Slow Wave Sleep-inducing Effects of First Generation H₁-Antagonists

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The present study was performed to see if first-generation histamine H₁-antagonists are useful sedative-hypnotic drugs. Increases in electroencephalogram (EEG) power spectra of the delta band (0—4 Hz) at the frontal cortex and theta band (4—8 Hz) at the hippocampus in rats were used as an indexes of sleep. The H₁-antagonists used in this study resulted in a decrease in sleep latency and an increase in sleep duration (slow wave sleep). The rate of REM (rapid eye movement) sleep during slow wave sleep was decreased by H₁-antagonists and brotizolam. The order of potency of H₁-antagonists for the reduction in sleep latency (from greatest to least) was promethazine > chlorpheniramine > diphenhydramine and pyrilamine, and that for the increase in sleep duration was chlorpheniramine > promethazine > diphenhydramine and pyrilamine. Brotizolam was more potent than these H₁-antagonists, with 14—18-fold and 4—14-fold greater effects on sleep latency and duration, respectively. These results clearly show that H₁-antagonists are effective in mild to moderate insomnia as sedative-hypnotic drugs.

Key words sleep; EEG power spectra; diphenhydramine; chlorpheniramine; promethazine; brotizolam

Insomnia has physical, physiological, psychological and psychiatric causes, and the symptoms can be divided into 3 types; i.e. inability to fall asleep upon retiring, intermittent waking after falling asleep and early awakening. In the past, barbiturate and miscellaneous sedative-hypnotic drugs were used to treat an inability to sleep under normal conditions. However, currently, benzodiazepine derivatives and nonbenzodiazepine sedative-hypnotic drugs (zolpidem, zopiclone) are commonly used.

On the other hand, first generation H₁-antagonists such as chlorpheniramine, diphenhydramine and promethazine have sedation-related clinical symptoms such as diminished alertness, slowed reaction time and somnolence. In animal studies, these drugs also resulted in a drowsy electroencephalogram (EEG) pattern from the cortex, hippocampus and amygdala in rats, decreased locomotor activity in mice, inhibition of active avoidance behavior in rats and radial maze performance in rats. Based on these findings, it is anticipated that first generation H₁-antagonists may be useful as sleep-inducers. However, little work has been done to see if first generation H₁-antagonists are useful as sedative-hypnotics. Therefore, the present study was undertaken in rats using power spectral analysis to clarify the sleep-inducing effect of H₁-antagonists compared with brotizolam.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 250—300 g (Nippon SLC, Shizuoka, Japan) were used. All animals were maintained in an air-conditioned room with controlled temperature (22—26°C) and humidity (40—70%). They were housed in aluminum cages with wood shavings 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.

Surgery The animals were anesthetized with pentobarbital sodium (35 mg/kg, i.p., Nembutal®, Abbott Laboratories, North Chicago, IL, U.S.A.), then fixed in a stereotaxic apparatus (Narishige, SR-5, Tokyo, Japan). Stainless-steel electrodes were implanted into the right frontal cortex (A: 6.9, L: 3.0) and hippocampus (A: 3.0, L: 2.5, H: 2.5) according to the atlas of de Groot. Electrodes were connected to a miniature receptacle, and the whole assembly was fixed to the skull with dental cement. At least 14 d were allowed for recovery from surgery.

Power Spectral Analysis The electroencephalogram (EEG) was recorded with an electroencephalograph (Nihon Kohden, Model EEG 5113, Tokyo, Japan). Power spectral analysis of the EEG was carried out according to the method described previously. The EEG signals were amplified, and the analogue EEG signals were converted into digital values by means of 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 (NEC, PC-9801 BX-2, 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 values of the power spectrum were displayed sequentially for 2-min periods. In most instances, the power spectrum densities, integrated and averaged for 5 min, could be divided into the 4 frequency areas: delta wave (0—4 Hz), theta wave (4—8 Hz), alpha wave (8—13 Hz) and beta wave (13—30 Hz).

Calculation for Sleep Latency and Duration of Sleep Experiments for EEG measurement were carried out after 20:00 to maintain the arousal level of the animals. When transferred from the home cage to the observation chamber, rats showed a low frequency power, especially at delta wave (0—4 Hz), in the frontal cortex in parallel with behavioral arousal for about 6 h. EEG spectral powers at the start of recording were taken as 100% (wakefulness). The sleep latency was defined as the time from the start of the recording to reaching values of more than 150% in EEG spectral powers (slow wave sleep) three times, and the sleep duration was the time from the end of latency to reaching values of less than 150% in EEG spectral powers three times. REM (rapid eye movement) sleep was defined as the time during which a clear theta wave (4—8 Hz) was observed in the hippocampus during the period of sleep duration (slow wave sleep).

Drugs The following drugs were obtained from the...
Fig. 1. Effects of Certain H₁-Antagonists and Brotizolam on Sleep Latency
Columns and vertical bars represent means±S.E.M. (n=8—10). *, **: Significantly different from control at p<0.05 and p<0.01, respectively.

Fig. 2. Effects of Certain H₁-Antagonists and Brotizolam on Sleep Duration
Columns and vertical bars represent means±S.E.M. (n=8—10). *, **: Significantly different from control at p<0.05 and p<0.01, respectively.

sources indicated in parentheses: diphenhydramine hydrochloride (Sigma, St. Louis, MO, U.S.A.), pyrilamine maleate (Sigma), chlorpheniramine hydrochloride (Yoshitomi, Osaka, Japan), promethazine hydrochloride (Sigma) and brotizolam (Nippon Boehringer Ingelheim, Hyogo, Japan). The drugs were dissolved in 0.2% carboxymethyl cellulose (CMC) solution, and administered orally. The animals were deprived of food 24 h prior to drug administration.

Data Analysis The data were analyzed statistically by ANOVA and Dunnett’s test. ED₅₀ values were calculated as those that showed a reduction to less than 1/2 (latency) or an increase of more than 2-fold (duration), compared with the control value, according to the probit method.

RESULTS

Effects on Sleep Latency In the control group treated with 0.2% CMC, sleep latency was 74.4±6.6 min (n=9). All the H₁-antagonists used in this study caused a dose-dependent decrease in sleep latency. Significant effects were observed with diphenhydramine (10 and 20 mg/kg), pyrilamine (20 and 50 mg/kg), chlorpheniramine (10 mg/kg) and promethazine (5, 10 and 20 mg/kg). Brotizolam caused a significant decrease in sleep latency at doses of 0.5, 1 and 2 mg/kg (Fig. 1).

Effects on Sleep Duration In the control group, sleep duration was 35.6±6.0 min (n=9). H₁-antagonists caused a dose-dependent increase in sleep duration. Significant effects were observed with diphenhydramine (10 and 20 mg/kg), pyrilamine (20 and 50 mg/kg), chlorpheniramine (5 and 10
mg/kg) and promethazine (10 and 20 mg/kg). Brotizolam also caused an increase in sleep duration, and a significant effect was observed at a dose of 2 mg/kg (Fig. 2). The rates of REM sleep during the slow wave sleep are shown in Table 1. All the H₁-antagonists used in this study caused dose-related decreases in the rate of REM sleep during slow wave sleep (sleep duration). Significant effects were observed with diphenhydramine (20 mg/kg), pyrilamine (50 mg/kg), chlorpheniramine (10 mg/kg) and promethazine (10 and 20 mg/kg). Brotizolam also caused a significant decrease in the rate of REM sleep at doses of 1 and 2 mg/kg.

**Comparison of the Effects on Sleep Latency and Duration Induced by H₁-Antagonists** Table 2 shows the ED₅₀ values of H₁-antagonists for sleep latency and sleep duration. Diphenhydramine, chlorpheniramine and promethazine showed similar potencies. Pyrilamine was less potent than the other H₁-antagonists used. On the other hand, brotizolam was 14—18-fold more potent than diphenhydramine, chlorpheniramine and promethazine. Similar results were observed for effects on sleep duration. However, chlorpheniramine was more potent than the other H₁-antagonists in increasing sleep duration. Brotizolam was 4—14-fold more potent than the H₁-antagonists.

**DISCUSSION**

There have been many reports that first generation H₁-receptor antagonists produce a drowsy EEG pattern and sedation in humans and animals. For instance, Monti et al.⁸ found that pyrilamine and diphenhydramine decreased wakefulness and increased slow wave sleep in rats when administered by intraperitoneal injection. Wauquier et al.⁹ also reported that NREM (non-rapid eye movement) sleep was increased in dogs when diphenhydramine and chlorpheniramine were administered p.o. at a dose of 10 mg/kg. We reported previously¹⁰ that some H₁-antagonists caused a drowsy EEG in the frontal cortex, hippocampus and amygdala and inhibited the EEG arousal response induced by acoustic and midbrain reticular formation stimulation. However, in these studies, the central nervous system (CNS) depressant effects of H₁-antagonists were recognized as side-effects, and there have been no animal studies of the application of H₁-antagonists as sedative-hypnotic drug. On the other hand, there have been some clinical reports that only diphenhydramine is effective as a sedative-hypnotic drug in humans. Rickels et al.¹¹ reported that diphenhydramine is useful as an OTC (over-the-counter) sleep aid in the treatment of temporary mild to moderate insomnia. Russo et al.¹² also found that diphenhydramine was significantly better than placebo in reducing sleep latency time and the number of incidences of awakening per night, while sleep duration was marginally increased.

In the present animal studies, it was found that not only diphenhydramine but also other H₁-antagonists caused a reduction in sleep latency and an increase in sleep duration. However, there is no close relationship between sleep latency and sleep duration for any of the drugs. At present, we are not prepared to offer an explanation of these findings. Lipid solubility, affinity for the central nervous system and biological half-life may play a role in the differences in the sleep latency and sleep duration produced by these drugs. On the other hand, Quach et al.¹³ reported that the order potency for inhibiting [³H]pyrimidazine binding is as follows: promethazine>chlorpheniramine>diphenhydramine and pyrilamine. This potency correlates well with the sleep latency of these drugs in the present study.

From these findings, it seems likely that not only diphenhydramine but also chlorpheniramine and promethazine would be effective as bedtime sleeping aids for insomniac patients. However, the potency of these 3 drugs was far less than that of brotizolam which is commonly used clinically. Therefore, these H₁-antagonists may be clinically useful as mild sleep-inducers. The H₁-antagonists used in this study were also effective in depressing the rate of REM sleep during slow wave sleep. Wauquier et al.¹⁴ also reported that diphenhydramine decreased REM sleep in dogs. It is well known that increases in the amount and frequency of REM sleep elicit intermittent waking. As shown in the present study, the H₁-antagonists increased the duration of slow wave sleep. Therefore, the H₁-antagonists used in this experiment may also be effective in preventing intermittent waking after falling asleep and early awaking.

Long-acting hypnotics cause residual daytime sleepiness. In the case of H₁-antagonists, however, the sleep duration was less than that observed following administration of the representative short-acting benzodiazepine, brotizolam. Therefore, it is reasonable to presume that H₁-antagonists will not cause any such residual daytime sleepiness.
REFERENCES