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Effects of short-acting hypnotics on sleep latency in rats placed on grid suspended over water

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Abstract

The present study was performed to develop a new sleep disturbance model for evaluating hypnotic potencies by placing rats on a grid suspended over water up to 1 cm under the grid surface. When rats were placed on the grid, significant increases in sleep latency and amount of wakefulness were observed compared with those of rats placed on sawdust. However, the amounts of non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep of rats placed on the grid were significantly decreased compared with those of rats placed on sawdust. Four short-acting hypnotics (triazolam, zopiclone, brotizolam, lormetazepam) caused significant decreases in sleep latency, and the effects of hypnotics in rats placed on the grid were more potent than those in rats placed on sawdust. In conclusion, the present model can serve as a new sleep disturbance model and may also be useful for evaluating the sleep-inducing effects of short-acting hypnotics.

Keywords: Insomnia; Sleep latency; Wakefulness; Non-rapid eye movement (non-REM) sleep; Rapid eye movement (REM) sleep; Short-acting hypnotic

1. Introduction

A number of short-acting benzodiazepines and benzodiazepine analogues have been developed for use as hypnotic drugs. These drugs are used extensively for clinical applications due to their rapid onset of action when taken at bedtime, sufficiently sustained action to facilitate sleep throughout the night and no residual action by the following morning (Charney et al., 2001). There have been a number of reports on the sleep-inducing effects of hypnotic compounds in rats and cats (Lancel et al., 1998; Lancel, 1999; Weber et al., 1985). Most of these studies were performed using normal animals and, therefore, it may be difficult to accurately estimate the sleep-inducing effects of these drugs. Halperin et al. (1981) attempted to produce a rat model of insomnia by lengthening the light phase of the light/dark cycle. In this latter model, the total awake time observed on electroencephalograms (EEG) in the dark period was increased. We have also measured rat EEG in the dark period to determine the arousal level of the animals (Saitou et al., 1999). The rat being a nocturnal animal made it seem likely that studies of the effects of drugs on sleep latency done in the daytime would be preferable to those done at night. In the daytime, however, rats show high-baseline sleep times. These findings suggested that it is necessary to develop a new sleep disturbance model that can be measured during the day.

In the present study, therefore, we developed a new sleep disturbance model that can be used for evaluation of hypnotic properties by placing rats on a grid suspended over water. In addition, sleep-inducing effects of certain short-acting hypnotics were studied using this model.

2. Materials and methods

2.1. Animals

Seventy-two male Wistar rats weighing 230–290 g (Japan SLC, Shizuoka, Japan) 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 aluminum cages with sawdust and kept under a light/dark cycle (lights on from 7:00 to 19:00). The

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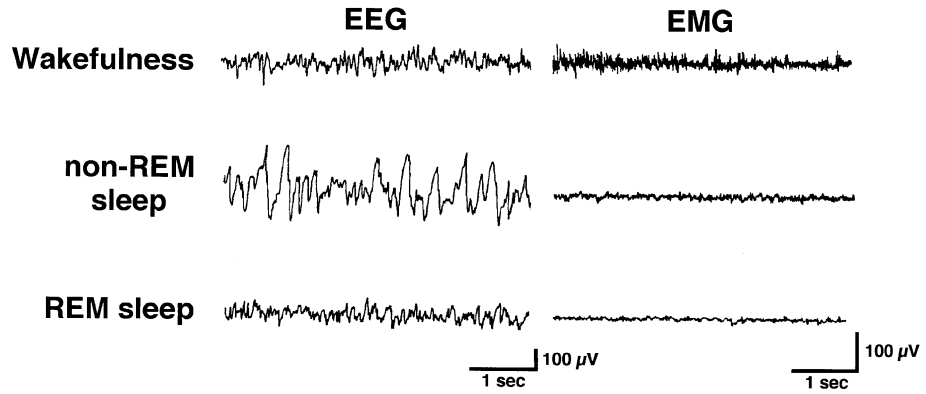

Fig. 1. Representative EEG and EMG tracings from a rat during wakefulness, slow-wave sleep and non-REM sleep.

animals were allowed free access to food and water except during the experiments.

2.2. Surgery

The animals were anesthetized with pentobarbital sodium (35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, USA) then fixed in a stereotaxic apparatus (SR-5, Narishige, Tokyo, Japan). For EEG recording, a stainless-steel screw electrode (200 μm) was chronically implanted into the right frontal cortex (A: 6.9, L: 3.0) according to the atlas of De Groot (1959). To record the electromyogram (EMG), stainless-steel wire electrodes (200 μm) were implanted into the dorsal neck muscle. Electrodes were connected to a miniature connector, and the whole assembly was fixed to the skull with dental cement. At least 7 days were allowed for recovery from the surgery.

2.3. Measurement of sleep

EEG and EMG of the rat were recorded in a plastic cage (30 × 18 × 24 cm); 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 cages were filled with water 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.

2.4. Analysis of EEG and EMG

EEG and EMG were recorded with an electroencephalograph (Model EEG 5113, Nihon Kohden, Tokyo, Japan). Power spectral analysis was carried out according to the method described previously (Saitou et al., 1999). The signals were amplified, and the analogue signals were converted into digital values using a multi-channel A–D converter (GENIUS, Medical Research Equipment, Tokyo, Japan) and fast Fourier transformer (FFT); spectral powers were calculated in real time using a personal computer (PC-9801 BX-2, NEC, Tokyo, Japan). In this system, data sampling was carried out at a rate of 50 Hz for 2.56 s. In the present study, the minimum frequency resolution was 0.2 Hz. The power spectrum densities were integrated and averaged for 60 s. Every 60-s epoch was classified as wakefulness, non-rapid eye movement (non-REM) sleep or rapid eye movement (REM) sleep according to a modified Witting's method (Witting et al., 1996). Each state was characterized as follows: wakefulness, low-voltage EEG and high-amplitude EMG activities; non-REM sleep, high-voltage slow EEG and low-EMG activities; REM sleep, low-amplitude EEG and EMG activities (Fig. 1). Sleep latency was defined as the time from drug administration up to the first five consecutive 60-s epochs of sleep.

2.5. Drugs

The following drugs were used: triazolam (Halcion®, Pharmacia & Upjohn, London, UK), zopiclone (Amoban®, Aventis Pharma, Tokyo, Japan), brotizolam (Lendormin®, Nippon Boehringer Ingelheim, Hyogo, Japan), and lormetazepam (Evamyl®, Nippon Shering, Osaka, Japan). The drugs were suspended in 0.5% carboxymethyl cellulose (CMC) solution and administered orally, and EEG and EMG were measured for 360 min after drug administration.

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

Sleep parameters	Sawdust	Grid
	Time (min) ± S.E.M.	
Sleep latency	24.5 ± 3.0	80.8 ± 12.9[a]
Wakefulness	123.8 ± 7.7	224.9 ± 6.5[a]
Non-REM sleep	203.6 ± 7.0	121.3 ± 6.2[a]
REM sleep	32.6 ± 3.6	13.9 ± 2.8[a]

Data represent means ± S.E.M. ($n = 8$).
[a] Significantly different from rats placed on sawdust at $P < 0.01$ (paired t-test).

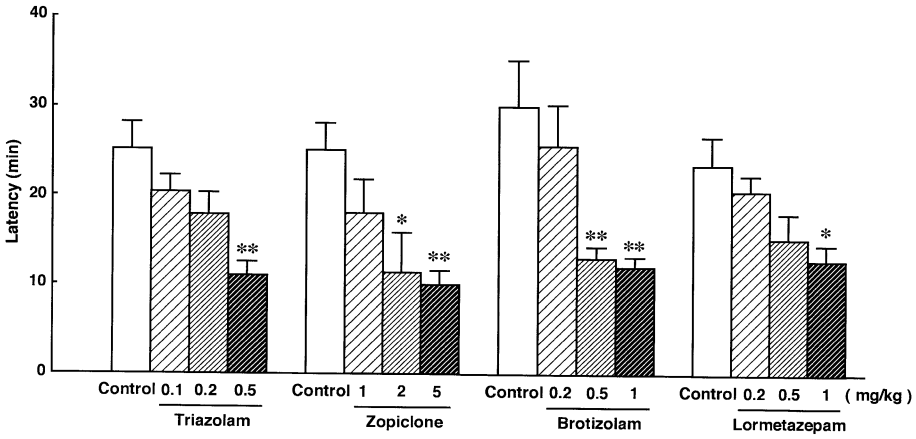

Fig. 2. Effects of hypnotics on sleep latency in rats placed on sawdust. Columns and vertical bars represent means ± S.E.M. ($n = 8$). Drugs were administered orally. Significantly different from control group at $^{*}P < 0.05$ and $^{**}P < 0.01$ (ANOVA with Dunnett's test).

2.6. Data analysis

Values shown are means ± S.E.M. A four-paired *t*-test was used for comparison of sleep parameters in rats placed on sawdust and on the grid suspended over water. One-way analysis of variance (ANOVA) with Dunnett's test was used for estimation of drug effects. ED_{50} values were calculated as those that showed a reduction to less than 1/2, compared with the control value, according to the probit method.

3. Results

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

As shown in Table 1, when rats were placed on the grid suspended over water, significant increases in sleep latency and total time of wakefulness were observed as compared with those of rats placed on sawdust. Total times of non-REM sleep and REM sleep in rats placed on the grid suspended over water were decreased significantly compared with those of rats placed on sawdust.

3.2. Effects of hypnotics on sleep latency

In rats placed on sawdust, significant shortening of sleep latency was observed with triazolam at a dose of 0.5 mg/kg, zopiclone at doses of 2 and 5 mg/kg, brotizolam at doses of 0.5 and 1 mg/kg, and lormetazepam at a dose of 1 mg/kg (Fig. 2). In rats placed on the grid suspended over water, the hypnotics showed increased potency to shorten sleep latency compared to that in rats placed on sawdust. That is, triazolam at doses of 0.05 and 0.1 mg/kg, zopiclone at doses of 1 and 2 mg/kg, brotizolam at doses of 0.2 and 0.5 mg/kg, and lormetazepam at doses of 0.1 and 0.2 mg/kg caused significant shortening of sleep latency (Fig. 3).

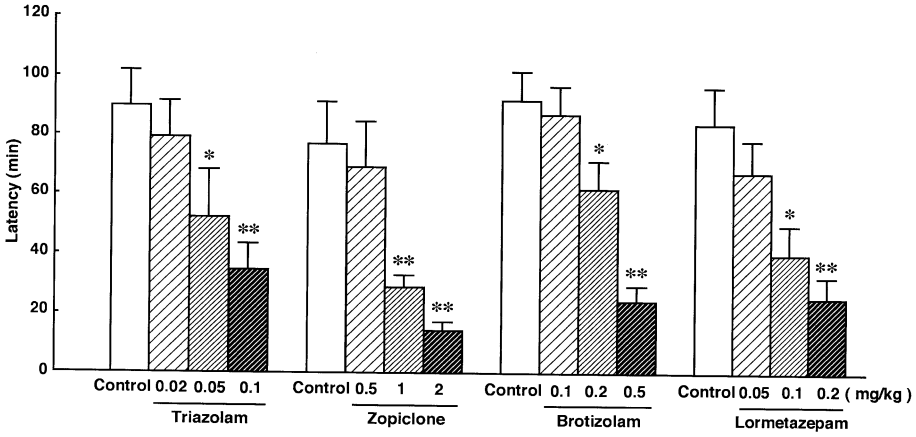

Fig. 3. Effects of hypnotics on sleep latency in rats placed on the grid suspended over water. Columns and vertical bars represent means ± S.E.M. ($n = 8$). Drugs were administered orally. Significantly different from control group at $^{*}P < 0.05$ and $^{**}P < 0.01$ (ANOVA with Dunnett's test).

Table 2
ED_{50} values of hypnotics for sleep latency in rats placed on sawdust or on the grid suspended over water

Drugs	ED_{50} (95% confidence limits) mg/kg, p.o.		Sawdust/ grid ratio
	Sawdust	Grid	
Triazolam	0.51 (0.34–0.98)	0.07 (0.05–0.10)	7.29
Zopiclone	2.43 (0.71–4.85)	1.00 (0.56–1.47)	2.43
Brotizolam	0.69 (0.46–1.04)	0.37 (0.22–0.52)	1.86
Lormetazepam	1.11 (0.67–3.31)	0.14 (0.09–0.24)	7.93

3.3. Comparison of sleep latency induced by hypnotics in rats placed on sawdust or on the grid suspended over water

Table 2 shows the ED_{50} values of hypnotics for sleep latency. ED_{50} values of all hypnotics for sleep latency for rats placed on the grid suspended over water were lower than those for animals placed on sawdust. Especially in the case of triazolam and of lormetazepam, the differences between the two groups were approximately seven- to eightfolds. On the other hand, the effects of zopiclone and brotizolam on decreases in sleep latency were greater by approximately twofolds at most in animals on the grid.

3.4. Effects of hypnotics on total time of each sleep state

No significant effects of any of the hypnotics examined were observed on total times of wakefulness, non-REM sleep or REM sleep in rats placed on sawdust. Findings were similar for rats placed on the grid (Fig. 4).

4. Discussion

In the present study, we developed a new insomnia model by placing rats on a grid in cages filled with water to 1 cm below the grid surface. As described in the text, rats placed on the grid showed significant increases in sleep latency and total time of wakefulness compared with those of rats placed on sawdust. In addition, total times of both non-REM sleep and REM sleep were significantly decreased compared with those of rats placed on sawdust. Insomnia is generally divided into five categories: that with a physical, physiological, psychological, psychiatric or pharmacological cause (Beetar et al., 1996; Waters et al., 1993; Wheatley, 1988). A new or unpleasant environment is considered to be a physical cause of insomnia. Michaud et al. (1982) developed a mild insomnia model caused by environmental perturbations, i.e. rats were moved from the breeding cage to individual plastic jars. Under these conditions, total times of non-REM sleep and REM sleep were significantly decreased. James and Piper (1978) found an increase in total time of wakefulness and decreases in total times of non-REM sleep and REM sleep in a model of consistent insomnia in rats using electric footshock. These two insomnia models seem to involve physical factors. Therefore, the sleep disturbance model described in the present study may also involve, to some extent, physical factors. On the other hand, Porkka-Heiskanen et al. (1995) reported an increase in percent waking and decreases in percent non-REM sleep and percent REM sleep in a water tank method (Morden et al., 1967). In this latter water tank method, the rats were placed on a platform,

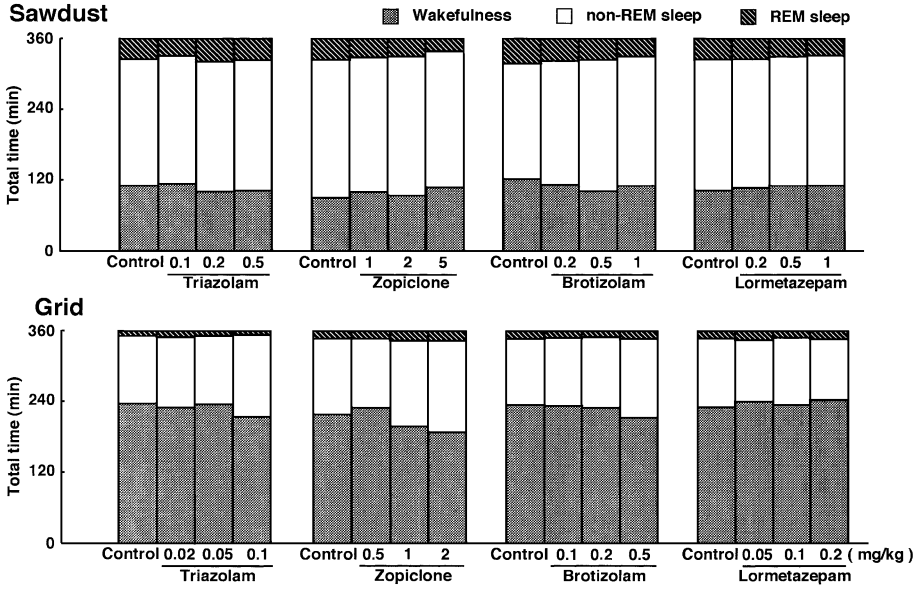

Fig. 4. Effects of hypnotics on total time of each sleep state in rats placed on sawdust or the grid suspended over water. Columns represent the means of each sleep state ($n = 8$). Drugs were administered orally, and EEG was measured for 6 h.

11 cm in diameter, fixed to a chamber, filled so that the platform was 5 cm above the water. Rats fall into a state of anxiety when placed on a platform surrounded by water (Fratta et al., 1987; Kovalzon and Tsibulsky, 1984; Vogel, 1975). Therefore, the present insomnia model seems to involve not only physical but also psychological factors.

To confirm whether this model is suitable for testing the sleep-inducing effects of hypnotics, the effects of four short-acting hypnotics (triazolam, zopiclone, brotizolam and lormetazepam) were studied. All the hypnotics used in the present study decreased sleep latency, and the effects were more potent in rats placed on the grid suspended over water than in rats placed on sawdust. Triazolam and lormetazepam were approximately seven- to eightfolds more potent in rats placed on the grid suspended over water than in those placed on sawdust as determined from comparison of ED_{50} values. In contrast, the effects of zopiclone and brotizolam were at most approximately twofolds more potent in the animals on the grid than in those on sawdust. It seems likely that these differences were due to differences in the potency of anxiolytic activities of these hypnotic drugs. There have been reports that the anti-conflict effects, widely used as an index of anxiolytic activity in rats, of triazolam, and lormetazepam, are more potent than those of brotizolam and zopiclone (Böke-Kuhn et al., 1986; Griebel et al., 1998; Shibata et al., 1989; Young et al., 1987). These findings, thus, confirmed that the present new model of insomnia is associated with not only physical but also psychological causes.

On the other hand, Suchecki and Tufik (2000) reported that corticosterone levels of rats placed on a grid were less than those in rats placed on a narrow platform on the tank floor. From these findings, it is reasonable to presume that the animals were subjected to a relatively powerful stress in the new insomnia model developed in our present study because the animals were exposed to two stressors, i.e. grid and water. As shown in the present study, the total times of wakefulness, non-REM sleep and REM sleep were not changed significantly by the hypnotics used. Short-acting hypnotics decrease sleep latency without affecting total times of wakefulness, non-REM sleep and REM sleep. The drugs now used were classified as short-acting benzodiazepines and their analogues, and the durations of action of these drugs in clinical use were reported to be 2–10 h (Wheatley, 1992). In rats, the plasma half-lives of these drugs were reported to be very short (Bechtel et al., 1986; Gaillot et al., 1983; Girkin et al., 1980; Kitagawa et al., 1979). This explains why the hypnotics used in the present study did not significantly alter the total times of wakefulness, non-REM sleep or REM sleep.

In conclusion, the present model that involves placing rats on a grid in a cage filled with water to 1 cm below the grid surface can be used as a new sleep disturbance model, and this model may be useful for evaluating the sleep-inducing effects of short-acting hypnotics.

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