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Enhancing Effects of Ro 15-1788 on Straw-Climbing Behavior as Measured With the Straw-Suspension Method: Reversal by Diazepam

HIROSHI NISHIMURA, YOSHISHIGE IDA AND MASATOSHI TANAKA

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

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NISHIMURA, H., Y. IDA AND M. TANAKA. *Enhancing effects of Ro 15-1788 on straw-climbing behavior as measured with the straw-suspension method: Reversal by diazepam.* PHARMACOL BIOCHEM BEHAV **36**(1) 183–186, 1990.—Ro 15-1788 (0.5, 1, 5, or 20 mg/kg), a pure benzodiazepine receptor antagonist, was studied for its effect on the duration of immobility and the number of straw-climbing attempts in a modified forced-swim test with straw-suspension of rats. A single dose of 20 mg/kg of Ro 15-1788 injected IP prolonged only the duration of immobility with no effect on straw-climbing behavior, whereas both doses of 1 and 5 mg/kg of this compound significantly enhanced the number of straw-climbing attempts in an inverted U-shaped manner. Ro 15-1788 at 1 mg/kg significantly reversed the inhibitory effect of 1 mg/kg of diazepam on the number of straw-climbing attempts. It is suggested that the enhancing effect of low doses of Ro 15-1788 on straw-climbing behavior can be regarded as an index of its anxiogenic effect, by acting via central benzodiazepine receptors.

Modified forced-swim test Straw-suspension Straw-climbing behavior Ro 15-1788 Diazepam Anxiogenic effect

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 tank. Since an increase in immobility occurs in the presence of suspended straw, in association with a decrease in straw-climbing behavior after acute administration of the anxiolytic drug, diazepam (0.5, 1, 5 mg/kg, IP), this finding is interpreted as an anxiolytic effect concomitantly with its sedative effect (16). Thus, the object-directed behavior is characterized as anxiety- or fear-motivated escape behavior from an aversive situation such as water (13,15).

Pharmacologically, it is known that anxiety-related drugs such as the anxiolytic diazepam, and the anxiogenic agent ethyl β-carboline-3-carboxylate (β-CCE), bind with high affinity to a specific benzodiazepine (BZD) receptor in the central nervous system (2, 11, 22). In a preliminary study, both of these BZD ligands with opposite effects on anxiety have been found to have opposite effects on each of these two behavioral measures (i.e., immobility and straw-climbing behavior) in a modified forced-swim test (16).

Ro 15-1788, the imidazobenzodiazepine derivative, has repeatedly been reported to be devoid of BZD-like effects and to antagonize the various effects of classical BZDs in a number of behavioral test procedures in animals (1, 2, 10–12, 16, 17), and also in man (3). However, several reports have demonstrated that Ro 15-1788 by itself has a weak anxiogenic activity in a specific behavioral test of anxiety in rodents [see, e.g., the social interaction test (5–7), the punished drinking test (6), the modified open-field test (9) and three different measures (licking, plasma corticosterone, exploratory rearing) of response to novelty (6, 19, 20)], and in humans (4). The pharmacological activity of this drug is currently at variance with animal test conditions of anxiety (18). Clearly, the most significant difference between the pharmacological profile and the behavioral effect of Ro 15-1788 is that the former is typically conducted in vitro, whereas the latter is tested in vivo. As such, the possibility exists that the challenge of a stressful situation may activate the release of an endogenous ligand which is displaced by the exogenous administration of Ro 15-1788 (7). The purpose of the present study was to assess the putative anxiogenic effect of Ro 15-1788 on the behavior of rats in a modified forced-swim test employing straw-suspension.

METHOD

Subjects

Male Sprague-Dawley rats (160–200 g) were housed 4 per cage. Rats had access to food and water freely, under constant temperature (25 ± 1°C) and humidity (60%) conditions in a room illuminated for 12 hr per day (lights on: 0700 hr).

Drugs

The drugs used were as follows: Ro 15-1788 (flumazenil) and

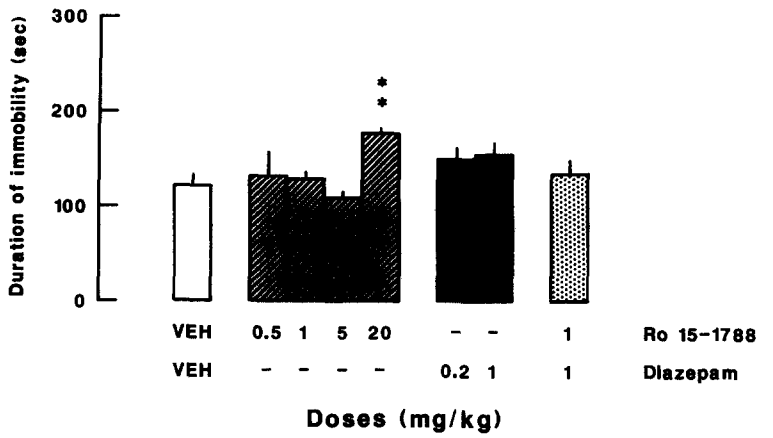

FIG. 1. Effects of Ro 15-1788 and/or diazepam on the duration of immobility during a 5-min test session without straw-suspension in forced swimming rats. Each value indicates the mean ± S.E.M. of 7 rats. All drugs were administered IP. Statistical significance: $^{**}p<0.01$ vs. vehicle (VEH) control group.

diazepam (a gift from Nippon Roche K.K.). Ro 15-1788 was suspended by ultrasonic dispersion in vehicle (one drop of Tween 80 per 3 ml of distilled water). Diazepam was suspended in 0.3% carboxymethyl cellulose. All drugs were injected intraperitoneally (IP) at a fixed volume of 0.2 ml/100 g of body weight.

Apparatus

The apparatus used was a vertical glass cylinder (height: 40 cm; diameter: 18 cm) equipped with 4 pieces of straw (length: 24 cm; diameter: 0.4 cm), which 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 (13,16). The apparatus was filled to a height of 15 cm with water maintained at 25°C.

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 eight groups (N = 7 per group). Either diazepam or its vehicle and Ro 15-1788 or its vehicle were injected IP 30 min and 10 min before a test, respectively, and the rats were replaced into the apparatus without the straw suspension and the total duration of immobility for 5 min (nonstraw-suspending period) was measured by an observer equipped with a quartz stopwatch. Immediately after this 5-min observation period, 4 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 (16).

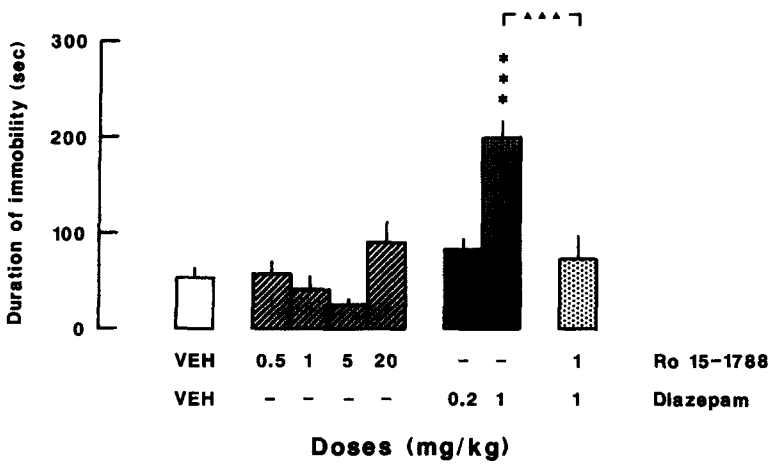

FIG. 2. Effects of Ro 15-1788 and/or diazepam on the duration of immobility during a 5–10-min test session with straw-suspension in forced swimming rats. Each value indicates the mean ± S.E.M. of 7 rats. All drugs were administered IP. Statistical significance: $^{***}p<0.001$ vs. vehicle (VEH) control group. ▲▲▲$p<0.001$ (Student's *t*-test).

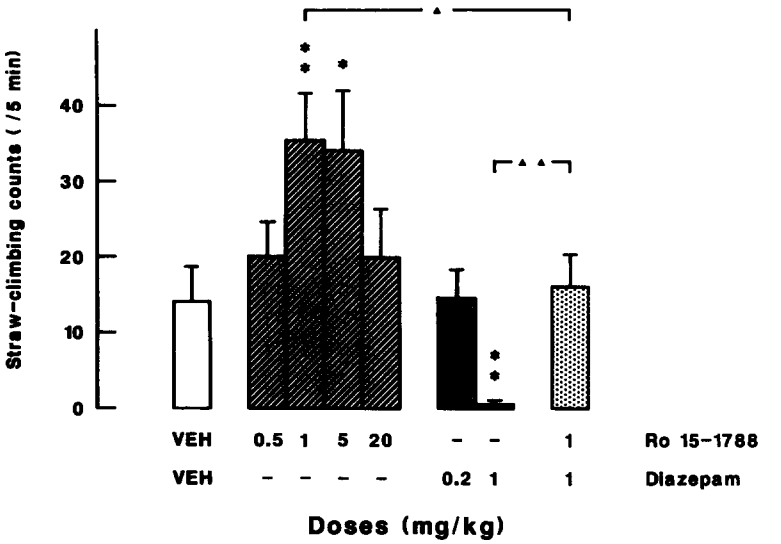

FIG. 3. Effects of Ro 15-1788 and/or diazepam on straw-climbing counts during a 5–10-min test session with straw-suspension in forced swimming rats. Each value indicates the mean ± S.E.M. of 7 rats. All drugs were administered IP. Statistical significance: *$p<0.05$, **$p<0.02$ vs. vehicle (VEH) control group. ▲$p<0.05$, ▲▲ $p<0.01$ (Student's t-test).

Statistical Evaluation

The results are expressed as the mean ± S.E.M. and were analyzed statistically by one-way analysis of variance and post hoc Tukey test for multiple comparisons.

RESULTS

Nonstraw-Suspending Period

In the first 5-min period of the forced-swim test without straw-suspension, diazepam did not affect the duration of immobility, $F(2,18)=1.39$, $p>0.05$; Ro 15-1788 prolonged immobility time, $F(4,30)=4.76$, $p<0.01$; post hoc analysis showed that the drug at 20 mg/kg significantly prolonged the duration of immobility relative to other treatment conditions (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 when compared with that seen in the first 5-min period (Figs. 1 and 2). Diazepam caused a significant prolongation of immobility time with straw-suspension, $F(2,18)=27.85$, $p<0.01$; post hoc analysis showed that at 1 mg/kg there was a significant prolongation of immobility, whereas diazepam caused a significant inhibition of straw-climbing counts, $F(2,18)=3.78$, $p<0.05$, as compared to the vehicle control group. No dose of Ro 15-1788 examined significantly affected immobility time with straw-suspension. This drug did not cause a significant enhancement of straw-climbing counts, $F(4,30)=2.69$, $p>0.05$; however, post hoc analysis showed that at 1 ($p<0.02$) and 5 ($p<0.05$) mg/kg there was a significant enhancement of straw-climbing counts, respectively. Ro 15-1788 at 0.5 and/or 20 mg/kg did not induce a significant increase in straw-climbing counts. Combined administration of Ro 15-1788 at 1 mg/kg and diazepam at 1 mg/kg antagonized each other's drug effect on straw-climbing counts.

DISCUSSION

Previous work indicates that both a forced-swim test and the modified forced-swim test with straw-suspension (i.e., straw-suspension test) are capable of detecting anxiogenic actions of both β-CCE and yohimbine, as well as the anxiolytic action of diazepam, mainly by acting via BZD receptors (13, 15, 16). In addition, Havoundjian *et al.* (8) showed that even brief exposure to a forced-swim test for 5 min elicited robust changes in the BZD/GABA receptor chloride ionophore complex in the central nervous system of rats. These findings suggest that both of these tests may be useful to assess the validity of the stressful swimming situation in the measurement of anxiety (14).

In the first 5-min period without straw-suspension (i.e., forced-swim test), Ro 15-1788, a specific brain BZD receptor antagonist, prolonged the duration of immobility only at a high dose of 20 mg/kg, which was not likely high enough to elicit sedation in the holeboard test (5). This prolongation of immobility duration by Ro 15-1788 in rats is in the same direction as that of diazepam's (1–2 mg/kg) prolonging effect on immobility, and may be partially related to a BZD-like activity.

In the second 5-min period with straw-suspension, climbing behavior occurred frequently and the duration of immobility was reduced as compared with that seen in the nonsuspending situation. Although no dose of Ro 15-1788 further shortened the immobility duration which was already decreased by the presence of straw-suspension, Ro 15-1788 (1 and 5 mg/kg) caused marked enhancement of the climbing counts. Furthermore, File *et al.* (5) have reported some partial agonistic activity for a dose of 10 mg/kg Ro 15-1788 in the social interaction test. Thus, it is not surprising to note that the enhancing effects of Ro 15-1788 on straw-climbing behavior in this test were observed at doses less than those used in other animal tests of anxiety (5, 6, 9). In the case of man following sleep deprivation, it has also been reported that scores on an anxiety self-rating scale increased after injection of Ro 15-1788, whereas this was not the case after placebo (4). These results suggest that an 'enhancement' of straw-climbing

behavior in this test may depend upon the anxiogenic effect of Ro 15-1788 by itself. Neither the lowest dose of 0.5 mg/kg nor the highest dose of 20 mg/kg of Ro 15-1788 showed an enhancing effect on straw-climbing counts (an inverted U-shape relationship, Fig. 3), reflecting its anxiogenic action in the same direction as that of β-CCE (16). This finding is consistent with other reports that the weak anxiogenic action of Ro 15-1788 is lost at 20 mg/kg (5,20). It should be noted, furthermore, that Ro 15-1788 at 20 mg/kg has been reported to have no significant sedative effect in the holeboard test (5).

Diazepam at a dose as low as 0.2 mg/kg failed to inhibit straw-climbing counts, although the nonsedative dose of diazepam, 0.5 mg/kg, significantly attenuated straw-climbing counts (16). However, diazepam at 1 mg/kg prolonged the reduced immobility duration by straw-suspension without prolonging the immobility duration in the first 5-min test period, and markedly inhibited straw-climbing counts. It is generally assumed that this 1 mg/kg dose of diazepam reflects the anxiolytic (concomitantly with its sedative) drug effect without producing motor inhibition in other behavioral tests of anxiety (2, 21, 23). The prolonging effect of diazepam at 1 mg/kg on the duration of immobility with straw-suspension was reversed by the combined administration of Ro 15-1788 at 1 mg/kg. Moreover, both Ro 15-1788 (1 mg/kg) and diazepam (1 mg/kg) reversed each other's effect on straw-climbing counts.

In summary, these data suggest that the prolonging effects of Ro 15-1788 at a high dose of 20 mg/kg on immobility duration may depend upon BZD-like activity, and that the enhancing effects of this compound at low doses (1 or 5 mg/kg) on straw-climbing counts in the straw-suspension test might be related to the anxiogenic actions of the drug mediated via central BZD receptors under these straw-suspending conditions.

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REFERENCES

1. Bonetti, E. P.; Pieri, L.; Cumin, R.; Schaffner, R.; Pieri, M.; Gamzu, E. R.; Müller, R. K. M.; Haefely, W. Benzodiazepine antagonist Ro 15-1788: Neurological and behavioral effects. Psychopharmacology (Berlin) 78:8–18; 1982.
2. Braestrup, C.; Nielsen, M. Benzodiazepine receptor binding in vivo and efficacy. In: Olsen, R. W.; Venter, J. C., eds. Receptor biochemistry and methodology, vol. 5. New York: Alan R. Liss, Inc.; 1986:167–184.
3. Darragh, A.; Lambe, R.; Kenny, M.; Brick, I.; Taaffe, W.; O'Boyle, C. Ro 15-1788 antagonises the central effects of diazepam in man without altering diazepam bioavailability. Br. J. Clin. Pharmacol. 14:677–682; 1982.
4. Emrich, H. M.; Sonderegger, P.; Mai, N. Action of the benzodiazepine antagonist Ro 15-1788 in humans after sleep withdrawal. Neurosci. Lett. 47:369–373; 1984.
5. File, S. E.; Lister, R. G.; Nutt, D. J. The anxiogenic action of benzodiazepine antagonists. Neuropharmacology 21:1033–1037; 1982.
6. File, S. E.; Pellow, S. The benzodiazepine receptor antagonist Ro 15-1788 has anxiogenic activity in four animal tests of anxiety. Br. J. Pharmacol. 84:103; 1985.
7. File, S. E.; Pellow, S. Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. Psychopharmacology (Berlin) 88:1–11; 1986.
8. Havoundjian, H.; Paul, S. M.; Skolnick, P. Acute, stress-induced changes in the benzodiazepine/γ-aminobutyric acid receptor complex are confined to the chloride ionophore. J. Pharmacol. Exp. Ther. 237:787–793; 1986.
9. Hoffman, D. K.; Britton, D. R. Anxiogenic-like properties of benzodiazepine antagonists. Soc. Neurosci. Abstr. 9:129; 1983.
10. Hunkeler, W.; Möhler, H.; Pieri, L.; Polc, P.; Bonetti, E. P.; Cumin, R.; Schaffner R.; Haefely, W. Selective antagonists of benzodiazepines. Nature 290:514–516; 1981.
11. Möhler, H.; Okada, T. Benzodiazepine receptor: Demonstration in the central nervous system. Science 198:849–851; 1977.
12. Nagatani, T.; Yamamoto T.; Sugihara T.; Ueki, S. The effect of agonists at the GABA-benzodiazepine receptor complex on the duration of immobility of mice in the forced swimming test. Eur. J. Pharmacol. 142:17–22; 1987.
13. 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.
14. Nishimura, H.; Tsuda, A.; Oguchi, M.; Ida, Y.; Tanaka, M. Is immobility of rats in the forced swim test "behavioral despair?" Physiol. Behav. 42:93–95; 1988.
15. Nishimura, H.; Tsuda, A.; Oguchi, M.; Shirao, I.; Ida, Y.; Tanaka, M. Effects of rope-suspending and drug administration (yohimbine, diazepam) on behavior in forced swimming rats. Jpn. J. Pharmacol. 43(Suppl.):220; 1987.
16. 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.
17. Nutt, D. J.; Cowen, P. J.; Little, H. J. Unusual interactions of benzodiazepine receptor antagonists. Nature 295:436–438; 1982.
18. Pellow, S.; File, S. E. Multiple sites of action for anxiogenic drugs: Behavioural, electrophysiological and biochemical correlations. Psychopharmacology (Berlin) 83:304–315; 1984.
19. Pellow, S.; File, S. E. The effects of putative anxiogenic compounds (FG 7142, CGS 8216 and Ro 15-1788) on the rat corticosterone response. Physiol. Behav. 35:587–590; 1985.
20. Rodgers, R. J.; Waters, A. J.; Rosenfield, S. Evidence for intrinsic behavioural activity of the benzodiazepine antagonist, Ro 15-1788, in male mice. Pharmacol. Biochem. Behav. 19:895–898; 1983.
21. Sepinwall, J.; Cook, L. Behavioral pharmacology of anti-anxiety drugs. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology, vol. 13. New York: Plenum Press; 1978:345–393.
22. Squires, R. F.; Braestrup, C. Benzodiazepine receptors in rat brain. Nature 266:732–734; 1977.
23. Treit, D; LoLordo, V. M.; Armstrong, D. E. The effects of diazepam on "fear" reactions in rats are modulated by environmental constraints on the rat's defensive repertoire. Pharmacol. Biochem. Behav. 25:561–565; 1986.