

RAPID COMMUNICATION

Effects of Alprazolam on Anxiety-Related Behavior of Rats in a Modified Forced-Swim Test Employing Straw Suspension

HIROSHI NISHIMURA¹ AND MASATOSHI TANAKA

Department of Pharmacology, Kurume University School of Medicine, Kurume 830, Japan

Received 17 September 1991

NISHIMURA, H. AND M. TANAKA. *Effects of alprazolam on anxiety-related behavior of rats in a modified forced-swim test employing straw suspension.* PHARMACOL BIOCHEM BEHAV 41(2) 425-427, 1992. — The present study was undertaken to examine how the triazolobenzodiazepine derivative, alprazolam, which possesses anxiolytic activity in man and anticonflict effects in animals, could affect both the duration of immobility and the incidence of straw-climbing behavior of rats in a modified forced-swim (MFS) test. After a 5-min test of forced swimming, four straws were suspended just above the surface of the water and subsequently the straw-climbing trials were counted for 5 min as an index of escape behaviors induced by negative emotionality (anxiety and/or fear). Rats were injected IP with either alprazolam (0.1, 0.2, 1, and 2 mg/kg) or its vehicle 30 min before testing. Alprazolam prolonged the duration of immobility and inhibited the straw-climbing counts in a dose-dependent manner. This effect is in the same direction as the effect shown by an anxiolytic benzodiazepine such as diazepam. The results suggest that alprazolam may possess anxiolytic effects at lower doses, whereas at a high dose of 2 mg/kg this compound might elicit sedation, concomitantly with its anxiolytic and/or antipanic effects. In addition, it appears that alprazolam is more potent than diazepam in the MFS test following a single-injection protocol.

Modified forced-swim test Straw suspension Immobility Straw-climbing behavior Alprazolam
Anxiolytic effect

PREVIOUS research has shown that rats generally display an immobile response when exposed to forced-swimming stress; however, this response is easily replaced by rope- or straw-climbing behavior when these objects are suspended above the swimming cylinder [i.e., modified forced-swim (MFS) test (7-9)]. Immobility time increases in the presence of suspended straws in association with an inhibition of straw-climbing behavior after acute administration of the anxiolytic benzodiazepine (BZD) diazepam (0.5, 1, and 5 mg/kg, IP). This finding was interpreted as an anxiolytic effect in conjunction with a sedative effect of diazepam (8). Thus, object-directed behavior is characterized as anxiety-related escape behavior from an aversive situation such as water.

Pharmacologically, it is well known that ethyl β -carboline-3-carboxylic acid (β -CCE) and yohimbine have anxiogenic and/or panicogenic properties. In a preliminary study, both these agents were found to have opposite effects compared to diazepam: They decreased immobility time and enhanced straw-climbing behavior in a MFS test (7,8).

Alprazolam, a triazolobenzodiazepine derivative, has recently been reported to show anxiolytic effects in animals (3,4,11) and in man (1,2,10). In addition, it has been found to have anti-panic-attack properties unique among the BZD's (1,6,10), although alprazolam acts *in vitro* as a specific ligand for BZD receptors in the rat brain like the other BZD's (5). The purpose of the present study was to assess the putative anxiolytic effect of alprazolam (0.1, 0.2, 1, and 2 mg/kg, IP), at lower doses than diazepam tested in a previous report (8), on the behavior of rats in an MFS test employing straw suspension.

METHOD

Subjects

Male Sprague-Dawley rats (160-220 g) were housed four per cage. Rats had free access to food and water and were kept under constant temperature ($25 \pm 1^\circ\text{C}$) and humidity (60%) conditions in a room illuminated for 12 h per day (lights on: 0700 h).

¹ Requests for reprints should be addressed to Dr. Hiroshi Nishimura, Department of Pharmacology, Kurume University School of Medicine, Kurume 830, Japan.

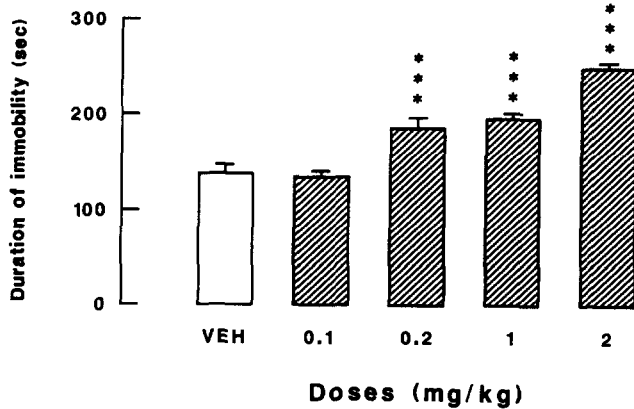


FIG. 1. Prolonging effects of alprazolam on the duration of immobility during a 5-min test session without straw suspension in forced-swimming rats. Each value indicates the mean \pm SEM of six or eight rats. All drugs were administered IP. Statistical significance: *** p < 0.001 vs. vehicle (VEH) control group.

Drugs

The drugs used were as follows: Alprazolam (Solanax[®], a gift from Japan Upjohn). Alprazolam was suspended in 0.3% carboxymethyl cellulose, with a corresponding vehicle or saline used for control injection. This drug was injected IP at a fixed volume of 0.2 ml/100 g body weight.

Apparatus

The apparatus used was a vertical glass cylinder (height: 40 cm; diameter: 18 cm) equipped with four pieces of straw (length: 24 cm; diameter: 0.4 cm) that were suspended from above. The cores of these straws were filled with cotton rope. These straws were painted black from the surface of the water to a height of 10 cm as described earlier (7-9). The apparatus was filled to a height of 15 cm with water maintained at 25°C.

Behavioral Procedure

Individual experimental rats were forced to swim in the apparatus without straw suspension (pretest session). After 15 min in the water, they were removed and allowed to dry for 15 min at 32°C before being returned to their home cages. Twenty-four hours later, they were randomly divided into four groups ($N = 6-8$ per group). One dose of alprazolam or its vehicle were injected IP 30 min before the rats were replaced into the apparatus without the straw suspension. The total duration of immobility for 5 min (non-straw-suspending period) was measured by an observer equipped with a quartz stopwatch. Immediately after this 5 min observation period, four pieces of straw were suspended and the total duration of immobility in the following 5-min period with the straw suspension (straw-suspending period) was again measured. The straw-climbing behavior was defined as escape-directed movements from the water such that the rat grasped at the straw with both forelimbs and attempted to lift its body up the straw. Each straw-climbing attempt was counted as described previously (9).

Statistical Evaluation

The results are expressed as the mean \pm SEM and were analyzed statistically by one-way analysis of variance (ANOVA) and posthoc Ryan test for multiple comparisons.

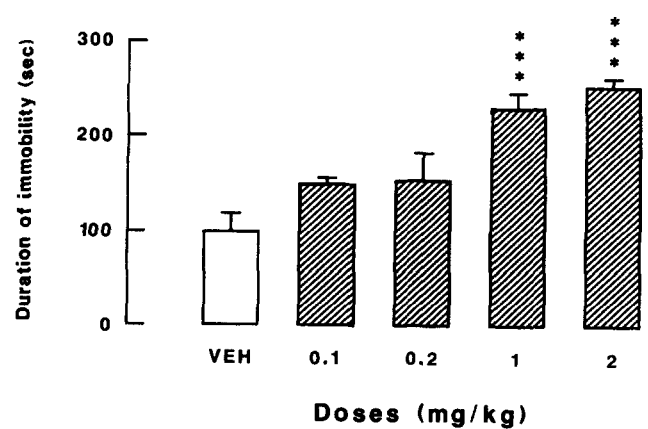


FIG. 2. Prolonging effects of alprazolam on the duration of immobility during a 5- to 10-min test session with straw suspension in forced-swimming rats. Each value indicates the mean \pm SEM of six or eight rats. All drugs were administered IP. Statistical significance: *** p < 0.001 vs. vehicle (VEH) control group.

RESULTS

Non-Straw-Suspending Period

In the first 5-min period of the forced-swim test without straw suspension, alprazolam caused a significant prolongation in immobility time, $F(4,27) = 69.4$, $p < 0.01$; posthoc analysis showed that the drug at 0.2, 1, and 2 mg/kg significantly prolonged immobility time as compared with vehicle control (Fig. 1).

Straw-Suspending Period

In the second 5-min period of the forced-swim test with straw suspension, the duration of immobility was shorter (mean value: from 139.3 to 99.9 s) when compared with that in the first 5-min period (see Figs. 1 and 2). Alprazolam caused

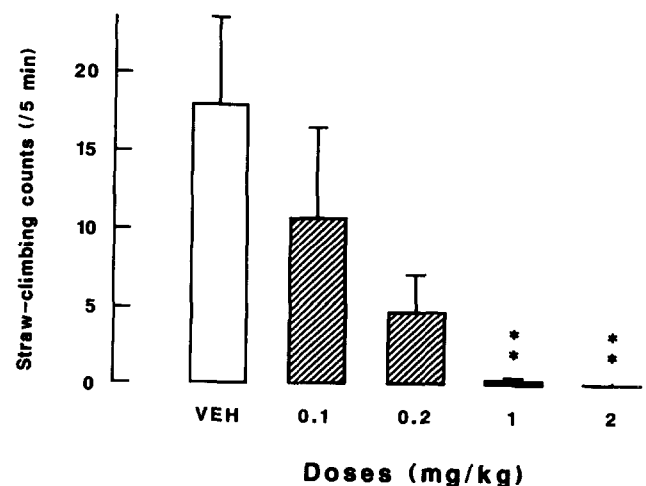


FIG. 3. Inhibitory effects of alprazolam on straw-climbing counts during a 5- to 10-min test session with straw suspension in forced-swimming rats. Each value indicates the mean \pm SEM of six or eight rats. All drugs were administered IP. Statistical significance: ** p < 0.02 vs. vehicle (VEH) control group.

a significant prolongation of immobility time with straw suspension, $F(4,27) = 11.56$, $p < 0.01$; posthoc analysis showed that the drug at 1 or 2 mg/kg showed a significant prolongation in immobility as compared to the vehicle control group (Fig. 2). Alprazolam significantly decreased straw-climbing counts, $F(4,27) = 3.23$, $p < 0.05$; posthoc analysis showed that the drug at 1 mg/kg ($p < 0.02$) and 2 mg/kg ($p < 0.02$) showed a significant inhibition of straw-climbing counts (Fig. 3).

DISCUSSION

In the first 5-min period without straw suspension (i.e., forced-swim test), alprazolam significantly prolonged the duration of immobility at 0.2, 1, and 2 mg/kg. This effect caused by alprazolam is in the same direction as that of diazepam at 5 mg/kg, and may be partially related to an anxiolytic/sedative activity of these drugs (8). In addition, this finding is consistent with a previous report that one of the triazolobenzodiazepines, U-43,465F, tended to prolong immobility time at a dose of 16 mg/kg, which produced an anxiolytic response in the four-plate test (12).

In the control rats seen in the second 5-min period with straw suspension (i.e., straw-suspension test), climbing behavior occurred frequently and the duration of immobility was reduced as compared with that seen in the nonsuspending situation. In contrast, alprazolam at 1 and 2 mg/kg significantly prolonged the immobility duration with straw suspension. Like diazepam, straw-climbing counts are decreased with low doses of 0.2 and 1 mg/kg of alprazolam and completely inhibited with only a high dose of 2 mg/kg, which also induces sedation (7,8). On the other hand, it has been reported that

higher doses (1–2.5 mg/kg) of alprazolam produce positive anxiolytic effects in other animal tests of anxiety such as the elevated plus-maze (4), ultrasonic vocalization (3), and defeat studies (11). In the case of humans, oral administration of alprazolam has been used in patients diagnosed as suffering from generalized anxiety disorder, panic disorder, and agoraphobia (1,2,10). Furthermore, a dosage range of 3–9 mg/day (approximately equivalent to 0.2 mg/kg) is necessary to achieve a therapeutic effect in the early stages of anxiety disorders in man (10). Thus, it is important to note that the inhibitory effects of alprazolam on straw-climbing behavior accompanied by an increase in immobility in this test may depend upon the anxiolytic effect of this agent—effects that have been observed at doses lower than those of diazepam used in a previous MFS test (7,8). However, caution is required in interpreting the effects of alprazolam at doses higher than 2 mg/kg as representing an anxiolytic action since such doses have been reported to have side effects such as behavioral sedation in the holeboard test (4).

In summary, these data suggest that the inhibitory effects of alprazolam on straw-climbing behavior at doses that prolong the duration of immobility may be indicative of anxiolytic activity (including sedation at higher doses). The results also indicate that alprazolam is more potent than diazepam as an anxiolytic drug in the MFS test employing straw suspension.

ACKNOWLEDGEMENTS

The authors thank Prof. G. B. Glavin of the Department of Pharmacology and Therapeutics, University of Manitoba, Canada, for his kind reviewing of an earlier version of this manuscript. Gratitude is due to Japan Upjohn for the generous supply of alprazolam.

REFERENCES

- Ballenger, J. C.; Burrows, G. D.; DuPont, R. L.; Lesser, I. M.; Noyes, R.; Pecknold, J. C.; Rifkin, A.; Swinson, R. P. Alprazolam in panic disorder and agoraphobia: Results from a multicenter trial. I. Efficacy in short-term treatment. *Arch. Gen. Psych.* 45:413–422; 1988.
- Chouinard, G.; Annable, L.; Fontaine, R.; Solyom, L. Alprazolam in the treatment of generalized anxiety and panic disorders: A double-blind placebo-controlled study. *Psychopharmacology (Berl.)* 77:229–233; 1982.
- Cuomo, V.; Cagiano, R.; De Salvia, M. A.; Maselli, M. A.; Renna, G.; Racagni, G. Ultrasonic vocalization in response to unavoidable aversive stimuli in rats: Effects of benzodiazepines. *Life Sci.* 43:485–491; 1988.
- File, S. E.; Pellow, S. The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. *Br. J. Pharmacol.* 86:729–735; 1985.
- McCabe, R. T.; Mahan, D. R.; Smith, R. B.; Wamsley, J. K. Characterization of [3 H]alprazolam binding to central benzodiazepine receptors. *Pharmacol. Biochem. Behav.* 37:365–370; 1990.
- Møller, M.; Lorentzen, K.; Bech, P.; Ottosson, J.-O.; Rosenberg, R. A trend analysis of changes during treatment of panic disorder with alprazolam and imipramine. *Acta Psych. Scand. (Suppl. 365)*:28–32; 1991.
- Nishimura, H.; Ida, Y.; Tanaka, M. Enhancing effects of Ro 15-1788 on straw-climbing behavior as measured with the straw-suspension method: Reversal by diazepam. *Pharmacol. Biochem. Behav.* 36:183–186; 1990.
- Nishimura, H.; Ida, Y.; Tsuda, A.; Tanaka, M. Opposite effects of diazepam and β -CCE on immobility and straw-climbing behavior of rats in a modified forced-swim test. *Pharmacol. Biochem. Behav.* 33:227–231; 1989.
- Nishimura, H.; Tsuda, A.; Ida, Y.; Tanaka, M. The modified forced-swim test in rats: Influence of rope- or straw-suspension on climbing behavior. *Physiol. Behav.* 43:665–668; 1988.
- Sheehan, D. V. Current perspectives in the treatment of panic and phobic disorders. *Drug Ther.* 12:179–193; 1982.
- Shepherd, J. K.; Rodgers, R. J. Acute and chronic effects of the triazolobenzodiazepine, alprazolam, on defeat and analgesia evoked by conspecific attack in male mice. *Behav. Pharmacol.* 1: 75–83; 1989.
- Von Voigtlander, P. F.; Puech, A. J. U-43,465F: A triazolobenzodiazepine with pronounced antidepressant-like as well as anxiolytic activities in animals. *Arch. Int. Pharmacodyn. Ther.* 266: 60–76; 1983.