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Effect of Tandospirone on Sleep Latency in Rats Placed on a Grid Suspended Over Water

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Abstract. The present study was performed to examine the effect of tandospirone on sleep latency in a new insomnia animal model by placing rats on a grid suspended over water. For investigating the mechanism of tandospirone, the effect of tandospirone on sleep latency was also studied using rats that were depleted with neuronal serotonin (5-HT) after *p*-chlorophenylalanine administration. Tandospirone caused a shortening of sleep latency dose-dependently, and a significant effect was observed at 20 mg/kg, p.o. or more. A shortening of sleep latency was observed by administration of *p*-chlorophenylalanine (300 mg/kg, i.p.) for 2 days. On the other hand, tandospirone exerted no potentiating effect on the shortening of sleep latency induced by *p*-chlorophenylalanine. From these findings, a shortening of sleep latency induced by tandospirone may occur through the pre-synaptic 5-HT_{1A} receptors in rats.

Keywords: tandospirone, sleep latency, sleep disturbed model, antianxiety, 5-HT_{1A} receptor

Introduction

We have demonstrated a new insomnia animal model by placing rats on a grid suspended over water up to 1 cm under the grid surface (1, 2). In addition, sleep-inducing effects of certain short-acting hypnotics were studied in this model (1). As a result, it was found that triazolam, zopiclone, brotizolam, and lormetazepam caused decreases in sleep latency. Among these drugs, triazolam and lormetazepam showed potent effects compared with zopiclone and brotizolam. It is generally recognized that both triazolam and lormetazepam showed more potent anxiolytic activity than zopiclone and brotizolam by measuring anti-conflict effects in rats (3, 4). In addition, in this model, the rats were subjected to a relatively powerful stress, that is, grid and water (1). From these findings, it is reasonable to presume that our model is correlated with anxiety.

Tandospirone is a new anxiolytic agent specific for a special subtype (5-HT_{1A} receptors) of serotonin (5-HT) receptors. Therefore, the present study was designed to examine the effect of tandospirone on sleep latency using this model. In addition, for investigating the mechanism of tandospirone, the effect of tandospirone on sleep latency in rats with depletion of 5-HT from neurons induced by treatment with *p*-chlorophenylalanine was also studied.

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Materials and Methods

Animals

Wistar male rats weighing 220 – 300 g (Japan SLC, Shizuoka) were used. All animals were maintained in an air-conditioned room with controlled temperature (24 ± 2°C) and humidity (55 ± 15%). They were housed in an aluminum cage with sawdust and kept under a light/dark cycle (lights on from 7:00 to 19:00). The animals were allowed free access to food and water except during the experiments. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery

The animals were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p.; Abbott Laboratories, North Chicago, IL, USA) and then fixed to a stereotaxic apparatus (SR-5; Narishige, Tokyo). For electroencephalogram (EEG) recording, a stainless steel screw electrode was chronically implanted into the right frontal cortex (A: 0.5, L: 3.0) according to the atlas of Paxinos and Watson (5). A stainless steel screw fixed in the left frontal bone served as a reference electrode. To record the electromyogram (EMG), stainless steel wire electrodes (0.2 mm) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. At least 7 days were allowed for recovery from surgery.

EEG and EMG recording

EEG and EMG were recorded with an electroencephalogram (Model EEG 5113; Nihon Koden, Tokyo) from 10:00 to 16:00. The recording was carried out according to the method described previously (1, 6 – 8). The signals were amplified and filtered (EEG, 0.5 – 30 Hz; EMG, 16 – 128 Hz), then digitized at a sampling rate of 128 Hz, and recorded using the data acquisition program SleepSign ver.2.0 (Kissei Comtec, Nagano). EEG and EMG of the rat were measured in a plastic cage (30 × 18 × 24 cm), with its floor covered with sawdust or placed on a stainless steel grid. A grid floor (29 × 15 × 7 cm) was placed inside the plastic cage. The cage was filled with water up to 1 cm below the grid surface. The stainless steel rods of the grid (3 mm wide) were set 2 cm apart from each other. The observation cage was placed in a soundproof and electrically shielded box (70 × 60 × 60 cm). The rats were housed in a home cage (with sawdust) and then at the time of experiment, the rats were placed in another new cage with sawdust or placed on a stainless steel grid.

Sleep-wake state analysis

Sleep-wake states were automatically classified by 10-s epochs as awake, non-rapid eye movement (non-REM), or REM sleep by SleepSign ver.2.0, according to the previously described criteria (1, 7, 8). 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; REM sleep, low-voltage EEG and EMG activities.

Drugs

The following drugs were used: tandospirone (Sediel®; Sumitomo, Osaka) and *p*-chlorophenylalanine (Sigma-Aldrich, St. Louis, MO, USA). Tandospirone was suspended in 0.5% carboxymethyl cellulose (CMC) solution and were administered orally at 10:00. EEG and EMG were measured for 6 h after drug administration. *p*-Chlorophenylalanine was dissolved in saline and administered intraperitoneally.

Method for 5-HT depletion

5-HT depletion was induced by the 5-HT synthesis inhibitor *p*-chlorophenylalanine. *p*-Chlorophenylalanine was administered intraperitoneally. On the first day, EEG and EMG were measured and then *p*-chlorophenylalanine (300 mg/kg) was administered daily for 2 days. After *p*-chlorophenylalanine treatment, a significant decrease in 5-HT contents in hippocampus was reported (9). Thereafter, on day 4, EEG and EMG were also measured.

Data analysis and statistics

Values shown are means ± S.E.M. One-way analysis of variance (ANOVA) with Dunnett's test was used for estimating the drug effects. Sleep latency was defined as the time from drug administration up to the first 12 consecutive 10-s epochs of sleep.

Results

Effect of tandospirone on sleep latency

In rats placed on the grid suspended over water, a significant shortening of sleep latency was observed with tandospirone at doses of 20 and 50 mg/kg, p.o. However, in rats placed on sawdust, no significant shortening of sleep latency was observed with tandospirone even at a dose of 50 mg/kg (Fig. 1).

Effect of tandospirone on sleep parameters

In the present study, measurements of EEG and EMG were performed 6 h (10:00 – 16:00). In order to analyze the sleep parameter in detail, analysis of the data were performed every 3 h. As shown in Fig. 2, no significant effects were observed with awake, non-REM, and REM sleep times after administration of tandospirone in rats placed on the sawdust and grid suspended over water in the first period. Moreover, no significant effects were also observed with sleep parameters in the second period (data not shown).

Effect of tandospirone on sleep latency in 5-HT depleted rats

In rats placed on the grid, administration of *p*-chlorophenylalanine caused a significant shortening of sleep latency. However, no significant effect was

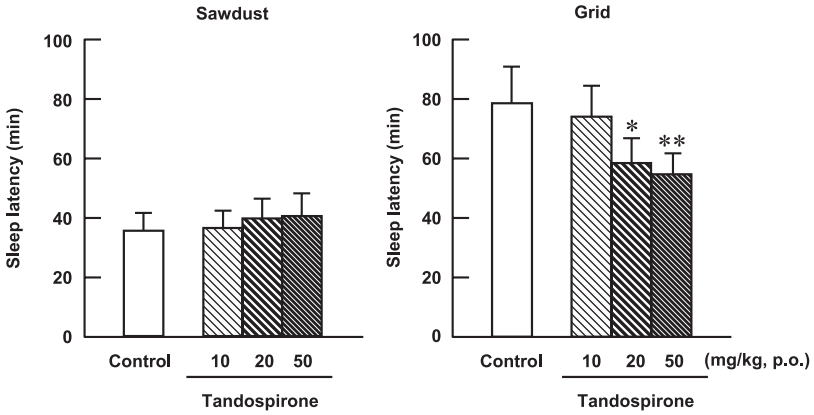

Fig. 1. Effect of tandospirone on sleep latency in rats placed on the sawdust or grid suspended over water. Columns and vertical bars represent values of the mean ± S.E.M. (n = 8). Drugs were administered orally. Significantly different from the control group at **P*<0.05 and ***P*<0.01 (ANOVA with Dunnett's test), respectively.

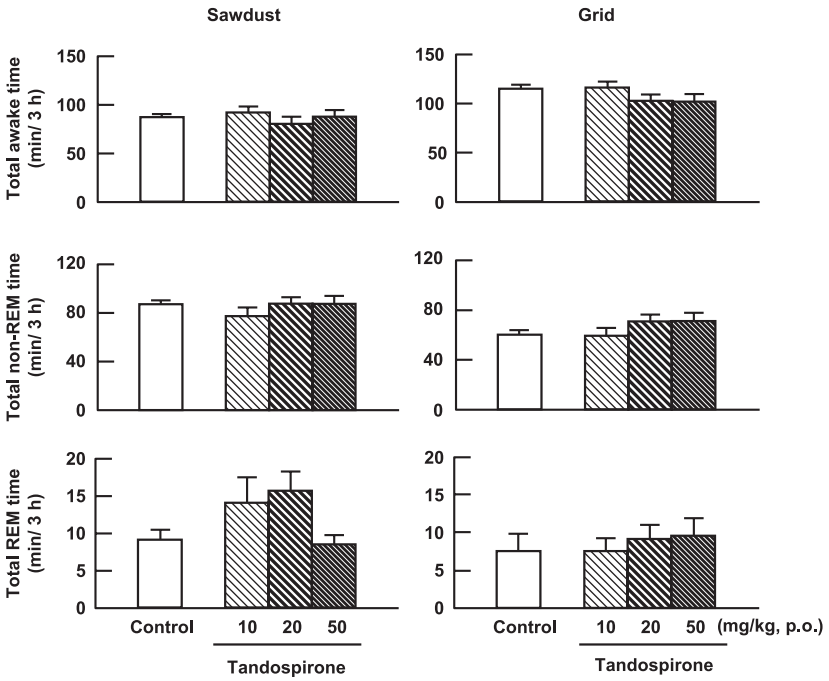

Fig. 2. Effect of tandospirone on total time of each sleep state in rats placed on the sawdust and grid suspended over water. Columns and vertical bars represent values of the mean ± S.E.M. (n = 8). Drugs were administered orally.

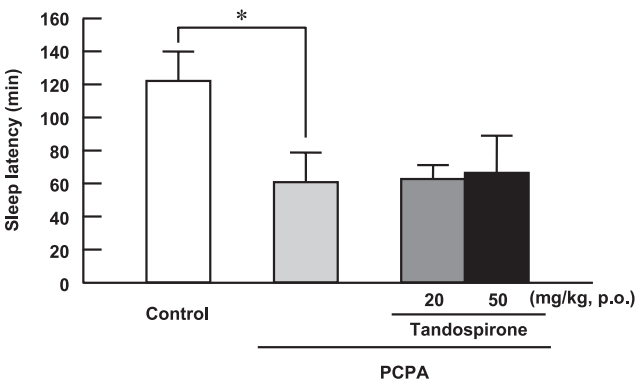

Fig. 3. Effect of tandospirone on sleep latency in 5-HT-depleted rats placed on the grid suspended over water. Columns and vertical bars represent values of the mean ± S.E.M. (n = 8). Drugs were administered orally. Significantly different from the control group at **P*<0.05 (ANOVA with Dunnett's test).

observed with tandospirone at doses of 20 and 50 mg/kg in 5-HT depleted rats (Fig. 3).

Effect of tandospirone on the sleep parameters in 5-HT depleted rats

In rats placed on the grid, administration of *p*-chlorophenylalanine caused a significant decrease in awaking time and an increase in non-REM sleep time. However, no significant effect was observed with REM sleep time. Tandospirone caused no significant effect on the sleep parameters in rats with 5-HT depletion by the administration of *p*-chlorophenylalanine (Fig. 4).

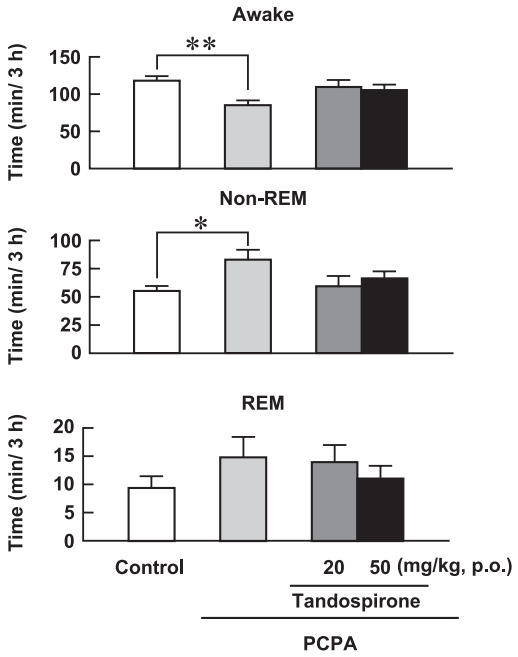

Fig. 4. Effect of tandospirone on total time of each sleep state in 5-HT-depleted rats placed on the grid suspended over water. Columns and vertical bars represent values of the mean ± S.E.M. (n = 8). Drugs were administered orally. Significantly different from the control group at **P*<0.05 and ***P*<0.01 (ANOVA with Dunnett's test), respectively.

Discussion

As described in the text, tandospirone caused a significant shortening of sleep latency on the grid suspended over water. On the other hand, tandospirone caused no significant shortening of sleep latency in rats placed on sawdust. We have reported that some benzodiazepine derivatives caused a shortening of sleep latency, and the drugs showing anti-anxiety properties had a potent shortening effect on sleep latency. On the other hand, the 5-HT_{1A} agonist tandospirone is known to show an anxiolytic effect similar to benzodiazepines (10). From these findings, a shortening of sleep latency caused by tandospirone may due to its anxiolytic effect.

In the present study, it was also found that tandospirone caused no significant changes in the total times of awake, non-REM or REM sleep in rats. Even in some benzodiazepines, findings similar to ours were also observed (1, 11). *p*-Chlorophenylalanine administration (300 mg/kg, i.p., for 2 days) resulted in a significant shortening of sleep latency in rats placed on the grid suspended over water. Nazar et al. (9) reported that 5-HT contents in the hippocampus were significantly decreased after 2 days administration of *p*-chlorophenylalanine (300 mg/kg) compared with those of control animals. Engel et al. (12) have reported that 5-HT depletion caused by *p*-chlorophenylalanine resulted in an anti-conflict effect measured by a modified Vogel's conflict test. Furthermore, in this sleep disturbed model, the rats received powerful stress induced by both the grid and water. Therefore, sleep disturbance of this model is closely related to anxiety. From these reasons, it seems likely that a shortening of sleep latency and changes in the sleep parameters induced by *p*-chlorophenylalanine may be due to its anti-anxiety effect. On the other hand, tandospirone caused no significant effect on shortening of sleep latency in 5-HT-depleted rats. The 5-HT_{1A} receptors are located postsynaptically in the hippocampus areas and located pre-synaptically on 5-HT cell bodies or dendrites in the raphe nuclei (13, 14). The 5-HT_{1A} receptors, which are expressed pre-synaptically, are known to regulate 5-HT release or synthesis as an autoreceptor (15). Tandospirone is a well-known selective 5-HT_{1A} agonist (16), and therefore, the drug showed anti-anxiety activity (17). Under the conditions of 5-HT depletion by *p*-chlorophenylalanine, 5-HT_{1A} pre-synaptic receptors cannot play a role to synthesize 5-HT or release inhibition (18). This is the reason why tandospirone showed no effect on sleep latency in 5-HT-depleted rats induced by *p*-chlorophenylalanine.

From the above findings, it maybe concluded that a shortening of sleep latency induced by tandospirone may occur through the pre-synaptic 5-HT_{1A} receptors in rats.

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