

*Full Paper***Effects of Some H₁-Antagonists on the Sleep-Wake Cycle in Sleep-Disturbed Rats**Shin Tokunaga¹, Yasuhiro Takeda¹, Kazuaki Shinomiya¹, Masahiro Hirase¹, and Chiaki Kamei^{1,*}¹*Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima-naka 1-1-1, Okayama 700-8530, Japan**Received September 26, 2006; Accepted December 10, 2006*

Abstract. The present study was undertaken to investigate the effects of some H₁-antagonists on the sleep-wake cycle in sleep-disturbed rats in comparison with those of nitrazepam. Electrodes were chronically implanted into the frontal cortex and the dorsal neck muscle of rats for the electroencephalogram (EEG) and electromyogram (EMG), respectively. EEG and EMG were recorded with an electroencephalograph. SleepSign ver. 2.0 was used for EEG and EMG analysis. The total times of waking, non-rapid eye movement (non-REM), and rapid eye movement (REM) sleep were measured from 10:00 to 16:00. Nitrazepam showed a significant decrease in sleep latency, total waking time, and delta activity and an increase in the total non-REM sleep time. A significant decrease in the sleep latency was observed with diphenhydramine, chlorpheniramine, and cyproheptadine. Cyproheptadine also caused a significant decrease in the total waking time and increases in total non-REM sleep time, REM sleep time, slow wave sleep, and delta activity, although no remarkable effects were observed with diphenhydramine and chlorpheniramine. In conclusion, cyproheptadine can be useful as a hypnotic, having not only sleep inducing-effects, but also sleep quantity- and quality-increasing effects.

Keywords: sleep-disturbed rat, H₁-antagonist, benzodiazepine hypnotic, sleep-wake cycle, delta activity

Introduction

About 10%–30% of the population in advanced countries has a sleep disorder complaint (1–3). Insomnia is the most prevalent and it is characterized by difficulty in falling asleep, intermittent waking after falling asleep and early morning awakening. Benzodiazepine hypnotics are widely used to treat insomnia due to both their safety and effectiveness; however, benzodiazepines have troublesome side effects such as rebound insomnia, amnesia, muscle relaxation, and drug dependence (4, 5); therefore, particular attention should be paid when benzodiazepines are used clinically. In addition, these drugs can induce unusual sleep patterns, increasing stage 2 sleep, characterized by sleep spindles, and reducing slow wave sleep (stage 3 and 4 sleep, characterized by delta waves), and changing delta

activity, used as an indicator of sleep quality (6–8).

On the other hand, H₁-antagonists have been used as nonprescription sleep aids in the USA (9). Although these drugs are widely used clinically, literature dealing with the hypnotic effects of H₁-antagonists in animals is limited. Moreover, most of these reports were performed using normal awake animals (10). It is inappropriate to estimate hypnotic effects from the waking state because insomniacs are prescribed hypnotics at bedtime.

In the present study, therefore, we used a sleep-disturbed model recently developed by Shinomiya et al. (11), which is useful for evaluating the hypnotic effects of drugs in the sleep state. To detect the characteristics of H₁-antagonists, the effects of some H₁-antagonists on the sleep-wake cycle were studied in comparison with those of a representative benzodiazepine hypnotic, nitrazepam.

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Materials and Methods

Animals

Male Wistar rats weighing 240–320 g (Japan SLC, Shizuoka) were used. All animals were maintained in an air-conditioned room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). They were housed in aluminum cages with sawdust and kept under a light-dark cycle (lights on from 07:00 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-5N; Narishige, Tokyo). For electroencephalogram (EEG) recording, a stainless steel screw electrode (800 μm in diameter) was chronically implanted into the right frontal cortex (A: 0.5, L: 3.0), according to the atlas of Paxinos and Watson (12). A stainless steel screw fixed in the left frontal bone served as a reference electrode. To record the electromyogram (EMG), stainless steel wire electrodes (200 μm in diameter) 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 the surgery.

EEG and EMG recordings

EEG and EMG were recorded with an electroencephalograph (Model EEG 4314; Nihon Kohden, Tokyo) for 6 h (10:00–16:00). Recording was carried out according to a method described previously (13–15). 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 rats were measured in a cylindrical plastic cage (diameter, 26 cm; height, 31 cm), with the floor covered with sawdust, or placed on a stainless steel grid. The grid floor was placed inside the plastic cage. The stainless steel rods of the grid (3-mm-wide) were set 2-cm apart. The cage was filled with water up to 1-cm below the grid surface. The observation cage was placed in a sound-proof and electrically shielded box (70 \times 60 \times 60).

Sleep-wake state analysis

The sleep-wake states were automatically classified by 10-s epochs as waking, non-rapid eye movement (non-REM) or rapid eye movement (REM) sleep by SleepSign ver. 2.0, according to the criteria previously described (11, 15). As a final step, the defined sleep-wake stages were examined visually and corrected if necessary. Each state was characterized as follows: waking, low-amplitude EEG and high-voltage EMG activities; non-REM sleep, high-amplitude slow or spindle EEG and low-voltage EMG activities; REM sleep, low-voltage EEG and EMG activities. Slow-wave sleep was defined as the state excluding spindle sleep from non-REM sleep.

Conclusion of delta activity during non-REM sleep

Delta activity during non-REM sleep was determined using SleepSign ver. 2.0. The power spectrum densities, integrated and averaged, could be divided into 4 frequency areas: delta wave (0.5–4 Hz), theta wave (4–8 Hz), alpha wave (8–13 Hz), and beta wave (13–30 Hz). Delta power data were calculated as the ratio of average delta activity during non-REM sleep in the drug administration group versus during non-REM sleep in the control group.

Drugs

Diphenhydramine hydrochloride, chlorpheniramine maleate, and cyproheptadine hydrochloride were from Sigma (St. Louis, MO, USA). Nitrazepam (Nelbon®) was from Sankyo (Tokyo). Diphenhydramine, chlorpheniramine, and cyproheptadine were dissolved in distilled water. Nitrazepam was suspended in 0.5% carboxymethyl cellulose solution. The drugs were administered orally at 10:00, and EEG and EMG were measured for 6 h after drug administration. Drugs were administered at intervals of 7 days when the same rats were used for repeated experiments. Each rat was used 4 times in this experiment.

Data analysis and statistics

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

Results

Comparison of sleep parameters for rats placed on a grid suspended over water or sawdust

When rats were placed on the grid suspended over water, significant increases in sleep latency and the

Table 1. Comparison of the sleep parameters for rats placed on a grid suspended over water or sawdust

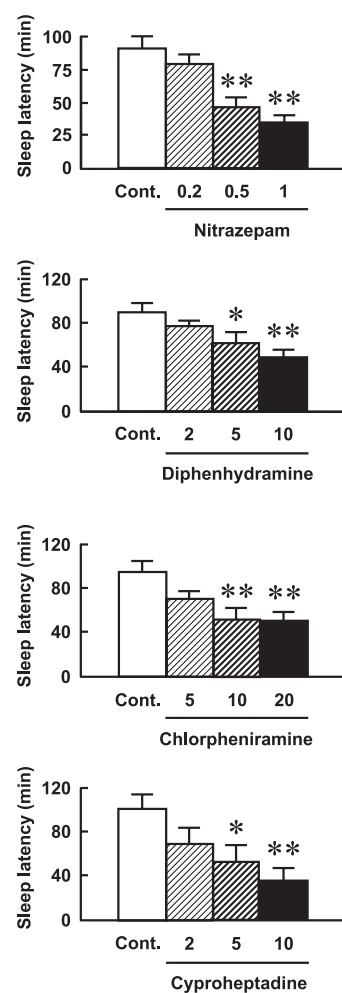
Sleep parameters	Time (min)	
	Sawdust	Grid
Sleep latency	44.3 ± 4.3	87.6 ± 8.3**
Wake	158.7 ± 7.6	230.5 ± 7.5**
NREM sleep	174.3 ± 7.7	119.0 ± 8.0**
REM sleep	27.0 ± 2.5	10.5 ± 2.8**

Each reported value is a mean ± S.E.M. (n = 8). **: Significantly different from the sawdust group at $P < 0.01$.

waking time and decreases in the non-REM and REM sleep time were observed compared with those of rats placed on sawdust (Table 1).

Effects of nitrazepam and H₁-antagonists on sleep latency

Significant shortening of sleep latency was observed with nitrazepam at doses of 0.5 and 1 mg/kg. Diphenhydramine at doses of 5 and 10 mg/kg, chlorpheniramine at doses of 10 and 20 mg/kg, and cyproheptadine at doses of 5 and 10 mg/kg also caused significant shortening of the sleep latency (Fig. 1).

Fig. 1. Effects of nitrazepam and H₁-antagonists on sleep latency in sleep-disturbed rats. Columns and vertical bars represent the means ± S.E.M. (n = 8). Drugs were administered orally. *, **: Significantly different from the control group at $P < 0.05$ and $P < 0.01$, respectively.**Table 2.** Effects of nitrazepam and H₁-antagonists on the sleep-wake cycle in sleep-disturbed rats

Drugs	Dose (mg/kg)	Wake (min)	non-REM sleep (min)	REM sleep (min)
Nitrazepam	—	227.9 ± 6.9	127.4 ± 7.1	4.8 ± 1.0
	0.2	210.2 ± 6.8	145.0 ± 7.1	4.8 ± 1.2
	0.5	200.0 ± 7.4*	156.1 ± 7.0*	3.9 ± 1.2
	1	184.6 ± 6.1**	170.1 ± 6.0**	5.4 ± 0.7
Diphenhydramine	—	230.5 ± 7.5	119.0 ± 8.0	10.5 ± 2.8
	2	238.3 ± 6.2	113.8 ± 6.1	8.0 ± 1.8
	5	219.1 ± 8.8	133.5 ± 8.7	7.4 ± 1.8
	10	204.6 ± 9.7	144.8 ± 9.0	10.6 ± 1.9
Chlorpheniramine	—	218.7 ± 10.0	134.5 ± 10.0	6.8 ± 2.3
	5	198.9 ± 9.8	154.0 ± 9.9	7.0 ± 1.6
	10	215.0 ± 11.3	139.5 ± 11.3	5.6 ± 1.4
	20	220.5 ± 16.8	136.8 ± 17.0	2.8 ± 0.6
Cyproheptadine	—	245.4 ± 8.7	107.5 ± 8.2	7.0 ± 1.3
	2	218.0 ± 10.3	135.7 ± 10.2	6.3 ± 1.0
	5	183.9 ± 12.7**	159.6 ± 12.2**	16.5 ± 2.4**
	10	188.1 ± 8.0**	155.6 ± 6.8**	16.4 ± 2.3**

Each reported value is a mean ± S.E.M. (n = 8). Drugs were administered orally. *, **: Significantly different from the control group at $P < 0.05$ and $P < 0.01$, respectively.

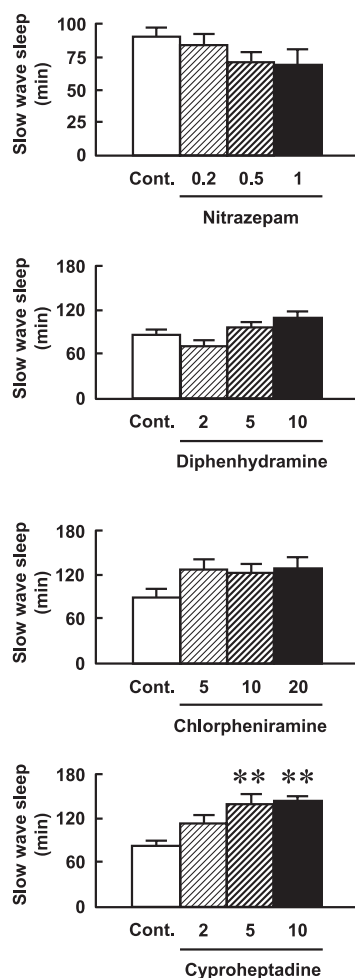


Fig. 2. Effects of nitrazepam and H_1 -antagonists on slow-wave sleep in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n=8$). Drugs were administered orally. **: Significantly different from the control group at $P<0.01$.

Effects of nitrazepam and H_1 -antagonists on the sleep-wake cycle

Nitrazepam at doses of 0.5 and 1 mg/kg caused a significant decrease in the total wake time and an increase in total non-REM sleep time. On the other hand, no significant effect was observed on total REM sleep time. No remarkable effects were observed with diphenhydramine and chlorpheniramine on the total wake time, non-REM sleep time, and total REM sleep time. On the other hand, cyproheptadine at doses of 5 and 10 mg/kg caused a significant decrease in the total wake time and an increase in total non-REM sleep time and total REM sleep time (Table 2).

Effects of nitrazepam and H_1 -antagonists on slow-wave sleep

No significant differences were observed with nitra-

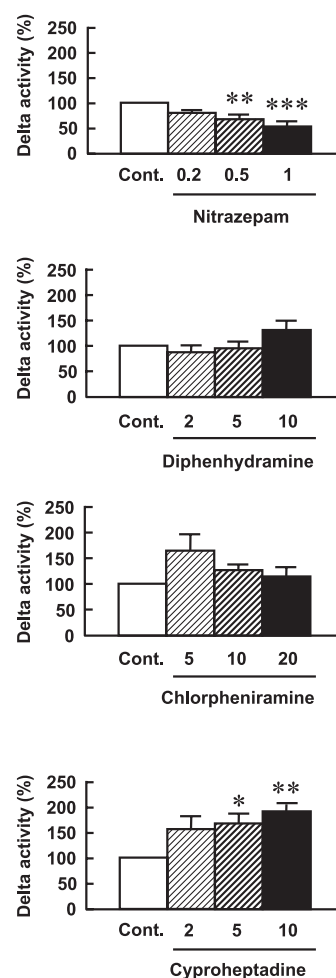


Fig. 3. Effects of nitrazepam and H_1 -antagonists on delta activity during non-REM sleep in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n=8$). Drugs were administered orally. *, **, ***: Significantly different from the control group at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

zepam, diphenhydramine, and chlorpheniramine on slow-wave sleep. On the other hand, cyproheptadine at doses of 5 and 10 mg/kg caused a significant increase in slow-wave sleep (Fig. 2).

Effects of nitrazepam and H_1 -antagonists on delta activity during non-REM sleep

The peak time of the appearance of the delta activity was from 1 to 2 h after drug administration; therefore, in this study, delta activity was calculated for 2 h.

As shown in Fig. 3, nitrazepam at doses of 5 and 10 mg/kg caused a significant decrease in delta activity during non-REM sleep. No significant effects were observed with diphenhydramine and chlorpheniramine on the delta activity during non-REM sleep even at doses of 10 and 20 mg/kg, respectively. On the other hand, cyproheptadine at doses of 5 and 10 mg/kg caused a

significant increase in delta activity during non-REM sleep.

Discussion

In the present study, it was confirmed that nitrazepam caused a significant shortening of sleep latency in sleep-disturbed rats. Nitrazepam also caused a significant decrease in the total waking time and an increase in the total non-REM sleep time. Almost the same finding was reported by Noguchi et al. (16) that nitrazepam lengthened the total sleep and shortened the onset time to the sleep stage in rats. On the other hand, it was found that nitrazepam caused a significant decrease in delta activity during non-REM sleep. Delta activity is thought to reflect sleep quality (17). It is well known that delta activity is an indicator of depth within non-REM sleep (18–20). A decrease in delta activity has been also reported for other benzodiazepines, such as triazolam, flunitrazepam and midazolam, during non-REM sleep (17, 21, 22). From these findings, it became clear that benzodiazepines produced an increase in the quantity of sleep, but the sleep quality deteriorated. Although the mechanism of the sleep quality deterioration induced by benzodiazepine is not clear, it is almost certain that the decrease in delta activity by benzodiazepines may not be mediated by benzodiazepine receptors (17, 23).

Diphenhydramine, chlorpheniramine, and cyproheptadine caused a significant shortening of sleep latency in sleep-disturbed rats. Saitou et al. (10) also reported that H₁-antagonists caused a reduction of sleep latency in rats, although the experiment time and conditions were quite different from those in our study. In insomniacs, it was demonstrated that diphenhydramine and chlorpheniramine decreased sleep latency and it has been widely used clinically (24, 25). Therefore, it is reasonable to presume that cyproheptadine is also an effective sleep inducer in the treatment of insomnia, classified as difficulty in falling asleep, similar to diphenhydramine and chlorpheniramine.

As shown in the present study, it is worthy of special mention that cyproheptadine also caused an increase in non-REM, REM, and slow-wave sleep in sleep-disturbed rats different from those of diphenhydramine and chlorpheniramine. In addition, cyproheptadine caused an increase in delta activity during non-REM sleep, indicating that this drug showed sleep quality-enhancement effects. Different from diphenhydramine and chlorpheniramine, cyproheptadine is well known to have a potent anti-serotonergic effect through 5-HT₂ receptors besides an anti-histaminergic effect (26). Viola et al. (27) and Kantor et al. (28) reported that ritanserin, a 5-HT₂-receptor antagonist, induced an

increase in slow-wave sleep, REM sleep, and delta activity in poor sleepers and rats. Stutzmann et al. (29) also demonstrated that RP 62203, a 5-HT₂-receptor antagonist, increased the duration of deep non-REM sleep, whereas DOI, a 5-HT₂ agonist, induced an increase in the waking state. These findings strongly suggest that cyproheptadine enhances sleep quantity and quality through the 5-HT₂ receptor.

In conclusion, cyproheptadine can be an effective hypnotic in the treatment of insomnia, not only for difficulty in falling asleep, but also intermittent waking after falling asleep.

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