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Sedative and anxiolytic effects of zopiclone's enantiomers and metabolite

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Abstract

We evaluated racemic zopiclone, its (*S*)- and (*R*)-enantiomers and a metabolite, (*S*)-desmethylzopiclone, for their actions on locomotor activity, rotarod performance, the elevated plus maze and the Vogel conflict test of anxiety, and electroconvulsive shock-induced seizures duration. Zopiclone and its (*R*)- and (*S*)-enantiomers reduced locomotor activity, and zopiclone and its (*S*)-enantiomer disrupted rotarod performance at 10 mg/kg. (*S*)-desmethylzopiclone did not alter these measures at doses of less than 200 mg/kg. (*S*)-desmethylzopiclone altered plus maze performance at the lowest dose of all the zopiclone derivatives tested, caused a dose-related effect on the Vogel conflict test and caused a dose-related reduction of electroconvulsive shock-induced seizure durations. The data indicate that (*S*)-desmethylzopiclone can bring about an anxiolyic effect without a substantial degree of central nervous system depression, and suggest that the agent may be particularly useful clinically in the treatment of anxiety. © 2001 Published by Elsevier Science B.V.

Keywords: Zopiclone; (*S*)-Desmethylzopiclone; Anxiolytic effect

1. Introduction

The sleep-improving drug zopiclone, a cyclopyrrolone, is a member of a group of nonbenzodiazepine structures that has been shown to have affinity for benzodiazepine receptors. Non-benzodiazepine agents appear to produce comparable anxiolytic effects, but less sedation and muscle relaxation (Piot et al., 1990), and seem to have a lower propensity to induce physical dependence in animals (Sanger et al., 1994) and humans (Dorian et al., 1983) than the benzodiazepines. These distinctive behavioral actions may involve a differential interaction with benzodiazepine receptor subtypes. Agents that interact with the benzodiazepine receptor exert their pharmacological effects by allosterically modulating the action of γ-amino butyric acid (GABA), by binding to specific benzodiazepine sites on the $GABA_A$/benzodiazepine receptor complex. $GABA_A$/benzodiazepine receptors have a pentameric structure made up of a co-assembly of subunits (α_{1-6}, β_{1-3}, γ_{1-3}, ρ_{1-2} and δ_1) that are differentially expressed throughout the brain. Two types of benzodiazepine receptors, designated ω_1 or benzodiazepine 1 and ω_2 or benzodiazepine 2 (Langer and Arbilla, 1988; Squires et al., 1979), have been identified. $GABA_A$/benzodiazepine receptors that express the ω_1/benzodiazepine 1 site have a structure that contains the α_1 subunit, while those that express the ω_2/benzodiazepine 2 site have a heterogeneous set of structures that contain the α_2, α_3 or α_5 subunits. Recent reports (Rudolph et al., 1999; McKernan et al., 2000) have indicated that the sedative, but not anxiolytic, properties of drugs that interact with the benzodiazepine receptor may be mediated by $GABA_A$/benzodiazepine receptors that express the α_1 subtype. They suggest the possibility that future drug development strategies might focus on the idea that agonists acting on the α_2, α_3, and/or α_5, but not the α_1, subunits might be non-sedating anxiolytics.

Of the non-benzodiazepine hypnotics currently available, two (zolpidem and zaleplon) appear to have a selective high affinity for $GABA_A$/benzodiazepine receptors

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containing the α_1 subunit (ω_1 subtype of the benzodiazepine receptor), whereas the cyclopyrrolones, including zopiclone and suriclone, like the benzodiazepines, are not subunit specific (Blanchard and Julou, 1983; Concas et al., 1994). Nonetheless, the cyclopyrrolones appear to exhibit a number of properties that distinguish them from the benzodiazepines. It has been demonstrated, for example, that these agents do not exhibit full competitive antagonism at the benzodiazepine site (Trifiletti and Snyder, 1984). Studies with protein-modifying agents and photoaffinity labeling suggest that zopiclone's action is either mediated by a different recognition site allosterically coupled to the benzodiazepine site (Trifiletti and Snyder, 1984), or by one of several benzodiazepine sites on the $GABA_A$/benzodiazepine receptor complex, to cause an allosteric change in the ligand affinity for the remaining sites (Sieghart, 1995). Presumably, the unique pharmacology of zopiclone and similar agents confers them with a set of behavioral actions that differ from those of benzodiazepine structures, and may indicate a potential for anxyolitic effects without sedation. However, relatively little work has been performed to characterize the behavioral effects of compounds derived from zopiclone and similar agents.

In the present studies, we evaluated a number of derivatives of zopiclone that have been developed by Sepracor, Marlborough, MA, for their behavioral actions. They included its (*S*)- and (*R*)-enantiomers as well as a metabolite, (*S*)-desmethylzopiclone. We compared the effects of these agents to those of the benzodiazepines, diazepam and alprazolam. The tests included measures of the effects of these agents on sedation as indicated by changes in spontaneous locomotor activity (Feldman et al., 1997), motor coordination on the rotarod (Watzman and Barry, 1968), anxiety-related behaviors using the elevated plus maze (Pellow et al., 1985) and the Vogel conflict test (Vogel et al., 1971), and duration of electroconvulsive shock-induced seizures (Gulati et al., 1986; Bowdler and Green, 1982).

2. Materials and methods

2.1. Animals

Male Long–Evans rats (weighing 250–275 g; Harlan Blue Spruce, Indianapolis, IN, USA) were housed in groups of three, with water available ad libitum on a 12-h (7:00–19:00) light/dark cycle. They were given at least 1 week of acclimatization to housing in the animal facility before commencement of experiments. Animals used in these studies were maintained in accordance with guidelines established by the Institutional Animal Care and Use Committee of Albany Medical College. Albany Medical College maintains a centralized animal research facility, which is licensed by the United States Department of Agriculture and the New York State Department of Health, Division of Laboratories and Research, and is accredited by the American Association for the Accreditation of Laboratory Animal Care.

2.2. Drugs

Racemic zopiclone, its (*S*)- and (*R*)- enantiomers, (*S*)-desmethylzopiclone (Sepracor), diazepam (Sigma/RBI) and alprazolam (Sigma/RBI) were used in these studies. The compounds were dissolved in 0.9% saline by solubilization in one or two drops of 1 N HCl. Drug solution pH was then adjusted to approximately 7 with 1 N NaOH. Control subjects were injected with an identical vehicle. Initial doses of all agents were 10 mg/kg. Further studies with (*S*)-desmethylzopiclone were performed using doses of 10, 20, 50, 100, 200 and 400 mg/kg. All drugs were injected i.p. in a final volume of 1 ml/kg.

2.3. Locomotor activity measurement

A measure of general locomotor activity was obtained by measuring horizontal movement in a novel, open field. Animals were placed individually in one of the eight opaque black Plexiglas arenas (40-cm diameter) that were housed in a quiet isolated room. Gross locomotor activity was detected by three infrared photo cells located along the walls of the arena at equal distance intervals. The animals' beam breaks were transmitted via a digital interface (Med Associates, Fairfield, VT) to a 486 IBM PC computer. Cumulative photocell breaks were recorded at 10-min intervals for 120 min. This provided an automated measure of gross locomotor activity.

2.4. Rotarod motor coordination test

Performance measures of motor behavior were conducted using a commercially designed and constructed accelerating rotarod (Rotamex 4/4, Columbus Instruments, Columbus, OH). Motor coordination was quantified as the animal's ability to remain in place on the rod.

Before drug-dosing, rats were gradually trained to stay on the rod by providing them with six to eight daily 3-min training periods. The rats were trained at increasing rod speeds of 3, 6, 9, 12 and 15 rpm. Electric shock (2 mA, AC) was present on the apparatus floor to encourage the animal to stay on the rod. The animal's time of falling was recorded automatically by photocell detection. An animal was considered trained when it reached a criterion of two successive trials at 15 rpm, wherein it stayed on the rod for the full 3-min session. Only those rats that met this criterion after 2 days of training were used in this experiment. All testing was conducted at least 2 h after the final training trial. Drugs were administered i.p. 30 min before testing. Animals were placed on the rod while it was rotating at 10 rpm. Upon placement, the rod's rotational speed was increased at a rate of 8.3 rpm/min. Total

possible test session duration was 3 min. Time of falling and rpm at time of fall were recorded for each animal.

2.5. Plus maze procedures

The apparatus was made of black Plexiglas and consisted of two runways that intersected the center at right angles. Each arm of the maze measured 40 cm (length) by 10 cm (width). Two of the arms that were opposed to each other had walls that measured 40 cm in height (closed arms), whereas the other two arms had no walls (closed arms). The maze was elevated 52 cm above the floor. It was located in a darkened room so that only the open arms were illuminated, each with its own 40-W incandescent light.

Animals were placed in the center of the maze (10×10 cm) and the number of entries into each type of arm was counted (all four paws in the arm defining an entry) as was the time spent in each type of arm. The test was terminated 5 min after the animal was placed in the center.

The following measures were calculated: total number of arm entries, entries into open and closed arms, time in open and closed arms and percent of total time spent in open arms. Changes in the total number of arm entries reflect a general index of activity, whereas changes in the percent measure constitutes an index of anxiety. Increased open arm time reflects an anxiolytic state, while decreased open arm time reflects an anxiogenic state. Animals' movements were recorded by using an overhead video camera and video cassette recorder. They were subsequently scored by a "blind" observer.

2.6. Anticonflict test

The procedure used was a modification of that used by Vogel et al. (1971). Testing took place in a $25.5 \times 30.5 \times 30.5$-cm modular test cage (E10-18TC, Coulbourn Instruments) enclosed in a sound and light attenuating chamber. The cage was equipped with an optical lickometer (E24-01M Coulbourn Instruments) that was used to measure licking at a tube attached to a drinking bottle positioned on the chamber wall and accessible through a hole 2 cm above the floor. The chamber floor was a grid formed of 0.5-cm steel bars spaced 1-cm apart. Shocks were delivered through the lick tube of the drinking bottle by an E13-14 universal shocker set at 0.25 mA. Maximal shock duration was 2 s. Rats were deprived of water for 48 h prior to the testing session. Approximately 24 h after water was removed, rats were placed in the boxes, without water being available, and allowed to habituate for 10 min. Then, approximately 6 h before the test session, animals were again placed in the boxes and water-licks were recorded; rats were allowed to drink without receiving shock for 1 min following the first lick, and rats that failed make at least 100 licks during this session were dropped from the experiment.

Rats were injected with the test drug 30 min before the test session. Each subject was placed in the apparatus and allowed to complete 20 licks before a shock of 2-s maximum duration was delivered through the tube. The animal terminated the shock by withdrawing its tongue from the tube. For 3 min after the first shock, shocks were delivered following each 20th lick. The number of shocks received during the session was recorded for each rat.

2.7. Electroconvulsive shock (ECS) seizure duration measures

ECS was administered through alligator-clip electrodes attached to both ears. An AC sine wave generator based on the original design of Hayes (1948) was used; it consisted of a 1145-V transformer connected in series to a 0–26-kΩ variable resistor. Applied current was adjusted to 35 mA through appropriate resistor settings, and a stimulus duration of 0.35 s was employed. This produced tonic–clonic convulsions lasting 5–30 s. Observed seizure characteristics included a rigid arched spinal column, falling to the side, quick convulsions of the limbs, and a rigid tail. Seizures were considered terminated when the rat's rigid spinal column began to relax. Seizure durations were recorded.

2.8. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using drug dose as a factor. Significant effects were then further assessed using post hoc Neuman–Keuls tests or the least significant difference (LSD) planned comparison test as appropriate (Kirk, 1968). Dose response data from the ECS experiment were also analyzed using trend analysis (Kirk, 1968). A level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Locomotor activity

The effects of each of the four zopiclone derivatives, at 10 mg/kg, on total activity during the first hour of testing are depicted in Fig. 1 (top). A significant difference was observed among these drugs [$F(4,28) = 4.71$; $P < 0.0049$]. Post hoc analyses indicated that only (S)- and racemic zopiclone caused a significant reduction in locomotor activity. These effects were no longer present during the second hour of testing (data not shown).

Fig. 1 (bottom) depicts the effects of various doses of (S)-desmethylzopiclone on locomotor activity counts during the first hour of testing. There was a significant main effect of dose [$F(5,31) = 4.76$; $P < 0.0024$, $P < 0.05$]. Post hoc analyses revealed that only the 200 and 400

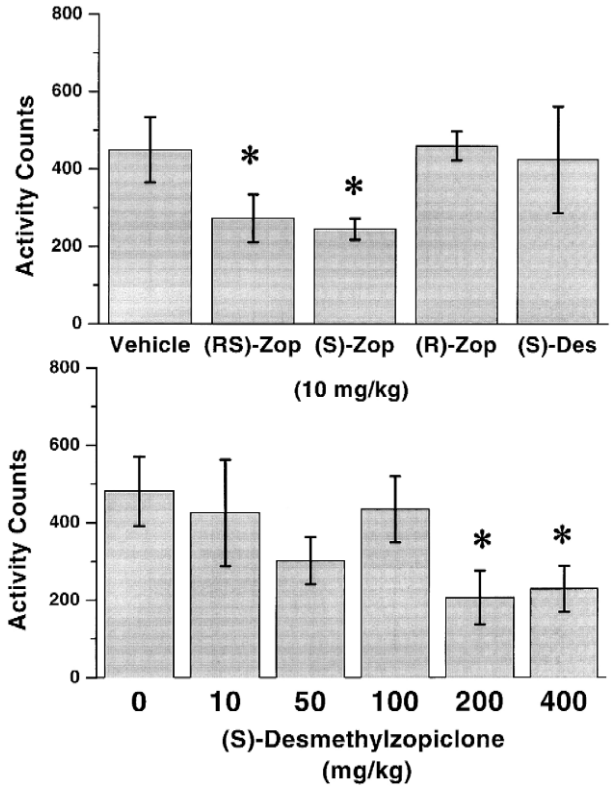

Fig. 1. The effects of racemic zopiclone [(RS)-Zop], (*S*)-zopiclone [(*S*)-Zop], (*R*)-zopiclone [(*R*)-Zop] and (*S*)-desmethylzopiclone [(*S*)-Des] (all doses 10 mg/kg) (top) and the effects of various doses of (*S*)-desmethylzopiclone (bottom) on mean (±SEM) locomotor activity (activity counts) during the first hour of testing ($Ns = 6$/group). $^{*}P < 0.05$ vs. vehicle control.

mg/kg doses differed from the vehicle control. These effects were no longer evident during the second hour of testing (data not shown).

3.2. Rotarod performance

The effects of each of four zopiclone derivatives (10 mg/kg) on time spent on the rotarod are depicted in Fig. 2 (top). A statistically reliable difference was observed [$F(4,15) = 6.05$; $P < 0.0042$]. Racemic zopiclone and both of its enantiomers caused a significant reduction in time spent on the rod. The 10 mg/kg dose of (*S*)-desmethylzopiclone did not affect performance.

Fig. 2 (bottom left) depicts the effects of various doses of diazepam on time spent on the rotarod. A statistically reliable effect was observed [$F(3,12) = 26.54$; $P < 0.00001$]. Post hoc analyses revealed that 5 and 10 mg/kg diazepam caused a significant reduction in time spent on the rotarod.

Fig. 2 (bottom left) depicts the effects of various doses of alprazolam on time spent on the rotarod. A statistically reliable effect was observed [$F(3,12) = 4.52$; $P < 0.0243$]. Post hoc analyses revealed that only 5 mg/kg alprazolam caused a significant reduction in time spent on the rotarod.

Fig. 2 (bottom center) depicts the effects of various doses of (*S*)-desmethylzopiclone on time spent on the rotarod. A statistically reliable dose-related effect was observed [$F(5,18) = 9.34$; $P < 0.0002$]. Post hoc analyses revealed that only the 200 and 400 mg/kg doses of (*S*)-desmethylzopiclone caused significant reductions in time spent on the rotarod.

3.3. Plus maze preformance

The effects of various doses of racemic zopiclone on total, closed arm and open arm entries are shown in Fig. 3A. Significant effects were noted for total arm entries [$F(4,44) = 2.86$; $P < 0.0344$] and open arm entries [$F(4,44) = 4.78$; $P < 0.0027$], but not closed arm entries [$F(4,44) = 0.45$; $P < 0.7734$]. Post hoc analyses revealed that the significant effects were attributable to the 10 mg/kg dose.

Fig. 3B shows the effects of (*S*)-zopiclone on total, closed arm and open arm entries. Significant effects were noted for closed arm entries [$F(4,44) = 4.03$; $P < 0.0072$], total arm entries [$F(4,44) = 7.82$; $P < 0.0001$] and open arm entries [$F(4,44) = 5.59$; $P < 0.0010$]. Subsequent post hoc analyses revealed that 10 mg/kg (*S*)-zopiclone caused a significant increase in total and open arm entries.

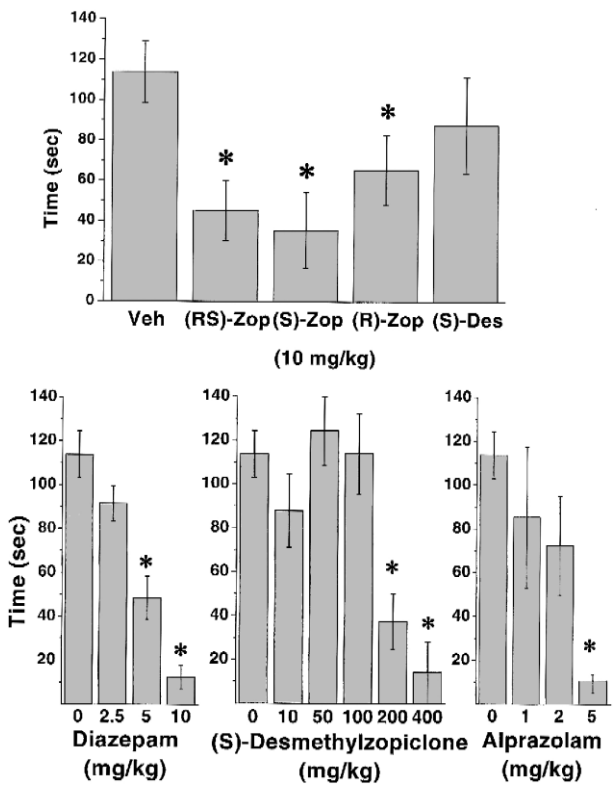

Fig. 2. The effects of racemic zopiclone [(RS)-Zop], (*S*)-zopiclone [(*S*)-Zop], (*R*)-zopiclone [(*R*)-Zop] and (*S*)-desmethylzopiclone [(*S*)-Des)] (all doses 10 mg/kg) (top) and the effects of various doses of diazepam, (*S*)-desmethylzopiclone and alprazolam (bottom) on the mean (±SEM) time spent on the rotarod ($Ns = 6$/group). $^{*}P < 0.05$ vs. vehicle control.

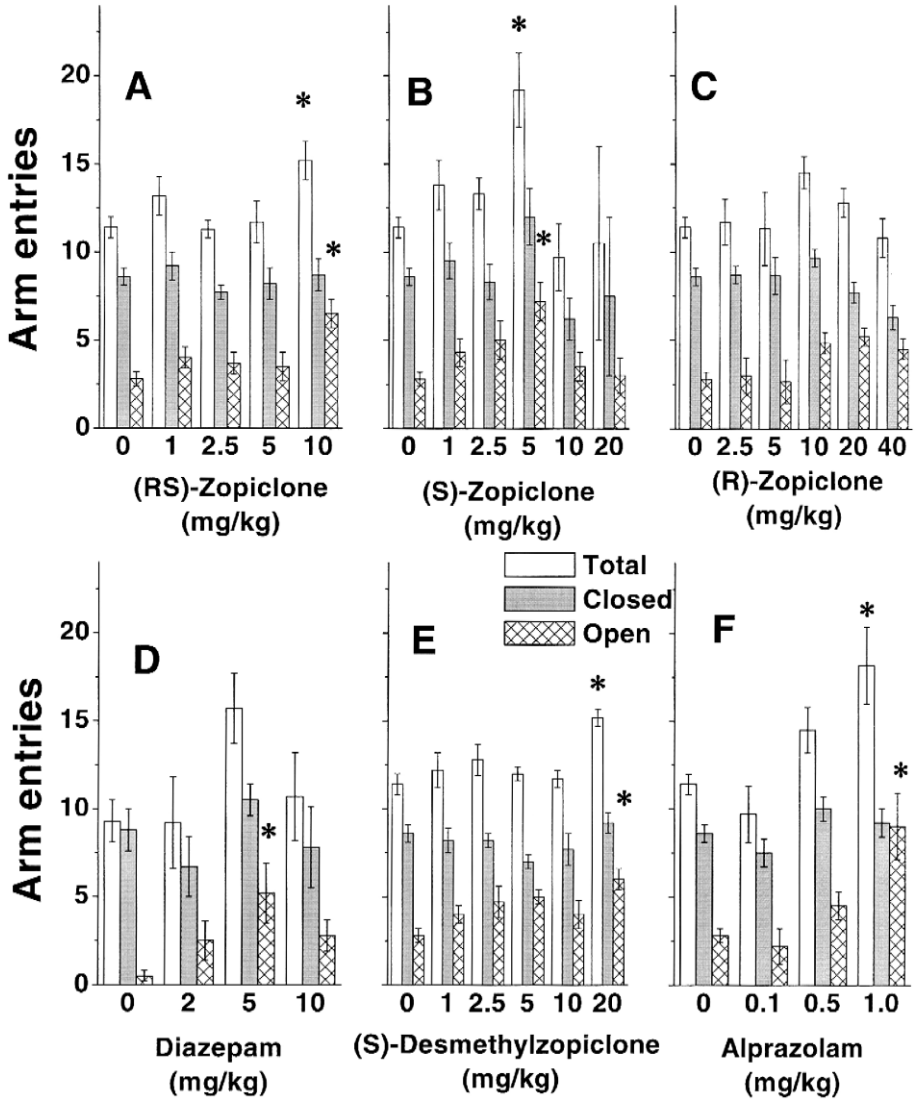

Fig. 3. The effects of various doses of the four zopiclone derivatives (A, B, C, E), diazepam (D) and alprazolam (F) on the means (±SEMs) of total, closed arm and open arm entries of the plus maze ($Ns = 6$/group). * $P < 0.05$ vs. vehicle control.

An effect was significant for open arm entries [$F(5,49) = 2.56$; $P < 0.0388$], but not for closed arm entries [$F(5,49) = 2.03$; $P < 0.0908$] or total arm entries [$F(5,49) = 1.34$; $P < 0.2641$], over a range of doses (0, 2.5, 5.0, 10.0 and 20.0 mg/kg) of (*R*)-zopiclone. However, as indicated in Fig. 3C, no statistically reliable differences between individual dose groups and the vehicle control were found.

As shown in Fig. 3D, diazepam had no significant effects on total arm entries [$F(3,20) = 2.02$; $P < 0.1431$] or closed arm entries [$F(3,20) = 0.99$; $P < 0.4188$]. A predicted increase in open arm entries just missed significance [$F(3,20) = 2.99$; $P < 0.0553$] and subsequent post hoc analyses revealed that 5 mg/kg diazepam did produce a significant and selective increase in open arm entries.

The effects of various doses of (*S*)-desmethylzopiclone on total, closed arm and open arm entries are shown in Fig. 3E. Significant effects were observed for total arm entries [$F(5,49) = 2.75$; $P < 0.0288$] and open arm entries [$F(5,49) = 4.27$; $P < 0.0027$], but not closed arm entries [$F(5,49) = 1.05$; $P < 0.3985$]. Subsequent post hoc analyses revealed that 20 mg/kg (*S*)-desmethylzopiclone caused significant increases in open and total arm entries as compared to vehicle control levels.

Alprazolam had significant effects on total arm entries [$F(3,39) = 8.45$; $P < 0.0002$] and open arm entries [$F(3,39) = 10.96$; $P < 0.00001$], but not on closed arm entries [$F(3,39) = 1.52$; $P < 0.2257$]. As indicated in Fig. 3F, post hoc analyses revealed that the 1 mg/kg dose of alprazolam caused a significant increase in open and total arm entries compared to the same measure in vehicle controls.

Fig. 4A shows the effects of various doses of racemic zopiclone on the percentage of time spent in the open

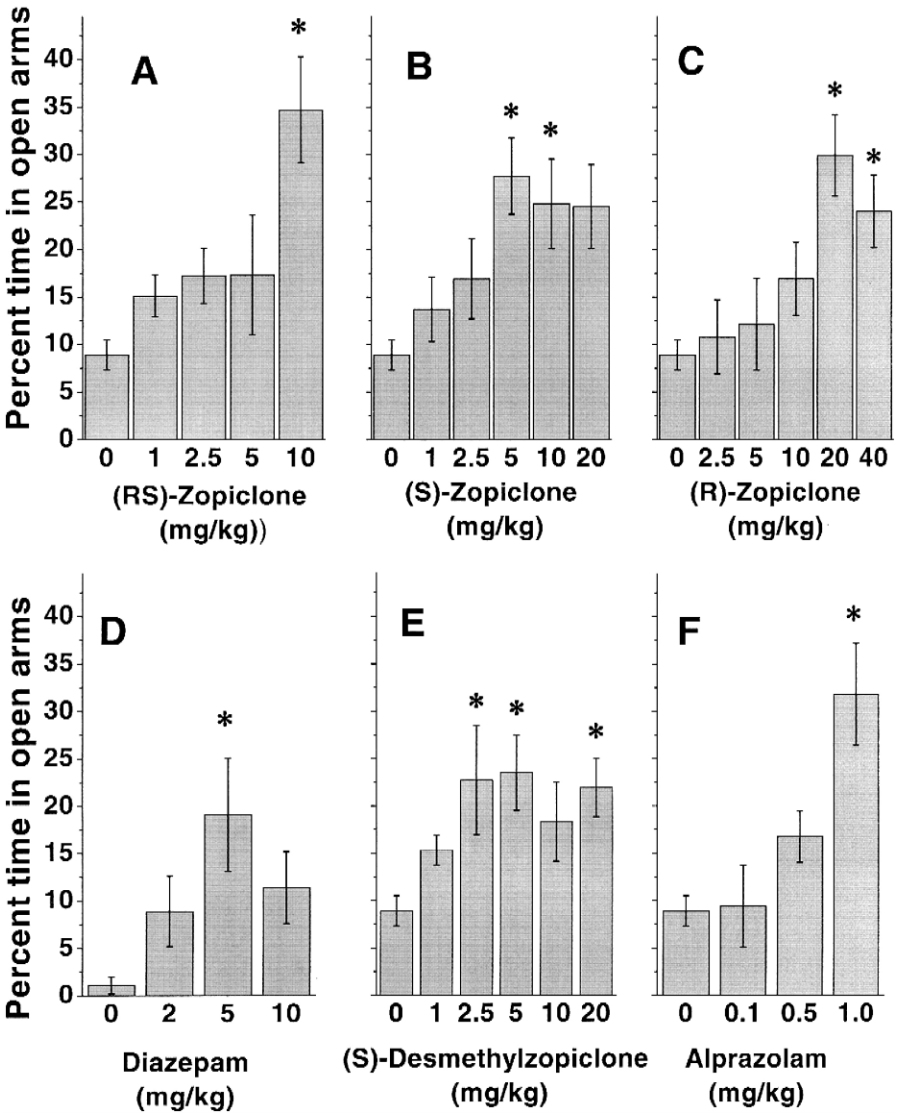

Fig. 4. The effects of various doses of the four zopiclone derivatives (A, B, C, E), diazepam (D) and alprazolam (F) on the mean (±SEM) percent of time spent in the open arms of the plus maze ($Ns = 6$/group). $^{*}P < 0.05$ vs. vehicle control.

arms. A statistically significant effect was observed [$F(4,44) = 9.17$; $P < 0.00001$]. Subsequent post hoc analyses revealed that 10 mg/kg racemic zopiclone caused a significant increase in time spent in the open arms.

Fig. 4B shows the effects of various doses of (*S*)-zopiclone on percentage of time spent in the open arms. ANOVA revealed a significant effect of the drug [$F(4,44) = 7.98$; $P < 0.0001$]. Post hoc analyses revealed that both 5 and 10 mg/kg (*S*)-zopiclone significantly increased the percent of time spent in the open arms.

Fig. 4C shows that (*R*)-zopiclone also had a significant effect on the percentage of time spent in the open arms [$F(5,49) = 7.04$; $P < 0.00001$]. Subsequent post hoc analyses revealed that 20 and 40 mg/kg (*R*)-zopiclone caused significant increases in percent of time spent in the open arms.

Diazepam had a statistically significant effect [$F(3,20) = 3.42$; $P < 0.0373$] on time spent in the open arms of the

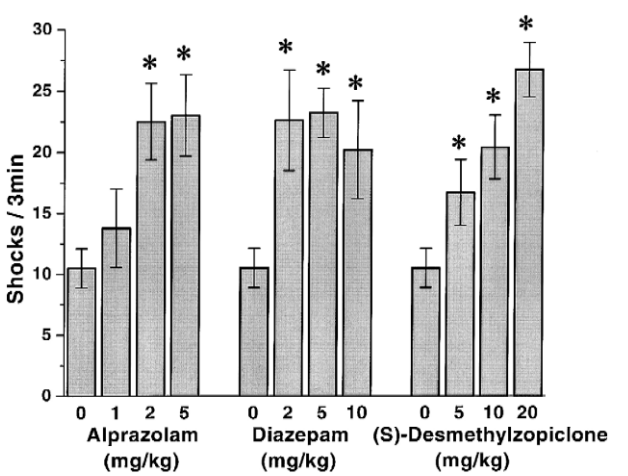

Fig. 5. The effects of various doses of alprazolam, diazepam and (*S*)-desmethylzopiclone ($Ns = 6$/group) on the mean (±SEM) number of shocks received during the 3-min testing session of the conflict procedure ($Ns = 6$/group). $^{*}P < 0.05$ vs. vehicle control.

plus maze. Post hoc analyses revealed that, as indicated in Fig. 4D, 5 mg/kg diazepam caused a significant increase in time spent in the open arms.

The effects of various doses of (*S*)-desmethylzopiclone on time spent in the open arms are shown in Fig. 4E. Statistically significant effects were observed [$F(5,49) = 5.26$; $P < 0.0006$]. Post hoc analyses revealed that 2.5, 5 and 20 mg/kg (*S*)-desmethylzopiclone increased the percent of time spent in the open arms.

As shown in Fig. 4F, alprazolam produced a statistically significant increase in time spent in the open arms of the plus maze [$F(3,39) = 11.43$; $P < 0.00001$]. Post hoc analyses revealed that the1 mg/kg dose caused a significant effect.

3.4. Conflict test

When vehicle-treated rats received a mild electric shock to the tongue following every 20 licks of the water tube, they reduced their drinking. Non-drugged animals made an average of approximately 200 licks (and received approximately 10 shocks) during the 3-min test period. Fig. 5 depicts the effects of various doses of alprazolam, diazepam and (*S*)-desmethylzopiclone on shocks received during the 3-min session. Alprazolam at 2 and 5 mg/kg [$F(3,30) = 5.66$ $P < 0.0034$], diazepam at 2, 5 and 10 mg/kg [$F(3,26) = 6.60$ $P < 0.0018$] and desmethylzopiclone at 5, 10 and 20 mg/kg [$F(3,31) = 9.30$ $P < 0.0002$] each increased punished drinking.

3.5. ECS seizure duration

Fig. 6 shows the effects of various doses of (*S*)-desmethylzopiclone, diazepam and alprazolam on the duration of electroconvulsive shock-induced seizures. All three drugs caused a significant reduction in seizure duration. A statistically reliable effect of dose was observed for the effects of (*S*)-desmethylzopiclone [$F(4,28) = 17.75$; $P < 0.00001$]; there was a strong linear dose-related effect as indicated by a significant [$F(1,28) = 64.235$; $P < 0.000001$] linear trend, with no significant quadratic [$F(1,28) = 2.046$; $P = 0.1636$] or cubic [$F(1,28) = 0.048$; $P = 0.827$] components.

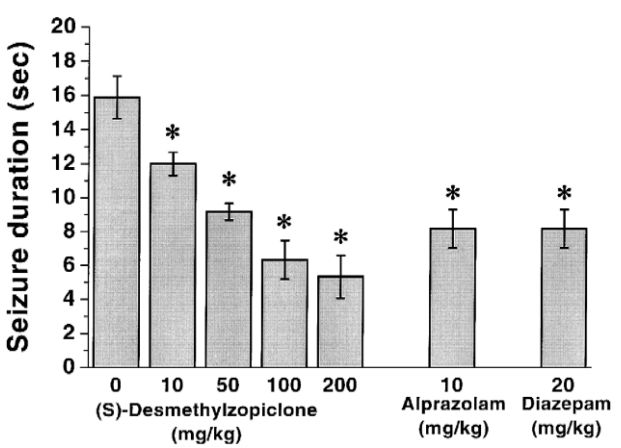

Fig. 6. The effects of various doses of (*S*)-desmethylzopiclone, alprazolam at 10 mg/kg and diazepam at 20 mg/kg (*Ns* = 6/group) on the mean (±SEM) duration of electroconvulsive shock-induced seizures. * $P < 0.05$ vs. vehicle control.

4. Discussion

In the present study, zopiclone and its derivatives displayed a number of properties that were qualitatively similar to those of the benzodiazepines diazepam and alprazolam. As in previous studies that focused on racemic zopiclone (Julou et al., 1983; Ueki, 1987), its derivatives exerted varying degrees of sedative–hypnotic, anticonflict, antianxiety and anticonvulsant effects. At the same time, we noted a number of quantitative differences among these agents, and not all of them had the same effects.

As in previous work (Liu et al., 1985), racemic zopiclone at 10 mg/kg reduced locomotor activity in the open field during the first hour after administration. Similar effects were seen for (*S*)-zopiclone, but not for the other two derivatives tested. No significant effects were observed for any of the agents during the second hour. The effects observed with racemic zopiclone and (*S*)-zopiclone were essentially similar to those reported in previous studies (Karle and Nielsen, 1998), i.e., effects on locomotor behavior that peak at 30–60 min after administration and are absent at 2 h. The lack of effects of (*R*)-zopiclone and (*S*)-desmethylzopiclone indicated that these agents differ from the parent compound in their ability to reduce locomotor activity.

A full dose response characterization of the effects of (*S*)-desmethylzopiclone on locomotor activity indicated a minimally effective dose of 200 mg/kg. Previous reports (Liu et al., 1985) have shown that the locomotor activity reducing effect of racemic zopiclone is similar to that of nitrazepam, and more potent than that of flurazepam. The present findings thus indicate that (*S*)-desmethylzopiclone is at least 20-fold less potent in reducing locomotor activity than either racemic zopiclone or various other benzodiazepine agents.

Previous studies have shown that racemic zopiclone exerts effects that are somewhat weaker than diazepam, nitrazepam, and flurazepam in disrupting performance on the rotarod test (Ueki, 1987). Other studies of this agent indicate that its muscle-relaxing properties are similar to those of chlordiazepoxide and weaker than those of diazepam on a variety of tests (Julou et al., 1983; Karle and Nielsen, 1998). In the present study, racemic zopiclone as well as the (*S*)- and (*R*)-enantiomers, at 10 mg/kg, caused a significant reduction in time spent on the rotarod. The data suggest that each of these agents is effective at disrupting the animal's ability to successfully perform coordinated motor movement. In contrast, (*S*)-de-

smethylzopiclone did not exhibit effects on rotarod performance until very high doses (200 and 400 mg/kg) were reached. The data show that (*S*)-desmethylzopiclone is far less potent at disrupting coordinated motor movement than racemic zopiclone, its enantiomers and alprazolam.

Racemic zopiclone has been shown to have relatively strong anticonvulsant properties. It antagonizes pentylenetetrazol (Metrazol) induced seizures in rats with an order of potency that is similar to that of chlordiazepoxide and nitrazepam. The agent is also active against electrically induced convulsions with a similar relative degree of potency (Julou et al., 1983). In the present study, a dose-related effect of (*S*)-desmethylzopiclone on ECS seizure duration was observed. Seizure duration was significantly reduced by the drug at doses from 10 to 200 mg/kg. The 50 mg/kg dose of (*S*)-desmethylzopiclone caused a seizure reduction that was about equal to that brought about by 10 mg/kg of alprazolam. While (*S*)-desmethylzopiclone did not reduce locomotor activity or impair rotarod performance at doses below 200 mg/kg, alprazolam impaired rotarod performance at the 5 mg/kg dose. The present findings thus show that, in contrast to alprazolam, (*S*)-desmethylzopiclone exhibits anticonvulsant actions at doses that are much lower that those producing motor incapacitation.

All of the tested drugs significantly increased the percent of time spent in the open arms of the plus maze, indicating that each exerts anxiolytic-like activity. Previous reports have shown that racemic zopiclone displayed anxiolytic-like activity in the plus maze only, at doses close to those producing behavioral impairment (Griebel et al., 1998). The results of the present study were similar. Racemic zopiclone and (*R*)-zopiclone increased open arm time only at doses that also impaired rotorod performance, while (*S*)-zopiclone increased this measure at 5 mg/kg and disrupted rotarod performance at 10 mg/kg. Alprazolam was similar in that while it increased open arm time at 1 mg/kg, it also impaired rotorod performance at 5 mg/kg. The findings with (*S*)-desmethylzopiclone indicate that it is unique among these agents in that its anxiolytic effect occurred at a dose of 2.5 mg/kg, but it did not impair rotorod performance at doses of less than 200 mg/kg—an 80-fold separation of doses.

The most widely used behavioral tests of anxiolytic properties of drugs are the conflict models. Drug effects in conflict models are positively correlated with clinical anxiolytic actions and benzodiazepine receptor binding (Haefely et al., 1990; Lippa et al., 1978). In order to test the generality of the effects observed on the plus-maze, we compared the effects of (*S*)-desmethylzopiclone to those of diazepam and alprazolam on the Vogel water-lick suppression test. Findings from other laboratories (Ueki et al., 1987) indicate that racemic zopiclone is more potent than diazepam in the Vogel test. In the present study, it was found that (*S*)-desmethylzopiclone exerted an anxiolytic effect at all doses tested. The data suggest that (*S*)-desmethylzopiclone induces an anxiolyic effect without a substantial degree of central nervous system depression, indicating that this agent has the potential to be particularly useful clinically in the treatment of anxiety. In order for this potential to be realized, a long half-life preparation of the drug would be desirable. The elimination half-life of the parent compound zopiclone in humans is relatively short (3.5–6.5 h) (Fernandez et al., 1995). Further studies will be necessary to assess this parameter for (*S*)-desmethylzopiclone.

While they are similar to the benzodiazepines in many ways, the cyclopyrrolones also appear to differ in many respects. It has been suggested (Karle and Nielsen, 1998) that the cyclopyrrolones and benzodiazepines exert their differing actions because they bind to different, yet closely related and overlapping domains of the $GABA_A$/benzodiazepine receptor complex. The unique behavioral pharmacology of (*S*)-desmethylzopiclone, as compared to racemic zopiclone, suggests that its actions may result from yet another binding difference. Recent findings (Rudolph et al., 1999; McKernan et al., 2000) indicate that benzodiazepine receptor-active agents produce sedation through interactions with α_1-containing $GABA_A$/benzodiazepine receptor complexes, and that compounds without this activity at this population of $GABA_A$/benzodiazepine receptors may act as anxyolytic agents with diminished sedative and motor-impairing side effects. In light of the present findings, further investigation of the specificity of (*S*)-desmethylzopiclone for $GABA_A$/benzodiazepine receptor subtypes is warranted.

The relatively low sedative and muscle-relaxant effects of (*S*)-desmethylzopiclone may also come about because the agent has low intrinsic activity (i.e., is a partial agonist). Benzodiazepine agents, such as triazolam and diazepam, have comparably high intrinsic activity at the $GABA_A$/benzodiazepine receptor complex, as indicated by their ability to increase GABA-stimulated chloride influx (Facklam et al., 1992a). These agents also induce sedation at doses close to those inducing anxiolytic effects (Facklam et al., 1992b). Radioligand binding studies have shown that for a full agonist, a smaller fraction of receptor occupancy is required for antianxiety efficacy than is necessary to produce sedation and muscle relaxation (Facklam et al., 1992b; Jones et al., 1994). A partial agonist with low intrinsic activity may therefore elicit anxiolytic effect with adequate receptor occupancy at low doses, but require much larger doses to produce muscle relaxation and sedation (Haefely et al., 1990). Current work in this laboratory is investigating whether the differential anxiolytic and sedative potencies of (*S*)-desmethylzopiclone seen in the current studies could be attributed to partial agonist actions.

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