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Opposite Effects of Diazepam and β-CCE on Immobility and Straw-Climbing Behavior of Rats in a Modified Forced-Swim Test

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NISHIMURA, H., Y. IDA, A. TSUDA AND M. TANAKA. *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**(1) 227–231, 1989.—The present study was undertaken to examine how two ligands of the benzodiazepine receptor, which possess anxiolytic or anxiogenic actions, affect both the duration of immobility and the incidence of straw-climbing behavior in rats in a modified forced-swim test. Rats were injected IP with either vehicle, diazepam (0.5, 1, 5 mg/kg), or β-carboline-3-carboxylic acid ethyl ester (β-CCE; 0.5, 1, 2, 5 mg/kg), or a combination of diazepam at 1 mg/kg and β-CCE at 2 mg/kg. In addition, Ro 15-1788 (1 mg/kg), a specific benzodiazepine antagonist, was injected IP 20 min after diazepam injection and immediately after β-CCE injection, respectively. In the first 5-min period of the forced-swim test, diazepam at 5 mg/kg prolonged the duration of immobility, whereas β-CCE at 1, 2 and 5 mg/kg reduced its duration. Immediately after the first 5-min test period, 4 straws were suspended above the surface of the water, and the number of straw-climbing attempts and the duration of immobility were measured for a subsequent 5-min test period. Straw-suspension elicited straw-climbing behavior in forced swimming rats, resulting in a shortening of the duration of immobility in this period. All doses of diazepam inhibited straw-climbing attempts and prolonged the duration of immobility in a dose-dependent manner. β-CCE at 1 or 2 mg/kg enhanced straw-climbing attempts, but did not significantly affect the duration of immobility. Furthermore, the combined administration of diazepam and β-CCE antagonized the respective drug effects on the duration of immobility and the number of straw-climbing attempts. Moreover, the inhibitory effect of diazepam (1 mg/kg) and the enhancing effect of β-CCE (2 mg/kg) on straw-climbing attempts were reversed by Ro 15-1788 at 1 mg/kg, respectively. These results suggest that the two benzodiazepine ligands, which possess opposite effects on anxiety, could affect both the duration of immobility and straw-climbing attempts of rats in a modified forced-swim test via benzodiazepine receptors. It is further suggested that the immobility in this test may reflect coping behavior related to emotionality (i.e., anxiety and/or fear) and that the straw-climbing behavior may represent escape attempts from the water. Therefore these measures may be new indices of anxiety-related behavior.

Immobility Straw-climbing behavior Straw-suspension Benzodiazepine receptor Diazepam β-CCE Ro 15-1788 Anxiety

IN general, immobility in the forced-swim test has been considered to reflect a state of "behavioral despair" (15). However, we have recently reported that rats in a modified forced-swim test, where a rope or straw is suspended above the surface of water, try to climb up to the suspended objects, which results in reducing the immobility duration (10,12). From these findings, we proposed a hypothesis that immobility in forced-swimming rats might reflect behavioral coping and/or adaptation following an emotional reaction (anxiety and/or fear) to an inescapable stressful situation. If this hypothesis is correct, anxiety-related drugs should affect not only the duration of immobility but also the appearance of escape behavior.

Anxiety-related drugs acting at benzodiazepine receptor binding sites are widely believed to possess one of two pharmacological profiles: benzodiazepine receptor agonists such as benzodiazepines, are anxiolytic; while benzodiazepine receptor 'inverse' agonists such as β-carbolines, are anxiogenic (2). In particular, β-carboline-3-carboxylic acid ethyl ester (β-CCE) is one of the β-carbolines classified as a benzodiazepine-receptor 'inverse' agonist (1), which has been reported to elicit an anxiogenic action in several species including humans (6), monkeys (9), and rats (7). In the present study, we examined the effects of two anxiety-related drugs, i.e., diazepam, an anxiolytic benzodiazepine and β-CCE, on the duration of immobility and the number of straw-climbing attempts in forced-swimming rats with or without straw-suspension. In addition, we examined whether effects of two kinds of benzodiazepine receptor ligands with opposite actions could be reversed by Ro 15-1788, a benzodiazepine receptor antagonist, respectively.

METHOD

Subjects

Male Sprague-Dawley rats (190–220 g) were housed 4–5 per

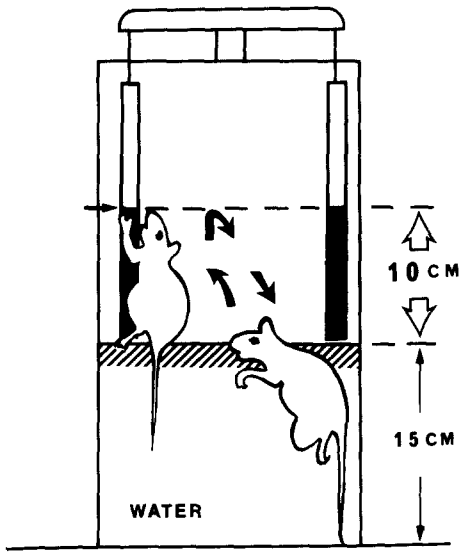

FIG. 1. Straw-climbing behavior of a rat in the forced-swim test with the straw-suspending apparatus. See the text for details.

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 (light on at 0700 hr).

Drugs

The drugs used were: β-carboline-3-carboxylic acid ethyl ester (β-CCE, Research Biochemicals Inc., Wayland, MA), diazepam and Ro 15-1788 (a gift from Nippon Roche K.K.). β-CCE and Ro 15-1788 were 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. In our previous study (10), the ropes were suspended, however, climbing counts could not be correctly assessed because the rats were sometimes able to climb and hang on to the ropes. Therefore, in the present study, we used slippery straws to count the climbing trials correctly as a useful quantitative index of escape behavior from water. The straws were placed at equal spaces so as to be situated with their lower tips just above the surface of the water (Fig. 1). These straws were painted black from the surface of the water to a height of 10 cm. 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 nine groups (N = 8 per group) in Experiment 1 and five groups (N = 7–8 per group) in Experiment 2. In Experiment 1, either diazepam or its vehicle and β-CCE or its vehicle were injected IP 30 min and 10 min before a test, respectively. In Experiment 2, Ro 15-1788 was injected IP 20 min after diazepam injection and immediately after β-CCE injection, respectively. 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. 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, when the rat climbed up a straw and reached a height of 10 cm and then slid down again to the water, as illustrated in Fig. 1.

Statistics

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

Experiment 1

Nonstraw-suspending period. In the first 5-min period of the forced-swim test without straw-suspension, diazepam caused a significant prolongation in immobility time, $F(3,28) = 14.4$, $p<0.01$; post hoc analysis showed that the drug at 5 mg/kg significantly prolonged immobility time as compared with vehicle control, and, conversely, β-CCE significantly reduced immobility time, $F(4,35) = 7.36$, $p<0.01$; post hoc analysis showed that at 1, 2, and 5 mg/kg there was a significant reduction in immobility (Fig. 2). The reducing effect of β-CCE at 2 mg/kg was eliminated when given in combination with diazepam at 1 mg/kg.

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 122.0 to 53.9 sec) when compared with that in the first 5-min period (Figs. 2 and 3). Diazepam caused a significant prolongation of immobility time with straw-suspension, $F(3,28) = 30.3$, $p<0.01$; post hoc analysis showed that at 0.5, 1 or 5 mg/kg there was a significant prolongation in immobility, whereas diazepam caused a significant inhibition of straw-climbing counts, $F(3,28) = 6.04$, $p<0.01$, in a dose-dependent manner as compared to the vehicle control group. Although no dose of β-CCE examined significantly affected immobility time with straw-suspension, this drug did cause a significant enhancement of straw-climbing counts, $F(4,35) = 6.49$, $p<0.01$; post hoc analysis showed that at 1 and 2 mg/kg there was a significant enhancement of straw-climbing counts. β-CCE at 0.5 and/or 5 mg/kg did not induce a significant increase in straw-climbing counts. Coadministration of β-CCE at 2 mg/kg and diazepam at 1 mg/kg antagonized not only the prolonging effect of diazepam on immobility duration but also antagonized each other's effects on straw-climbing counts.

Experiment 2

Nonstraw-suspending period. In the first 5-min period of the forced-swim test without straw-suspension, diazepam at 1 mg/kg did not cause a significant prolongation in immobility as compared with vehicle control. β-CCE at 2 mg/kg caused a significant

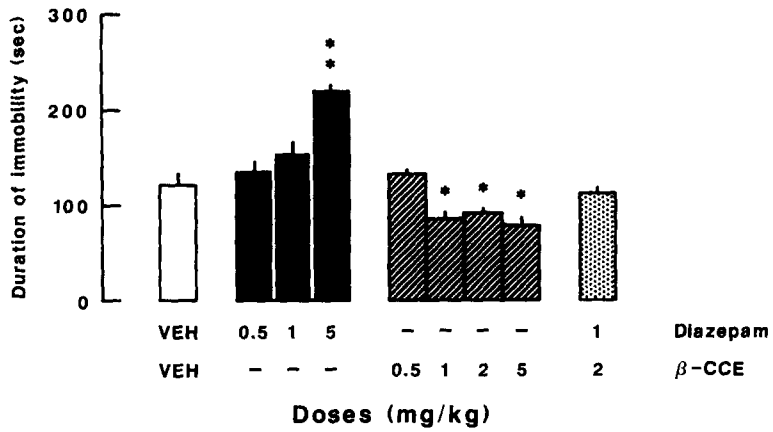

FIG. 2. Effects of diazepam and/or β-CCE on duration of immobility during a 5-min test session without straw-suspension in forced-swimming rats. Each value indicates the mean ± S.E.M. of 8 rats. All drugs were administered IP. Statistical significance: $*p<0.05$; $**p<0.01$ vs. vehicle (VEH) control group.

reduction in immobility time as compared to the vehicle control group. The reducing effect of β-CCE at 2 mg/kg was eliminated when given in combination with Ro 15-1788 at 1 mg/kg (Table 1).

Straw-suspending period. In the second 5-min period of the forced-swim test with straw-suspension, diazepam at 1 mg/kg significantly prolonged immobility time as compared to the vehicle control group. The prolonging effect of diazepam at 1 mg/kg was eliminated when given in combination with Ro 15-1788 at 1 mg/kg. β-CCE at 2 mg/kg did not reduce immobility time as compared with the vehicle control group. Diazepam at 1 mg/kg caused a significant inhibition of straw-climbing counts, as compared to the vehicle control group. Coadministration of diazepam at 1 mg/kg and Ro 15-1788 at 1 mg/kg antagonized the inhibitory effect of diazepam on straw-climbing counts. β-CCE at 2 mg/kg caused a significant enhancement of straw-climbing counts. Coadministration of β-CCE at 2 mg/kg and Ro 15-1788 at 1 mg/kg antagonized the enhancing effect of β-CCE on straw-climbing counts (Table 1).

DISCUSSION

In the first 5-min period without straw-suspension, diazepam prolonged immobility duration only at 5 mg/kg. Previous work has shown that diazepam at 2 mg/kg also prolonged the duration of immobility [e.g., (12)]. These doses are likely high enough to elicit sedative and ataxic effects concomitantly with its anxiolytic effects. Although Porsolt *et al.* (17) reported that diazepam at various doses from 0.5 mg/kg to 4 mg/kg had no significant effects on immobility duration, a trend toward an increasing effect was shown in their data. A recent study reported that the duration of

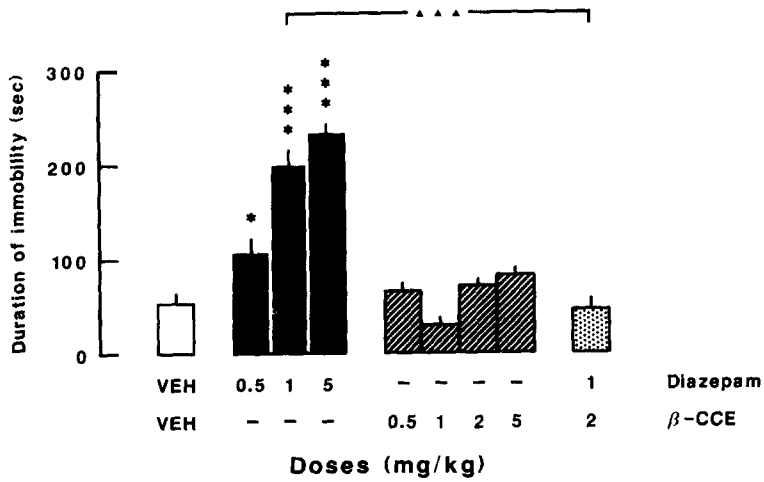

FIG. 3. Effects of diazepam and/or β-CCE on duration of immobility during a 5–10-min test session with straw-suspension in forced-swimming rats. Each value indicates the mean ± S.E.M. of 8 rats. All drugs were administered IP. Statistical significance: $*p<0.05$; $***p<0.001$ vs. vehicle (VEH) control group. ▲▲▲$p<0.001$ (Student's *t*-test).

TABLE 1

ANTAGONISM OF DIAZEPAM (DZP)- OR β-CCE-INDUCED EFFECTS BY Ro 15-1788 (RO)

Drug (dose, mg/kg)	N	Immobility (sec) 0–5 min	Immobility (sec) 5–10 min	Straw-Climbing (counts) 5–10 min
Vehicle	8	129 ± 12	54 ± 10	14 ± 5
DZP (1)	7	159 ± 15	194 ± 19[a]‡	0.2 ± 0[a]†
DZP (1) + RO (1)	7	135 ± 14	75 ± 21[b]‡	9 ± 5[b]†
β-CCE (2)	7	86 ± 5[a]*	68 ± 8	36 ± 5[a]*
β-CCE (2) + RO (1)	7	145 ± 6[c]‡	83 ± 22	15 ± 4[c]†

Each value represents the mean ± S.E.M. Statistical significances were made by Student's *t*-test; a: vs. vehicle. b: vs. DZP (1) value. c: vs. β-CCE (2) value. Significant levels are: *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

behavior occurred frequently and the duration of immobility was reduced as compared with that seen in the nonsuspending situation. Diazepam prolonged the reduced immobility duration by straw-suspension in a dose-dependent manner with a negative correlation to the straw-climbing counts, although the effect of drug on muscle relaxation rather than emotion may be largely involved in behavioral inhibition when administered at a high dose. Although no dose of β-CCE further shortened the immobility duration which was already decreased by the presence of straw-suspension, this anxiogenic drug at 1 and 2 mg/kg caused marked enhancement of the climbing counts. These data suggest that the appearance of straw-climbing behavior in the forced-swim test may depend upon the opposite effects of anxiety-related drugs, and support our previous hypothesis (10) that straw-climbing behavior may represent escape from an inescapable stressful situation. Immobility thus reflects behavioral coping and/or adaptation following an emotional reaction [see also (10,11)]. Neither the lowest dose of 0.5 mg/kg nor the highest dose of 5 mg/kg of

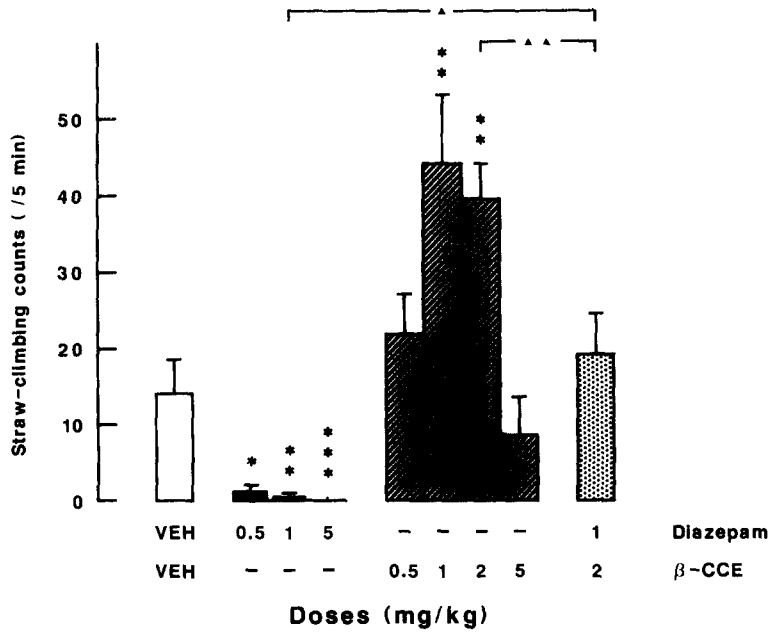

FIG. 4. Effects of diazepam and/or β-CCE on straw-climbing counts during a 5–10-min test session with straw-suspension in forced swimming rats. Each value indicates the mean ± S.E.M. of 8 rats. All drugs were administered IP. Statistical significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. vehicle (VEH) control group. ▲$p<0.05$, ▲▲ $p<0.01$ (Student's *t*-test).

immobility of mice is increased by diazepam at doses from 0.3 to 5 mg/kg, but not by a muscle relaxant drug, mephenesin, which suggests that these effects of diazepam may be related to the anxiolytic but not ataxic action of diazepam (8).

On the other hand, the anxiogenic agent β-CCE reduced the duration of immobility at all doses over 1 mg/kg used in the present study. Porsolt *et al.* (16) and our laboratory (12) have reported that another drug, yohimbine, which has been reported to have an anxiogenic action in humans (4), monkeys (18) and rats (14), reduced the duration of immobility in rats. Therefore, a reduction in immobility might be related to aversive hyperemotional reactions (anxiety and/or fear). Taken together, the present data suggest that anxiety-related drugs can affect immobility duration.

In the second 5-min period with straw-suspension, climbing β-CCE showed an enhancing effect on straw-climbing counts (inverted U-shape relationship). When β-CCE at 5 mg/kg was administered, the rats showed pronounced struggling in the water rather than climbing the straw, although subconvulsive states were not observed as reported in a previous study (3, 5, 13). A dose of 5 mg/kg of β-CCE may probably be too high to elicit climbing behavior, which might be a more complicated behavior than struggling.

The prolonging effect of diazepam at 1 mg/kg on the duration of immobility with straw-suspension was reversed by the combined administration of β-CCE at 2 mg/kg. Furthermore, these two drugs antagonized each other's effects on straw-climbing counts. The reducing effect of β-CCE on the duration of immobility without straw-suspension was reversed by the combined administration of Ro 15-1788. In the straw-suspension situation,

the prolonging effect of diazepam on the duration of immobility and opposite effects of diazepam and β-CCE on straw-climbing counts were reversed in combination with Ro 15-1788, respectively. Therefore, behavioral effects of benzodiazepine receptor ligands with opposite (anxiolytic and anxiogenic) effects in the modified forced-swim test may be specific actions mediated via benzodiazepine receptors. These data strongly support our hypothesis that immobility and straw-climbing behavior might be related to an altered level of emotionality (anxiety and/or fear). However, to further develop our hypothesis, further investigations will be needed in this modified forced-swim test, specifically using other kinds of anxiety-related drugs such as nonbenzodiazepines without muscle relaxant effects and other psychotropic agents such as antidepressants and antipsychotics.

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