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One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze

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Abstract. Chlordiazepoxide (CDP 7.5 mg/kg) had a significant anxiolytic effect in rats tested on the plus-maze for the first time. On a second trial the control scores did not change, but those of the CDP group did and they no longer differed from controls. Rats previously tested undrugged or after flumazenil (4 mg/kg) also failed to show an anxiolytic response to CDP. Thus this phenomenon of one-trial tolerance depended on prior experience with the plus-maze. It also depended on CDP acting at the benzodiazepine receptors on trial 2, since the joint administration of CDP and flumazenil on trial 2 reversed the phenomenon.

Key words: Learned tolerance – Anxiety – Benzodiazepines

Tolerance develops at different rates to the different behavioural effects of the benzodiazepines and develops more slowly to the anxiolytic than to the sedative or anticonvulsant effects (File 1985). In general, 3 weeks of daily injections are needed to demonstrate tolerance in animal tests of anxiety (File and Baldwin 1989), including the elevated plus-maze (File et al. 1987). However, there is evidence that a single (undrugged) experience of the plus-maze can significantly reduce the anxiolytic effects of chlordiazepoxide in the mouse, even though the scores of control animals remained unchanged by prior experience (Lister 1987).

This experiment was designed to investigate the conditions under which the anxiolytic effects of chlordiazepoxide in the plus-maze might be modified in the rat.

Methods

Male hooded Lister rats (Olac, Bicester) were housed in groups of five in a room with lights on from 0700 to 1800 hours; food and water were freely available.

Chlordiazepoxide hydrochloride (CDP 7.5 mg/kg, Roche Products Ltd) was dissolved in distilled water and flumazenil (FLU 4 mg/kg, Hoffmann-La Roche) was suspended in water with a drop of Tween, control (CON) rats received water injections. All injections were IP in a volume of 2 ml/kg, 30 min before testing in the plus-maze, where appropriate.

The plus-maze was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with walls 40 cm high; it was elevated 50 cm above the ground. Each rat was placed in the central square (10 × 10 cm) and observed on a video camera for the number of entries into each type of arm (all four paws defining an entry) and the time spent in open and closed arms. Each trial lasted 5 min and the maze was cleaned after each trial. The rats were tested in an order randomised for drug treatment between 1330 and 1630 hours. When rats were tested twice in the plus-maze the inter-trial interval was 24 h. When the initial treatment was an injection alone, the interval between first and second treatments was 24 h. The rats were randomly allocated to the experimental groups shown in Table 1.

Results

The total number of arm entries was not changed by this dose of CDP (CON = 12.0 ± 0.9, CDP = 10.7 ± 1.4).

Figure 1a illustrates the phenomenon of one-trial tolerance to the anxiolytic effects of CDP in the plus-maze. The scores of the control animals did not change from trial 1 to 2, whereas CDP had an anxiolytic effect (increase in % number of entries onto open arms and increase in % time spent on open arms) on trial 1, but by trial 2 the scores were no different from controls [Split-plot ANOVA for groups *A* and *B*, drug × trial interaction $F(1,16) = 6.4$ and 4.9 for % number and % time, respectively, $P < 0.05$). Thus, one previous experience of the plus-maze with CDP was sufficient to produce tolerance to its anxiolytic effects.

Figure 1b shows that this tolerance cannot be attributed

Table 1. Treatments received by the various experimental groups. Animals received injections of water (CON), chlordiazepoxide (CDP, 7.5 mg/kg) or flumazenil (FLU 4 mg/kg). On the first treatment some groups (Inject) received an injection only (no test); the others were injected and tested in the plus-maze (Plus-maze)

Group	n/grp	1st treatment	2nd treatment
Experiment 1			
A	9	CON Plus-maze	CON Plus-maze
B	9	CDP Plus-maze	CDP Plus-maze
C	8	CDP Inject	CDP Plus-maze
D	9	CON Plus-maze	CDP Plus-maze
E	8	CON Inject	CDP Plus-maze
F	9	FLU Plus-maze	CDP Plus-maze
G	8	FLU Inject	CDP Plus-maze
Experiment 2			
H	7	CDP Plus-maze	CDP Plus-maze
I	7	CDP Plus-maze	CDP + FLU Plus-maze
J	9	CDP Plus-maze	CON Plus-maze
K	9	CON Plus-maze	FLU Plus-maze

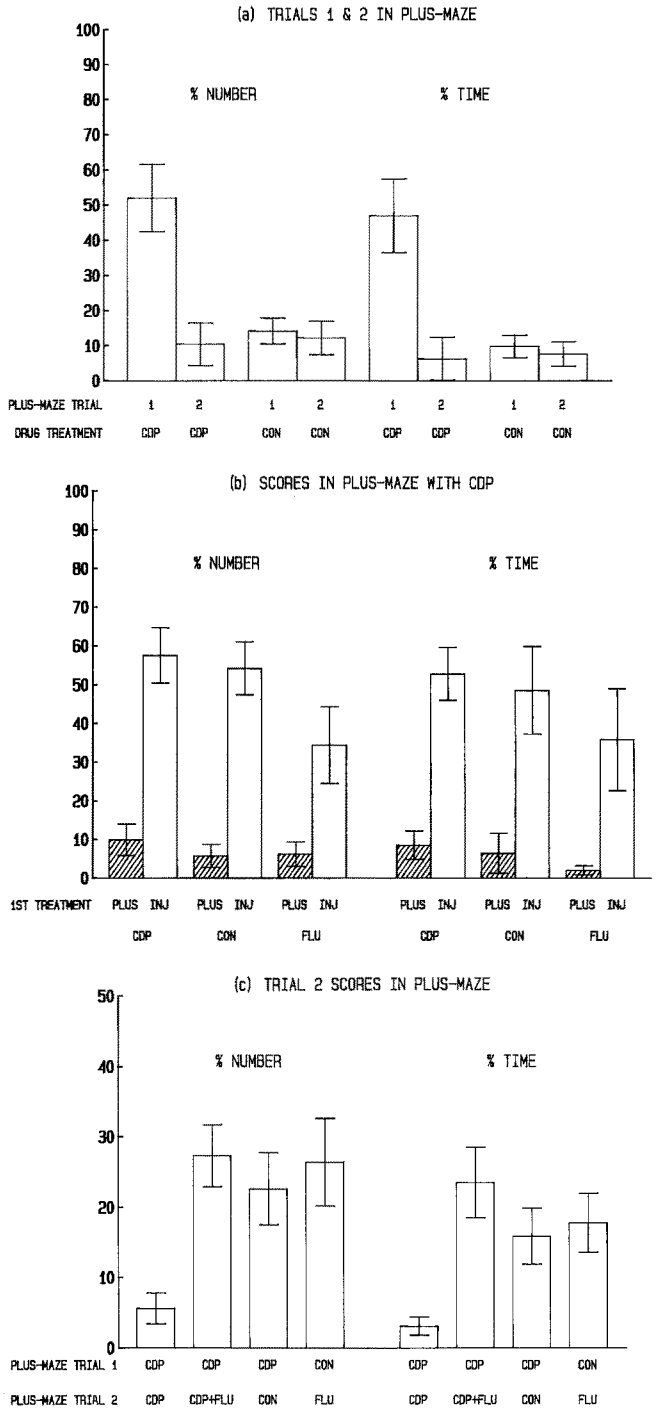

Fig. 1a–c. Mean (±SEM) scores in the plus-maze for control rats (*CON*) and for those tested with chlordiazepoxide (7.5 mg/kg *CDP*) or flumazenil (4 mg/kg *FLU*). **a** Scores on trials 1 and 2 in the plus-maze for rats injected with water (*CON*) on both trials (group *A*) and for rats injected with CDP on both trials (group *B*). **b** Scores for rats tested in plus-maze with CDP, after a previous plus-maze trial (*PLUS*) or after only a previous injection (*INJ*), with chlordiazepoxide (*CDP*), water (*CON*) or flumazenil (*FLU*). **c** Trial 2 scores in the plus-maze, for rats from groups *H, I, J* and *K*

to prior experience of CDP; thus the rats that had a previous plus-maze experience with CDP (group *B*) had significantly lower scores than those with just a prior injection of CDP, but no test (group *C*) [$F(1,15)=36.2$ for % number and 34.6 for % time, $P<0.0001$]. The phenomenon depends on a previous plus-maze experience, but it can also be seen from Fig. 1b that the drug state of the rats on trial 1 is unimportant to the phenomenon. Thus, rats with a previous undrugged trial in the plus-maze (group *D*) had significantly lower scores when they were subsequently tested with CDP, than did the group that had received only a prior control injection (group *E*) [$F(1,15)=46.4$, $P<0.001$ for % number and $F(1,15)=12.5$, $P<0.005$ for % time]. Similarly, rats with a previous plus-maze trial with flumazenil (group *F*) had significantly lower scores than those with only a previous flumazenil injection (group *G*) [$F(1,15)=8.0$, $P<0.1$ for % number and $F(1,15)=7.4$, $P<0.02$ for % time].

While the drug state of the rats on trial 1 was not important for the development of "one trial tolerance", the phenomenon did depend on chlordiazepoxide acting at the benzodiazepine receptor on trial 2. Thus, it can be seen from Fig. 1c that the rats tested on trial 2 with CDP *plus* flumazenil (group *I*) had significantly higher scores than those tested with CDP alone (group *H*) [$F(1,12)=18.9$, $P<0.001$ for % number and $F(1,12)=15.5$, $P<0.002$ for % time]. The scores of group *I* (tested on trial 2 with CDP plus flumazenil) were close to those of group *J* (tested after a control injection on trial 2), indicating that the flumazenil treatment had completely antagonised the chlordiazepoxide, and thereby reversed the phenomenon of "one trial tolerance". There was no evidence that flumazenil was having an anxiolytic effect once tolerance had developed to the effects of CDP, since group *I* did not differ significantly from either group *J* or group *K*, see Fig. 1c.

Discussion

The phenomenon of "one trial tolerance" to the effects of CDP in the plus-maze was clearly dependent on prior experience of the plus-maze, regardless of the drug state in which it was experienced on trial 1. It cannot be explained simply by assuming that the plus-maze had become less anxiogenic with repeated testing, since this should have been reflected in a change in the control scores over trials. The phenomenon depends on an interaction between the experience on trial 1 and an action at the benzodiazepine receptors on trial 2. Behaviourally, one 5 min exposure to the plus-maze produces the same apparent tolerance as 21 daily CDP injections; whether the same neurochemical mechanism underlies the two phenomena remains to be explored.

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