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The Effect of Chlordiazepoxide on the Habituation of Exploration: Interactions With the Benzodiazepine Antagonist RO 15-1788

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LISTER, R G AND S E FILE *The effect of chlordiazepoxide on the habituation of exploration Interactions with the benzodiazepine antagonist RO 15-1788* PHARMACOL BIOCHEM BEHAV 26(3) 631–634, 1987 —Rats tested on two occasions in a holeboard apparatus showed between-session habituation of exploratory activity No habituation was observed on the measure of locomotor activity. Administration of chlordiazepoxide before the first test reduced exploratory behavior in this test and also reduced the degree of between-session habituation Administration of RO 15-1788 reversed the effect of chlordiazepoxide on exploration in the first test, but failed to reverse the drug's effect on between-session habituation It is unlikely that state-dependent retrieval could account for these results. The results are discussed in relation to the effects of benzodiazepines on learning and memory

Benzodiazepine Exploration Locomotor activity Habituation Learning RO 15-1788 Benzodiazepine antagonist

THE habituation of exploratory behavior has been used as a paradigm to investigate animal learning [12]. The paradigm relies on the observation that in certain environments animals show higher levels of exploratory activity when the environment is novel than when it is familiar. For a detailed discussion of the factors affecting exploratory behavior the reader is referred to recent reviews [1,4]. The holeboard apparatus is particularly suitable for investigating the habituation of exploration since it allows exploratory behavior to be measured independently of locomotor activity [5] Chlordiazepoxide has been found to retard the habituation of exploration that occurs between an animal's first and second exposures to a holeboard apparatus [3]. The aim of the present experiment was to investigate this phenomenon further by examining whether the benzodiazepine receptor ligand RO 15-1788, a drug that reverses many of the behavioral effects of the benzodiazepines [2], also reverses the effect of chlordiazepoxide on the habituation of exploration. The experiment was also designed to examine whether state-dependent learning effects might account for any of the results. The dose of chlordiazepoxide (5 mg/kg) was the lowest that consistently produced behavioral effects in our previous studies. A two week interval between the first and second exposures to the holeboard was chosen on the basis of pilot studies. At this time chlordiazepoxide and its active metabolites would all have been eliminated and, therefore, could not alter performance during the second holeboard test.

METHOD

Male hooded-rats from Olac Bicester, weighing approximately 200 g at the start of the experiment were housed in groups of 8, allowed ad lib access to food and water, and maintained on a 11 hr light-13 hr dark cycle (lights on 06:30).

Chlordiazepoxide (CDP) was dissolved in distilled water to a concentration of 2.5 mg/ml. RO 15-1788 was suspended in distilled water to which a drop of Tween 20 had been added to give a concentration of 5 mg/ml. All injections were made intraperitoneally using an injection volume of 2 ml/kg.

The experimental design is illustrated in Table 1. Eighty-two rats were divided into 3 groups each containing 28 or 27 animals. One group received an IP injection of chlordiazepoxide (5 mg/kg), another received CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) and the third group received the drug vehicles. Thirty minutes after the injections the animals were placed in a holeboard for a 7.5 minute test. A further 9 animals received the vehicle 30 minutes before the holeboard test and an injection of CDP (5 mg/kg) immediately after removal from the holeboard.

Exactly two weeks after the first test all animals were

TABLE 1

EXPERIMENTAL DESIGN THE DOSE OF CHLORDIAZEPOXIDE (CDP) USED WAS 5 mg/kg AND THAT OF RO 15-1788 WAS 10 mg/kg

Group	Treatment on Day 1: 30 Min Before Holeboard Test	Treatment on Day 1: Immediately After Holeboard Test	Treatment on Day 15: 30 Min Before Holeboard Test
1	Vehicle	Vehicle	Vehicle
2	Vehicle	Vehicle	CDP
3	Vehicle	Vehicle	CDP + RO 15-1788
4	CDP	Vehicle	Vehicle
5	CDP	Vehicle	CDP
6	CDP	Vehicle	CDP + RO 15-1788
7	CDP + RO 15-1788	Vehicle	Vehicle
8	CDP + RO 15-1788	Vehicle	CDP
9	CDP + RO 15-1788	Vehicle	CDP + RO 15-1788
10	Vehicle	CDP	Vehicle

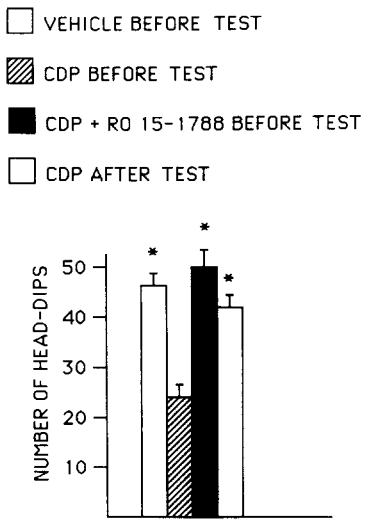

FIG 1 The number of head-dips made by apparatus-naive rats during a 7 5 min holeboard test 30 min after treatment with CDP (5 mg/kg), CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) or the drug vehicles (n=27 or 28 per group) A final group of 9 animals received CDP (5 mg/kg) immediately after the holeboard test Scores are means±SEM *Significantly different from animals that received CDP alone before the test, $p<0$ 01

TABLE 2

THE LOCOMOTOR ACTIVITY SCORES OF RATS DURING TWO EXPOSURES TO A HOLEBOARD APPARATUS

(A) Behavior During First Test

Treatment Before First Test	
Vehicle (n=28)	564 ± 10
CDP (n=27)	404 ± 24*
RO 15-1788 + CDP (n=27)	613 ± 15†
CDP after test (n=9)	589 ± 16

(B) Behavior During Second Test

Treatment Before First Test	Treatment Before Second Test: Vehicle	CDP	CDP + RO 15-1788
Vehicle	626 ± 35	307 ± 46*	728 ± 41†
CDP	711 ± 37	440 ± 48*	669 ± 28†
CDP + RO 15-1788	654 ± 25	366 ± 70*	683 ± 38†
CDP after test	618 ± 21	not tested	not tested

(A) For the first test animals were placed in the holeboard 30 min after treatment with CDP (5 mg/kg), CDP (5 mg/kg) + RO 15-1788 (10 mg/kg), or the vehicles A final group received CDP (5 mg/kg) immediately after their first test (B) Two weeks later animals were tested 30 min after treatment with vehicle, chlordiazepoxide (CDP 5 mg/kg), or CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) Scores are means ± SEM, n=9 or 10 per group, unless stated

*Significantly different from vehicle treated animals, $p<0$ 01

†Significantly different from animals that received CDP alone, $p<0$ 01

tested again. Approximately one third of the animals from each group received CDP (5 mg/kg), a third received CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) and the remaining animals received the vehicle The animals treated with CDP after the first test all received the vehicles. The animals were tested individually for 7.5 min in the holeboard 30 min after treatment.

The data from the first test were analysed using analysis of variance with drug treatment during the first test as the independent measure Data from the second test were also analysed using analysis of variance, drug treatment on the first test day and drug treatment on the second test day were the independent factors Between group comparisons were made using Dunnett's test unless otherwise stated.

RESULTS

In the first test, there were significant drug effects on both the number of head-dips, $F(2,80)=25$ 4, $p<0.0001$, and locomotor activity, $F(2,80)=38.7$, $p<0.0001$. Rats that received chlordiazepoxide made fewer head-dips ($p<0.01$) and had lower locomotor activities ($p<0.01$) than vehicle-treated animals. RO 15-1788 reversed the effect of chlordiazepoxide on both measures ($p<0$ 01), see Fig 1 and Table 2 Although animals that received the drug combination had mean head-dipping and activity scores that were above those of vehicle-treated animals, the differences were not significant As expected, animals that received CDP after the first test did not differ from controls in their behavior.

The animals that received the vehicles before both the first and second tests made fewer head-dips, $t(9)=6$ 45, $p<0$ 001, during the second test than during the first. There was no significant difference in locomotor activity between the first and second test sessions, see Fig. 2

Drug treatment before the second test in the holeboard significantly altered both locomotor activity, $F(2,73)=52.8$,

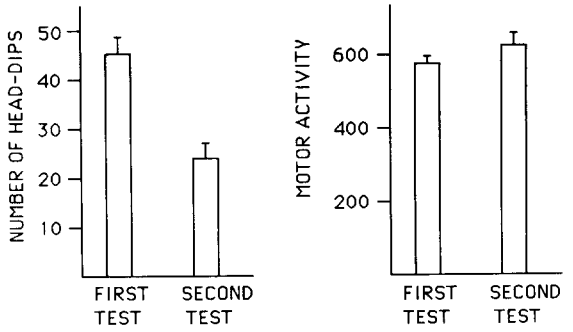

FIG 2 The number of head-dips made by rats during a 7 5 min holeboard test (left) and their locomotor activities (right) 30 min after treatment with both the CDP and RO 15-1788 vehicles The animals were tested on two occasions, separated by two weeks Scores are means±SEM

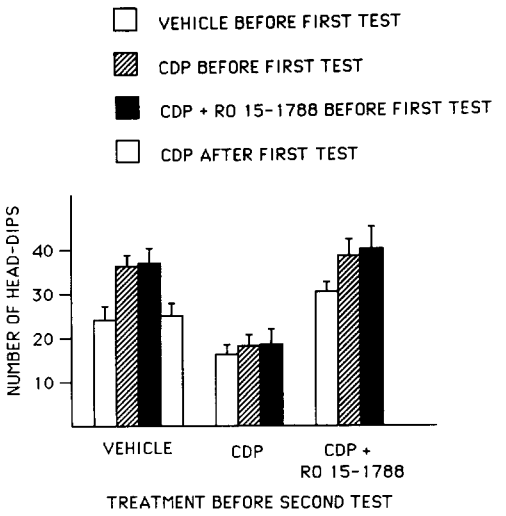

FIG 3 The number of head-dips made by rats during their second 7 5 min test in a holeboard apparatus, 30 min after treatment with CDP (5 mg/kg), CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) or the vehicles The animals had been tested in the apparatus for 7.5 min two weeks earlier, and had received CDP (5 mg/kg), CDP (5 mg/kg) + RO 15-1788 (10 mg/kg) or the vehicles 30 min before the first test, or CDP (5 mg/kg) immediately after the test Scores are means±SEM See text for statistics

$p<0$ 0001, and the number of head-dips, $F(2,73)=28$ 0, $p<0$ 0001 CDP reduced both these measures, and RO 15-1788 completely reversed the effect ($p<0$ 01), see Table 2 and Fig. 3. There was also a significant effect of treatment on day 1 on the number of head-dips, $F(2,73)=6.0$, $p<0.005$, see Fig 3 Animals that received CDP during the first test made more head-dips during the second test than animals that received the vehicle, $F(1,49)=10$ 8, $p<0.002$. Surprisingly there was no indication that RO 15-1788 reversed this effect In fact animals that received the drug combination also made significantly more head-dips than those that received the vehicle, $F(1,49)=9$ 1, $p<0.005$. Moreover, during their second test, animals that received CDP immediately after their first test made significantly fewer head-dips, $t(16)=3.38$, $p<0$ 005, than those that received CDP before their first test The animals that received CDP after their first test did not differ in their behavior from the vehicle-treated controls on either of the behavioral measures.

DISCUSSION

The administration of chlordiazepoxide 30 min before the first test in the holeboard caused reductions in both exploratory head-dipping and locomotor activity reflecting the sedative action of this drug. RO 15-1788 completely reversed chlordiazepoxide's effect on both measures, consistent with previous reports that it completely reverses the sedative action of benzodiazepines, both in the holeboard test [6] and in other experimental paradigms [2]. We have previously found that the combination of RO 15-1788 and CDP caused increases in head-dipping above the level of vehicle treated controls [6] In the present experiment, although the mean exploratory head-dipping scores of animals receiving the drug combination were consistently above those receiving the vehicles, the differences failed to reach significance

Our results clearly show that an animal's behavior on a second exposure to a holeboard apparatus can be markedly influenced by the drug-treatment before its first exposure. Animals that received CDP before the first test had higher exploratory activities in the second test than the animals that had received the vehicle before the first test. The animals that received CDP in combination with RO 15-1788 before the first test also showed increased exploration in the second test and behaved like those that received CDP alone This is surprising because, as noted above, in the first test RO 15-1788 completely reversed the sedative effects of CDP. It might be argued that since CDP-treated animals explored less than controls during the first test, the environment was less familiar to them at the start of the second test and this caused levels of exploration higher than those of controls. However, the animals that received the drug combination before the first test, and that explored no less than the controls in test 1, also had increased exploratory activity during the second test The increased exploration in the second test cannot be due solely to a single injection of CDP two weeks earlier because the animals that received the drug immediately after the holeboard test did not show such an effect and behaved in the second test like those that had received the vehicle

Since animals that received the vehicle before the first and the second test showed between session habituation, one possible explanation of the data is that animals that received a drug before their first test could not 'remember' their previous experience as well as vehicle-treated animals Benzodiazepine-induced impairments in acquisition have been well documented in human studies (see [8]) and similar effects may be observed in laboratory animals [13]. Prior to accepting this explanation, however, several points need to be made Firstly, there is evidence that RO 15-1788 can reverse the amnesic action of benzodiazepines in humans [10], although the reversal in rodents may not be complete [14]. Secondly, this would provide evidence for a dissociation between the sedative and the amnesic effects of the benzodiazepines, since in the current experiment RO 15-1788 totally reversed the sedative effect but showed no indication of reversing the amnesic effect. The relationship between the sedative and amnesic effects of benzodiazepines in humans has been the subject of some debate [8]. Thirdly, it is unlikely that CDP impaired memory consolidation because the animals that received the drug immediately after the first test

behaved like vehicle-treated controls. A final point concerns whether the amnesia resulted from a change in state between the first and second tests

State-dependent learning, or more correctly, state-dependent retrieval (SDR), has been extensively investigated in many behavioral paradigms. Overton [11] has discussed in some detail the difficulties in demonstrating unequivocally that a drug causes SDR if it also affects acquisition processes or performance In the present study CDP alone altered exploration the first time animals were exposed to the holeboard, and the effects of benzodiazepines on acquisition have already been mentioned. It is, therefore, impossible to address the issue of SDR for animals that received CDP alone. However, the combination of CDP and RO 15-1788 did not significantly alter performance Further, there was no indication that animals that received the drug combination before both tests had better retention (reduced exploration) than animals who received different treatments before each test, see Fig. 2. Hence, it seems unlikely that state-dependent retrieval can account for our findings

Whatever the exact mechanisms involved, the results of this experiment suggest that the benzodiazepine antagonist RO 15-1788, at a dose that clearly reverses the sedative effect of chlordiazepoxide, fails to reverse the drug's effect on between-session habituation of exploration.

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