortex; MPO, medial pre-optic area; non-REM, non-rapid eye movement; REM, rapid eye movement; SS-rBF, subarachnoid space underlying the rostral basal forebrain; TMN, tuberomammillary nucleus; VLPO, ventrolateral pre-optic area.

knockout mice, lacking histamine in the brain, show a deficit in waking, attention, and interest in a new environment (Parmentier *et al*. 2002; Dere *et al*. 2004).

Adenosine is proposed to be an endogenous sleep-promoting substance. The adenosine A_{2A} receptor ($A_{2A}R$) plays an important role in the regulation of the sleep process, as demonstrated by the fact that intracerebroventricular infusion of an $A_{2A}R$ agonist, 2-p-(2-carboxyethyl)phenyl-ethylamino-5′-*N*-ethylcarboxyamidoadenosine (CGS21680), promotes sleep (Satoh *et al*. 1996, 1998, 1999; Gerashchenko *et al*. 2000; Scammell *et al*. 2001; Urade *et al*. 2003). The largest increase in sleep occurred when CGS21680 was administered to the subarachnoid space underlying the rostral basal forebrain (SS-rBF), the sleep-promoting zone of PGD2, which is another endogenous somnogen (Satoh *et al*. 1999). The CGS21680-induced sleep is associated with an increase in the expression of c-Fos in the ventrolateral pre-optic area (VLPO) and a reduced expression of it in the histaminergic TMN (Scammell *et al*. 2001; Satoh *et al*. 1998, 1999). Projection of histaminergic fibers from the TMN throughout the brain, especially to the cerebral cortex, basal forebrain, and anterior hypothalamus (Haas and Panula 2003) has been characterized in detail, whereas afferents to the TMN are less well understood (Ericson *et al*. 1991). Sleep-promoting cells are recorded in the large pre-optic area (McGinty and Szymusiak 2003) including the VLPO, one of the sleep centers (Scammell *et al*. 2001; Hayaishi and Urade 2002). The VLPO sends inhibitory GABAergic and galaninergic projections to the histaminergic TMN in the wake-promoting posterior hypothalamic area (Sherin *et al*. 1996, 1998). Moreover, the release of GABA in the posterior hypothalamus is increased during slow-wave sleep and is lowered while awake (Nitz and Siegel 1996). However, the role of histamine and GABA in $A_{2A}R$ agonist-induced sleep remains to be elucidated.

In the present study, we used electroencephalogram (EEG) and electromyogram (EMG) recordings coupled with *in vivo* microdialysis to investigate the effects of CGS21680 on sleep and histamine release in freely moving rats. Then we revealed the involvement of GABA in the effect of CGS21680 on the histaminergic system in anesthetized rats to further elucidate the mechanism for adenosine $A_{2A}R$ in the promotion of sleep.

Materials and methods

Animals

Male Sprague-Dawley rats, weighing 280–320 g (8–10 weeks old), were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. They were housed at a constant temperature (24 ± 0.5°C) with a relative humidity (60 ± 2%) on an automatically controlled 12 : 12 h light/dark cycle (light on at 08:00 h), and they had *ad libitum* access to food and water. The experimental protocols were approved by the Animal Care Committee of Osaka Bioscience Institute.

Drugs and chemicals

CGS21680 and picrotoxin were purchased from Research Biochemicals International (Natick, MA, USA) and Sigma-Aldrich Company (St Louis, MO, USA), respectively. GABA and histamine were from Wako Pure Chemical Industries Ltd (Osaka, Japan). All other chemicals were of analytical grade.

EEG and EMG recordings coupled with *in vivo* microdialysis in freely moving rats

Under pentobarbital anesthesia [50 mg/kg intraperitoneal (i.p.)], rats underwent surgery for implantation of electrodes for EEG and EMG recordings and placement of a guide cannula for the microdialysis probe and a stainless steel cannula for infusion of CGS21680, as described earlier (Satoh *et al*. 1996; Chu *et al*. 2004). Briefly, a guide cannula (outer diameter 0.6 mm) with an in-dwelling stylet and a stainless steel cannula (outer diameter, 0.2 mm) were inserted stereotaxically into the FrCx and SS-rBF, respectively. The stereotaxic coordinates were anteroposterior (AP) + 3.2 mm; left–right (LR) − 1.0 mm; dorsoventral (DV) − 1.8 mm for the guide cannula and AP + 1.0 mm; LR 0 mm; DV − 8.0 mm for the stainless steel cannula from bregma according to the atlas of Paxinos and Watson (1997), as shown in Fig. 1. To ensure correct placement of the stainless steel cannula, we attached a plastic tube filled with saline to it; a drop in the meniscus would indicate that the cannula tip was in the subarachnoid space. The two cannulae and EEG electrodes were fixed to the skull with dental cement and four stainless steel screws for anchorage to the skull. Two stainless steel wire electrodes were placed into the neck muscles for EMG recordings. Post-operatively, each rat was allowed 10 days of recovery and was then transferred to a sound-proof recording chamber where it was connected to an EEG/EMG recording cable for a 3-day period of habituation to the experimental conditions.

At least 20 h before the recording session, the stylet of the microdialysis guide cannula was replaced with a microdialysis probe

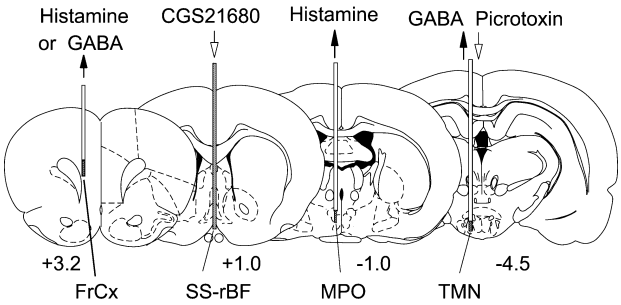

Fig. 1 Schematic representation of the implantation sites for microdialysis probes and cannulae in the rat brain. In freely moving rats, CGS21680 was infused into the SS-rBF through a stainless steel cannula; and histamine release was monitored in the FrCx. In anesthetized rats, CGS21680 was also infused into the SS-rBF through a cannula, and two microdialysis probes were inserted, one into the FrCx or MPO for monitoring histamine and/or GABA, and the other into the TMN for determining GABA or for perfusion of picrotoxin. Figures of coronal sections were obtained from the stereotaxic atlas of Paxinos and Watson (1997). FrCx, frontal cortex; SS-rBF, subarachnoid space underlying the rostral basal forebrain; MPO, medial pre-optic area; TMN, tuberomammillary nucleus.

(CMA/Microdialysis, Stockholm, Sweden) consisting of a semipermeable membrane with a tip length of 3 mm, an outer diameter of 0.5 mm, and a molecular cut-off size of 20 kDa. The microdialysis probe and the stainless steel cannula were continuously perfused and infused, respectively, with artificial cerebrospinal fluid (aCSF; composition: 140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, 2 mM Na_2HPO_4, and 0.2 mM NaH_2PO_4, pH 7.4] at a flow rate of 2 μL/min and 0.4 μL/min, respectively. The EEG/EMG signals were amplified, filtered (EEG, 0.5–30 Hz; EMG, 16–128 Hz), digitized at a sampling rate of 128 Hz, and recorded by using the data acquisition program SLEEPSIGN (Kissei Comtec, Nagano, Japan), as described earlier (Huang *et al*. 2001, 2003). The recordings and dialysates collected from the FrCx at 20-min intervals (40 μL each) were taken in each rat for two consecutive 14-h periods, starting at 20:00 h. On the experimental day, CGS21680, at a dose of 10 pmol/min, was infused for 6 h from 23:00 to 05:00 h. The dialysates were kept at −20°C until they could be assayed for their histamine levels by high-performance liquid chromatography (HPLC)-fluorometry.

The vigilance states were automatically classified off-line by 10-s epochs, into wakefulness, non-REM sleep, and REM sleep by SLEEPSING software, according to the standard criteria (Huang *et al*. 2001, 2003). As a final step, defined sleep–wake stages were examined visually, and corrected, if necessary.

In vivo microdialysis to monitor GABA and histamine in anesthetized rats

Under urethane anesthesia (1.2 g/kg, i.p.), rats underwent surgery for implantation of two microdialysis probes (outer diameter, 0.22 mm; Eicom, Kyoto, Japan) and a stainless steel cannula as described above. Briefly, two microdialysis probes were inserted stereotaxically, one into the FrCx (membrane length, 3 mm) or the medial pre-optic area (MPO: AP −1 mm; LR − 0.8 mm; DV −8.6 mm; membrane length, 2 mm) for monitoring extracellular histamine or GABA (the latter only in the FrCx, not MPO) and the other into the TMN (AP −4.5 mm; LR − 0.8 mm; DV −9.2 mm; membrane length, 2 mm) for collecting samples to measure extracellular GABA (Fig. 1). A stainless steel cannula (outer diameter, 0.2 mm) was inserted into the SS-rBF for administration of CGS21680. The two microdialysis probes and stainless steel cannula were perfused with CSF at flow rates of 2 μL/min and 0.4 μL/min, respectively. CGS21680 and picrotoxin were administered to the SS-rBF and the TMN through the cannula and microdialysis probe, respectively. Two hours after insertion of the microdialysis probes, dialysates were continuously collected from the FrCx or MPO and TMN at 20-min intervals (40 μL each), for 1 h before infusion of CGS21680, during the 2-h infusion, and for 2 h after the infusion had ended. The dialysates were kept at −20°C until they could be assayed by HPLC-fluorometry for histamine or GABA.

Histological verification

When an experiment was over, rats were killed with an overdose of pentobarbital sodium and injected through the implanted cannula, or perfused through the microdialysis probes with a microquantity of pontamine sky-blue dye solution (0.5% wt/vol) to verify the site of cannula placement for CGS21680 administration, and the sites of microdialysis probes implanted in the FrCx, MPO, and TMN.

Measurements of GABA and histamine

The GABA level in a dialysate was determined by an HPLC-fluorometry (Lindroth and Mopper 1979) based on pre-column derivatization of a 40-μL microdialysis perfusate sample with 6 μL of *o*-phtaldialdehyde reagent and separation by a reverse-phase analytical column (EICOMPAK SC-5ODS, Ø2.1 mm × 150 mm, Eicom, Kyoto, Japan) eluted at a flow rate of 0.3 mL/min at 30°C with 20% acetonitrile and 0.1 M sodium acetate/citric acid (pH 5.4) containing 0.1 mM EDTA-2Na. For preparing the *o*-phtaldialdehyde reagent, 1 mg *o*-phthalaldehyde was dissolved in 200 μL of ethanol; and the solution was then mixed with 5 μL of 2-mercaptoethanol and diluted with 0.1 M $NaHCO_3/Na_2CO_3$ (pH 9.6) to 1 mL. The fluorescence detector (Hitachi, Tokyo, Japan) was set at 340 and 445 nm wavelengths for excitation and emission, respectively.

Histamine level in the dialysate was determined by HPLC-fluorometry also (Yamatodani *et al*. 1985; Huang *et al*. 1999). Briefly, 35 μL of the dialysate sample was injected into a column packed with cation exchanger, TSK gel SP2SW (Ø 6 mm × 150 mm; Tosoh, Tokyo, Japan) and eluted with 0.25 M KH_2PO_4 at a flow rate of 0.9 mL/min. The histamine elute was postlabeled with 0.1% *o*-phthalaldehyde under alkaline conditions, and detected fluorometrically in an F1080 fluorometer (Hitachi) using excitation and emission wavelengths of 360 and 450 nm, respectively.

The chromatograms for GABA and histamine were recorded and analyzed with a computer using Millennium32 Chromatography Manager software (Waters, Milford, MA, USA).

Statistical analysis

All data were expressed as the mean ± SEM ($n = 5$ or 6). For vigilance studies, amounts of the different sleep–wake states were expressed in minutes. Statistical analyses were performed by use of Student's *t*-test. For the microdialysis data, the difference between groups was analyzed by two-way analysis of variance (ANOVA) followed by post-hoc Newman–Keuls test except as otherwise stated. In all cases, $p < 0.05$ was taken as the level of significance.

Results

CGS21680 inhibited histamine release in the FrCx of freely moving rats during sleep induction

To investigate the role of the histaminergic system in sleep-promotion, we induced sleep by infusing CGS21680 into the SS-rBF in freely moving rats for 6 h from 23:00 to 05:00 h during the dark period, and then monitored histamine release in the FrCx.

As shown in Fig. 2(a), when the vehicle was infused, the extracellular histamine level was high; and the time spent in non-REM and REM sleep was low, similar to those we found in freely moving rats without any treatments (Chu *et al*. 2004). However, when CGS21680 was infused at a rate of 10 pmol/min, the histamine release was significantly decreased in the FrCx immediately after the infusion; and

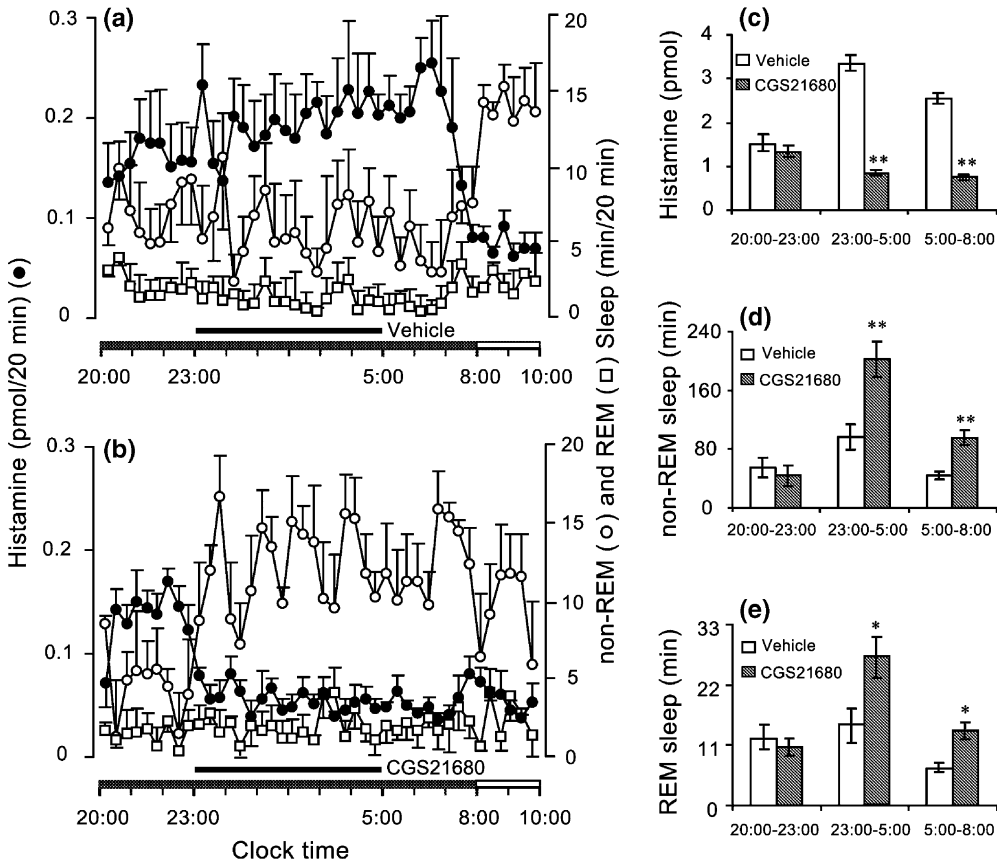

Fig. 2 Effects of CGS21680 on extracellular histamine level in the FrCx and on the amount of non-REM and REM sleep in freely moving rats. Experiments were carried out during the dark period. Time-courses of non-REM (○) and REM (□) sleep amounts and histamine level in the FrCx (●) on the vehicle-treated day (a) and on the CGS21680-infused day (b) are shown. The horizontal solid bar indicates the duration of vehicle or CGS21680 infusion. (c, d, and e) represent the total amounts of histamine released in the FrCx, total times spent in non-REM sleep and REM sleep before, during, and after CGS21680 infusion, respectively. Each value represents the mean ± SEM of five rats. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from the control, as assessed by Student's *t*-test.

the effect lasted until 140 min after the infusion had ended. The suppression of histamine release was concomitant with an increase in non-REM and REM sleep (Fig. 2b). As shown in Fig. 2(c), CGS21680 decreased the total amount of histamine released about 74% and 70% during the 6-h infusion and 3 h after ending the infusion, respectively, as compared with the amount on the vehicle-treated day. On the other hand, CGS21680 increased the amount of non-REM sleep by 2.1- and 2.2-fold and that of REM sleep by 1.8- and 2.0-fold during the 6-h infusion and 3 h after ending administration, respectively, as compared with those on the vehicle-treated day (Figs 2d and e).

We then compared the histamine release in the FrCx with the amount of wakefulness, non-REM, and REM sleep in 27 sequential samples taken at 20-min intervals during the 6-h infusion (18 samples) and 3 h after the infusion had ended (9 samples). Regression analysis revealed that the extracellular histamine level was strongly and positively correlated with wakefulness ($r = 0.691$, $p < 0.001$), and negatively correlated with the time spent in non-REM sleep ($r = -0.652$, $p < 0.001$), respectively (Fig. 3). These results clearly indicate that the histaminergic system was inhibited during CGS21680-promoted sleep.

CGS21680 increased GABA release in the TMN and inhibited histamine release in the FrCx and MPO of anesthetized rats

Due to the technical difficulty in doing experiments with two sets of microdialysis probes and a cannula in a freely moving rat, we monitored the release of GABA and of histamine in different brain regions after the infusion of CGS21680 into the SS-rBF in urethane-anesthetized rats to examine the mechanism underlying which CGS21680 inhibited histamine release.

As shown in Fig. 4(a), infusion of the SS-rBF with CGS21680 for 2 h at doses of 2.5 and 5 pmol/min significantly inhibited the histamine release in the FrCx to 60% and 40% of the basal release at 100 and 120 min, respectively,

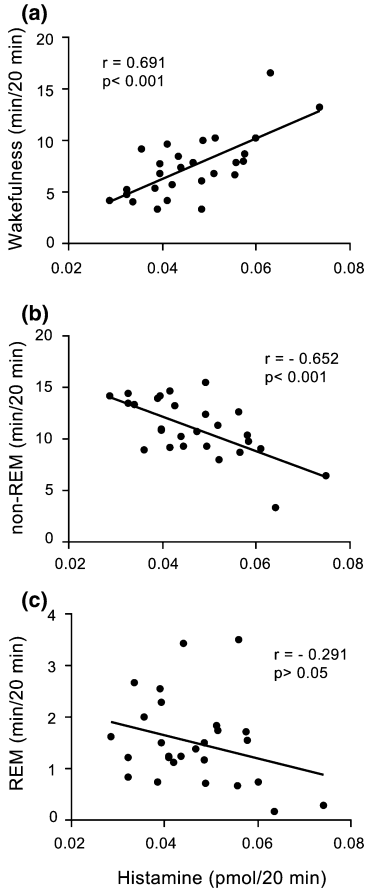

Fig. 3 Correlation of extracellular level of histamine in the FrCx with time spent in wakefulness (a), non-REM (b), and REM (c) sleep during the 6-h CGS21680 infusion and 3 h after the infusion in rats. The time for each vigilance stage and the histamine concentration were measured every 20 min. Each value represents the mean of five rats.

after starting the infusion. The decreased level of histamine then gradually returned to the basal level. When the agonist was given at a dose of 10 pmol/min, the histamine release was decreased more rapidly than with the two lower doses and reached its minimal level of 16% of the basal release at 120 min after the administration had ended. Similar results were obtained for the suppression of histamine release in the MPO after CGS21680 infusion (Fig. 4b). The minimal levels of histamine release were 66%, 62%, and 37% of the basal release after the CGS21680 infusion at doses of 2.5, 5, and 10 pmol/min, respectively, indicating that infusion of CGS21680 into the SS-rBF inhibited histamine release not only in the FrCx but also in the MPO in a dose-dependent manner.

When GABA release was monitored in the FrCx after the CGS21680 infusion, we did not detect any significant change in it (Fig. 4c). However, the infusion of CGS21680 significantly increased GABA release in the TMN in a dose-dependent manner. As shown in Fig. 4(d), CGS21680 at 2.5 and 5 pmol/min increased the release with the maximal magnitude being about 185% and 243% of the control, respectively; and then the GABA level gradually returned to the basal level. When given at the highest dose of 10 pmol/min, CGS21680 significantly enhanced GABA release to the maximal level being 320% of the control at 140 min after dosing; and this high level was sustained to the end of the experiment. These results suggest that CGS21680 increased GABA release in a TMN-specific manner.

As summarized in Figs 4(e and f), the infusion of CGS21680 into the SS-rBF inhibited histamine release in the FrCx and MPO and simultaneously increased GABA release in the TMN in a dose-dependent manner.

GABA antagonist blocked the CGS21680-induced inhibition of histamine release

To clarify the role of GABA release in the TMN in the CGS21680-induced inhibition of histamine release, we perfused the TMN with the GABA receptor antagonist picrotoxin at doses of 100 and 200 pmol/min and monitored the histamine release in the FrCx with and without CGS21680 infusion into the SS-rBF. As shown in Fig. 5, without CGS21680 infusion, the picrotoxin perfusion of the TMN increased the histamine release in the FrCx in a dose-dependent manner. The CGS21680 infusion into the SS-rBF at the dose of 10 pmol/min inhibited the histamine release from the FrCx rapidly and significantly, and this inhibition was markedly attenuated by the picrotoxin perfusion of the TMN at the dose of 100 pmol/min and completely antagonized at the dose of 200 pmol/min. These results clearly indicate that the CGS21680-induced inhibition of histamine release was mediated by GABA released in the TMN.

Discussion

Our present study has provided direct evidence that CGS21680 infused into the SS-rBF significantly increased sleep and inhibited histamine release in the both MPO and FrCx (Figs 2 and 4), both regions having been implicated in the arousal effect of histamine (Lin *et al.* 1994, 1996; Lin 2000). These findings suggest that a decrease in histaminergic transmission contributed to the sleep promoted by the $A_{2A}R$ agonist. The inhibition of histamine release was observed in several brain regions, whereas a concomitant increase in GABA release was found only specifically in the TMN, where histaminergic neurons are located, but not in the FrCx (Fig. 4c). Furthermore, we also found that perfusion of the TMN with the $GABA_A$ antagonist picrotoxin blocked CGS21680-induced inhibition of histamine release in the FrCx, indicating that the inhibition of histamine release is mediated specifically by an increasing endogenous GABA release in the TMN.

The GABA released in the TMN may originate from the VLPO. Although GABA (Airaksinen *et al.* 1992), the

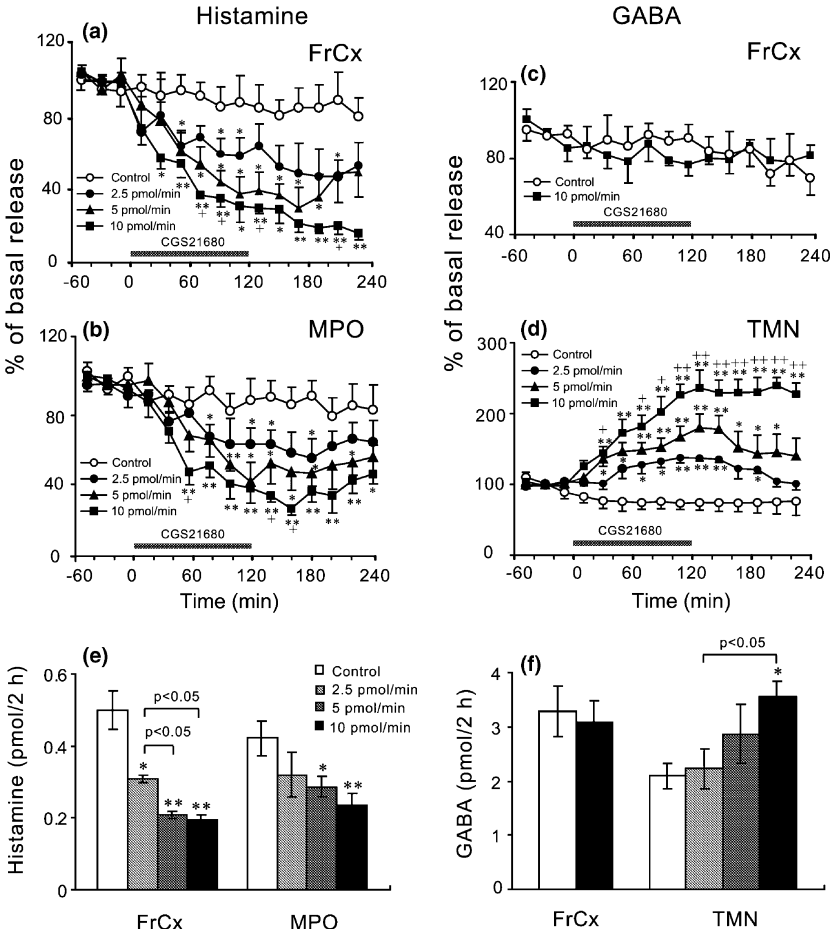

Fig. 4 Effects of CGS21680 on the histamine release in the FrCx (a) and MPO (b) as well as on the GABA release in the FrCx (c) and TMN (d) in anesthetized rats. The horizontal solid bar indicates the duration of CGS21680 infusion. Because the output of either histamine or GABA became stable at 2 h after implantation of the probes, the mean value of histamine or GABA found during the next 1 h was defined as the basal release, and the subsequent fractions were expressed as percentages of this value. The total amounts of histamine (e) and GABA (f) released during the 2-h CGS21680 infusion are shown. Each value represents the mean ± SEM of five or six rats. $^{*}p < 0.05$, $^{**}p < 0.01$ and $+p < 0.05$, $++p < 0.01$, significantly different from the control and the group treated with CGS21680 at 2.5 pmol/min, respectively, as assessed by ANOVA followed by post-hoc Newman–Keuls test.

GABA-synthesizing enzyme glutamic acid decarboxylase (Senba *et al*. 1985), and its mRNA (Esclapez *et al*. 1994) are localized in histaminergic neurons, Eriksson *et al*. (2004) found that the histaminergic neurons in the posterior hypothalamus did not release GABA within the TMN, suggesting that increased GABA in the TMN may come from outside the TMN. With respect to neuroanatomy, the VLPO provides an intense and specific GABAergic set of inputs to the cell bodies and proximal dendrites of the TMN (Sherin *et al*. 1996, 1998), and a small percentage of GABAergic neurons from the pontomesencephalic tegmentum (1%) projects to the posterior lateral hypothalamus (Ford *et al*. 1995). C-Fos studies showed that CGS21680 infusion into the SS-rBF induces sleep with the activation of the VLPO and suppression of the TMN (Scammell *et al*. 1998, 2001; Satoh *et al*. 1999). In addition, stimulation of large forebrain areas including the VLPO and diagonal band of Broca, induces inhibitory post-synaptic potential in the TMN (Yang and Hatton 1997). These findings indicate that the VLPO is an important GABAergic source to inhibit the histaminergic neurons in the TMN. Because the VLPO also sends galaninergic fibers to the TMN, the role of this system in the inhibition of the TMN remains to be clarified. Because the GABA antagonist blocked the CGS21680-induced inhibition of histamine release in a dose-dependent manner (Fig. 5), the GABAergic system may be the dominant factor mediating this inhibition.

The mechanism by which CGS21680 activates the VLPO neurons remains to be further studied. Satoh *et al*. (1999) found that infusion of CGS21680 into the subarachnoid space induced the largest increases in non-REM and REM sleep, which were accompanied by intense c-Fos expression

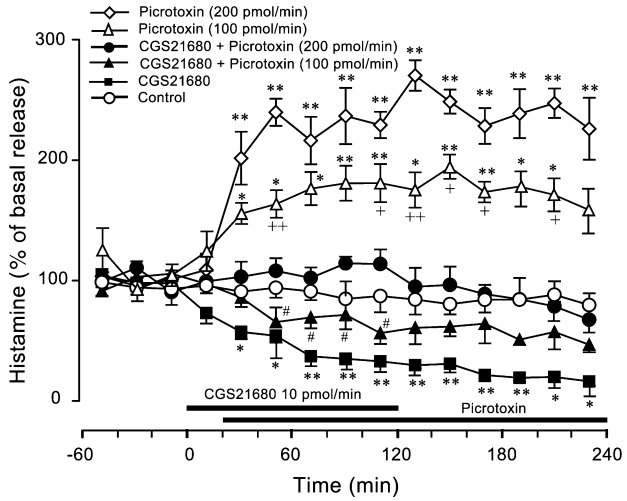

Fig. 5 Effect of picrotoxin on CGS21680-induced inhibition of histamine release in the FrCx of anesthetized rats. The short and long solid bars indicate the duration of CGS21680 infusion and picrotoxin perfusion, respectively. Each value represents the mean ± SEM of five or six rats. $*p < 0.05$, $**p < 0.01$ and $\#p < 0.05$, significantly different from the control and the group treated with CGS21680 plus 200 pmol/min picrotoxin, respectively; $+p < 0.05$, $++p < 0.01$, significantly different from the group treated with 200 pmol/min picrotoxin, as assessed by ANOVA followed by post-hoc Newman–Keuls test.

in the VLPO (Scammell *et al*. 2001) as well as in the shell of the nucleus accumbens and in the medial portions of the olfactory tubercle (Satoh *et al*. 1999). Interestingly, $A_{2A}R$ mRNA is abundant in the nucleus accumbens, olfactory tubercle, and striatum, as indicated by *in situ* hybridization (Fink *et al*. 1992; Weaver 1993), and $A_{2A}R$ immunoreactivity is also prominent in these brain regions (Rosin *et al*. 1998). Neuroanatomically, the shell and rostral pole of the nucleus accumbens project efferents to putative state-regulatory regions including the VLPO (Chou *et al*. 2002), indicating that there is an anatomical connection between the nucleus accumbens and the VLPO. Thus, the cell signal produced by CGS21680 infused into the SS-rBF may be transferred to the VLPO, or CGS21680 may activate $A_{2A}R$-bearing accumbens neurons that directly or indirectly increase the activity of sleep-producing neurons in the VLPO to induce sleep through releasing GABA to inhibit the TMN.

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References

Airaksinen M. S., Alanen S., Szabat E., Visser T. J. and Panula P. (1992) Multiple neurotransmitters in the tuberomammillary nucleus: comparison of rat, mouse, and guinea pig. *J. Comp. Neurol.* **323**, 103–116.

Chou T. C., Bjorkum A. A., Gaus S. E., Lu J., Scammell T. E. and Saper C. B. (2002) Afferents to the ventrolateral preoptic nucleus. *J. Neurosci.* **22**, 977–990.

Chu M., Huang Z. L., Qu W. M., Eguchi N., Yao M. H. and Urade Y. (2004) Extracellular histamine level in the frontal cortex is positively correlated with the amount of wakefulness in rats. *Neurosci. Res.* **49**, 417–420.

Dere E., De Souza-Silva M. A., Spieler R. E., Lin J. S., Ohtsu H., Haas H. L. and Huston J. P. (2004) Changes in motoric, exploratory and emotional behaviours and neuronal acetylcholine content and 5-HT turnover in histidine decarboxylase-KO mice. *Eur. J. Neurosci.* **20**, 1051–1058.

Ericson H., Blomqvist A. and Kohler C. (1991) Origin of neuronal inputs to the region of the tuberomammillary nucleus of the rat brain. *J. Comp. Neurol.* **311**, 45–64.

Eriksson K. S., Sergeeva O. A., Selbach O. and Haas H. L. (2004) Orexin (hypocretin)/dynorphin neurons control GABAergic inputs to tuberomammillary neurons. *Eur. J. Neurosci.* **19**, 1278–1284.

Esclapez M., Tillakaratne N. J., Kaufman D. L., Tobin A. J. and Houser C. R. (1994) Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. *J. Neurosci.* **14**, 1834–1855.

Fink J. S., Weaver D. R., Rivkees S. A., Peterfreund R. A., Pollack A. E., Adler E. M. and Reppert S. M. (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. *Brain Res. Mol. Brain Res.* **14**, 186–195.

Ford B., Holmes C. J., Mainville L. and Jones B. E. (1995) GABAergic neurons in the rat pontomesencephalic tegmentum: co-distribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *J. Comp. Neurol.* **363**, 177–196.

Gerashchenko D., Okano Y., Urade Y., Inoue S. and Hayaishi O. (2000) Strong rebound of wakefulness follows prostaglandin D2- or adenosine A2a receptor agonist-induced sleep. *J. Sleep Res.* **9**, 81–87.

Haas H. and Panula P. (2003) The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.* **4**, 121–130.

Hayaishi O. and Huang Z. L. (2004) Role of orexin and prostaglandin E(2) in activating histaminergic neurotransmission. *Drug News Perspect.* **17**, 105–109.

Hayaishi O. and Urade Y. (2002) Prostaglandin D2 in sleep–wake regulation: recent progress and perspectives. *Neuroscientist* **8**, 12–15.

Huang Z. L., Mochizuki T., Watanabe H. and Maeyama K. (1999) Activation of sensory nerves participates in stress-induced histamine release from mast cells in rats. *Neurosci. Lett.* **270**, 181–184.

Huang Z. L., Qu W. M., Li W. D., Mochizuki T., Eguchi N., Watanabe T., Urade Y. and Hayaishi O. (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl Acad. Sci. USA* **98**, 9965–9970.

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

Itowi N., Yamatodani A., Kiyono S., Hiraiwa M. L. and Wada H. (1991) Effect of histamine depletion on the circadian amplitude of the sleep–wakefulness cycle. *Physiol. Behav.* **49**, 643–646.

Kiyono S., Seo M. L., Shibagaki M., Watanabe T., Maeyama K. and Wada H. (1985) Effects of α-fluoromethylhistidine on sleep–waking parameters in rats. *Physiol. Behav.* **34**, 615–617.

Lin J. S. (2000) Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med. Rev.* **4**, 471–503.

Lin J. S., Sakai K., Vanni-Mercier G. and Jouvet M. (1989) A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. *Brain Res.* **479**, 225–240.

Lin J. S., Sakai K., Vanni-Mercier G., Arrang J. M., Garbarg M., Schwartz J. C. and Jouvet M. (1990) Involvement of histaminergic neurons in arousal mechanisms demonstrated with H3-receptor ligands in the cat. *Brain Res.* **523**, 325–330.

Lin J. S., Sakai K. and Jouvet M. (1994) Hypothalamo-preoptic histaminergic projections in sleep–wake control in the cat. *Eur. J. Neurosci.* **6**, 618–625.

Lin J. S., Hou Y., Sakai K. and Jouvet M. (1996) Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. *J. Neurosci.* **16**, 1523–1537.

Lindroth P. and Mopper K. (1979) High-performance liquid chromatography determination of subpicomole amounts of amino acids by pre-column fluorescence derivatization with *o*-phthaldialdehyde. *Analyt. Chem.* **51**, 1667–1674.

McGinty D. and Szymusiak R. (2003) Hypothalamic regulation of sleep and arousal. *Front Biosci.* **8**, s1074–s1083.

Monti J. M. (1993) Involvement of histamine in the control of the waking state. *Life Sci.* **53**, 1331–1338.

Nelson L. E., Guo T. Z., Lu J., Saper C. B., Franks N. P. and Maze M. (2002) The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat. Neurosci.* **5**, 979–984.

Nicholson A. N., Pascoe P. A. and Stone B. M. (1985) Histaminergic systems and sleep. Studies in man with H1 and H2 antagonists. *Neuropharmacology* **24**, 245–250.

Nitz D. and Siegel J. M. (1996) GABA release in posterior hypothalamus across sleep–wake cycle. *Am. J. Physiol.* **271**, R1707–R1712.

Parmentier R., Ohtsu H., Djebbara-Hannas Z., Valatx J. L., Watanabe T. and Lin J. S. (2002) Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep–wake control. *J. Neurosci.* **22**, 7695–7711.

Paxinos G. and Watson C., eds. (1997) *The Rat Brain in Stereotaxic Coordinates*. Academic, San Diego.

Rosin D. L., Robeva A., Woodard R. L., Guyenet P. G. and Linden J. (1998) Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. *J. Comp. Neurol.* **401**, 163–186.

Satoh S., Matsumura H., Suzuki F. and Hayaishi O. (1996) Promotion of sleep mediated by the A2a-adenosine receptor and possible involvement of this receptor in the sleep induced by prostaglandin D2 in rats. *Proc. Natl Acad. Sci. USA* **93**, 5980–5984.

Satoh S., Matsumura H. and Hayaishi O. (1998) Involvement of adenosine A2A receptor in sleep promotion. *Eur. J. Pharmacol.* **351**, 155–162.

Satoh S., Matsumura H., Koike N., Tokunaga Y., Maeda T. and Hayaishi O. (1999) Region-dependent difference in the sleep-promoting potency of an adenosine A2A receptor agonist. *Eur. J. Neurosci.* **11**, 1587–1597.

Scammell T. E., Gerashchenko D., Urade Y., Onoe H., Saper C. B. and Hayaishi O. (1998) Activation of ventrolateral pre-optic neurons by the somnogen prostaglandin D2. *Proc. Natl Acad. Sci. USA* **95**, 7754–7759.

Scammell T. E., Gerashchenko D. Y., Mochizuki T., McCarthy M. T., Estabrooke I. V., Sears C. A., Saper C. B., Urade Y. and Hayaishi O. (2001) An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. *Neuroscience* **107**, 653–663.

Senba E., Daddona P. E., Watanabe T., Wu J. Y. and Nagy J. I. (1985) Co-existence of adenosine deaminase, histidine decarboxylase, and glutamate decarboxylase in hypothalamic neurons of the rat. *J. Neurosci.* **5**, 3393–3402.

Sherin J. E., Elmquist J. K., Torrealba F. and Saper C. B. (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J. Neurosci.* **18**, 4705–4721.

Sherin J. E., Shiromani P. J., McCarley R. W. and Saper C. B. (1996) Activation of ventrolateral preoptic neurons during sleep. *Science* **271**, 216–219.

Strecker R. E., Nalwalk J., Dauphin L. J., Thakkar M. M., Chen Y., Ramesh V., Hough L. B. and McCarley R. W. (2002) Extracellular histamine levels in the feline pre-optic/anterior hypothalamic area during natural sleep–wakefulness and prolonged wakefulness: an *in vivo* microdialysis study. *Neuroscience* **113**, 663–670.

Tasaka K., Chung Y. H., Sawada K. and Mio M. (1989) Excitatory effect of histamine on the arousal system and its inhibition by H1 blockers. *Brain Res. Bull.* **22**, 271–275.

Urade Y., Eguchi N., Qu W. M., Sakata M., Huang Z. L., Chen J. F., Schwarzschild M. A., Fink J. S. and Hayaishi O. (2003) Sleep regulation in adenosine A2A receptor-deficient mice. *Neurology* **61**, S94–S96.

Weaver D. R. (1993) A2a adenosine receptor gene expression in developing rat brain. *Brain Res. Mol. Brain Res.* **20**, 313–327.

Yamatodani A., Fukuda H., Wada H., Iwaeda T. and Watanabe T. (1985) High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. *J. Chromatogr.* **344**, 115–123.

Yang Q. Z. and Hatton G. I. (1997) Electrophysiology of excitatory and inhibitory afferents to rat histaminergic tuberomammillary nucleus neurons from hypothalamic and forebrain sites. *Brain Res.* **773**, 162–172.