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Electroencephalographic Properties of Zaleplon, a Non-Benzodiazepine Sedative/Hypnotic, in Rats

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Abstract. The encephalographic (EEG) properties of zaleplon were investigated in comparison with those of other sedative hypnotics in conscious rats with chronically implanted electrodes. The oral administration of zaleplon (0.25 – 1.0 mg/kg), triazolam (0.0625 – 0.25 mg/kg), zopiclone (1.0 – 4.0 mg/kg), brotizolam (0.0625 – 0.25 mg/kg), and nitrazepam (0.125 – 0.5 mg /kg) lengthened the total sleep in a dose-dependent manner. On distribution of sleep-wakefulness stages, zaleplon, in particular, increased the slow wave deep sleep (SWDS), whereas triazolam, brotizolam, and nitrazepam increased the slow wave light sleep (SWLS) in a dose-dependent manner. Zopiclone significantly increased the SWDS at a dose of 2 mg/kg and both the SWLS and the SWDS at a dose of 4 mg/kg. All tested hypnotics caused no influence on fast wave sleep (FWS) at doses tested. The appearance of the sleep-inducing activity of zaleplon was more rapid than those of any compounds tested, and zaleplon significantly increased the relative EEG power density in the delta frequency band over that of triazolam at 20 and 30 min after the administration in the spectral analysis. Therefore, the present findings suggest that the non-benzodiazepine zaleplon can be expected to exhibit high practical potential as a hypnotic and is characterized by an increase in SWDS with rapid onset of hypnotic action.

Keywords: zaleplon, ω_1 receptor, EEG, spectra analysis, hypnotic

Introduction

Sleep disorders are a frequent complaint. Regional and nationwide surveys show that about one-third of the adult population currently complains of some difficulty in sleeping: difficulty falling asleep, difficulty staying asleep, or waking up too early; most subjects have difficulty falling asleep (1, 2). Zaleplon (*N*-[3-(3-cyanopyrazolo[1,5-*a*]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide), a pyrazolopyrimidine, is a non-benzodiazepine sedative-hypnotic. A benzodiazepine receptor binding assay suggested that zaleplon is a selective full agonist of the ω_1-receptor subtype within the γ-aminobutyric acid $(GABA)_A$ receptor ionophore complex in the brain, enhancing the function of GABA as a result of an allosteric interaction with the $GABA_A$-receptor chloride anion channel complex (3). It has been reported that zaleplon, although not benzodiazepine-like in chemical structure, induces sedative-hypnotic, anticonvulsant and anticonflict effects mediated by its binding to the central nervous system (CNS) type benzodiazepine receptors (4 – 6). In our previous electrocorticographic recordings of rabbits, a qualitative difference between the effects of zaleplon and benzodiazepines was observed. Triazolam and zopiclone significantly increased the energy of the beta frequency band, whereas zaleplon did not (3). A striking difference between zaleplon and benzodiazepines may be due to selectivity for the ω_1-receptor subtype. Furthermore, we have also reported that zaleplon is characterized by a reduced amnesic liability, which may be due to its low affinity for the benzodiazepine site of the $GABA_A$ receptor in the hippocampus (7). Additionally, it would seem that zaleplon may promote melatonin secretion, suggesting an influence of zaleplon on chronobiology (8).

The hypnotic effect of zaleplon has been established in humans. The elimination half-life of zaleplon is approximately 1 h, regardless of dose and the absorption is rapid (9). Therefore, as the hypnotic action of zaleplon can be characterized by rapid onset and short duration

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of action, it has been suggested that zaleplon should be classified as an ultra-short-acting sedative/hypnotic agent, and it may be used for insomnia in patients whose main difficulty is in falling asleep. In the present study, we carried out polygraphic recording of electroencephalogram (EEG) and electromyogram (EMG) in free-moving rats chronically implanted with cortical and muscle electrodes, and examined the effects of zaleplon on the distribution of sleep-wakefulness stages as well as the power spectral analysis of EEG in comparison with those of other sedative hypnotics.

Materials and Methods

All animals used in the present study were treated according to the guidelines of the National Institutes of Health on the welfare of laboratory animals.

Animal

Male Sprague-Dawley rats (130 – 210 g) were obtained from Charles River Japan (Atsugi). The animals were kept under standard laboratory conditions of temperature and humidity (23 ± 1°C, 50 ± 5% humidity), and a 12-h light-dark cycle (lights on 7:00 AM and off at 7:00 PM) with food and water freely available. Experiments were conducted following after a minimum 7 days adaptation period to laboratory conditions. All EEG and EMG measurements were collected between 11:00 AM and 3:00 PM.

Surgery

Rats were anesthetized with an intraperitoneal injection of 35 mg/kg of pentobarbital Na (Nembutal®; Abbott Laboratories, Abbot Park, IL, USA) and fixed to a stereotaxic apparatus. For the EEG recording, monopolar electrodes were implanted into the right frontal cortex and right occipital cortex regions following the stereotaxic atlas for rats (10). For the EMG recording, enamel electrodes with bare tips were used to record the muscular activity of the dorsal muscles of the neck (11). The cortical reference electrode was screwed into the interparietal bone of the animals. All electrodes were connected to a miniature receptacle and were fixed on the skull with dental cement. After the surgical implantation, rats were housed for at least 1 week for recovery from surgery.

EEG recordings

Recordings of EEG and EMG in conscious rats (250 – 300 g) with chronically implanted electrodes were carried out by electroencephalograph (EEG-4113; Nihon Kohden Co., Tokyo) immediately after the oral administration of each compound at 11:00 AM, and continued for 4 h in an observation chamber.

Visual analysis of the recordings differentiated four sleep-wakefulness stages as awake, slow wave light sleep (SWLS), slow wave deep sleep (SWDS), and fast wave sleep (FWS) (11, 12) at 20-s intervals. Sleep stages must have had a duration of longer than 1 min to be counted. The hypnotic activities of zaleplon and the reference compounds were estimated as ED_{25} values and their 95% confidence limits. The dose required to produce a 25% increase in the total sleep (SWLS + SWDS + FWS) as compared to that of the vehicle-treated group (ED_{25}) was calculated.

Spectral analyses

Power spectral analysis of the EEG began immediately after the oral administration of zaleplon and triazolam and continued for 2 h. The analog signals of the EEG were sampled 256 times at 10-ms intervals. After fast fourier transformation (FFT), the power spectrum was calculated from the FFT data collected every 2.56 s. The power spectrum, averaged 16 times, was distributed into four frequency bands, namely as delta wave (1 – 4 Hz), theta wave (4 – 8 Hz), alpha wave (8 – 13 Hz), and beta wave (13 – 30 Hz). Relative power (percent of total) was calculated every 2 min.

Drugs

Zaleplon were supplied by Wyeth Ayerst Laboratories, Philadelphia, PA, USA. Triazolam (Halcion®; Sumitomo Pharmaceutical Co., Tokyo), zopiclone (Chugai Pharmaceutical Co., Tokyo), brotizolam (Lendormin®; Boehringer Ingelheim, Hyogo), and nitrazepam (Nerbon®; Sankyo Co., Ltd., Tokyo) were purchased from their respective suppliers (Fig. 1). Zaleplon and the reference drugs were suspended in 2% starch and were administered at a volume of 5 ml/kg.

Statistical analyses

Data were expressed as the mean ± S.E.M. or ED value. The ED_{25} value was calculated by the linear regression method. Between-group differences were analyzed statistically using ANOVA followed by the Dunnett's multiple range test. In the rat spectra analysis study, the Tukey's multiple range test was used. The statistical significance level was set at $P<0.05$.

Results

Effect on sleep-wakefulness stages

The oral administration of zaleplon (0.25 – 1.0 mg/kg), triazolam (0.0625 – 0.25 mg/kg), zopiclone (1.0 – 4.0 mg/kg), brotizolam (0.0625 – 0.25 mg/kg), and nitrazepam (0.125 – 0.5 mg/kg) lengthened the total

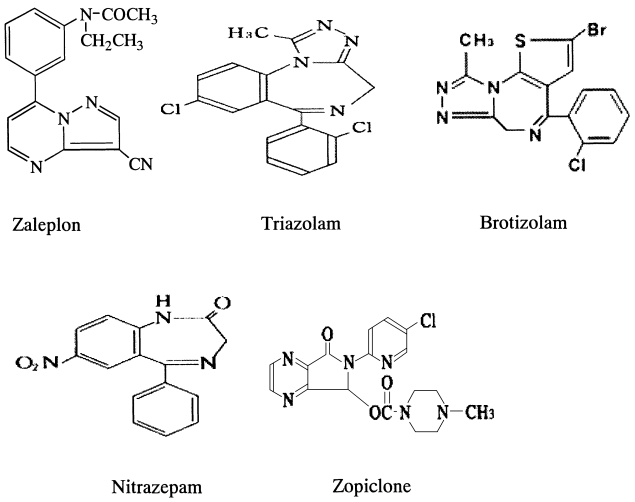

Fig. 1. Chemical schematics.

sleep in a dose-dependent manner with ED_{25} values of 0.33, 0.18, 1.41, 0.21, and 0.26 mg/kg, respectively (Table 1). On distribution of sleep-wakefulness stages, zaleplon, in particular, increased the SWDS, whereas triazolam, brotizolam, and nitrazepam increased the SWLS in a dose-dependent manner. In addition, zopiclone significantly increased the SWDS at a dose of 2 mg/kg and both the SWLS and the SWDS at a dose of 4 mg/kg. All tested hypnotics caused no influence on FWS at doses tested (Table 1). Moreover, all tested compounds lessened the latency of sleep onset depending on dosage. The onset time to sleep stage of zaleplon was more rapid than those of zopiclone, brotizolam, and nitrazepam and was equipotent with that of triazolam at the minimum effective dose (MED), which lengthened the total sleep time (Table 2).

Effect on power spectral analyses

Zaleplon (0.5 mg/kg) and triazolam (0.25 mg/kg) significantly increased the relative EEG power density in the delta frequency band over that of vehicle at 10 min and 40 min after the oral administration, respectively (Fig. 2). Furthermore, zaleplon significantly increased the relative EEG power density in the delta frequency band over that of triazolam at 20 and 30 min after administration (Fig. 2).

Table 1. Effect on distribution of sleep-wakefulness stages in rats

Compound	Dose (mg/kg, p.o.)	Distribution of sleep-wakefulness stages (%)				
		Awake	SWLS	SWDS	FWS	Total sleep
Zaleplon	0	52.3 ± 2.8	31.8 ± 3.0	10.1 ± 1.9	5.8 ± 0.6	47.7 ± 2.8
	0.25	46.0 ± 3.5	34.4 ± 2.3	13.5 ± 2.7	6.1 ± 1.8	54.1 ± 3.5
	0.5	31.7 ± 3.9**	38.5 ± 1.9	24.0 ± 2.4**	5.9 ± 1.1	68.4 ± 3.9**
	1.0	24.5 ± 2.4**	37.8 ± 2.5	31.3 ± 2.1**	6.5 ± 1.2	75.6 ± 2.4**
Triazolam	0	49.3 ± 3.2	35.2 ± 3.0	12.7 ± 1.3	2.8 ± 0.4	50.7 ± 3.2
	0.0625	48.1 ± 5.2	38.1 ± 4.5	10.3 ± 1.4	3.5 ± 1.0	51.9 ± 5.2
	0.125	37.8 ± 2.7	49.8 ± 2.4*	9.8 ± 1.8	2.8 ± 0.8	62.3 ± 2.7
	0.25	34.9 ± 4.4*	48.0 ± 4.9*	14.2 ± 1.5	3.0 ± 1.0	65.1 ± 4.4*
Zopiclone	0	50.9 ± 4.7	33.7 ± 2.6	10.5 ± 1.6	5.0 ± 1.2	49.2 ± 4.6
	1.0	42.3 ± 4.1	35.4 ± 3.2	16.7 ± 3.1	5.6 ± 1.4	57.7 ± 4.1
	2.0	35.2 ± 4.2*	38.3 ± 2.5	20.2 ± 2.7*	6.2 ± 1.0	64.8 ± 4.2*
	4.0	24.8 ± 3.6**	43.0 ± 2.6*	23.8 ± 2.6**	8.4 ± 1.6	75.2 ± 3.6**
Brotizolam	0	51.8 ± 3.7	39.6 ± 3.1	5.8 ± 1.3	2.8 ± 1.1	48.2 ± 3.7
	0.0625	48.5 ± 3.1	45.4 ± 2.8	3.6 ± 0.9	2.5 ± 0.9	51.5 ± 3.1
	0.125	44.4 ± 3.2	47.6 ± 3.4	5.0 ± 1.0	3.1 ± 0.4	55.6 ± 3.2
	0.25	38.3 ± 2.2**	55.6 ± 2.4**	3.3 ± 0.7	2.9 ± 0.8	61.7 ± 2.2**
Nitrazepam	0	55.1 ± 3.9	35.3 ± 3.6	7.0 ± 0.8	2.6 ± 0.7	45.0 ± 3.9
	0.125	52.7 ± 5.6	39.8 ± 4.6	4.9 ± 0.9	2.5 ± 0.5	47.3 ± 5.6
	0.25	43.9 ± 3.3	44.8 ± 2.6	7.8 ± 1.5	3.6 ± 1.0	56.1 ± 3.3
	0.5	35.7 ± 5.1**	56.2 ± 4.0**	4.1 ± 0.8	4.0 ± 0.9	64.3 ± 5.1**

Results are shown as the mean ± S.E.M. of eight animals per group. Total sleep is shown as the sum of the slow wave light sleep (SWLS), slow wave deep sleep (SWDS), and fast wave sleep (FWS). The EEG measurement was performed immediately after administration of each test compound and continued for 4 h in an observation chamber. *,**: Significantly difference from the corresponding vehicle control group at $P<0.05$ and $P<0.01$, respectively (Dunnett's multiple range test).

Table 2. Effect on onset time of sleep stage in rats

Compound	Dose (mg/kg, p.o.)	Onset time to sleep stage
Zaleplon	0	60.9 ± 4.8
	0.25	44 ± 4.2*
	0.5	13.8 ± 5.5**
	1.0	6.6 ± 2.6**
Triazolam	0	62.3 ± 9.2
	0.0625	56.7 ± 18.0
	0.125	15.6 ± 3.0**
	0.25	11 ± 1.7**
Zopiclone	0	57 ± 8.2
	1.0	46.3 ± 9.5
	2.0	24.9 ± 6.3**
	4.0	17.8 ± 4.1**
Brotizolam	0	67.5 ± 6.5
	0.0625	68.5 ± 12.1
	0.125	46.9 ± 9.4
	0.25	30.7 ± 6.9**
Nitrazepam	0	74.1 ± 8.9
	0.125	52.3 ± 9.9
	0.25	54.8 ± 8.1
	0.5	22.5 ± 4.3**

Each test compound was orally administered at 11:00 AM, and the EEG and EMG were continuously recorded for 4 h. The onset time to sleep stage lasting longer than 1 min for sleep wave was measured. Each value represents the mean ± S.E.M. of eight rats per group. *,**: Significantly difference from the corresponding vehicle control group at $P<0.05$ and $P<0.01$, respectively (Dunnett's multiple range test).

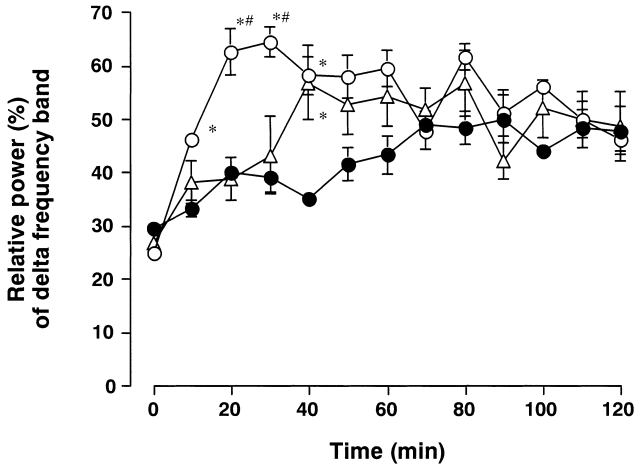

Fig. 2. Effects of zaleplon and triazolam on the power spectra of frontal cortex EEG in conscious rats. EEG was measured immediately after the oral administration of zaleplon or triazolam over a 2-h period. Results are shown as the mean ± S.E.M. of five animals per group. Closed circle: vehicle (2% starch), 5 ml/kg; open circle: zaleplon, 0.5 mg/kg; open triangle: triazolam, 0.25 mg/kg. *$P<0.05$ *vs* the vehicle control group, $^{\#}P<0.05$ *vs* the triazolam-treated group (Tukey's multiple comparison test).

Discussion

In the rat EEG study, zaleplon showed an increase in the percentage of total sleep with ED_{25} value of 0.33 mg/kg (Table 1). It was estimated that the hypnotic activity of zaleplon was approximately equipotent with that of triazolam, nitrazepam, and brotizolam and 4.3 times more potent than that of zopiclone. Thus, it is suggested that zaleplon possesses a potent sleep-inducing activity that would be evident on EEG. The latency for sleep onset of zaleplon was shorter than that of zopiclone, brotizolam, and nitrazepam and was equipotent with that of triazolam at the MED, which lengthened the total sleep time in the EEG visual study. As for the doses of each drug, they were established based on the results of our preliminary study. That is, we used the doses that are able to detect the MED and their ED values on hypnotic effect on EEG in rats. In the rat spectral analysis, zaleplon and triazolam showed an increase in the energy of the delta frequency band and a decrease in the energy of the theta frequency band (data not shown). Moreover, zaleplon showed a faster onset of action than triazolam in increasing the energy of the delta frequency band. Doses of 0.5 mg/kg for zaleplon and 0.25 mg/kg for triazolam were chosen because these doses were the MEDs of test drugs for hypnotic action in the EEG study (Table 1). It is well known that an increase in the relative EEG power density at the delta frequency range of rats is consistent with an increase in the sleep stage (13). Thus, it is suggested that the hypnotic effect of zaleplon is characterized by rapid onset, as compared with that of triazolam and the other hypnotics tested. Elimination of zaleplon in rats was rapid with a terminal half-life of approximately 1 h, and absorption was rapid, similar to what has been observed in humans (5, 9). Thus, the EEG recording and spectral analysis study in rats support the pharmacokinetics characterized by rapid absorption of zaleplon, and the rapid sleep onset of zaleplon may be solely due to rapid absorption. Interestingly enough, zaleplon increased SWDS especially without affecting SWLS and FWS, whereas triazolam, brotizolam, and nitrazepam increased the SWLS without affecting SWDS and FWS. Zopiclone showed an increase in the SWDS at a low dose, but increased both SWLS and SWDS at the high dose.

We have reported that zaleplon, triazolam, and zopiclone caused a drowsy pattern of spontaneous EEG characterized by high-voltage slow waves and desynchronization of hippocampal theta waves and an increase in the energy of the delta frequency band on the spectral analysis of the electroencephalogram in rabbits. In our previous EEG study, some interesting

results, namely that intravenous administration of triazolam and zopiclone caused a burst of high-amplitude sleep spindles in the cortical EEGs and significantly increased the energy of the beta frequency band, whereas zaleplon did not, were observed (3). Similar properties have been previously described for zolpidem and CL218,872, a non-benzodiazepine ω_1-receptor-selective agonist, that also significantly increased the energy of the delta frequency band and decreased the energy of the theta frequency band, without affecting the beta frequency band (3, 14). Furthermore, the different action of the ω_1-receptor-selective agonist on EEG has been reported in humans (15, 16). Therefore, these results suggest that binding selectively to ω_1 receptors might be a crucial factor responsible for an increasing in SWDS.

Zopiclone showed an increase in the only SWDS at a dose of 2 mg/kg, but increased both of SWLS and SWDS at a dose of 4 mg/kg in the present study. It is unclear why these phenomenon were observed due to zopiclone treatment. However, there are some supportive studies. Nakajima et al. have reported that zopiclone increased both stage 2 and slow wave sleep (SWS), while zolpidem, a ω_1-selective compound, increased only SWS and caused no effect on stage 2 in clinical study (17). They have suggested that the difference in the action to the $GABA_A$-receptor subtypes might be related to the difference in the effects on the sleep architecture between the compounds. Our previous binding study demonstrated that zopiclone is less selective to the ω_1 site and has higher affinity to the ω_2 site than zaleplon and has higher selectivety to the ω_1 site than conventional BZPs including triazolam. This could be related to the action of zopiclone at the dose of 4 mg/kg to increase SWLS as well as SWDS. The present study also suggested that zopicone at a dose of 2 mg/kg might have ω_1-selectivity since it had no effect on SWLS. In our previous animal EEG study, the relative power of the beta frequency band induced by zopiclone was lower than that by triazolam (3). The present study results supported our previous EEG study and the above clinical report.

Previous clinical EEG studies show that most benzodiazepines commonly used for the treatment of insomnia affect the distribution and composition of the different stages of sleep, as well as sleep duration and efficacy (18, 19). The EEG profile of such compounds is characterized by an appearance of sleep spindles and K-complexes in stage 2, but also in interfering with the production of EEG slow waves (18 – 20). The facts indicate that benzodiazepines induce a light sleep and, conversely, suppress a deep sleep. The reduction of SWS can be detected by visual analysis in the form of decreased stages 3 and 4. As individuals age, there is an increase in complaints of insomnia. The elderly obtain less total sleep and have very little of the deeper sleep stages 3 and 4 (21, 22). Thus, an imbalance of the sleep-wakefulness distribution is considered to be an important factor in the origin of insomnia. Therefore, it is suggested that a non-benzodiazepine sedative /hypnotic zaleplon that produces increases in SWDS without affecting SWLS and FWS may be effective for individual suffering from insufficient deep sleep stages.

In conclusion, the present studies demonstrate that zaleplon has a pharmacological profile similar to benzodiazepines that is characterized by an increase in SWDS with rapid onset of hypnotic action.

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