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BRIEF COMMUNICATION

Evidence That the Increased Anxiety Detected in the Elevated Plus-Maze During Chlordiazepoxide Withdrawal Is Not Due to Enhanced Noradrenergic Activity

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BALDWIN, H. A., P. K. HITCHCOTT AND S. E. FILE. *Evidence that the increased anxiety detected in the elevated plus-maze during chlordiazepoxide withdrawal is not due to enhanced noradrenergic activity.* PHARMACOL BIOCHEM BEHAV **34**(4) 931–933, 1989.—Rats displayed a reduction in the percentage of time spent on the open arms of the elevated plus-maze 24–30 hours after withdrawal from chronic chlordiazepoxide treatment (10 mg/kg/day IP for 4 weeks). This indicated an anxiogenic response in this test. This anxiogenic response was not significantly reversed by DL-propranolol (5 and 10 mg/kg IP) or clonidine (0.02 and 0.04 mg/kg IP). These results provide no evidence to suggest that the anxiogenic effects of chlordiazepoxide withdrawal are mediated by an increase in noradrenergic activity. The possible involvement of multiple transmitter systems in benzodiazepine withdrawal symptomology is discussed.

Anxiety Elevated plus-maze Chlordiazepoxide Withdrawal Noradrenergic activity

IT is now generally acknowledged that the benzodiazepines (BDZs) can induce dependence in man and that the cardinal symptom of BDZ withdrawal is increased anxiety. Using 2 animal models of anxiety, the elevated plus-maze test and the social interaction test, we have recently demonstrated that rats display anxiogenic-like activity after withdrawal from chronic chlordiazepoxide (CDP) treatment (3,6).

There is evidence that the turnover rate of brain noradrenaline is increased during withdrawal from chronic BDZ treatment (7,12). Furthermore, the hyperactivity and diarrhoea seen during diazepam withdrawal in rats was significantly antagonized by administration of various α_2-adrenoceptor agonists (8). These results suggest that these BDZ withdrawal responses are due to increased noradrenergic activity. There is evidence that drugs that increase noradrenergic activity, such as yohimbine and caffeine, increase anxiety in man and display anxiogenic activity in animal tests of anxiety (4). The purpose of the present experiments was, therefore, to investigate whether the increased activity during BDZ withdrawal was due to an increase in noradrenergic activity. Thus, the effects of a β-adrenoceptor antagonist, DL-propranolol, and an α_2-adrenoceptor agonist, clonidine, were examined during CDP withdrawal in the elevated plus-maze test of anxiety (11). Doses of clonidine were selected on the basis of studies reporting reversals of the anxiogenic effect of yohimbine (10) and the hyperactivity and diarrhoea seen during diazepam withdrawal (8). The doses of propranolol were selected on the basis of a study reporting a reversal of the anxiogenic effect of caffeine (5).

METHOD

Animals

Male hooded Lister rats (Olac Ltd., Bicester, U.K.), weighing 200–250 g at the start of the experiment, were used. The rats were housed in groups of 5–6, in a room with a 13-hr light:11-hr dark cycle (lights on at 06.00), and allowed free access to food and water.

Drug Treatment

Rats were randomly allocated to both the chronic and acute

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drug treatments. Rats received once daily intraperitoneal (IP; 2 ml/kg) injections of CDP hydrochloride in distilled water (Sigma; 10 mg/kg/day), or vehicle (distilled water alone), for 4 weeks. They were then tested in the elevated plus-maze test 24–30 hr after their last dose. In one experiment, rats received an IP (2 ml/kg) injection of DL-propranolol (Sigma; 5 or 10 mg/kg) or vehicle (distilled water) 30 min before testing. In a second experiment, rats received an IP (2 ml/kg) injection of clonidine (Sigma; 0.02 or 0.04 mg/kg) or vehicle (distilled water) 30 min before testing.

Procedure

The elevated plus-maze. The plus-maze is described in more detail in (11). The maze consisted of 2 opposite open arms and 2 enclosed arms and was elevated 50 cm from the floor. Rats were placed individually in the centre of the maze and the following measures were scored, for 5 min, by an observer who was blind to drug treatment: number of entries onto 1) open and 2) enclosed arms; the time spent on 1) open and 2) enclosed arms. The values obtained were converted into percentages of open/open + enclosed. The total number of arm entries provided a measure of general activity.

In this test, anxiolytics increase, and anxiogenics decrease, the % of time spent on, the open arms, independently of any change in general activity (11).

Statistics

The % of time spent on the open arms and the total number of arm entries were analyzed using Analysis of Variance (ANOVA). When a reduction occurred in the total number of arm entries, Analysis of Covariance (ANCOVA) was performed to see whether the changes in the % of time spent on the open arms was independent of the reduction in general activity. Post hoc comparisons were made using Duncan's Multiple Range tests.

RESULTS

Effect of DL-Propranolol on CDP Withdrawal

CDP withdrawal had no significant effect on total number of arm entries (see Table 1) on the plus-maze. However, DL-propranolol (10 mg/kg) produced a significant reduction in the total number of arm entries [ANOVA, $F(1,20)=6.9$, $p<0.05$], indicating a reduction in general activity on the plus-maze (see Table 1).

CDP withdrawal significantly reduced the % of time spent on the open arms [ANOVA, $F(1,55)=4.7$, $p<0.05$] (see Fig. 1). This is indicative of an anxiogenic response during CDP withdrawal (11). DL-propranolol (5 and 10 mg/kg) also significantly reduced the % of time on the open arms [ANOVA, $F(2,55)=6.9$, $p<0.01$] (see Fig. 1), but this reduction was not independent of the decrease in total number of arm entries and therefore this effect cannot be interpreted as an anxiogenic response. There was no significant withdrawal × DL-propranolol interaction for % of time spent on the open arms. This indicates that propranolol failed to affect the anxiogenic CDP withdrawal response.

Effect of Clonidine on CDP Withdrawal

CDP withdrawal had no significant effect on total number of arm entries, whilst clonidine (0.02 and 0.04 mg/kg) significantly reduced this measure [ANOVA, $F(2,69)=11.8$, $p<0.001$] (see Table 1). Thus, clonidine produced a reduction in general activity on the plus-maze.

As in the DL-propranolol experiment, CDP withdrawal again significantly reduced the % of time spent on the open arms compared with controls (Duncan's tests after ANOVA: $p<0.05$) (see Fig. 1). Clonidine (0.04 mg/kg) also significantly reduced the % of time spent on the open arms [ANOVA, $F(2,69)=6.7$, $p<0.01$], followed by Duncan's tests: $p<0.01$ (see Fig. 1), but this reduction was not independent of the decrease in total number of arm entries. There was no significant withdrawal × clonidine interaction for % of time spent on the open arms showing that clonidine did not significantly reverse the withdrawal response. However, the % of time spent on the open arms by rats given 0.02 mg/kg of clonidine during CDP withdrawal was no different from the control or withdrawal group (see Fig. 1).

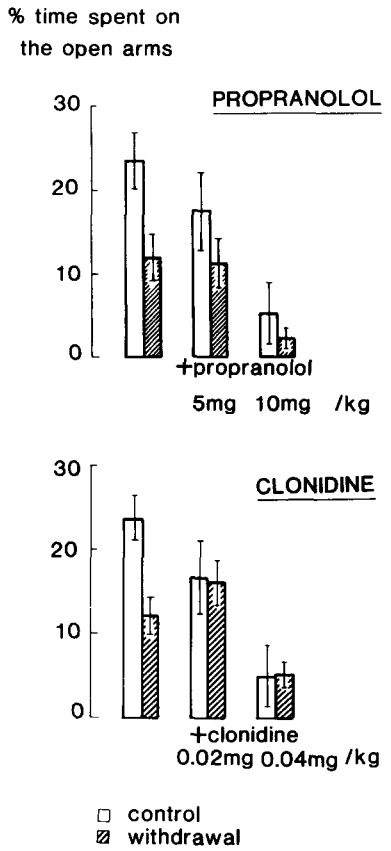

FIG. 1. Values are mean (±S.E.M.) % of time spent on the open arms of the elevated plus-maze. Top: the effect of DL-propranolol (5 and 10 mg/kg) during CDP withdrawal. Bottom: the effect of clonidine (0.02 and 0.04 mg/kg) during CDP withdrawal. See text for statistical details.

DISCUSSION

In agreement with previous studies (2,6), CDP withdrawal reduced the % of time spent on the open arms of the elevated plus-maze compared with controls, indicating an increase in anxiety. DL-propranolol and clonidine also reduced the % of time spent on the open arms, but this was not independent of reductions in general activity. Thus, the effects of DL-propranolol and clonidine cannot be interpreted as indicating increased anxiety. Neither DL-propranolol nor clonidine significantly reversed the anxiogenic response during CDP withdrawal. In fact, the effects of CDP withdrawal and DL-propranolol on the % of time spent on the open arms were additive (see Fig. 1). Although the effects of clonidine and CDP withdrawal were not additive (see Fig. 1), there was no significant clonidine × withdrawal interaction indicating that clonidine did not significantly reverse the withdrawal response.

TABLE 1

EFFECT OF DL-PROPRANOLOL (5 AND 10 mg/kg) AND CLONIDINE (0.02 AND 0.04 mg/kg) ON ARM ENTRIES OF RATS TESTED ON THE ELEVATED PLUS-MAZE DURING CDP WITHDRAWAL

Drug Treatment	Total No. of Arm Entries	n
Experiment 1		
control	13.1 ± 1.2	16
propranolol (5 mg/kg)	10.8 ± 1.2	5
propranolol (10 mg/kg)	7.0 ± 1.9	6
withdrawal	10.9 ± 1.0	19
withdrawal + propranolol (5 mg/kg)	10.7 ± 1.6	7
withdrawal + propranolol (10 mg/kg)	10.6 ± 1.8	8
Experiment 2		
control	14.5 ± 0.9	21
clonidine (0.02 mg/kg)	9.9 ± 1.3	14
clonidine (0.4 mg/kg)	7.3 ± 1.8	6
withdrawal	11.6 ± 0.9	14
withdrawal + clonidine (0.02 mg/kg)	12.9 ± 1.6	15
withdrawal + clonidine (0.04 mg/kg)	5.2 ± 1.2	5

Values are mean (±S.E.M.) total number of arm entries during the 5 min trial. n = number of rats per group.

The inability of clonidine and propranolol to significantly reverse the anxiogenic effect of CDP withdrawal suggests that in this test and at these doses, there is no evidence of noradrenergic involvement in the anxiogenic BDZ withdrawal response. This is in contrast to the hyperactivity and diarrhoea during diazepam withdrawal that were prevented by administration of α_2-adrenoceptor agonists such as clonidine (8). In our study, we failed to observe changes in the total number of arm entries during CDP withdrawal, but the plus-maze is not the ideal test for measuring changes in motor activity. However, both propranolol (10 mg/kg) and clonidine (0.02 and 0.04 mg/kg) reduced total arm entries in control-treated animals, but not in CDP withdrawn animals and, thus, the loss of the sedative effects of propranolol and clonidine during CDP withdrawal may indicate that hyperactivity was present.

Our results are in agreement with clinical studies showing that whilst DL-propranolol was effective against the cardiovascular symptoms of BDZ withdrawal, it was ineffective against the anxiety and dysphoria (1). Thus, the autonomic symptoms, but not the increased anxiety, during BDZ withdrawal may be mediated by increased noradrenergic activity. If positive results are obtained in other tests then we will evidence that different withdrawal symptoms are controlled by different neurochemical mechanisms.

We recently demonstrated that the BDZ-antagonist, flumazenil (Ro 15-1788), completely reverses the anxiogenic effect of CDP withdrawal in 2 animal tests of anxiety (2,3). However, a problem with flumazenil is that it can precipitate autonomic withdrawal symptoms such as diarrhoea and vomiting (9). Thus, an ideal way of protecting patients from BDZ withdrawal symptoms on stopping treatment may be the administration of flumazenil for the anxiety and propranolol or clonidine for the autonomic symptoms.

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