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Ethological Comparison of the Effects of Diazepam and Acute/Chronic Imipramine on the Behaviour of Mice in the Elevated Plus-Maze

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COLE, J. C. AND R. J. RODGERS. *Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behaviour of mice in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **52**(3) 473–478, 1995. – Recent clinical evidence suggests that the tricyclic antidepressant imipramine is effective against not only panic disorder but also generalized anxiety disorder. Although most animal models of anxiety appear to be insensitive to this agent, such work has almost invariably employed an acute treatment regimen. In the present study, ethological methods have been used to assess in detail the effects of acute and chronic imipramine treatment on the behaviour of male DBA/2 mice in the elevated plus-maze test. In contrast to acutely administered diazepam (1 mg/kg), which produced a significant anxiolytic profile on standard and ethological measures, neither acute nor chronic (daily, 15 days) treatment with imipramine (0–20 mg/kg) was associated with anxiety reduction. Data are discussed in relation to test sensitivity factors and the nonspecific mechanism of action of imipramine.

Anxiety Elevated plus-maze Diazepam Antidepressant Imipramine Mice

SINCE the turn of the century, psychiatrists have debated whether anxiety and depression constitute different aspects of the same disorder or distinct, yet overlapping, conditions (23). Contemporary discussions on this issue emphasize not only the high degree of symptom overlap and comorbidity of these disorders (31), but also the absence of a clear therapeutic demarcation (30,50). For example, tricyclic antidepressants and monoamine oxidase inhibitors have been used clinically for many years to treat panic disorder (27,31,32,39,48), with similar results now being reported for serotonin-selective antidepressants (1,30,35,51). Furthermore, although tricyclics were initially considered not to be effective in generalized anxiety disorder (27,39), more recent research has indicated that chronic imipramine treatment may be at least as effective as benzodiazepines in such patients (23,25,38,50).

Against this background, it is pertinent to note that antidepressant drugs in general and imipramine in particular have been found not to exert positive effects in rodent models of anxiety. For example, negative results have been reported in traditional conflict/conditioned suppression procedures (18, 19,26,29) as well as light/dark exploration (10,11,33,52), potentiated startle (7), separation- and shock-induced ultrasonic calling (13,21), and elevated plus-maze (5,14,28,36) paradigms. However, it must be emphasized that these animal studies involved acute drug treatment, whereas agents such as imipramine are normally only effective in treating generalized anxiety disorder when given chronically. Indeed, clinical experience with acute effects of these agents in anxious patients (1,31,51) is supported by the anxiogenic-like reactions seen in some animal studies (4,22,34,37).

Although negative effects in animal models of anxiety have also been reported following chronic administration of antidepressants (7,16,37), results have generally been much more positive using this clinically relevant approach. For example, anxiolytic-like profiles have been seen with chronic imipramine, amitriptyline and phenelzine in a conditioned suppres-

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sion of drinking task (18,19), with chronic imipramine, phenelzine, and mianserin in the mouse social interaction test (20), with chronic imipramine in novelty-suppressed feeding (3) and antipredator defense (2) tests, and with chronic tianeptine in the rat social interaction test (17). In the elevated plus-maze, positive results have been obtained following chronic paroxetine treatment (6), and similar trends have been seen with chronic phenelzine (24). To our knowledge, only one study has reported the effects of chronic imipramine in the elevated plus-maze test. Although no significant effects were obtained, the authors commented on a tendency toward an anxiolytic effect in rats at one of the doses tested (16).

The present study was undertaken to assess more fully the effects of imipramine on anxiety in the murine elevated plus-maze test. For reasons given earlier, the effects of acute and chronic imipramine treatment were contrasted, and acute diazepam treatment was included as a positive control. Ethological methods were used to provide comprehensive behavioural profiles, an approach that has recently proved very sensitive to both increases (40,44,45) and decreases (8,9,41,42) in anxiety.

METHODS

Subjects

Subjects were 12–15-week-old adult male DBA/2 mice (Biomedical Services, Leeds University, Leeds, UK), group housed (n = 10) for at least 4 weeks before testing (cage size: 45 × 28 × 13 cm). They were maintained under a reversed light cycle (lights off at 0700 h) in a temperature-controlled environment (21 ± 1°C). Food and water were freely available. All mice were experimentally naive.

Drugs

Imipramine hydrochloride (Sigma, Poole, UK) was dissolved in physiologic saline, which, alone, served for control injections. Diazepam (Roche Products, London, UK) was ultrasonically dispersed in saline to which Tween 80 (two drops/10 ml) had been added; a corresponding saline–Tween 80 mixture was used for control injections. Compounds were administered intraperitoneally (IP) in a volume of 10 ml/kg.

Apparatus

The elevated plus-maze was a modification of the one validated for NIH swiss mice by Lister (28) and was composed of two open (30 × 5 cm) and two enclosed (30 × 5 × 15 cm) arms that radiated from a central platform (5 × 5 cm) to form a plus sign. The entire apparatus was elevated to a height of 45 cm above floor level by a single central support. The maze floor was constructed of black Plexiglas; the side and end walls of the enclosed arms were made of clear Plexiglas. As previously reported [e.g., (9)], a slight raised edge on the open arms (0.25 cm) provided additional grip for the animals, whereas open arm activity was further encouraged by testing under dim red light (3 × 60 W).

Procedure

All testing was conducted during the dark phase of the light cycle and, to facilitate habituation, animals were transported to the laboratory and left undisturbed for at least 1 h before testing. In each study, mice were randomly allocated to treatment conditions and tested in a counterbalanced order. Testing commenced by placing a mouse on the central platform of the maze facing an open arm. A 5-min test duration was employed, and between subjects, the maze was thoroughly cleaned using damp and dry cloths. All sessions were recorded by a vertically mounted videocamera linked to a monitor and VCR in an adjacent laboratory.

Three experiments were conducted. In Experiment 1 (acute diazepam), mice were assigned to two treatment conditions (n = 17–20) and tested 30 min after a single injection of vehicle or 1.0 mg/kg diazepam. In Experiment 2 (acute imipramine), mice were randomly allocated to one of four treatment conditions (n = 10) and tested 30 min after a single injection of saline, 5.0, 10.0, or 20.0 mg/kg imipramine. In Experiment 3 (chronic imipramine), mice were randomly assigned to four treatment conditions (n = 10) in which they received daily injections of saline, 5.0, 10.0, or 20.0 mg/kg imipramine for 15 days. The side of the injection was alternated on a daily basis to reduce peritoneal irritation, and testing was conducted 30 min after the final injection.

Behaviour scored off videotape, by an observer blind to treatment condition, comprised both traditional and novel parameters (8,9,40–42). Traditional spatiotemporal measures were the number of open and closed arm entries (arm entry = all four paws into a maze arm), and time spent in the various sections of the maze (including the central platform). Derived measures were the number of total arm entries, percent open arm entries (open/total × 100), and time spent in the different sections of the maze expressed as a percentage of the test duration (percent open time, percent closed time, percent centre time). Ethologically derived measures were entry latency (time taken at the start of the session to move from the centre platform into an arm), nonexploratory behaviour (combined duration of nonexploratory elements, immobility and grooming), rearing, stretched attend postures (SAP; an exploratory posture in which the mouse stretches forward and retracts to its original position without locomoting forward), closed arm returns (exiting a closed arm with only two paws, and returning or doubling back into the same arm), and head-dipping (exploratory movement of the head or shoulders over the sides of the open arms). In view of the importance of thigmotactic cues in the maze (49), SAP and head-dips were further differentiated as protected (i.e., occurring in or from the relative security of the closed arms or central platform) or unprotected (occurring on or from the open arms). For these measures, data are expressed as totals and percent protected (protected/total × 100; percent pSAP and pHD) values.

Statistics

Data were analyzed by single-factor (treatment) or two-factor (treatment; location; repeated measures on second factor) analyses of variance (ANOVA). Where indicated, either by significant main effects or interactions, or F values approaching statistical significance, further comparisons were made using the appropriate error variance estimates from the ANOVA summary tables.

RESULTS

Experiment 1: Acute Diazepam

Data and corresponding ANOVA statistics are summarized in Table 1. Diazepam, 1 mg/kg, significantly increased total and open arm entries without altering closed arm entries. Percent open arm entries, percent time spent on the open arms, and total head-dipping were also significantly increased. Further analysis of the percent time data revealed a highly significant preference for different sections of the maze [$F(2, 70)$ =

TABLE 1

EFFECTS OF ACUTE DIAZEPAM TREATMENT (0-1.0 mg/kg) ON PLUS-MAZE BEHAVIOUR IN MALE DBA/2 MICE

Behaviour	Vehicle	1.0 mg/kg diazepam	$F(1, 35)$
Total arm entries	13.2 ± 0.9	16.9 ± 1.6	4.75, $p < 0.05$
Open arm entries	3.4 ± 0.4	6.4 ± 0.9	11.39, $p < 0.01$
Closed arm entries	9.8 ± 0.7	10.5 ± 1.1	0.28, NS
% Open arm entries	25.2 ± 2.7	37.3 ± 3.7	7.25, $p < 0.01$
% Open arm time	12.0 ± 2.9	23.8 ± 4.0	5.92, $p < 0.025$
% Centre time	32.9 ± 2.0	27.3 ± 2.3	3.42, NS
% Closed arm time	55.1 ± 3.3	48.9 ± 4.1	1.40, NS
Total rears	7.5 ± 1.0	5.1 ± 0.6	3.91, NS
Total head-dips	2.0 ± 0.4	7.5 ± 1.0	31.70, $p < 0.01$
% pDips	49.2 ± 9.8	45.2 ± 5.7	0.11, NS
Total SAP	16.5 ± 1.0	11.1 ± 1.0	14.88, $p < 0.01$
% pSAP	74.6 ± 3.9	57.6 ± 6.6	5.29, $p < 0.025$
Closed arm returns	1.5 ± 0.4	0.5 ± 0.2	4.53, $p < 0.05$
Entry latency (s)	6.3 ± 1.7	3.8 ± 1.9	1.02, NS
NEB (s)	19.3 ± 3.6	33.0 ± 6.4	3.77, NS

Data presented are mean values ± SEM. SAP, stretched attend posture; NEB, nonexploratory behaviour; %p, percent protected.

42.0, $p < 0.001$], with control mice showing a rank order preference for closed arms > centre platform > open arms. This profile was altered by diazepam [$F(2, 70) = 3.49$, $p < 0.05$] such that closed > centre = open. Although rearing was unaffected by drug treatment, total stretched attend postures and closed arm returns were significantly reduced. Percent protected head-dipping was not affected by diazepam, whereas percent protected SAP was substantially reduced. Neither entry latency nor nonexploratory behaviour was significantly affected by drug treatment.

Experiment 2: Acute Imipramine

Table 2 summarizes the data and ANOVA statistics. On the percent time measures, mice displayed a distinct pattern of activity in the maze [$F(2, 72) = 96.87$, $p < 0.005$], with control subjects showing a rank order preference for closed arms > centre platform > open arms. Drug treatment did not alter this profile [$F(6, 72) = 1.20$, NS]. ANOVA revealed a significant F value for closed arm entries, but follow-up tests showed that this was not due to differences between control and drug conditions. Although no other variables produced significant F values, several approached significance [F(crit0.05) = 2.84], including open arm entries (2.12), supported rears (2.36), percent time on the central platform (2.30), and entry latency (1.99). Follow-up tests on these measures showed an decrease in open entries at 5 mg/kg ($p < 0.05$), an increase in central platform time at 10 mg/kg ($p < 0.05$), and a reduction in entry latency at 20.0 mg/kg ($p < 0.05$).

Experiment 3: Chronic Imipramine

Data and ANOVA statistics are summarised in Table 3. Analysis revealed that chronic imipramine treatment had significant effects on rearing [$F(3, 36) = 3.16$, $p < 0.05$] and nonexploratory behaviour $F(3, 36) = 3.54$, $p < 0.05$]. Follow-up analyses indicated that both measures were reduced at the highest dose tested ($p < 0.05$–0.025). The F value for entry latency approached significance (2.40), reflecting an increase in this measure at the lowest dose tested ($p < 0.05$). On the percent time measures, control mice displayed a clear preference for different sections of the maze [$F(2, 72) = 41.05$, $p < 0.01$], with controls spending more time in the protected areas of the maze (equal time on the closed arms and central platform) than on the open arms. Drug treatment did not alter this profile [$F(6, 72) = 0.42$, NS]. No other analyses were significant.

DISCUSSION

The present results confirm the anxiolytic effects of acutely administered diazepam in the elevated plus-maze test [for a review, see (43)] and are entirely consistent with previous data obtained in this laboratory using other benzodiazepine receptor agonists such as chlordiazepoxide and bretazenil (8). They also provide a very much more comprehensive plus-maze profile for diazepam than was possible in earlier studies which, for design reasons, recorded only the standard spatiotemporal measures (46,47). In the current study, an anxiolytic effect of diazepam was indicated not only by significant increases in percent open entries and percent open time, but also by increases in exploratory head-dipping and reductions in all primary risk assessment measures (SAP, %pSAP, and closed returns). Although these effects were accompanied by a significant increase in total arm entries, a nonspecific stimulant action is unlikely, because a) if anything, rearing showed a trend toward inhibition, whereas b) no effects were seen on closed arm entries, the measure now accepted as the most valid index of general activity in this model (12,17).

Our findings with the tricyclic antidepressant imipramine stand in marked contrast to this typical benzodiazepine profile. In agreement with previous studies (5,28,36), acute imipramine treatment failed to produce anxiolytic-like effects in the plus-maze model. Indeed, nonsignificant changes in several anxiety-related parameters (e.g., open entries, percent open entries; percent open time, %pSAP), particularly at

TABLE 2

EFFECTS OF ACUTE IMIPRAMINE TREATMENT (0-20.0 mg/kg) ON PLUS-MAZE BEHAVIOUR IN MALE DBA/2 MICE

Behaviour	Saline	Imipramine (mg/kg) 5.0	10.0	20.0	$F(3, 36)$
Total arm entries	17.9 ± 2.0	17.3 ± 2.0	17.2 ± 1.3	13.0 ± 1.1	1.87, NS
Open arm entries	6.0 ± 1.2	3.4 ± 0.7*	3.6 ± 0.6	3.6 ± 0.7	2.12, NS
Closed arm entries	11.9 ± 1.0	13.9 ± 1.7	13.6 ± 1.0	9.4 ± 1.0	2.89, $p < 0.05$
% Open arm entries	30. ± 5.1	19.1 ± 3.6	20.1 ± 2.6	27.9 ± 6.5	1.37, NS
% Open arm time	14.6 ± 2.9	7.7 ± 2.0	7.3 ± 1.5	12.9 ± 4.7	1.47, NS
% Centre time	32.1 ± 2.2	41.5 ± 3.4	43.4 ± 3.9*	39.8 ± 3.2	2.30, NS
% Closed arm time	53.3 ± 3.8	50.8 ± 3.1	50.3 ± 3.5	48.3 ± 5.0	0.27, NS
Total rears	9.1 ± 1.4	7.2 ± 1.2	10.4 ± 1.9	5.3 ± 1.2	2.36, NS
Total head-dips	3.4 ± 0.6	3.0 ± 0.9	3.9 ± 0.9	4.1 ± 1.6	0.22, NS
% pDips	73.1 ± 8.5	70.7 ± 12.7	82.9 ± 7.7	74.7 ± 9.3	0.30, NS
Total SAP	19.5 ± 2.4	21.9 ± 2.3	23.0 ± 0.9	22.1 ± 2.1	0.56, NS
% pSAP	73.0 ± 6.1	80.4 ± 5.1	75.4 ± 5.2	76.3 ± 5.9	0.31, NS
Closed arm returns	1.3 ± 0.6	1.9 ± 0.6	1.2 ± 0.3	1.7 ± 0.5	0.41, NS
Entry latency (s)	12.6 ± 5.8	7.3 ± 1.7	6.5 ± 1.6	1.8 ± 0.4*	1.99, NS
NEB (s)	29.1 ± 8.4	21.4 ± 1.7	18.7 ± 3.6	21.5 ± 7.6	0.52, NS

Data are presented as mean values (± SEM). SAP, stretched attend postures; NEB, nonexploratory behaviour; %p, percent protected.

*$p < 0.05$ vs. saline.

lower doses (5–10 mg/kg), would be more indicative of anxiety enhancement. Although extremely weak, these trends would not be inconsistent with reports of anxiogenic-like reactions to acute imipramine in both animals (34,37) and humans (1,31). In contrast to expectation, chronic pretreatment with imipramine also failed to produce anxiety reduction under the present test conditions. Indeed, the only behavioural changes observed under this schedule were high-dose reductions in rearing and nonexploratory behaviour. Although these data generally agree with negative results obtained in rat plus-maze and social interaction studies (16,37), they are at variance with positive findings obtained in the mouse social interaction model (20), as well as antipredator defense (2), novelty-suppressed feeding (3), and conditioned suppression (18,19) tests in rats. Several explanations for such inconsistency may be considered.

TABLE 3

EFFECTS OF CHRONIC IMIPRAMINE TREATMENT (0-20.0 mg/kg: DAILY FOR 15 DAYS) ON PLUS-MAZE BEHAVIOUR IN MALE DBA/2 MICE

Behaviour	Saline	Imipramine (mg/kg) 5.0	10.0	20.0	$F(3, 36)$
Total arm entries	13.2 ± 1.8	14.1 ± 1.8	13.5 ± 1.7	12.1 ± 2.1	0.20, NS
Open arm entries	4.2 ± 0.9	3.9 ± 0.6	5.4 ± 1.1	3.2 ± 0.8	1.10, NS
Closed arm entries	9.0 ± 1.0	10.2 ± 1.5	8.1 ± 0.7	8.9 ± 1.9	0.40, NS
% Open arm entries	28.9 ± 3.8	27.8 ± 3.8	36.9 ± 4.0	30.7 ± 9.3	0.50, NS
% Open arm time	10.3 ± 2.8	9.3 ± 1.8	16.2 ± 3.8	15.9 ± 5.2	1.00, NS
% Centre time	40.1 ± 5.6	45.9 ± 3.8	40.7 ± 5.3	40.9 ± 6.2	0.326, NS
% Closed arm time	49.6 ± 5.2	44.8 ± 4.0	49.6 ± 3.8	43.3 ± 7.4	0.38, NS
Total rears	8.3 ± 1.3	6.7 ± 1.6	8.8 ± 1.2	3.8 ± 0.7*	3.16, $p < 0.05$
Total head-dips	1.6 ± 0.5	1.6 ± 0.4	2.0 ± 0.7	1.2 ± 0.6	0.36, NS
% pDips	50.0 ± 13.2	48.3 ± 15.0	58.5 ± 14.0	29.0 ± 13.2	0.81, NS
Total SAP	13.7 ± 1.2	17.0 ± 1.7	15.6 ± 1.5	15.1 ± 1.0	1.07, NS
% pSAP	70.7 ± 6.9	70.7 ± 7.9	59.6 ± 9.0	68.0 ± 8.9	0.41, NS
Closed arm returns	1.0 ± 0.5	1.0 ± 0.3	1.4 ± 0.5	2.2 ± 0.6	1.23, NS
Entry latency (s)	1.3 ± 0.3	14.6 ± 8.0*	2.5 ± 0.6	2.7 ± 1.1	2.40, NS
NEB (s)	23.7 ± 8.3	9.5 ± 3.2	22.1 ± 6.1	2.4 ± 1.3†	3.54, $p < 0.05$

Data are expressed as mean values (± SEM). SAP, streteched attend postures; NEB, nonexploratory behaviour; %p, percent protected.

*$p < 0.05$, †$p < 0.025$ vs. saline.

It is clear that the antipanic effects of imipramine are more well established than its effects in generalized anxiety disorder (1,38). As the plus-maze is considered to be relatively insensitive to antipanic and panic-promoting drugs [e.g., (17)], it may be that the interparadigm differences in imipramine's profile simply reflect differences in the degree to which these tests evoke panic-like reactions. However, this explanation alone would not account for the anxiolytic effects seen in the rat plus-maze with chronic paroxetine (6) or the trend toward anxiolysis reported for chronic phenelzine (24); both of these agents have been reported to have antipanic efficacy (1,35). In this context, Briley and colleagues (4) attributed differences in the acute effects of antidepressant reuptake inhibitors on plus-maze performance to variations in their neurochemical profiles. In particular, they suggest that anxiogenic responses to acute paroxetine and indalpine relate to the specific effects of these agents on 5-HT reuptake, whereas additional (i.e., noradrenergic) effects of less specific reuptake blockers (e.g., imipramine) may have a protective effect in this context. This proposal raises the possibility that the lack of neurochemical specificity of imipramine may have contributed to the present negative findings (acute and chronic), and suggests that more positive results might be obtained in the present model using chronic treatment with 5-HT-selective reuptake inhibitors. Studies are currently under way comparing the behavioural effects of chronic treatment with 5-HT-selective and NA-selective reuptake inhibitors in the plus-maze.

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