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Effects of second-generation histamine H_1 receptor antagonists on the sleep–wakefulness cycle in rats

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Abstract

The present study was performed to examine the sedative effects of second-generation histamine H_1 receptor antagonist using power spectrum analysis in the rat. Similar to ketotifen, olopatadine caused a decrease in sleep latency at a dose of 50 mg/kg, while epinastine and cetirizine showed no significant effect even at a dose of 50 mg/kg. On the other hand, no significant difference was observed in the total times of wakefulness, non-rapid eye movement sleep and rapid eye movement sleep by any drugs used in the experiments. The number of sleep phases and interval between sleep phases were also unchanged by these drugs. Ketotifen and olopatadine inhibited [^{3}H]mepyramine binding to rat brain homogenates in parallel with a decrease in sleep latency. No significant effect was observed with epinastine and cetirizine on [^{3}H]mepyramine binding. These findings suggest that the differences in the central nervous system (CNS) depressant effect observed in second generation H_1 receptor antagonists may be due to their liability to penetrate into the CNS.

Keywords: Second-generation histamine H_1 receptor antagonists; Ketotifen; Sleep–wakefulness cycle; Binding assay; (Rat)

1. Introduction

It is well known that a number of first-generation histamine H_1 receptor antagonists such as diphenhydramine, chlorpheniramine and promethazine cause central nervous system (CNS) depressant effects, such as sedation in rodents and humans (Kamei et al., 1981; Nicholson et al., 1991; Monti et al., 1986; Adam and Oswald, 1986) diminished attention, slowed reaction and drowsiness in humans (Wyngaarden and Seevers, 1951). These histamine H_1 receptor antagonists also showed a drowsy electroencephalogram (EEG) pattern characterized by slow waves with high voltage in the cortex, a decreased locomotor activity and an inhibition of active avoidance behavior or radial maze performance in rats (Kamei et al., 1990; Tasaka et al., 1989).

On the other hand, it has been recognized that second-generation histamine H_1 receptor antagonists are less sedative than first-generation histamine H_1 receptor antagonists (Seidel et al., 1990), and a number of second-generation histamine H_1 receptor antagonists have been developed and widely used clinically. Recently, however, Hindmarch and Shamsi (1999) found that some second-generation histamine H_1 receptor antagonists also showed sedative effects similar to diphenhydramine and chlorpheniramine using new cognition and psychomotor tests in humans. It is well recognized that ketotifen causes potent somnolence in humans (Hindmarch and Shamsi, 1999) and an EEG drowsy state in rats (Kamei et al., 1996). In animal studies, little work has been done to determine if second-generation histamine H_1 receptor antagonists cause the CNS sedative effect. On the other hand, it has been reported that histamine release was promoted under stressful condition (Endo et al., 2001); therefore, we used the new sleep disturbance model that is known to increase latency to sleep (Shinomiya et al., 2003) for easily detecting the CNS effects of histamine H_1 receptor antagonists. In the present study, we studied the sedative effects of some second-generation histamine H_1 receptor antagonists by measuring EEG and electromyogram (EMG). In order to elucidate the mechanism, the effects of these drugs on histamine H_1 receptor occupancy in the rat brain were also studied.

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2. Materials and methods

2.1. Animals

Male Wistar rats at 7 weeks old (body weight 220–260 g, Japan SLC, Shizuoka, Japan) were used. All animals were kept in an air-conditioned room controlled for temperature (24 ± 2 °C) and humidity (55 ± 15%). They were housed in aluminum cages with sawdust and kept under a light/dark cycle (light on from 7:00 to 19:00). The animals were given food and water ad libitum except during the experiment. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Surgery

The animals were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg i.p., Abbott Laboratories, North Chicago, IL, USA), then fixed in a stereotaxic apparatus (Narishige, Type SR-5, Tokyo, Japan). For EEG recording, a stainless-steel screw electrode 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 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 the surgery.

2.3. Apparatus for measurement of EEG and EMG

The experiment was carried out according to the method of Shinomiya et al. (2003). EEG and EMG of the rat were recorded in a plastic cage (30 × 18 × 24 cm). A grid floor (29 × 15 × 7 cm) was placed inside the plastic cage. The cage was 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. This observation cage was placed in a sound-attenuating and electrically shielded box (70 × 60 × 60 cm) equipped with a television monitor for observing the behavior of the rats.

2.4. Analysis of EEG and EMG

EEG and EMG were recorded with an electroencephalograph (Model EEG 4314, Nihon Kohden, Tokyo, Japan). Power spectral analysis was carried out according to the method described previously (Shinomiya et al., 2003). The signals were amplified, and the analogue signals were converted into digital values using a multichannel 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). The power spectrum densities were integrated and averaged for 60 s. Every 60-s epoch was classified as wakefulness, non-rapid eye movement sleep or rapid eye movement sleep according to a modified Witting's et al. (1996) method. Each state was characterized as follows: wakefulness, low voltage EEG and high amplitude EMG activities; non-rapid eye movement sleep, high-voltage slow EEG and low-EMG activities; rapid eye movement sleep, low-amplitude EEG and EMG activities.

2.5. Calculation of sleep latency, number of sleep phases and interval between sleep phases

Sleep latency was defined as the time from drug administration until the first five consecutive 60 s of sleep. Numbers of sleep phases were counted as one time when the sleep pattern of EEG more than 5 min was continued. Intervals between sleep phases were defined as the time from five consecutive 60 s of each sleep until the next five consecutive 60 s of each sleep.

2.6. Binding of [^{3}H]mepyramine to rat brain homogenates (ex vivo and in vitro studies)

The experiment was carried out according to the method of Ishii et al. (1993) with some modifications. All drugs were administered orally to five rats, and 60 min later, the rats were decapitated. The cerebral cortex was rapidly dissected on ice, and homogenized in ice-cold phosphate-buffered saline (PBS, pH: 7.4). The homogenate was used for the receptor assay. One hundred microliters of the homogenate (1 mg/ml protein), 50 μl of [^{3}H]mepyramine (50 nmol/l) and 350 μl of PBS were incubated at 25 °C for 30 min. In vitro study, 300 μl of PBS containing 50 μl of each drug (10^{-11}–10^{-6} M) was used instead of 350 μl of PBS. The incubation was terminated by rapid filtration through a Whatman GF/C glass filter under reduced pressure. The filter was washed three times with ice-cold PBS (3 ml) and transferred to scintillation vials that contained 5 ml of scintillation cocktail (Clear-sol I, Nacalai Tesque, Kyoto, Japan). The radioactivity was determined using a liquid scintillation counter (Type LSC-5100, Aloka, Tokyo, Japan). All determinations were done in duplicate.

2.7. Drugs

The following drugs were used: olopatadine hydrochloride (Allelock, Kyowa Hakko, Tokyo, Japan), 5, 10, 20 and 50 mg/kg (1.69, 3.37, 6.74 and 16.87 nmol/kg), epinastine hydrochloride (Alesion, Nippon Boehringer Ingelheim, Hyogo, Japan), 5, 10, 20 and 50 mg/kg (1.25, 2.49, 4.99 and 12.47 nmol/kg), cetirizine hydrochloride (Zyrtec, UCB Japan, Tokyo, Japan), 5, 10, 20 and 50 mg/kg (1.94, 3.89, 7.78, 19.44 nmol/kg) and ketotifen fumarate (Zaditen, Novartis Pharma, Tokyo, Japan), 2, 5, 10 and 20 mg/kg (0.62, 1.55, 3.09, 6.18 nmol/kg). All drugs were dissolved

in distilled water except for ketotifen fumarate, which was suspended in 0.5% carboxymethyl cellulose (CMC) solution. Vehicle control for olopatadine, epinastine or cetirizine was distilled water, and that for ketotifen was 0.5% CMC solution. They were administered orally and EEG and EMG were measured for 180 min after drug administration. Drugs were administered at intervals of 10 days when the same animals were used for repeated experiments.

2.8. Data analysis

Values shown are mean ± S.E.M. One-way analysis of variance (ANOVA) with the Dunnett's test was used for estimation of the 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. Effects of histamine H_1 receptor antagonists on sleep latency

Similar to ketotifen, olopatadine caused a significant shortening of sleep latency at a dose of 50 mg/kg. On the other hand, no significant differences were found in epinastine and cetirizine even at a dose of 50 mg/kg (Fig. 1). Among the histamine H_1 receptor antagonists used in this study, ketotifen showed the most potent effect with ED_{50} values of 4.5 mg/kg. Olopatadine was 10 times less potent than ketotifen with ED_{50} of 46.1 mg/kg.

Table 1
Binding of [^{3}H]mepyramine to histamine H_1 receptors in brain homogenates obtained from rats given histamine H_1-antagonists (ex vivo study)

Drugs	Dose (mg/kg, p.o.)	[^{3}H]mepyramine binding (CPM)
Control	–	921.9 ± 131.0
Olopatadine	50	502.9 ± 141.8[a]
Epinastine	50	671.1 ± 194.1
Cetirizine	50	557.1 ± 138.4
Ketotifen	20	317.1 ± 99.00[b]

Each drug was orally administered to rats and 60 min later, the brains were dissected. The binding of [^{3}H]mepyramine to histamine H_1 receptors in the brain homogenates was measured.
CPM: counts/min.
Data represent means ± S.E.M of five rats.
[a,b] Significantly different from control at $P<0.05$ and $P<0.01$, respectively.

3.2. Effects of histamine H_1 receptor antagonists on the sleep parameters

No significant effects were found in the total times of wakefulness, non-rapid eye movement sleep and rapid eye movement sleep for any histamine H_1 receptor antagonists used (data not shown). Also, no significant differences were observed in the number of sleep phases and interval between sleep phases for any histamine H_1 receptor antagonists (data not shown).

3.3. Effects of histamine H_1 receptor antagonists on [^{3}H]mepyramine binding to the brain homogenates (ex vivo study)

Olopatadine caused a significant inhibition of [^{3}H]mepyramine binding at a dose of 50 mg/kg. Ketotifen showed a

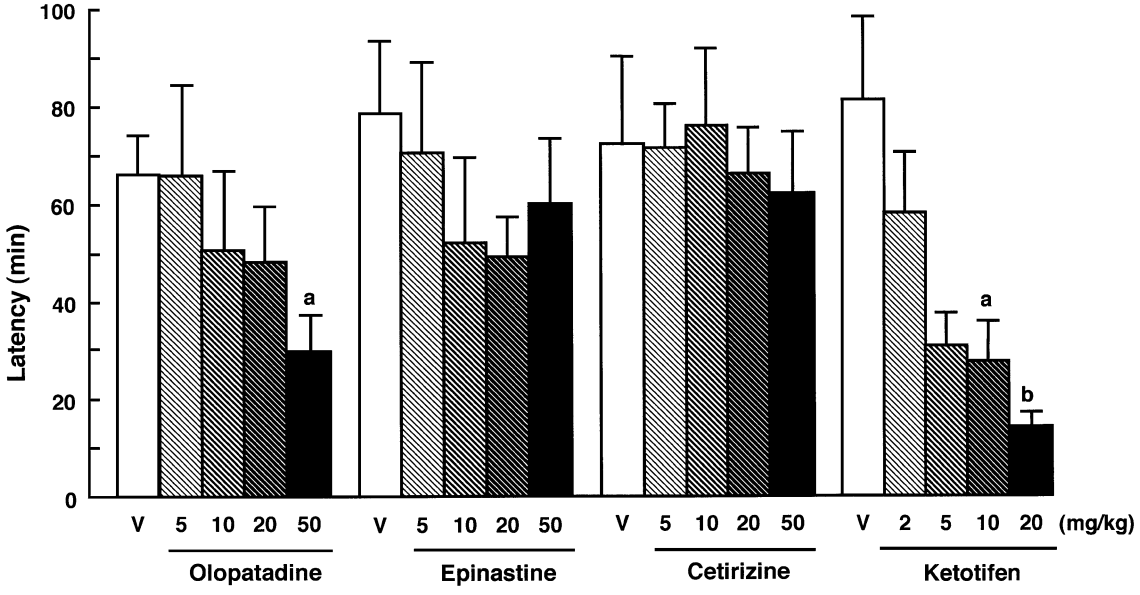

Fig. 1. Effects of olopatadine, epinastine, cetirizine and ketotifen on sleep latency. Drugs were administered orally, and EEG and EMG were measured for 180 min. V: vehicle. Columns and vertical bars represent means ± S.E.M of 10 rats. [a,b]Significantly different from control at $p<0.05$ and $p<0.01$, respectively.

Table 2
IC_{50} values of second-generation histamine H_1-antagonists on [^{3}H]mepyramine binding in rat brain homogenates (in vitro study)

Drugs	IC_{50} values (nM, 95% confidence limits)
Olopatadine	125 (44.2–534)
Epinastine	173 (73.2–480)
Cetirizine	119 (44.4–433)
Ketotifen	193 (77.6–553)

The IC_{50} is the concentration of inhibitor showing 50% inhibition of the receptor-specific binding of 50 nmol/l [^{3}H]mepyramine. Eight rats were used in each drug.

more potent effect in inhibiting [^{3}H]mepyramine binding than olopatadine. However, no significant effect was observed with epinastine and cetirizine at a dose of 50 mg/kg (Table 1).

3.4. Effects of histamine H_1 receptor antagonists on [^{3}H]mepyramine binding to the brain homogenates (in vitro study)

IC_{50} values of these second-generation histamine H_1 receptor antagonists on [^{3}H]mepyramine binding to the brain homogenates were almost the same, indicating that the affinity for the H_1 receptor was unchanged by any drugs used (Table 2).

4. Discussion

Similar to ketotifen, olopatadine was found to cause a decrease in sleep latency indicating that the drug has drowsy effects. We have reported that first-generation histamine H_1 receptor antagonists, such as diphenhydramine, mepyramine, chlorpheniramine and promethazine showed a dose-dependent decrease in sleep latency in rats, although the experimental conditions were somewhat different (Saitou et al., 1999). Doses of 5–20 mg/kg showed a significant effect on sleep latency of these first-generation histamine H_1 receptor antagonists (Saitou et al., 1999). As shown in the present study, a dose of 50 mg/kg was required for olopatadine to cause a significant effect. Therefore, it is reasonable to presume that the potency showing the drowsy effect of olopatadine was relatively weak. On the contrary, both epinastine and cetirizine caused no significant effect on the sleep latency even at a dose of 50 mg/kg. Seidel et al. (1990) and Tasaka et al. (1989) found that cetirizine and epinastine caused no significant effect on EEG in humans and rats, respectively. From these findings, it can be assumed that ketotifen and olopatadine cause CNS depressant effects, while epinastine and cetirizine have no such effects. Similar findings were observed by Nishiga et al. (2003) using a new protocol of active avoidance response in rats. That is, ketotifen and olopatadine caused a significant inhibition of response retrieval at doses of 10 and 20 mg/kg, respectively. Epinastine and cetirizine had weak or no effects on the active avoidance response.

On the other hand, no significant difference was observed in the total times of wakefulness, non-rapid eye movement sleep and rapid eye movement sleep by any drugs used in the experiments. Depoortere et al. (1995) reported that cetirizine caused no modification of the sleep–wakefulness pattern at a dose of 10 mg/kg, i.p. in rats. Epinastine was also reported to show no remarkable effect on EEG activity in rats or on the sleep–wakefulness patterns in cats (Fügner et al., 1988). The number of sleep phases and interval between the sleep phases were also unchanged by these drugs. These results indicate that both epinastine and cetirizine showed no CNS depressant effects even when a detailed examination of sleep parameters was studied.

To clarify the mechanism of non-drowsiness or drowsiness after oral administration of four histamine H_1 receptor antagonists, ex vivo and in vitro [^{3}H]mepyramine binding assays to brain homogenates were performed in rats. No significant difference was observed in [^{3}H]mepyramine binding in the in vitro study by the histamine H_1 receptor antagonists used, indicating that the affinity of these drugs to the brain is almost the same. However, as shown in the present study, it was found that both ketotifen and olopatadine inhibited the specific [^{3}H]mepyramine binding to the histamine H_1 receptors in brain homogenates obtained from rats given histamine H_1 receptor antagonists (ex vivo study) at doses of 20 and 50 mg/kg, respectively. On the other hand, no significant inhibition was found with epinastine and cetirizine. Ishii et al. (1993) also reported that ketotifen inhibited the binding of [^{3}H]mepyramine to rat brain homogenates. Epinastine has been found to show a very high affinity for histamine H_1 receptor binding, but it poorly penetrates into the CNS (Fügner et al., 1988). Kato et al. (1997) also demonstrated that ketotifen had extremely high ratios of drug concentration in the brain/plasma value, while epinastine and cetirizine had small brain/plasma values. These results are essentially consistent with the view that ketotifen and olopatadine are effective at decreasing sleep latency, while epinastine and cetirizine are not. From these findings, it was considered that both ketotifen and olopatadine can easily penetrate into the CNS, whereas epinastine and cetirizine cannot.

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