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Dizocilpine Prevents the Development of Tolerance to the Sedative Effects of Diazepam in Rats

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FILE, S. E. AND C. FERNANDES. *Dizocilpine prevents the development of tolerance to the sedative effects of diazepam in rats.* PHARMACOL BIOCHEM BEHAV 47(4) 823–826, 1994.—Acute treatment with diazepam (2 mg/kg) decreased locomotor activity, rearing, and the number of head dips made in a holeboard. After 3 days of diazepam treatment, tolerance developed to the decreases in locomotor activity and the number of head dips, and there was an emergence of an increased time spent head dipping, compared with controls. Two days of concomitant treatment with the noncompetitive NMDA receptor antagonist, dizocilpine (0.25 mg/kg) blocked the development of tolerance and the increased time spent head dipping. In some respects, the effects of dizocilpine resembled those of holeboard experience. Thus, the rats tested daily in the holeboard after diazepam treatment showed significantly less tolerance to the decrease in locomotor activity and did not show enhanced time spent head dipping after 3 days of treatment. Possible similarities between changes induced by experience and those occurring during the development of tolerance are discussed.

Benzodiazepines Tolerance Sensitization NMDA receptor antagonists

KHANNA et al. (9) have reported that two noncompetitive NMDA receptor antagonists [ketamine and dizocilpine (MK-801)] blocked the development of rapid tolerance to ethanol's hypothermic and motor impairing effects, without affecting ethanol metabolism. The experience of ethanol was associated with testing on a tilt-plane and body temperature measurement, and it was suggested that these were learned (or contingent) forms of tolerance and that the block of tolerance by the NMDA receptor antagonists reflected a general role of NMDA receptors in learning. However, Wu et al. (14) found that dizocilpine prevented the development of tolerance to the motor impairing effects of ethanol (as measured on a moving belt) after 14 days of treatment, regardless of whether testing on the moving belt occurred before or after daily ethanol administration. This suggests that NMDA receptors may play a role in the development of noncontingent, as well as contingent, tolerance. Indeed, in both experiments a higher dose of dizocilpine (0.25 mg/kg) was needed to significantly block the development of tolerance to ethanol effects than is needed to impair learning (6). Although dizocilpine had the same motor-impairing effects as ethanol, no tolerance developed to the effects of dizocilpine itself. Wu et al. (14), therefore, argued that the inhibition of tolerance resulted from an action of dizocilpine that was not shared by ethanol or other drugs that do produce tolerance.

Interestingly, whilst dizocilpine blocked crosstolerance between chlordiazepoxide and ethanol, it failed to prevent the development of rapid tolerance to the acute ataxic effects of chlordiazepoxide (8), suggesting that the mechanism underlying tolerance to benzodiazepine effects might be different. Rapid tolerance develops to the ataxic and sedative effects of benzodiazepines [see (4) for review] but, in contrast to the results with ethanol, there is little evidence for contingent tolerance. Indeed, Mackenzie-Taylor and Rech (10) found that chronically administered chlordiazepoxide, whilst leading to a dramatic noncontingent tolerance, actually nullified the development of contingent tolerance.

The purpose of the present experiment was to determine whether dizocilpine (0.25 mg/kg) prevented the rapid development of tolerance to diazepam's sedative effects in the holeboard test, which provides independent measures of locomotor activity and exploratory responses (5). The design followed that of Khanna et al. (9): i.e., diazepam pretreatment was given for 2 days ± dizocilpine; the rats were then tested in

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the holeboard the following day after diazepam, but dizocilpine was not given on the test day. Dizocilpine has a short elimination half-life (12) and motor impairment in the tilt-plane test was not found after 60 min (9). However, to assess any residual impairments in the holeboard, a control group was injected with dizocilpine for 2 days and then tested undrugged the following day. To assess the role of contingent tolerance following this dosing regime, additional vehicle, acute and 3-day diazepam treatment groups were tested that received holeboard experience after each day's injection.

METHOD

Animals

Male hooded Lister rats (Charles River, Margate) were housed in groups of four, in a room maintained at 22°, with lights on from 0700–1900 h. Food and water were freely available.

Apparatus

The holeboard was a wooden box 60 × 60 × 35 cm with four holes, each 6.5 cm in diameter, equally spaced on the floor. Head dipping was measured by the interruption of infrared beams from cells located immediately beneath the edges of the holes; locomotor activity and rearing were measured by the interruption of infrared beams from cells located in the walls of the box, 4.5 and 12.5 cm, respectively, from the floor.

Drugs

Diazepam (Roche Products) was suspended with a drop of Tween-20 in 20 ml of distilled water and left in an ultrasonic water bath for 20 min before injection. Rats were injected IP with vehicle or diazepam (2 mg/kg) in a volume of 2 ml/kg, as shown in Table 1. Dizocilpine hydrogen maleate (Research Biochemicals Inc.) was dissolved in water and injected IP in a volume of 2 ml/kg, 30 min before injection with diazepam or vehicle, as appropriate.

Procedure

Rats were randomly assigned to the eight experimental groups shown in Table 1. The holeboard-experienced groups received daily 5 min trials in the holeboard 1 h after a vehicle injection and 30 min after injection with diazepam or vehicle, as appropriate. All the other groups received only injections on days 1 and 2 and were tested in the holeboard for the first time on day 3, 30 min after diazepam or vehicle injection, as appropriate.

TABLE 1

TREATMENTS RECEIVED IN THE EIGHT EXPERIMENTAL GROUPS

n	Days 1 and 2	Day 3
14	Veh–INJ	Veh–HBD
8	MK + Veh–INJ	Veh–HBD
12	Veh–INJ	DZ–HBD
14	DZ–INJ	DZ–HBD
14	MK + DZ–INJ	DZ–HBD
8	Veh–HBD	Veh–HBD
8	Veh–HBD	DZ–HBD
8	DZ–HBD	DZ–HBD

n = number of rats in each group; INJ = injection only; HBD injection plus holeboard test. Veh = vehicle injections; DZ = diazepam (2 mg/kg); MK = dizocilpine [(+)-MK-801; 0.25 mg/kg].

Statistics

The effects of dizocilpine on the development of tolerance were assessed with two-way analyses of variance, with 3-day diazepam treatment as one factor and dizocilpine treatment as the second factor. The effects of holeboard experience were also assessed with two-way analyses of variance, with holeboard experience (1 or 3 days) as one factor and diazepam treatment (0, 1, or 3 days) as the second. Comparisons between individual groups were made using least significant difference tests.

RESULTS

Holeboard Naive Rats

In holeboard naive rats, acute administration of diazepam (2 mg/kg) significantly reduced locomotor activity, the number of rears, the number of head dips, but not the time spent head dipping (see Figs. 1 and 2). The only significant effect of the dizocilpine pretreatment was a reduction in motor activity.

Although the previous dizocilpine (0.25 mg/kg) treatment produced a decrease in motor activity, its ability to block the rapid development of tolerance to diazepam's effects on motor activity was shown by the significant diazepam × dizocilpine interaction, $F(1, 46) = 7.2$, $p < 0.01$. The 3-day diazepam treatment group differed significantly from both the acute diazepam group and the diazepam + dizocilpine group (see Fig. 1). The group treated for 3 days with diazepam made more rears than the acute treatment group, but on posthoc tests this failed to reach significance (see Fig. 1). There was, however, significant tolerance to the reduction in the number of head dips made after 3 days of diazepam treatment, and this group differed significantly from both the acute diazepam and the diazepam + dizocilpine groups (see Fig. 2).

The time spent head dipping was not reduced by acute diazepam, but after 3 days of treatment there was a significant increase in this measure, which was blocked by the dizocilpine treatment (see Fig. 2).

Effects of Holeboard Experience

Previous holeboard experience significantly modified the sedative effects of diazepam, as reflected in decreased locomotor activity [holeboard experience × diazepam interaction, $F(2, 58) = 7.2$, $p < 0.005$] and it can be seen from Fig. 1 that acute diazepam was more sedative in the holeboard experienced rats, and that there was no significant development of tolerance in the 3-day treatment group. Previous holeboard experience may have also retarded the development of tolerance to diazepam's effects on the number of head dips. In the holeboard-experienced rats, those treated for 3 days with diazepam were not significantly different from the acute treatment group, but nor were they significantly different from the control group (see Fig. 2). Holeboard experience also prevented the emergence of increased time spent head dipping in the group treated with diazepam for 3 days (see Fig. 2).

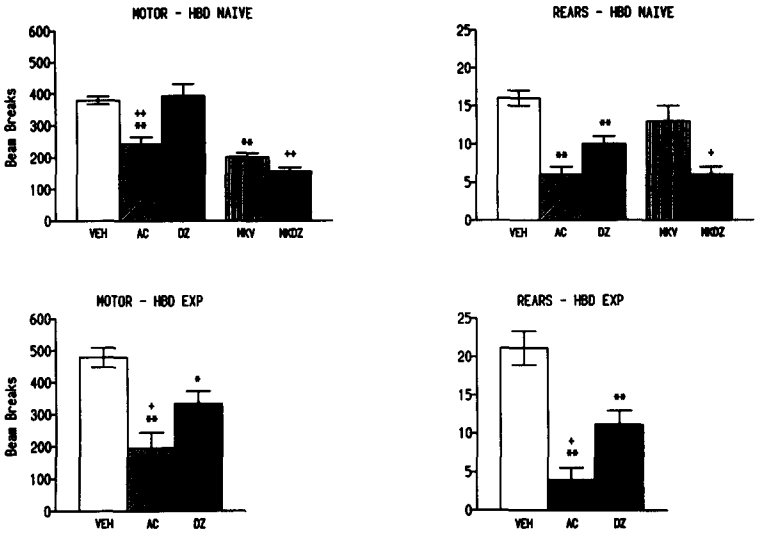

FIG. 1. Mean (±SEM) locomotor activity (beam breaks) and number of rears made by rats naive to (HBD NAIVE—top panels) or experienced with (HBD EXP—lower panels) the holeboard. Rats were tested after injection with vehicle (VEH), acute diazepam (AC) or 3 days of diazepam (DZ); tested after vehicle injection, but with two previous injections of dizocilpine (MKV); tested after diazepam, but with two previous injections of dizocilpine + diazepam (MKDZ). $*p < 0.05$, $**p < 0.01$ compared with VEH group $+p < 0.05$, $++p < 0.01$ compared with DZ group.

There was a significant interaction between holeboard ex pperience and diazepam in the number of rears made, $F(2, 58) = 3.4$, $p < 0.05$, because of a significantly greater development of tolerance in the holeboard-experienced group, and because of a greater acute sedative effect in this group (as reflected in the differences from their respective control groups) (see Fig. 1). Thus, holeboard experience *enhanced* the development of tolerance to the reduction in rearing, whereas dizocilpine did not do this.

DISCUSSION

The only effect of the 2 days of dizocilpine treatment in the control group was a reduction of motor activity. The rea-

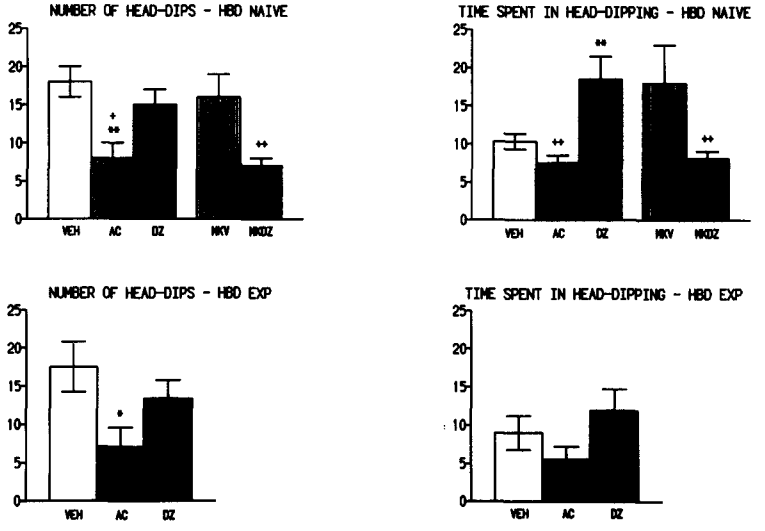

FIG. 2. Mean (±SEM) number of head dips and time spent head dipping for rats naive to (HBD NAIVE—top panels) or experienced with (HBD EXP—lower panels) the holeboard. Treatments were as described for Fig. 1. $*p < 0.05$, $**p < 0.01$ compared with VEH group $+p < 0.05$, $++p < 0.01$ compared with DZ group.

son for this is not known, and is most unlikely to be due to any residual drug presence, because dizocilpine has such a short half-life (9). This effect of dizocilpine complicates the interpretation of its effects on the development of tolerance. However, administration of dizocilpine during the 2 days of pretreatment with diazepam clearly blocked the development of tolerance to the decrease in the number of head dips, and probably also to the decrease in locomotor activity. The reasons for the difference between our results with diazepam in the holeboard test and those of Khanna et al. (8) with chlordiazepoxide in the tilt-plane test, where dizocilpine did not block the development of tolerance, are not known. However, the ability of dizocilpine to block the development of tolerance does seem to be a general phenomenon, and it has now been found for several drugs and behavioral responses. For example, it blocks the development of tolerance to the effects of cocaine (10 mg/kg) on locomotor activity (2), to the analgesic effects of morphine (11,13) and to the motor-impairing effects of ethanol (9,14). There have also been other reports of a block of the development of tolerance under experimental conditions in which there would be little, or no, contingent tolerance (8,1).

In addition, dizocilpine blocked the emergence of increased time spent head dipping after 3 days of diazepam treatment. There have also been other reports of dizocilpine blocking sensitisation or "reverse tolerance" to drug effects. Thus, it blocks the development of sensitization to the effects of cocaine (40 mg/kg) on locomotor activity (2), to the effects of amphetamine and cocaine on locomotor activity stereotypy and convulsions (7).

The effects on head dipping of previous holeboard experience were similar to those of dizocilpine. Thus, in holeboard-experienced rats there was no significant tolerance to the reduction in the number of head dips and no emergence of increased time head dipping after 3 days of diazepam treatment. The effects of holeboard experience on tolerance to the decreases in motor activity are less easy to interpret. There was a reduced development of tolerance to the reduction of locomotor activity in the holeboard-experienced rats, but this may have been partly due to the enhanced acute sedative effect. Holeboard experience also enhanced the acute effect of diazepam on rearing, but in this case, there seemed to be an enhancement of the development of tolerance. Only in this latter case was there any evidence for the development of contingent tolerance. The lack of development of contingent tolerance to the effects on locomotor activity and head dipping is in agreement with previous results with chlordiazepoxide (3). The actual retardation of the development of tolerance as a result of holeboard experience agrees with the results of Mackenzie-Taylor and Rech (10), and suggests that the retardation of the development of contingent tolerance may be a general property of benzodiazepines.

In conclusion, dizocilpine was able to block the changes that occurred after 3 days of treatment in diazepam's effects on responses in the holeboard. These effects were found for the measures reflecting motor activity and for those reflecting exploration, and were found for a response that increased with chronic treatment as well as for those to which tolerance developed. At least for the changes in time spent head dipping and locomotor activity, the effects of dizocilpine treatment were similar to those of holeboard experience with diazepam treatment. It is, perhaps, surprising that experience had effects in the same direction as those of a drug that blocks plasticity changes. One explanation is that the changes that had already occurred as a result of holeboard experience precluded those that normally occur during the development of tolerance (as would be the case if the two events resulted in the same plasticity changes). This hypothesis could be tested by examining the effects of dizocilpine on the development of tolerance in holeboard-experienced rats. Further experiments are also needed to examine why different results were obtained for the effects of holeboard experience on the development of tolerance to diazepam's effects on rearing.

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