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Evaluation of Anxiolytic-like Effects of Some Short-Acting Benzodiazepine Hypnotics in Mice

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Abstract. Anxiolytic-like effects of some short-acting benzodiazepine hypnotics were examined with experimental paradigms of anxiety using an elevated plus-maze in male ICR mice. Diazepam was used as a positive control. The drug at a dose of 1 mg/kg significantly increased the percentage of time spent in the open arms and percentage of the number of open arm entries in the elevated plus-maze. Triazolam, brotizolam, rilmazafone, and lormetazepam also showed an anxiolytic-like effect as indicated by the significant increase in the percentage of time spent in the open arms and percentage of the number of open arm entries. Effects of short-acting benzodiazepine hypnotics used in the study were more potent than those of diazepam. In addition, the doses affecting the elevated plus-maze by benzodiazepine hypnotics were much smaller than those that showed muscle-relaxant activity measured by the rotarod test, indicating that anxiolytic-like effects of benzodiazepine hypnotics had high specificity and selectivity.

Keywords: benzodiazepine, anxiolytic-like effect, hypnotics, plus maze, rotarod

Introduction

It is well recognized that benzodiazepines are useful and effective anti-anxiety drugs used worldwide (1, 2). On the other hand, short-acting benzodiazepines, such as triazolam and brotizolam, are widely used as hypnotics. Considered from their chemical structures, it is considered that these drugs are expected to have anti-anxiety properties. In fact, Kuribara and Asahi (3) reported that triazolam significantly increased lever-pressing behavior in the alarm period (punished responding) under the conflict test, which is used to estimate anti-anxiety activity. On the other hand, the elevated plus-maze paradigm is currently one of the most widely used animal models of anxiety. Using this elevated plus-maze test, Carr et al. (4) reported that diazepam at doses of 1 and 1.5 mg/kg, p.o. significantly increased both the percentage time spent in open arms and the percentage number of open arm entries. Gonález-Pardo et al. (5) also showed that diazepam (5 mg/kg, i.p.) and alprozolam (0.5 mg/kg, i.p.) caused a significant increase in the percentage time spent in the open arms in the elevated plus-maze test.

Benzodiazepine derivatives, however, showed numerous undesirable side effects such as sedation, muscle relaxation, and ataxia (6); therefore, it is considered that muscle relaxation and ataxia induced by benzodiazepine hypnotics may influence performance in the elevated plus-maze test. In view of the above findings, the muscle-relaxant activity of these drugs was also measured by the rotarod test in the present study.

The aim of this study was to estimate and compare the effects of short-acting benzodiazepine hypnotics (triazolam, rilmazafone, brotizolam, and lormetazepam) using the elevated plus-maze in mice. The selectivity and specificity of the anti-anxiety effect of benzodiazepine hypnotics were also examined in comparison with the muscle-relaxant activity of these drugs.

Material and Methods

Animals

Male ICR mice (body weight of 23 – 28 g) were purchased from Japan SLC, Shizuoka. Animals were maintained in an air-conditioned room with controlled temperature (24 ± 2°C) and humidity (55 ± 15%). They

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were housed in plastic cages with sawdust and kept under a light-dark cycle (lights on from 07:00 to 19:00). Food and water were freely available with the exception of test periods. Animals were handled gently every day for at least 3 days. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Experimental procedures in the elevated plus-maze test

The elevated plus-maze apparatus consisted of 4 arms elevated 30-cm above the floor, with each arm positioned at 90° relative to the adjacent arms. Two of the arms were enclosed with high walls (30 × 7 × 20 cm), and the other arms were connected via a central area (7 × 7 cm) to form a plus sign. The maze floor and the walls of enclosed arms were constructed of black Plexiglas. The room was illuminated with a desk lamp set to 200 lux at the central platform. These drugs were administered orally 60 min prior to the test. The experiments were performed between 9:00 and 14:00, and the mice became accustomed to the dimly lit experimental laboratory for 30 min prior to behavioral testing. Each mouse (5 weeks of age) was individually placed on the central platform facing an open arm. The frequency and duration of entries into the open and closed arms were measured using a CCD camera and computer system Lime Light 2. An entry was counted when all 4 paws of the mouse entered an open or closed arm. The percentage of time spent (duration) in open arms and percentage of the number (frequency) of open arm entries were counted during 5 min. Arm entry was defined as all 4 paws having crossed the dividing line between an arm and the central area. Subsequently, the percentage of time spent in the open arms [100 × open / (open + enclosed)] and percentage of the number of open arm entries (100 × open / total entries) (4) were calculated for each animal. The apparatus was thoroughly cleaned after each trial.

Experimental procedures in rotarod test

Mice were placed on a rotating rod (3-cm diameter, divided into five equal compartments, rotating at 15 rpm) (7). Animals remaining on the rod in two successive trials (24-h before the experiment) were selected for drug testing. In this study, at 15, 30, 60, and 90 min after drug administration, mice were placed on the spinning bar of the rotarod apparatus for 3 min. When the animal fell from the rotarod within 180 s, this was defined as a positive effect.

Drugs

The following drugs were used: diazepam (Cercin®; Takeda, Osaka), triazolam (Halcion®; Pfizer, New York, NY, USA), brotizolam (Lendormin®; Nippon Boehringer Ingelheim, Hyogo), rilmazafone (Rhythmy®; Shionogi, Osaka) and lormetazepam (Loramet®, Takeda). These drugs were suspended in 0.5% carboxymethylcellulose sodium solution. These drugs were prepared immediately before use and were administered orally in a volume of 10 ml/kg of body weight.

Statistical analyses

All data are expressed as the means ± S.E.M. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparisons between control- and drug-treatment groups were performed using Dunnett's tests. Fischer's exact test was used for the statistical analysis of the results from the rotarod test. $P<0.05$ was regarded as statistically significant. ED_{50} values were determined by the probit method.

Results

The elevated plus-maze test

The effect of diazepam used as an active control on anxiolytic-like behavior in the elevated plus-maze is shown in Fig. 1. The drug dose-dependently caused an increase in the duration in open arms. Significant differences were observed at doses of 1, 2, and 5 mg/kg, p.o. Almost the same findings were observed in the frequency of open arm entries, that is, significant effects were observed with 1, 2, and 5 mg/kg, p.o.

Figure 2 shows the effects of triazolam and rilmazafone on axiolytic-like behavior. Triazolam showed a significant effect of duration in open arms and frequency of open arm entries at doses of 0.05, 0.1, and 0.2 mg/kg, p.o. Rilmazafone at doses of 0.1, 0.2, and 0.5 mg/kg, p.o. also caused significant increases in the duration in open arms and frequency of open arm entries. Effects of brotizolam and lormetazepam on axiolytic-like behavior are shown in Fig. 3. Brotizolam and lormetazepam increased both the duration in open arms and frequency of open arm entries. Significant effects were observed for brotizolam at doses of 0.2, 0.5, and 1.0 mg/kg, p.o. and lormetazepam at doses of 0.02, 0.05, and 0.1 mg/kg, p.o. on both duration in open arms and the frequency of open arm entries.

The rotarod test

Diazepam dose-dependently caused muscle-relaxant activity measured by rotarod. Five of 10 mice fell from the rotarod when they were tested at 60 min after administration of diazepam at a dose of 5 mg/kg, and the ED_{50} was 3.11 (2.53 – 3.81) mg/kg. Four benzodiazepine hypnotics also showed muscle-relaxant activity,

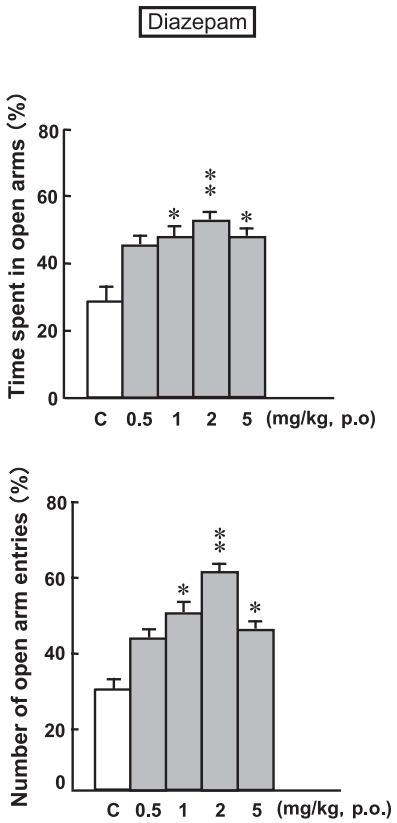

Fig. 1. Effect of diazepam on anxiolytic-like behavior in mice. C: Control. Columns and vertical bars represent the means ± S.E.M. (n = 10). Drugs were administered orally. Significantly different from the control group at **P*<0.05 and ***P*<0.01 (ANOVA with Dunnett's test).

with the following ED_{50} values: triazolam, 1.25 (1.00 – 1.49) mg/kg; rilmazafone, 9.55 (8.20 – 12.9) mg/kg; brotizolam, 5.76 (5.45 – 9.12) mg/kg; and lormetazepam, 3.39 (2.75 – 4.27) mg/kg.

Discussion

Diazepam used as an active control showed anxiolytic-like behavior in mice, and a significant effect was observed at a dose of 1 mg/kg. Almost the same findings were observed by Carr et al. (4) and Consoli et al. (8). They reported that diazepam at a dose of 1 mg/kg significantly increased the percentage of time spent in open arms and percentage of the number of open arm entries in the elevated plus-maze. These findings implied that the results obtained in our present study are highly reliable. In this study, we also found that short-active benzodiazepine hypnotics, such as triazolam, rilmazafone, brotizolam, and lormetazepam, showed potent anxiolytic-like effects in mice. The doses observed with significant effects were triazolam at 0.05 mg/kg, p.o.; rilmazafone at 0.1 mg/kg, p.o.; brotizolam at 0.2 mg/kg, p.o.; and lormetazepam at 0.02 mg/kg, p.o. The same findings were reported by Aburawi et al. (9) and Yasui et al. (10). On the other hand, triazolam, rilmazafone, brotizolam, and lormetazepam are short-acting hypnotics classified as benzodiazepines. We have reported the effects of these drugs on sleep latency in rats placed on a grid suspended over water (11, 12). These drugs induced a remarkable decrease in sleep latency. A significant difference compared with the control was observed for triazolam at 0.5 mg/kg, p.o.; rilmazafone at 1 mg/kg, p.o.; brotizolam at 0.5 mg/kg, p.o.; and lormetazepam at 1 mg/kg, p.o. Doses showing anxiolytic-like effects in these drugs are 2.5 times (brotizolam), 10 times (triazolam, rilmazafone), and 50 times (lormatazepam) more potent than the doses effective to decrease sleep latency, although there is a difference in mice and rats. These results suggest that anxiolytic-like effects of benzodiazepine hypnotics may contribute to their sleep-inducing effects. It has been reported by some researchers (13, 14) that compared to diazepam, lormetazepam and triazolam used in the present study had more potent $\omega 2$ ($\alpha 2$) receptor–agonistic activity, which is closely related to the anti-anxiety–like effects of benzodiazepines. Almost the same findings can be obtained with brotizolam and rilmazafone. This is the reason why short- and ultrashort-acting benzodiazepine hypnotics caused more potent antianxiety-like effect than diazepam. As shown in Fig. 1, the increase in the duration in open arms and frequency of open arm entries induced by 5 mg/kg, p.o. of diazepam was less than those of 2 mg/kg, p.o. This result suggests that diazepam showed muscle-relaxant activity or ataxia at a dose of 5 mg/kg, p.o. In fact, as shown in Table 1, 5 of 10 mice fell from the rotarod by diazepam administration at a dose of 5 mg/kg, indicating that the drug showed remarkable muscle-relaxant activity. Rorick-Kehn et al. (15) reported that diazepam caused impairment. On the other hand, the short-acting hypnotics used in this study also showed muscle-relaxant activity. Yasui et al. (10) also reported that rilmazefone and brotizolam caused muscle-relaxant activity in the rotarod test. Although, as shown before, short-acting hypnotics caused muscle relaxant activity, the doses causing these activities were larger than the doses effective in the elevated plus-maze test. Low et al. (16) reported that sleep-inducing, anxiolytic-like, and muscle relaxant effects of benzodiazepines are related with the $\alpha 1$-, $\alpha 2$-, and $\alpha 5$-receptor, respectively. Sanna et al. (17) found that short-acting benzodiazepines showed $\alpha 2$-receptor–binding activity more potent than $\alpha 1$- and $\alpha 5$-receptor–binding activity. From the above findings, it seems likely that the anxiolytic-like effect induced by benzodiazepine hypnotics showed specificity

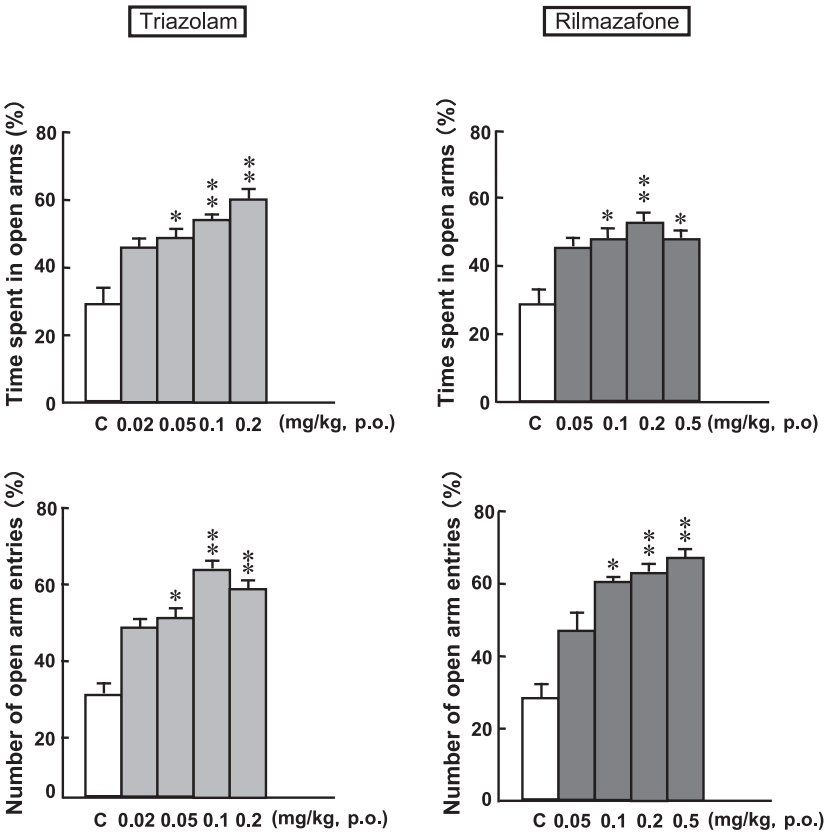

Fig. 2. Effects of triazolam and rilmazafone on anxiolytic-like behavior in mice. C: Control. Columns and vertical bars represent the means ± S.E.M. (n = 10). Drugs were administered orally. Significantly different from the control group at **P*<0.05 and ***P*<0.01 (ANOVA with Dunnett's test).

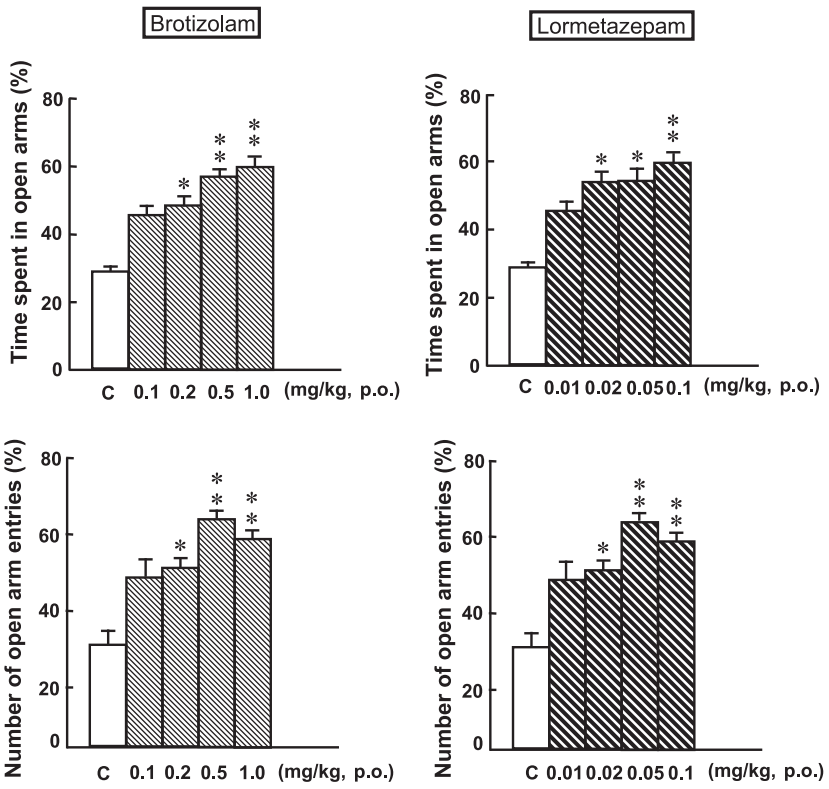

Fig. 3. Effects of brotizolam and lormetazepam on anti-anxiety–like behavior in mice. C: Control. Columns and vertical bars represent the means ± S.E.M. (n = 10). Drugs were administered orally. Significantly different from the control group at **P*<0.05 and ***P*<0.01 (ANOVA with Dunnett's test).

Table 1. Effects of benzodiazepine hypnotics on rotarod performance in mice

Drugs	Dose (mg/kg)	15	30	60	90 min
Control	–	0/10	0/10	0/10	0/10
Diazepam	2	1/10	3/10	4/10*	3/10
	5	2/10	4/10*	5/10*	4/10*
	10	4/10*	7/10**	9/10**	8/10**
	20	9/10**	10/10**	10/10**	9/10**
Triazolam	0.5	2/10	3/10	3/10	9/10
	1	3/10	4/10*	4/10*	2/10
	2	5/10*	8/10**	7/10**	5/10*
	5	8/10**	9/10**	9/10**	4/10*
Rilmazafone	5	1/10	2/10	2/10	2/10
	10	3/10	5/10*	5/10*	2/10
	20	6/10**	7/10**	8/10**	4/10*
	30	8/10**	9/10**	9/10**	6/10**
Brotizolam	2	0/10	2/10	2/10	1/10
	5	2/10	4/10*	4/10*	2/10
	10	5/10*	6/10**	7/10**	5/10*
	20	6/10**	6/10**	9/10**	5/10*
Lormetazepam	2	1/10	4/10*	4/10*	0/10
	5	2/10	5/10*	6/10**	2/10
	10	7/10**	9/10**	9/10**	5/10*
	20	9/10**	10/10**	10/10**	9/10**

Numeral indicates number of positive/tested mice. Significantly different from the control group at *P<0.05 and **P<0.01 (Fisher's exact test).

and selectivity.

In conclusion, the benzodiazepine hypnotics used in the present study had a potent anxiolytic-like effect, and the potency was much greater than that of the anxiolytic drug diazepam. In addition, the doses of benzodiazepine hypnotics affecting the elevated plus-maze were much smaller than those that showed muscle-relaxant activity as measured by the rotarod test, indicating that anxiolytic-like effects of benzodiazepine hypnotics had high specificity and selectivity.

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