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Binding and neuropharmacological profile of zaleplon, a novel nonbenzodiazepine sedative/hypnotic

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Abstract

The binding properties of CL284,846 (zaleplon), a novel nonbenzodiazepine sedative/hypnotic, at benzodiazepine receptor subtypes were evaluated. Zaleplon was 14.3 times more potent at inhibiting [^{3}H]flunitrazepam binding to membrane preparations of the cerebellum than to membrane preparations of the spinal cord. The γ-aminobutyric acid (GABA) ratio of zaleplon was 2.07. Zaleplon produced significant increases in muscimol binding similar to those of diazepam, and it was antagonized by flumazenil. Furthermore, zaleplon showed little affinity for other receptors. Spectral analysis of the electroencephalogram (EEG) of rabbits showed that zaleplon and 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4,-triazolo [4,3-β] pyridazine (CL218,872), an ω_1 receptor-selective compound (1 mg/kg, i.v., respectively), produced large increases in energy of the delta frequency band without affecting the energy of the alpha and beta frequency bands. In contrast, intravenous administration of triazolam and zopiclone increased the energy of the beta frequency band at doses of 0.1 and 2 mg/kg, respectively. In addition, the zaleplon-induced increase in the energy of the delta frequency band was antagonized by pretreatment with flumazenil (1 mg/kg, i.v.), which did not affect the spontaneous EEG alone. The present results clearly demonstrate that zaleplon is a selective full agonist of the ω_1 receptor subtype, and thus, zaleplon may induce responses closely resembling the physiological pattern of slow wave sleep.

Keywords: Zaleplon; Nonbenzodiazepine; Hypnotic; ω_1 receptor; Binding assay; EEG (electroencephalogram)

1. Introduction

Zaleplon (*N*-[3-(3-cyanopyrazolo [1,5-a] pyrimid-7-yl) phenyl]-*N*-ethylacetamide) (Fig. 1), a pyrazolopyrimideine, is a novel nonbenzodiazepine sedative-hypnotic. 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 central nervous system (CNS) type benzodiazepine receptors (Day et al., 1992; Gaudreault et al., 1995; Griebel et al., 1996). In clinical trials, it has been shown that zaleplon is a safe and effective hypnotic with clear advantages over lorazepam with respect to over lorazepam with respect to unwanted cognitive and psychomotor effects (Beer et al., 1994; Allen et al., 1993). However, the detailed mechanism of action of zaleplon has not been completely elucidated. Subtypes of benzodiazepine receptors have been classified into ω_1, ω_2, and ω_3 receptors in mouse, rat, monkey and human brains (Benavides et al., 1988; Dennis et al., 1988; Schoemaker et al., 1983). ω_1 and ω_2 receptors are associated with the $GABA_A$/benzodiazepine receptor Cl^- channel complex and modulated γ-aminobutyric acid (GABA) neuronal function. They exist in different areas in the brain (Olsen et al., 1990). Furthermore, the ω_3 receptor exist mainly in peripheral organs, particularly the kidney (Anholt et al., 1985; Schoemaker et al., 1983). These subtypes seem to play different functional roles in the brain, but the phamacological or physiological properties of their binding sites have not yet been defined. The cerebellum, spinal cord and kidney are examples of tissues that possess predominantly receptors of the ω_1, ω_2 and ω_3 subtypes, respectively (Niddam et al., 1987; Schoemaker et al., 1983). Zolpidem has marked selectivity for the ω_1 receptor subtype and is a clinically effective hypnotic drug with a pharmacological profile, which differs from that of benzodiazepines (Niddam et al., 1987; Dennis et al., 1988). In electrocorticographic recordings, a qualitative difference between the effects of zolpidem and benzodiazepines is observed. Zolpidem increases the energy peaks predominantly in the 2- to 4-Hz frequency band whereas other benzodiazepines increase energy in the 12- to 14-Hz bands (Depoortere et al., 1986). A striking difference between zolpidem and benzodiazepines

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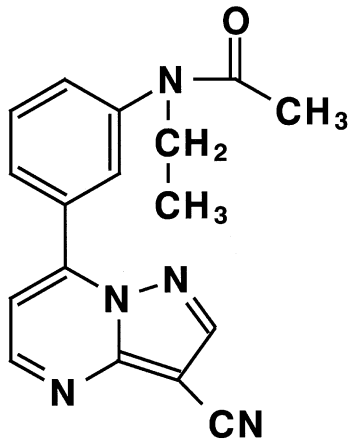

Fig. 1. Chemical structure of zaleplon (*N*-[3-(3-cyanopyrazolo [1,5-a] pyrimidin-7-yl) phenyl]-*N*-ethylacetamide.

may be due to selectivity for the ω_1 receptor subtype. One purpose of the present study is to clarify its selectivity for benzodiazepine receptor subtypes and GABAergic transmission in vitro. Thus, the effects of zaleplon on radioligand binding to membrane preparations from rat cerebellum, spinal cord and kidney were examined in comparison with those of drugs acting at benzodiazepine receptors. The second purpose of the present study is to characterize the hypnotic activity in vivo study. Therefore, we examined the electoencephalographic profile of zaleplon in rabbits with chronically implanted electrodes compared with that of some benzodiazepine-related compounds.

2. Materials and methods

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

2.1. Animals

Male Sprague–Dawley rats (250–300 g) and Japanese white rabbits (1.3–1.9 kg) were obtained from Charles River Japan and Kitayama Labes (Nagano, Japan), respectively. Rats were housed in groups of five; rabbits were individually housed with free access to food and water. All animals were kept in a controlled environment (23 ± 1 °C, 50 ± 5% humidity) with a 12-h light–dark cycle (lights on between 7:00 a.m. and 7:00 p.m.). Experiments were conducted following adaptation to laboratory conditions for at least 7 days (rats) and 14 days (rabbits), respectively. Electroencephalogram (EEG) and electromyogram (EMG) measurements were carried out between 11 h 00 min and 16 h 00 min.

2.2. Membrane preparations

After decapitation, the brain and kidney were removed rapidly, and the cerebellum and spinal cord were separated on an ice plate according to the method of Zukin et al. (1974) with the following modifications. These tissues were immediately stored at −80 °C until assay. They were homogenized gently in 0.32 M sucrose with a glass homogenizer. The cerebellum and the spinal cord homogenates were centrifuged (1000 × *g*) for 10 min at 4 °C, and the kidney homogenates were centrifuged (100 × *g*) for 5 min at 4 °C. The resulting supernatants were centrifuged at 20,000 × *g* for 20 min. The pellet from the second centrifugation was resuspende in 50 mM Tris–HCl buffer (pH 7.4) and again centrifuged at 20,000 × *g* for 20 min. This was done three times to remove endogenous GABA, The pellet was resuspended in 50 mM Tris–HCl buffer until a final concentration of 1 mg protein/ml was obtained. The protein content of tissue homogenates was determined according to Lowry et al. (1951), using bovine serum albumin as the standard.

2.3. Binding affinity for benzodiazepine receptor

Duplicate membrane preparations containing 0.5-mg protein from the cerebellum and spinal cord of rats were incubated for 2 h at 4 °C with [^{3}H]flunitrazepam (1 nM) in the presence of the test compounds. Kidney membrane preparations (0.5 mg) were incubated for 1 h at 4 °C with [^{3}H]Ro5-4864 (4-chloro-diazepam: 2.5 nM), a selective ligand of the ω_3 binding site (Schoemaker et al., 1983). Then, 5 ml of ice-cold 50 mM Tris–HCl buffer was added. The mixture was filtered immediately under vacuum through Whatman GF/B grass-fiber filters, which had been soaked in 50 mM Tris–HCl buffer (pH 7.4) containing 0.3% polyethylenimine at 4 °C, and then washed three times with 5-ml aliquots of ice-cold buffer. The nonspecific binding was defined as binding in the presence of 100 μM unlabeled diazepam. The specific binding was determined by subtracting nonspecific binding from total binding. Radioactivity was determined in a liquid scintillation counter.

2.4. Effects of GABA on binding affinity of benzodiazepine receptor

Rat cerebellar membranes were incubated for 2 h at 4 °C with [^{3}H]flunitrazepam (1 nM) and test compounds in the presence of 30 μM GABA or 50 μM bicuculline ($GABA_A$ receptor antagonist). Each concentration required to displace [^{3}H]flunitrazepam binding by 50% (IC_{50}) was calculated and the GABA ratio (IC_{50} value in the presence of $GABA_A$ receptor antagonist/IC_{50} value in the presence of GABA) was determined.

2.5. Effect on muscimol binding

Duplicate membrane preparations containing 0.5-mg protein from rat cerebellum were incubated for 2 h at 4 °C with [^{3}H]muscimol (16 nM) in the presence of the test compounds. The effects of zaleplon on [^{3}H]muscimol binding to the membrane preparations were estimated.

2.6. Affinity of zaleplon for other neurotransmitter recognition sites

The affinity of zaleplon for other neurotransmitter receptors was assayed with rat brain homogenates. Membrane preparations from rat brains were incubated with various neurotransmitter receptor radioligands in the presence of zaleplon. All studies were performed based on methods described previously: 5-HT_1 receptors (Bennett and Snyder, 1976), 5-HT_{1A} receptors (Peroutka, 1986), 5-HT_2 receptors (Leysen et al., 1982), 5-HT_3 receptors (Kilpatrick et al., 1987), α_1-adrenoceptors (Timmermans et al., 1981), α_2-adrenoceptors (Doxey et al., 1983), dopamine D1 receptors (Billard et al., 1984), D2 receptors (Imafuku, 1987), muscarine M_1 receptors (Watson et al., 1986) and μ-opioid receptors (Gillan and Kosterlitz, 1982). Details of the radioligands used, the methods for determining nonspecific binding and the results are summarized in Table 1.

2.7. EEG recording in rabbits

Male white rabbits were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and immobilized in a stereotaxic instrument. For EEG recording, monopolar electrodes were implanted into the right frontal cortex and right occipital cortex, hippocampus, amygdala and caudatus regions following the stereotaxic atlas for rabbits (Sawyer et al., 1954). 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. At least 14 days were allowed for recovery of the rabbits. Recording of EEG in conscious rabbits with chronically implanted electrodes was carried out by electroencephalography immediately after intravenous administration of zaleplon (1 mg/kg), triazolam (0.1 mg/kg), zopiclone (2 mg/kg) and 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4,-triazolo [4,3-β] pyridazine (CL218,872: 1 mg/kg) and continued for 2 h in an observation chamber. The analogue signals of EEG were converted into digital values 256 times at 10-ms intervals. After fast Fourier transformation, the power spectrum was calculated from the data of fast Fourier transformation collected every 2.56 s. The power spectrum averaged 16 times was distributed into the four frequency bands, such 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. Flumazenil (1 mg/kg, i.v.), a benzodiazepine receptor antagonist, was injected 10 min prior to the administration of zaleplon.

2.8. Drugs

Zaleplon, CL218,872 and flumazenil were supplied by Wyeth Lederle Japan. Triazolam and zopiclone were extracted from commercially available tablets (Halcion® from Upjohn Japan and Amoban® from Chugai Pharmaceutical, respectively). [^{3}H]flunitrazepam (86 Ci/mmol), [^{3}H]Ro5-4864 (86 Ci/mmol) and [^{3}H]muscimol (4.6 Ci/mmol) were obtained from Dupont. Diazepam, GABA (Wako, Osaka) and bicuculline (Sigma, St. Louis, MO, USA) were purchased from the suppliers indicated in parentheses. Other chemicals and reagents of an analytical grade were obtained from commercial suppliers. Zaleplon and the reference drugs were dissolved in dimethyl sulfoxide (DMSO) for the receptor binding study. These solutions were made up to the appropriate concentration with 50 mM Tris–HCl buffer (pH 7.4). The final concentration of DMSO in each preparation was less than 0.5%. For the EEG study of rabbits, zaleplon, triazolam and zopiclone were dissolved in physiological saline containing 5% DMSO and intravenously administered in an injection volume of 1 ml/kg. CL218,872 was dissolved in 100% propylene glycol and intravenously administered in an injection volume of 0.2 ml/kg.

2.9. Statistical analysis

Data are expressed as the means ± S.E. The concentration required to displace [^{3}H]flunitrazepam and [^{3}H]Ro5-4864 binding by 50% (IC_{50}) was calculated by linear regression analysis. In the EEG study, inter-group differences were analyzed statistically using Dunnett's multiple range test, and to determine the antagonism of flumazenil for hypnotic effects in rabbits, Turkey's multiple range test was used.

Table 1
Affinity of zaleplon for other neurotransmitter recognition sites

Receptor	Radioligand	Tissue	Nonspecific binding ligand	IC_{50} value (μM)	Assay reference
5-HT_1	[^{3}H]serotonin	cortex	serotonin	>100	Bennett and Snyder, 1976
5-HT_{1A}	[^{3}H]8-hydroxy-DPAT	cortex	8-hydroxy-DPAT	>100	Peroutka, 1986
5-HT_2	[^{3}H]ketanserin	cortex	methysergide	>100	Leysen et al., 1982
5-HT_3	[^{3}H]GR65630	cortex	MDL72222	>100	Kilpatrick et al., 1987
α_1 Adrenoceptor	[^{3}H]prazoisin	forebrain	phentolamine	>100	Timmermans et al., 1981
α_2 Adrenoceptor	[^{3}H]rauwolscine	cortex	phentolamine	>100	Doxey et al., 1983
D1	[^{3}H]SCH23390	striatum	SCH23390	>100	Billard et al., 1984
D2	[^{3}H]sulpiride	striatum	sulpiride	>100	Imafuku, 1987
μ-Opioid	[^{3}H]DAMGO	forebrain	naloxone	>100	Gillan and Kosterlitz, 1982
Muscarinic M_1	[^{3}H]pirenzepine	striatum	atropine	>100	Watson et al., 1986

3. Results

3.1. Effects of zaleplon on [^{3}H]flunitrazepam and [^{3}H]Ro5-4864 binding to rat membrane preparations

Zaleplon and the other hypnotics showed a concentration-dependent inhibition of [^{3}H]flunitrazepam binding to membrane preparations (Fig. 2A,B). In the membranes prepared from rat cerebellums and spinal cords, zaleplon displaced the binding [^{3}H]flunitrazepam with IC_{50} values of 167.0 and 2385.1 nM, respectively (Table 2). Therefore, zaleplon was 14.3 times more potent at inhibiting [^{3}H]flunitrazepam binding to membrane preparations of cerebellum enriched in ω_1 receptors than to membrane preparations of spinal cord enriched in ω_2 receptors. The IC_{50} value of nitrazepam in the cerebellum was found to be 58.8 nM, and a similar value was obtained in the case of the spinal cord. In the case of triazolam, brotizolam and zopiclone, these IC_{50} values in the cerebellum was found to be 58.8 nM, and a similar value was obtained in the case of the spinal cord. In the case of triazolam, brotizolam and zopiclone, these values in the cerebellum were 7.6, 7.1 and 112.5 nM, respectively, and similar values were obtained in the case of the of the spinal cord. CL218,872, a selective ligand for ω_1 receptors (Squires et al., 1979), also possessed a 15.5 times higher affinity for cerebellar benzodiazepine receptors than for spinal cord benzodiazepine receptors (Table 2). In the membranes prepared from rat kidneys, triazolam and brotizolam displaced the binding of [^{3}H]Ro5-4864, an ω_3 receptor ligand, with IC_{50} values of 449.9 and 107.4 nM, respectively, but zaleplon did not (Table 2, Fig. 2C).

3.2. Effects of GABA on binding affinity for benzodiazepine receptors

The ratio of the IC_{50} value obtained in the presence of 30 μM GABA and in the presence of 50 μM bicuculline (GABA ratio) for zaleplon was 2.07 (Table 3). The ratio was higher than that for CL218,872 (1.28), a benzodiazepine partial receptor agonist, and diazepam (1.84), a benzodiazepine full receptor agonist.

3.3. Effects on muscimol binding

Zaleplon (10^{-10}–10^{-5} M) showed concentration-dependent increases in [^{3}H]muscimol binding to membrane preparations of rat cerebellum, similar to diazepam (data not shown). Zaleplon and diazepam at concentrations of 10^{-6} M and 10^{-5} M produced significant increases in [^{3}H]muscimol binding with percent values of 115.2 ± 2.5 and 115.2 ± 0.5 for zaleplon and 117.4 ± 1.32 and 121.2 ± 1.2 for diazepam. However, in the presence of flumazenil, a benzodiazepine receptor antagonist, at a concentration of 10^{-5} M, the zaleplon- and diazepam-induced increases in [^{3}H]muscimol binding were antagonized to the vehicle control level (Fig. 3). In addition, flumazenil (10^{-5} M) alone produced no significant alteration in [^{3}H]muscimol binding.

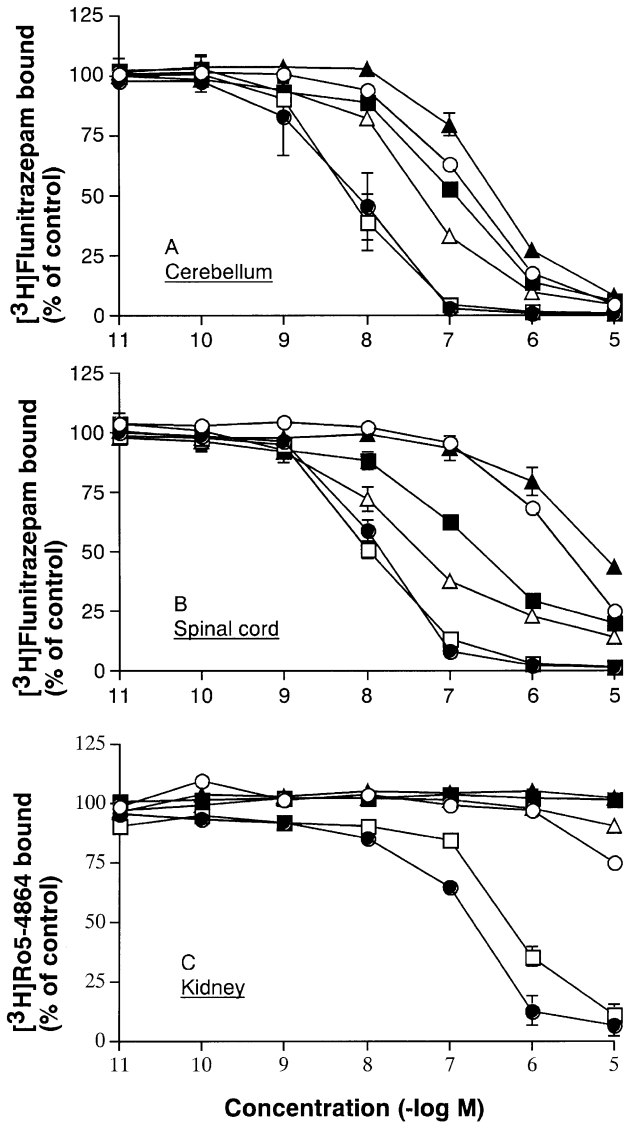

Fig. 2. Displacement curves of zaleplon (○), triazolam (□), zopiclone (■), brotizolam (●), nitrazepam (△) and CL218,872 (▲) against [^{3}H]flunitrazepam (A and B) and [^{3}H]Ro5-4864 (C) in rat cerebellum (A), spinal cord (B) and kidney (C) membranes, respectively. Aliquots of rat cerebellar (A) and spinal cord (B) membranes (0.5 mg protein) were incubated for 2 h at 4 °C with [^{3}H]flunitrazepam (1 nM) in the presence of zaleplon or various compounds. Aliquots of rat kidney (C) membrane (0.5 mg protein) were incubated for 2.5 h at 4 °C with [^{3}H]Ro5-4864 (2.5 nM) in the presence of zaleplon or various compounds. Each point represents the mean ± S.E. of three independent experiments done in duplicate. The data shown are from duplicate experiments.

3.4. Affinity of zaleplon at other neurotransmitter recognition sites

At a concentration of 100 μM, zaleplon did not displace 5-hydroxytryptamine (5-HT_1, 5-HT_{1A}, 5-HT_2, 5-HT_3) receptors, adrenoceptors (α_1, α_2), dopamine (D1, D2), acetylcho-

Table 2
Effects of zaleplon, triazolam, nitrazepam, zopiclone and CL218,872 on [^{3}H]flunitrazepam and [^{3}H]Ro5-4864 binding to membrane preparations of the cerebellum, spinal cord and kidney in rats

Compound	IC_{50} (95% Confidence Limits) (nM)			IC_{50} ratio (Spinal cord/ Cerebellum)
	[^{3}H]flunitrazepam		[^{3}H]Ro5-4864	
	Cerebellum	Spinal cord	Kidney	
Zaleplon	167.0 (136.4–207.0)	2385.1 (1819.3–3233.7)	>10000	14.3
Triazolam	7.6 (4.0–13.6)	12.1 (10.0–14.6)	449.9 (259.7–805.9)	1.6
Zopiclone	112.5 (93.6–135.6)	222.8 (160.3–324.9)	>10000	2.0
Brotizolam	7.1 (2.2–18.5)	12.7 (10.1–16.2)	107.4 (48.5–305.6)	1.8
Nitrazepam	58.8 (38.9–85.2)	59.8 (31.1–104.4)	>10000	1.0
CL218,872	472.7 (246.5–799.8)	7895.9 (3500.3–32945.8)	>10000	15.5

The IC_{50} values (concentration of the displacer that inhibited specific binding by 50%) were determined by linear regression analysis. The IC_{50} ratio (spinal cord/cerebellum) was calculated as the quotient IC_{50} values in the spinal cord/IC_{50} values in the cerebellum.

line (M_1) and μ-opioid receptors labeled with each specific radioligand (Table 1).

3.5. Power spectral analysis of EEG in rabbits

Intravenous administration of vehicle (5% DMSO) caused a temporary arousal pattern on the spontaneous EEG. However, after 1 h or so, a drowsy pattern of spontaneous EEG characterized by high-voltage slow waves in the cortical EEGs, and desynchronization of the hippocampal theta waves, indicative of sleep patterns, and no frequent bursts of high-amplitude sleep spindles were observed. In the power spectral analysis of the frontal cortex EEG, an increase in the energy of the delta frequency band and a decrease in the energy of the theta frequency band were observed, but the energy of the alpha and beta frequency bands did not change in the drowsy pattern in the spontaneous EEG (data not shown).

Intravenous administration of zaleplon at a dose of 1 mg/kg caused a drowsy pattern in the spontaneous EEG characterized by high-voltage slow waves in the cortical EEGs and desynchronization of the hippocampal theta waves (Fig. 4). Triazolam (0.1 mg/kg, i.v.) and zopiclone (2 mg/kg, i.v.) also induced a drowsy pattern in the EEG similar to zaleplon. However, triazolam and zopiclone caused frequent bursts of high amplitude in the drowsy EEG pattern (Fig. 4). In the power spectral analysis of the frontal cortex EEG, zaleplon, triazolam and zopiclone significantly increased the energy of the delta frequency band and decreased the energy of the theta frequency band. Triazolam and zopiclone significantly increased the energy of the beta frequency band, whereas zaleplon did not (Fig. 5). In particular, triazolam caused a long-lasting increase in the energy of the beta frequency band (Fig. 5). Intravenous administration of CL218,872, and ω_1 receptor-selective agonist, also significantly increased the energy of the delta frequency band, from $29.9 \pm 1.7\%$ in vehicle-treated control to $44.1 \pm 3.7\%$ in 1 mg/kg CL 218,872-treated group, and significantly decreased the energy of the theta frequency band, from $55.1 \pm 1.7\%$ in vehicle-treated control to $39.7 \pm 3.0\%$ in 1 mg/kg CL 218,872-treated group, at 10 min after administration, with-

Table 3
GABA ratio of zaleplon, diazepam, CL218,872 and flumazenil in rat cerebellar membranes

Compound	GABA ratio
Zaleplon	2.07
Diazepam	1.84
CL218,872	1.28
Flumazenil	0.89

The GABA ratio was calculated as the quotient IC_{50} values in the presence of 50 mM bicuculline/IC_{50} values in the presence of 30 mM GABA. The GABA ratio values shown are the mean from three separate experiments done in duplicate.

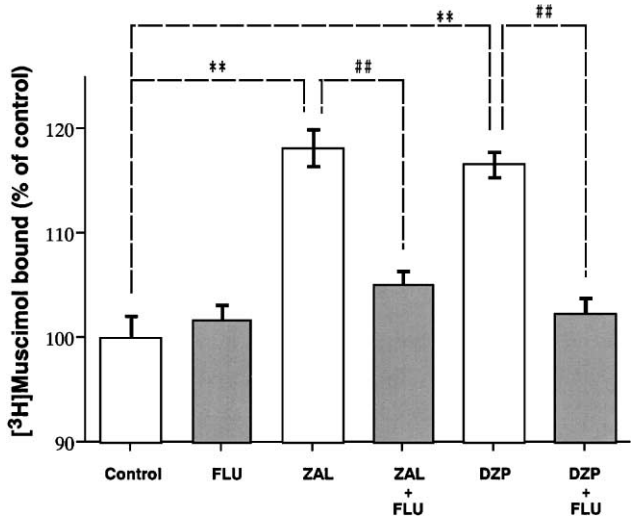

Fig. 3. Effects of flumazenil (10^{-5}M: FLU) on the enhancement of [^{3}H]muscimol binding induced by zaleplon (10^{-5}M: ZAL) and diazepam (10^{-5}M: DZP). Aliquots of rat cerebellar membranes (0.5 mg protein) were incubated for 2 h at 4 °C with [^{3}H]muscimol (16 nM) in the presence or absence of various compounds. Each value represents the mean ± S.E. of six experiments done in duplicate. ** $P < 0.01$, Dunnett's multiple range test, as compared with the control value. ## $P < 0.01$, Dunnett's multiple range test, as compared with values in the presence of ZAL or DZP.

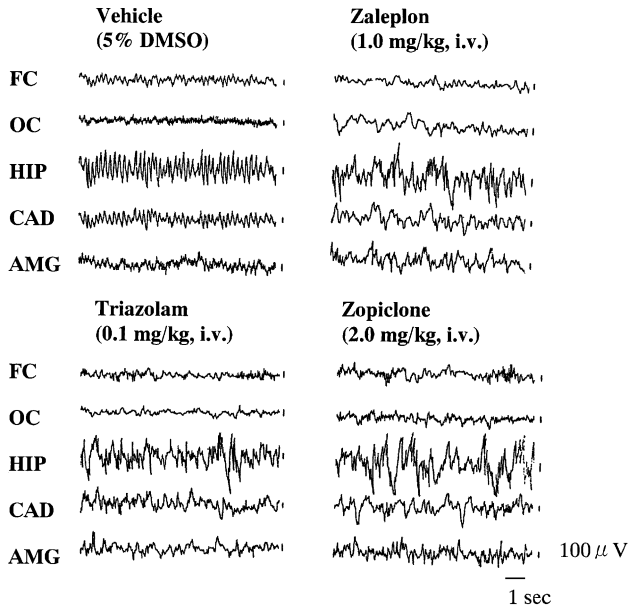

Fig. 4. Representative effect of zaleplon, triazolam and zopiclone on spontaneous EEG in conscious rabbits (10 min after intravenous administration). FC, frontal cortex; OC, occipital cortex; HIP, hippocampus; CAD, caudatus; AMG, amygdala.

out affecting the beta frequency band, similar to zaleplon (Dunnett's multiple range test). Furthermore, pretreatment with intravenous flumazenil (1 mg/kg) antagonized the increase in the energy of the delta frequency band induced by zaleplon (1 mg/kg, i.v.). The relative EEG power density in the delta frequency band significantly decreased from 51.8 ± 3.9% in the zaleplon-treated group to 36.2 ± 3.6% in the presence of flumazenil. Flumazenil (1 mg/kg, i.v.) alone was without significant effect on the power spectral analysis of the frontal cortex EEG.

4. Discussion

Zaleplon displaced [^{3}H]flunitrazepam binding from cerebellar benzodiazepine receptor with an about 14.3 times higher potency than for spinal cord benzodiazepine receptors. In contrast, triazolam and zopiclone showed no selectivity for these benzodiazepine receptors. It has been reported that the cerebellum and substantia nigra contain mostly ω_1 receptors, while the hippocampus, striatum and the spinal cord contain mostly ω_2 receptors (Niddam et al., 1987; Benavides et al., 1993). Furthermore, it has been reported that CL218,872 binds with high selectivity to the ω_1 receptor subtype in the brain but possesses low affinity for ω_2 and ω_3 receptor subtypes (Squires et al., 1979; Langer and Arbilla, 1988). In this study, CL218,872 displaced [^{3}H]flunitrazepam binding from cerebellar benzodiazepine receptors with an about 15.5 times higher potency than for spinal cord benzodiazepine receptors. These result suggest the preferential affinity of zaleplon for cerebellar benzodiazepine receptors, which is indicative of selectivity for ω_1 receptors. In addition, zaleplon did not displace [^{3}H]Ro5-4864 binding from kidney benzodiazepine receptors. It has been shown that Ro5-4864 is a selective ligand of the ω_3 receptor, which is rich in the kidney, and that it has little affinity for ω_1 and ω_2 receptors in the central nervous system (Schoemaker et al., 1983). Furthermore, zaleplon showed no affinity for the 5-hydroxytryptamine (5-HT$_1$, 5-HT$_{1A}$, 5-HT$_2$, 5-HT$_3$), adrenoceptor (α_1,

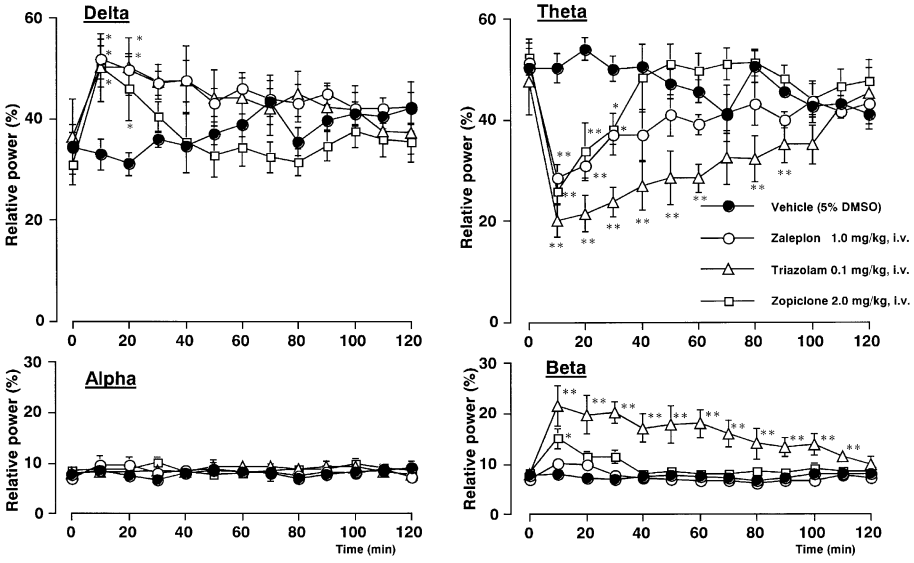

Fig. 5. Effect of zaleplon, triazolam and zopiclone on the power spectra of the frontal cortex EEG in conscious rabbits. EEG was measured immediately after oral administration of each test drug over a 2-h period. Results are shown as the mean ± S.E. for four to five animals per group. (●) Vehicle 1 ml/kg; (○) zaleplon 1 mg/kg; (△) triazolam 0.1 mg/kg; (□) zopiclone 2.0 mg/kg. * $P<0.05$; ** $P<0.01$, Dunnett's multiple range test, as compared with the control value.

α_2), dopamine (D1,D2), acetylcholine (M_1) and μ-opioid receptors even at a concentration of 100 μM. However, receptors of the ω_1 type correspond to those identified by agonist such as CL218,872, zolpidem and alpidem, inverse agonists such as β-carboline, ethyl-β-carboline-3-carboxylate (β-CCE) and antagonist pyrazoloquinolinone CGS8216 (Squires et al., 1979; Nielsen and Braestrup, 1980, Arbilla et al., 1985; Langer and Arbilla, 1988). It is known that the binding of benzodiazepine receptor ligands is influenced by the presence of $GABA_A$ receptor agonist such as GABA or muscimol. The binding of benzodiazepine receptor agonist is potentiated in the presence of muscimol or GABA, producing hypnotic, anxiolytic or anticonvulsant effects, while the opposite is true for inverse agonists, which produce anxiogenic and proconvulsant effects. The binding of benzodiazepine antagonist remains unchanged irrespective of the presence of GABA (Braestrup et al., 1984; Ehlert et al., 1983). Therefore, the GABA ratio can be used as an index of the intristic activity of benzodiazepine receptor ligands at the GABA/benzodiazepine Cl^- channel complex (Ehlert et al., 1983). Benzodiazepine receptor agonists have a GABA ratio of more than 1. Benzodiazepine inverse agonists possess a GABA ratio of less than 1. Benzodiazepine receptor antagonists have a GABA ratio of approximately 1. In the present study, zaleplon possessed a GABA ratio of 2.07. This ratio is more than that of the benzodiazepine partial agonist CL218,872 (1.28), the benzodiazepine full agonist diazepam (1.84) and the benzodiazepine receptor antagonist flumazenil (0,89). Thus, it is suggested that zaleplon is classified as a full agonist, but not a partial agonist, of benzodiazepine receptors. In the present study, zaleplon as well as diazepam produced a significant increase in muscimol binding. Furthermore, the zaleplon-induced increased in [^{3}H]muscimol biding was completely antagonized by flumazenil, a benzodiazepine receptor antagonist. In general, it has been shown that benzodiazepines, such as diazepam, enhance the function of GABA, as a result of an allosteric interaction with the $GABA_A$ receptor (Wood et al., 1984). Thereby, the results indicate that zaleplon also increase the affinity of GABA for $GABA_A$ receptors of the CNS.

On the spectral analysis of the electroencephalogram in rabbits, zaleplon, triazolam and zopiclone caused a drowsy pattern of spontaneous EEG characterized by high-voltage slow waves and desynchronization of hippocampal theta waves, and showed a nearly equal increase in the energy of the delta frequency band. It has been reported that the increase in the energy of the delta frequency band in the cortical EEG relates to the sleep stage of behavior. Thus, it is suggested that each dose of zaleplon, triazolam and zopiclone in the present study induced nearly equal hypnotic activity. Intravenous administration of triazolam caused a burst of high-amplitude sleep spindle in the cortical EEGs and significantly increased the energy of the beta frequency band, whereas zaleplon did not. It has been reported that the increases in beta activity are, in general, the most sensitive EEG parameters after administration of benzodiazepine receptor agonists (Creviosier et al., 1983; Saletu et al., 1983). The increase in beta activity appears to be a common effect of benzodiazepine receptor ligands, as it occurred upon administration of triazolam and zopiclone (Depoortere et al., 1988; Patat et al., 1994). Thus, it is suggested that the present results correlate with those of EEG reports. In the present spectral analysis study, physiological sleep patterns in the vehicle-treated group showed an increase in the energy of the delta frequency band and a decrease in the energy of the theta frequency band but not a change in the energy of the alpha and beta frequency bands. Thus, the sleep patterns induced by triazolam and zopiclone may be different from the physiological sleep pattern. Borbély (1995) has also reported that benzodiazepine hypnotics and zopiclone do not mimic physiological sleep on the basis of the sleep EEG study. In this respect, zaleplon can be differentiated from benzodiazepine and zopiclone by spectral analysis of sleep EEG, suggesting that the response elicited by zaleplon may more closely resemble the physiological sleep state than that elicited by triazolam and zopiclone. Furthermore, an increase in the energy of the delta frequency band induced by zaleplon was antagonized by pretreatment with the benzodiazepine antagonist flumazenil. These results suggest that the hypnotic action of zaleplon is mediated by its binding to CNS-type benzodiazepine receptors. In addition, intravenous administration of CL218,872, an ω_1 receptor selective agonist, also significantly increased the energy of the delta frequency band and decreased the energy of the theta frequency band, without affecting that of the frequency band. Furthermore, it is reported that zolpidem has the same affect as zaleplon and CL218,872 (Depoortere, et al., 1986). Therefore, these results suggest that ω_1 receptors may play an important role in zaleplon-induced natural sleep.

In conclusion, the results of the present benzodiazepine receptor binding assay suggest that zaleplon is a selective full agonist of the ω_1 receptor subtype within the $GABA_A$ receptor ionophore complex in the brain, and enhance the function of GABA as a result of an allosteric interaction with the $GABA_A$ receptor chloride anion channel complex. Therefore, zaleplon may induce a state closely resembling the physiological pattern of slow-wave sleep.

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