Effects of the 5-HT<sub>1A</sub> Receptor Agonist Tandospirone on ACTH-Induced Sleep Disturbance in Rats

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The aim of this study was to compare the effect of the serotonin (5-HT) <sub>1A</sub> receptor agonist tandospirone versus that of the benzodiazepine hypnotic flunitrazepam in a rat model of long-term adrenocorticotropic hormone (ACTH)-induced sleep disturbance. Rats implanted with electrodes for recording electroencephalogram and electromyogram were injected with ACTH once daily at a dose of 100 µg/rat. Administration of ACTH for 10 d caused a significant increase in sleep latency, decrease in non-rapid eye movement (non-REM) sleep time, and increase in wake time. Tandospirone caused a significant decrease in sleep latency and increase in non-REM sleep time in rats treated with ACTH. The effect of tandospirone on sleep patterns was antagonized by the 5-HT<sub>1A</sub> receptor antagonist WAY-100635. In contrast, flunitrazepam had no significant effect on sleep parameters in ACTH-treated rats. These results clearly indicate that long-term administration of ACTH causes sleep disturbance, and stimulating the 5-HT<sub>1A</sub> receptor by tandospirone may be efficacious for improving sleep in cases in which benzodiazepine hypnotics are ineffective.

Key words sleep disturbance; adrenocorticotropic hormone; serotonin (5-HT)<sub>1A</sub> receptor; gamma-aminobutyric acid

Eighty to ninety percent of patients with major depression have disturbed sleep patterns, and it is widely accepted that sleep disturbance may be a risk factor for and/or a prodromal symptom of depression. Furthermore, disturbed sleep is characteristic of individuals undergoing treatment for depression, as well as those exhibiting a relapse of depressive symptoms. Therefore the treatment of sleep disturbance is likely important in preventing the development of depression.

Benzodiazepines and their analogues are commonly effective in the clinical treatment of sleep disturbances via their effects on the gamma-aminobutyric acid (GABA)-ergic system. The GABAergic system plays an important role in the control of the sleep–wake cycle; however, sensitivity to benzodiazepines has been shown decreased in depressed patients with disturbed sleep. Therefore it is conceivable that sleep patterns in these individuals are influenced by non-GABAergic systems. For example, abnormal functioning of the serotonergic system was proposed to be closely associated with depressive systems. Benzodiazepines has been shown decreased in depressed patients with disturbed sleep. Therefore it is conceivable that sleep patterns in these individuals are influenced by non-GABAergic systems.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 240–260 g (Japan SLC, Shizuoka, Japan) were maintained in an air-conditioned room with controlled temperature (24±2°C) and humidity (55±15%) under a light–dark cycle (lights on from 07:00 to 19:00). The rats were housed in plastic cages with sawdust and allowed free access to food and water. All procedures involving these animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery Rats were anesthetized with pentobarbital sodium (Nembutal, 35 mg/kg intraperitoneally (i.p.); Abbott Laboratories, North Chicago, IL, U.S.A.) and fixed to a stereotaxic apparatus (SR-5N; Narishige, Tokyo, Japan). To record electroencephalogram (EEG), a stainless steel screw electrode (diameter, 0.5 mm; depth, 1.7 mm) was chronically implanted into the cortex (A, −4.5; L, +2.5), according to coordinates from the atlas of Paxinos and Watson. A stainless steel screw fixed in the left frontal bone served as a reference electrode. To record electromyogram (EMG), stainless steel wire electrodes (0.2 mm) were implanted into the dorsal neck muscle. These electrodes were connected to a miniature receptacle, and the entire assembly was fixed to the skull with

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At least 7 d was allowed for recovery from the surgery.

Animal Model Development  ACTH (Cortrosyn-Z; Daiichi-Sankyo & Co., Ltd., Japan) was subcutaneously administered once daily for 1, 5, or 10 d, at a dose of 100 µg/rat in a 0.2-mL injection volume.

EEG and EMG Recordings  EEG and EMG were recorded from 7:00 to 19:00. Recordings were conducted as described previously.12,13) The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–60 Hz), then digitized at a sampling rate of 128 Hz, and recorded by SleepSign ver. 2.0 (Kissei Comtec, Nagano, Japan) data acquisition program. Recordings were made in a cylindrical plastic cage (diameter, 26 cm; height, 31 cm) placed on sawdust with food and water. The observation cage was placed in a sound-proof and electrically shielded box (70×60×60 cm).

Sleep–Wake State Analysis  Sleep–wake states were automatically classified in 10-s epochs as wake, non-rapid eye movement (non-REM) sleep, or rapid eye movement (REM) sleep by SleepSign ver. 2.0, according to criteria described previously.14,15) As a final step, defined sleep–wake stages were examined visually and corrected, if necessary. Each state was characterized as follows: wake, low-amplitude EEG and high-voltage EMG activities; non-REM sleep, high-amplitude slow or spindle EEG and low-EMG activities; and REM sleep, low-voltage EEG and EMG activities. Sleep latency was defined as the time from drug administration until the first 12 consecutive 10-s epochs of sleep.

Drugs  The following drugs were used: tandospirone citrate (Sediel; Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan); flunitrazepam (Rohypnol; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan); and WAY-100635 (Sigma, St. Louis, MO, U.S.A.). Tandospirone and flunitrazepam were suspended in a 0.5% carboxymethyl cellulose solution and administered orally. WAY-100635 was dissolved in saline and administered intraperitoneally. All drugs were administered at 7:00, and EEG and EMG were measured for 12 h after drug administration. Eight rats were used in each group, and a counterbalanced design was used for drug dosing.

Data Analysis and Statistics  Values shown are mean±S.E.M. A one-way ANOVA with Dunnett’s test was used to estimate the effects of each drug. Probability values <0.05 were considered significant.

RESULTS  Changes of Sleep Patterns in ACTH-Treated Rats  Rats were administered ACTH for 10 d, and EEG and EMG were
measured prior to and on days 1, 5, and 10 following treatment. Administration of ACTH for 10 d resulted in a significantly longer sleep latency and wake time (Figs. 1A, C), and shorter non-REM sleep time (Fig. 1B) compared with pretreatment values. ACTH decreased REM sleep time, but not significantly (Fig. 1D). We also examined the hourly effects of ACTH on non-REM sleep time over 12 h. ACTH caused significant decreases in non-REM sleep time on day 10 for 7 to 10 h and 16 to 18 h (Fig. 1E).

**Effect of Tandospirone on Sleep Parameters in Rats Treated with ACTH**

We used rats treated with ACTH to study the effect of tandospirone on sleep parameters. Tandospirone caused significant decreases in sleep latency at 10, 20, and 50 mg/kg (Fig. 2A). Moreover, this agent caused a significant increase in non-REM sleep time at 50 mg/kg (Fig. 2B). Further analysis revealed that tandospirone caused an increase in non-REM sleep time (20 mg/kg, 7 to 9 h; 50 mg/kg, 7 to 11 h) (Fig. 2C).

**Effect of Flunitrazepam on Sleep Parameters in Rats Treated with ACTH**

We also investigated the effects of flunitrazepam on non-REM sleep time at any time-point (Fig. 3C).

**Effect of WAY-100635 on Tandospirone-Induced Improvements in Sleep Disturbance Induced by ACTH**

To investigate the relation between the effect of tandospirone and the 5-HT1A receptor, we examined the effects of WAY-100635. WAY-100635, at doses of 0.3 and 1 mg/kg i.p., antagonized the hypnotic effect of tandospirone on sleep latency (Fig. 4A) and non-REM sleep time (Fig. 4B). Further investigation revealed that WAY-100635 antagonized the hypnotic effect of tandospirone on hourly non-REM sleep times (0.1 mg/kg, 9 to 11 h; 0.3 mg/kg, 9 to 11 h and 13 to 14 h; 1 mg/kg, 9 to 12 h and 13 to 15 h) (Fig. 4C).

**Effect of Tandospirone, Flunitrazepam, and WAY-100635 on Sleep Parameters in Normal Rats**

We also investigated the effects of tandospirone, flunitrazepam, and WAY-100635 on sleep parameters in normal rats. Tandospirone (50 mg/kg p.o.), flunitrazepam (3 mg/kg p.o.), and WAY-100635 (1 mg/kg i.p.) had no significant effect on sleep latency, wake time, non-REM sleep time, or REM sleep time (data not shown).

**DISCUSSION**

It has been recognized that sleep disturbance in depressive patients is characterized by an increase in sleep latency and a decrease in non-REM sleep time.1,16,17 It is also well known that an increase in immobilized time after chronic administration of ACTH in rat swim test is useful as a depression model in human beings.18 In the present study, a significant increase in sleep latency and decrease in non-REM sleep time were
observed following chronic administration of ACTH in rats. From the above findings, it may be hypothesized that ACTH-induced sleep disturbance in rats is similar to depression-related sleep disturbance in human beings.

Next, we examined the effects of flunitrazepam and tandospirone on sleep disturbance in rats treated with ACTH. Flunitrazepam had no significant effect on any of the parameters examined, even at the highest dose tested (3 mg/kg). Contrariwise, Shinomiya et al.\(^1\) reported that flunitrazepam at 3 mg/kg caused a decrease in sleep latency, a decrease in wake time, and an increase in non-REM sleep time in rats whose sleep was disturbed by both water- and altitude-induced stress. Flunitrazepam is a benzodiazepine hypnotic that binds to the GABA\(_A\) receptor and enhances the effect of GABA. In recent years, patients with sleep disturbances in conjunction with psychiatric illness, especially depression, have been prescribed multiple and/or high doses of benzodiazepine hypnotics.\(^{19,20}\) This indicates that sleep disturbance associated with psychiatric diseases exhibits tolerance or resistance to benzodiazepine hypnotics and is related to abnormalities not only with GABAergic neurotransmission, but also with 5-HT, noradrenaline, and dopamine.

Why flunitrazepam had no hypnotic effect on sleep disturbance induced by the long-term administration of ACTH remains unclear; however, we believe that the mechanism may be closely related to upregulation of alpha5-GABA\(_A\) receptors. Flunitrazepam has been shown to produce a hypnotic effect by binding to GABA\(_A\) receptors, which comprise various alpha, beta, and gamma subunits. It has been reported that the hypnotic effects of benzodiazepines are mediated by the alphal subunit. Conversely, alpha5-GABA\(_A\) receptors were insensitive and developed tolerance to the sedative actions of benzodiazepines.\(^{21–23}\) Verkuyl et al.\(^{24}\) reported that expression of alpha5, not alphal-GABA\(_A\) receptors, was increased in patients with hyperfunctional HPA axes. Therefore the absence of a flunitrazepam-induced hypnotic effect may be the result of tolerance developed by upregulation of alpha5-GABA\(_A\) receptors in ACTH-treated rats.

The 5-HT\(_{1A}\) receptor is related to depression, and 5-HT\(_{1A}\) receptor agonists have been shown to exhibit antidepressant-like effects. Kitamura et al.\(^{25}\) reported that the 5-HT\(_{1A}\) agonist 8-hydroxy-2-di-n-propylamino tetralin (8-OH-DPAT) decreased immobility time for ACTH-treated rats in the forced swim test. Our results suggest that tandospirone caused a significant decrease in sleep latency and an increase in total non-REM sleep time in rats administered ACTH, which were different to those observed with flunitrazepam. This effect was significantly inhibited by the 5-HT\(_{1A}\) receptor antagonist WAY-100635. Therefore tandospirone had a clear, 5-HT\(_{1A}\)-mediated hypnotic effect in this sleep-disturbance model.

Previous studies reported that stimulation of 5-HT\(_{1A}\) receptors produced a sleep-inducing effect in animal models of sleep disturbance whereas tandospirone had no significant effect on non-REM sleep time.\(^{26,27}\) As shown in the present data, however, tandospirone increased non-REM sleep time in rats treated with ACTH. This difference may be due to upregulation of 5-HT\(_{2A}\) receptors in ACTH-treated rats. We previously reported that chronic treatment with ACTH increased expression of 5-HT\(_{2A}\) receptor mRNA in the frontal cortex, and enhanced the function of 5-HT\(_{2A}\) receptors in rats.\(^{28,29}\) Some studies in rats and humans have shown that 5-HT\(_{2A}\) receptor agonists increase wakefulness and inhibit slow-wave sleep (SWS), whereas 5-HT\(_{2A}\) receptor antagonists enhance duration of SWS and EEG low-frequency activity in non-REM sleep.\(^{30–32}\) On the other hand, 5-HT\(_{1A}\) receptors were regulated to report the activity of the serotonin nervous system, which inhibited the function of 5-HT\(_{2A}\) receptors.\(^{33,34}\) Kitamura et al.\(^{29}\) demonstrated that 8-OH-DPAT, a potent agonist of 5-HT\(_{1A}\) receptors, inhibited ACTH-induced 5-HT\(_{2A}\) receptor hyperfunction. This led us to conclude that long-term administration of ACTH upregulates 5-HT\(_{2A}\) receptors, and the effect of the partial 5-HT\(_{1A}\) agonist tandospirone on sleep disturbance in ACTH-treated rats may be attributable to 5-HT\(_{1A}\)-receptor-mediated inhibition of 5-HT\(_{2A}\) receptors.

In summary, sleep was disturbed in rats repeatedly administered ACTH. The sleep disturbance observed in the present model was markedly improved in rats treated with tandospirone, but not flunitrazepam, which suggests a close association between disturbed sleep and the serotonin nervous system, especially the 5-HT\(_{1A}\) receptor. We also confirmed that a 5-HT\(_{1A}\) agonist was useful for treating sleep disturbance in cases in which benzodiazepine hypnotics were ineffective.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES


