

*Full Paper***Hypnotic and Sleep Quality–Enhancing Properties of Kavain in Sleep-Disturbed Rats**

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Abstract. The present study was performed to investigate the effects of kavain on the sleep–wake cycle in comparison with that of rilmazafone and diphenhydramine using sleep-disturbed rats. Electrodes for the electroencephalogram (EEG) and electromyogram (EMG) were implanted into Wistar rats. Total awake time, non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep were measured for 6 h. Kavain and rilmazafone showed a significant shortening in sleep latency, decreased awake time, and increased non-REM sleep time. On the other hand, significant shortening of the sleep latency was observed following the administration of diphenhydramine, while no effects were observed on the awake and non-REM sleep time. Moreover, kavain showed a significant increase in delta activity during non-REM sleep in sleep-disturbed rats, whereas a significant decrease in delta power during non-REM sleep was observed with rilmazafone. These results clearly indicate that kavain is a compound with not only hypnotic effects, but also sleep quality–enhancement effects.

Keywords: kavain, hypnotic effect, kava-kava, sleep

Introduction

It is well known that about 20% of the population has insomnia (1–3). Insomnia often induces daytime sleepiness, impairs judgment and memory, and increases human errors (4, 5). Moreover, it has been reported that insomnia may increase the risk of lifestyle-related and psychiatric diseases (6, 7); therefore, it is of considerable importance to treat insomnia.

Recently, a number of benzodiazepines and their analogues have been widely used to treat insomnia as hypnotic drugs in clinical practice; however, benzodiazepines have various side-effects such as rebound insomnia, anterograde amnesia, and tolerance. Furthermore, it has been reported that these drugs also decrease the quality of sleep; therefore, it seems likely that these hypnotics should be used cautiously (8–11). On the other hand, diphenhydramine and chlorpheniramine are

used to treat disorders of falling asleep as self-medication; however, these drugs are unsuitable for long-term use since it is easy to develop tolerance (12). For these reasons, new drugs are expected.

Kava-kava is an herbal medicine used to treat insomnia or anxiety in the South Pacific islands. Shinomiya et al. (13) reported that kava-kava extract significantly shortened the sleep latency in rats; furthermore, there are reports on the hypnotic effects of kava-kava extract in humans. On the other hand, it is suspected that long-term administration of kava-kava supplement causes fulminant hepatic failure (14).

Kavain is one of the components of kava-kava and also has a hypnotic effect (15). In addition, there are no reports about hepatic failure in the *in vivo* experiments using this drug. With respect to these findings, however, little work has been done using an animal model. Therefore, we investigated the effect of kavain on the sleep–wake cycle using sleep-disturbed rats in comparison with rilmazafone, a benzodiazepine hypnotic, and diphenhydramine.

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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) was chronically implanted into the right frontal cortex (A: 0.5, L: 3.0) according to the atlas of Paxinos and Watson (16). 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) 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 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 the method described previously (17, 18). The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–60 Hz), 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 cylindrical plastic cage (diameter, 26 cm; height, 31 cm), with its floor placed on a stainless steel grid. A 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 cm).

Sleep-wake state analysis

Sleep-wake states were automatically classified by 10-s epochs as awake, non-rapid eye movement (non-

REM), or rapid eye movement (REM) sleep by SleepSign ver. 2.0, according to the criteria previously described (19, 20). As a final step, defined sleep-wake stages were examined visually and corrected, if necessary. Each state was characterized as follows: awake, 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.

Calculation 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 to non-REM sleep in the control group (21).

Drugs

The following drugs were used: (+/-)-kavain (Biomol, Plymouth Meeting, PA, USA), rilmazafone hydrochloride hydrate (Rhysmy; Shionogi & Co., Ltd., Osaka), and diphenhydramine hydrochloride (Sigma, St. Louis, MO, USA). Kavain and rilmazafone were suspended in 0.5% carboxymethyl cellulose solution, diphenhydramine was dissolved in distilled water and administered orally at 10:00, and EEG and EMG were measured for 6 h after drug administration. Eight rats were used in each group, and a counterbalanced design for drug dosage was used. Drugs were administered at intervals of 7 days when the same rats were used for repeated experiments.

Data analyses

Values shown are the means \pm S.E.M. One-way analysis of variance (ANOVA) with Dunnett's test was used to estimate drug effects.

Results

Effects of kavain, rilmazafone, and diphenhydramine on sleep latency

A significant shortening of sleep latency was observed with kavain at doses of 30 and 100 mg/kg. Rilmazafone at doses of 1 and 3 mg/kg, and diphenhydramine at doses of 3 and 10 mg/kg also showed a significant shortening of sleep latency (Fig. 1).

Effects of kavain, rilmazafone, and diphenhydramine on awake time

Kavain at a dose of 100 mg/kg and rilmazafone at a

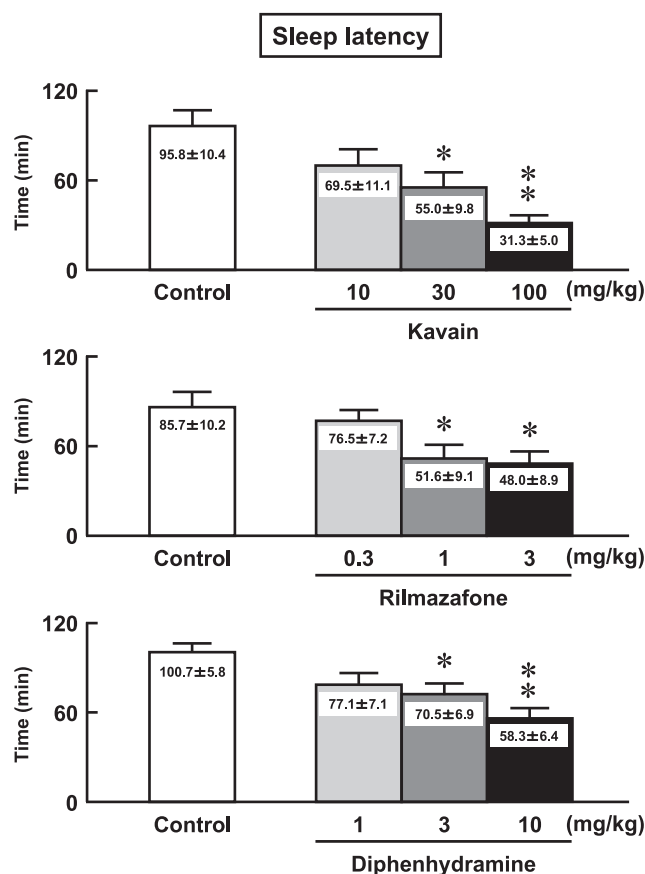


Fig. 1. Effect of each drug on sleep latency in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n = 8$). Drugs were administered orally. * $P < 0.05$, ** $P < 0.01$: Significantly different from the control group.

dose of 3 mg/kg caused a significant decrease in awake time. On the other hand, no significant difference was observed with diphenhydramine in awake time, even at a dose of 10 mg/kg (Fig. 2).

Effects of kavain, rilmazafone, and diphenhydramine on non-REM sleep time

Kavain at doses of 30 and 100 mg/kg and rilmazafone at a dose of 3 mg/kg caused a significant increase in non-REM sleep time. On the other hand, no significant difference was observed with diphenhydramine in non-REM sleep time, even at a dose of 10 mg/kg (Fig. 3).

Effects of kavain, rilmazafone, and diphenhydramine on REM sleep time

No significant effects were shown in total REM sleep time with kavain, rilmazafone, and diphenhydramine at any doses used (Fig. 4).

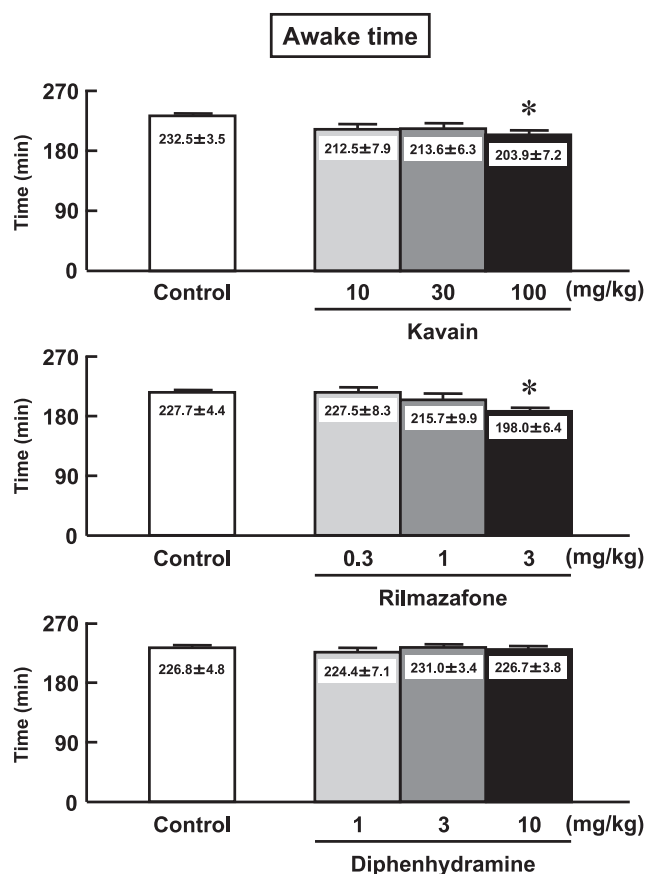


Fig. 2. Effect of each drug on awake time in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n = 8$). Drugs were administered orally. * $P < 0.05$: Significantly different from the control group.

Effects of kavain, rilmazafone, and diphenhydramine on delta activity during non-REM sleep

Kavain at doses of 30 and 100 mg/kg caused a significant increase in delta activity during non-REM sleep. On the other hand, rilmazafone at doses of 1 and 3 mg/kg caused a significant decrease in delta activity during non-REM sleep. No significant effects were observed with diphenhydramine on delta activity during non-REM sleep, even at a dose of 10 mg/kg (Table 1).

Discussion

In the present study, we investigated the effect of kavain on the sleep-wake cycle using sleep-disturbed rats. As a result, kavain caused a significant decrease in sleep latency and the total awake time and an increase in total non-REM sleep time. Kavain also increased delta activity during non-REM sleep. Kavain is one of the components of the herbal medicine kava-kava, and it has a hypnotic effect. Shinomiya et al. (13) reported recently that kava-kava extract caused a significant decrease in

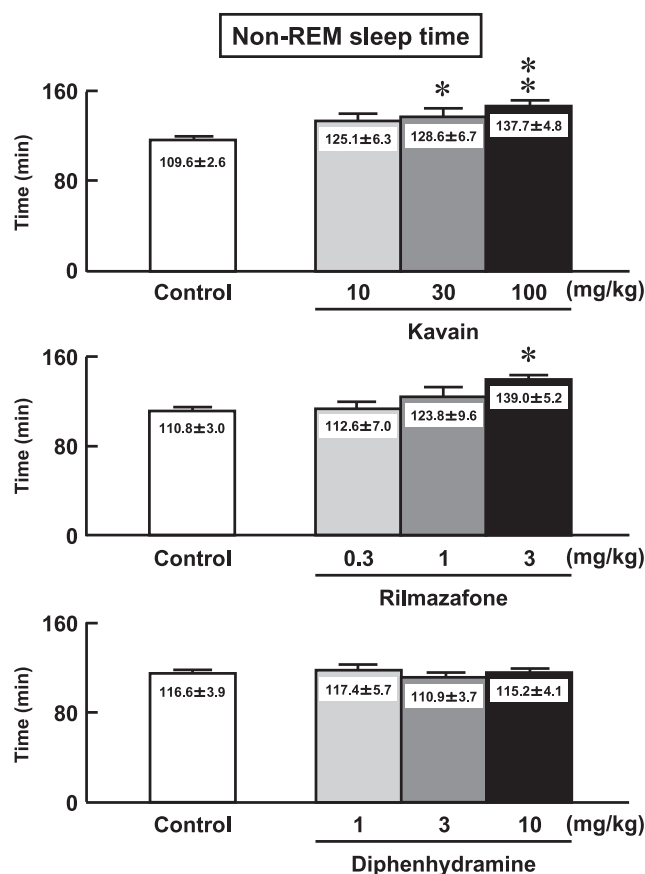


Fig. 3. Effect of each drug on non-REM sleep time in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n = 8$). Drugs were administered orally. * $P < 0.05$, ** $P < 0.01$: Significantly different from the control group.

sleep latency in sleep-disturbed rats at a dose of 300 mg/kg, p.o. In this study, significant shortening of sleep latency was observed with kavain at doses of 30 and 100 mg/kg, p.o. Cote et al. (22) reported that kavain is included as no more than 10% by weight of kava-kava. In the past year, similar results were published by Holm et al. (15) on the hypnotic effect of kavain using cats. From these findings, it was thought that the hypnotic effect of kava-kava may be due to kavain. Furthermore, it has been reported that kava-kava extract had no effect on total awake time and non-REM sleep time. On the other hand, kavain caused not only shortening of sleep latency but also decreased the awake time and increased non-REM sleep. Based on these points, we expect that kavain is more effective than kava-kava extract as a hypnotic when used clinically. In addition, there are few reports about side effects of kavain in in vivo experiments.

Next, we compared the effects of kavain with those of rilmazafone, a benzodiazepine hypnotic. Although rilmazafone showed similar hypnotic effects to kavain,

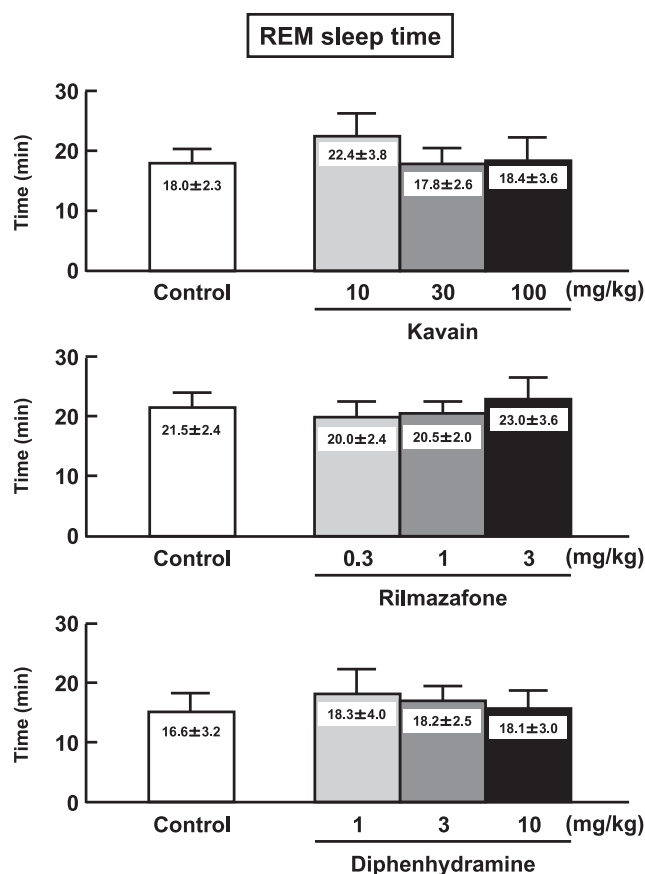


Fig. 4. Effect of each drug on REM sleep time in sleep-disturbed rats. Columns and vertical bars represent the means \pm S.E.M. ($n = 8$). Drugs were administered orally.

rilmazafone caused a significant decrease in delta activity during non-REM sleep. We have reported in previous papers that the hypnotic effects of benzodiazepines had potent hypnotic effects and decreased delta activity during non-REM sleep (13, 18). Moreover, in clinical practice, benzodiazepines induced 2nd stage sleep, light sleepiness, and inhibited 3rd and 4th stage sleep, deep sleepiness (23); therefore, it is reasonable to presume that rilmazafone induces light sleepiness and decreases the quality of sleep. On the other hand, the drug induces and maintains sleep, like other benzodiazepines. As is clear from the above findings, kavain has an effective hypnotic effect, similar to benzodiazepines, and ameliorates the quality of sleep, different from benzodiazepines.

On the other hand, diphenhydramine, a histamine H_1 receptor antagonist, showed significant shortening of sleep latency. Tokunaga et al. (24) also reported that diphenhydramine only had a sleep-inducing effect on sleep-disturbed rats. Furthermore, it has been reported that diphenhydramine shortened the sleep latency in

Table 1. Effect of each drug on delta activity in sleep-disturbed rats

	Delta activity (%)					
	0 – 1 h	1 – 2 h	2 – 3 h	3 – 4 h	4 – 5 h	5 – 6 h
Kavain (mg/kg)						
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
10	134.2 ± 14.6	135.8 ± 13.3	110.3 ± 12.5	101.3 ± 18.4	100.7 ± 8.9	121.2 ± 13.7
30	118.5 ± 21.6	141.1 ± 13.4*	123.4 ± 14.9	122.5 ± 14.9	116.8 ± 13.3	130.5 ± 18.6
100	131.5 ± 20.8	138.5 ± 8.9*	109.1 ± 8.3	124.0 ± 15.2	104.4 ± 10.8	119.7 ± 17.2
Rilmazafone (mg/kg)						
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.3	79.7 ± 9.8	98.1 ± 6.2	97.1 ± 16.7	100.2 ± 18.6	101.3 ± 23.0	94.9 ± 20.1
1	77.5 ± 6.3*	90.6 ± 6.3	90.6 ± 19.6	91.8 ± 12.8	97.7 ± 15.0	95.5 ± 14.0
3	75.6 ± 4.7*	79.6 ± 6.1*	83.9 ± 8.8	90.5 ± 15.3	88.5 ± 16.3	94.2 ± 16.0
Diphenhydramine (mg/kg)						
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
1	103.1 ± 18.6	123.1 ± 25.6	116.2 ± 19.6	93.7 ± 16.6	97.9 ± 14.1	99.1 ± 14.2
3	89.7 ± 8.3	92.4 ± 8.5	86.0 ± 7.1	76.7 ± 10.1	89.8 ± 7.8	98.0 ± 12.3
10	118.2 ± 14.3	114.2 ± 12.2	96.4 ± 7.3	94.1 ± 12.3	100.2 ± 15.4	101.0 ± 14.0

Data each represent a mean ± S.E.M. (n = 8). Drugs were administered orally. *Significantly different from the control group at $P < 0.05$.

humans (25). In addition, self-medication drugs, including diphenhydramine, are regarded as unsuitable for patients with chronic insomnia in Japan because tolerance develops easily (12). On the other hand, kavain has a sleep-maintaining effect as well as a sleep-inducing effect, different from diphenhydramine.

In conclusion, our present study clearly indicates that kavain is more effective than kava-kava extract, since kavain has a strong hypnotic effect. Furthermore, kavain is an effective component showing not only sleep induction and maintenance effects, but also ameliorates the quality of sleep, different from benzodiazepines and histamine H_1 -receptor antagonists.

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