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Dopamine D_4 receptor and anxiety: Behavioural profiles of clozapine, L-745,870 and L-741,742 in the mouse plus-maze

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Abstract

The dopamine D_4 receptor has been implicated in the therapeutic effects of the atypical antipsychotic, clozapine. As it has been proposed that anxiolytic-like activity may contribute to the efficacy of this agent in ameliorating the negative symptoms of schizophrenia, the current study employed ethological methods to fully characterize the acute behavioural profiles of clozapine and two more selective dopamine D_4 receptor antagonists, L-745,870 (3-[{4-(4-chlorophenyl)piperazin-1-yl)]methyl}-1*H*-pyrrolo[2,3b]pyridine) and L-741,742 (5-(4-chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole), in the mouse elevated plus-maze test. Results showed that while clozapine (0.3–6.0 mg/kg) dose-dependently inhibited all active behaviours (arm entries, exploration, rearing) and increased grooming and immobility, it failed to alter the major anxiety indices (percent open entries and open time). In contrast, L-745,870 (0.02–1.5 mg/kg) and L-741,742 (0.04–5.0 mg/kg) did not produce any significant behavioural changes under present test conditions. These data, which contrast markedly with the robust anxiolytic profile of the reference compound, chlordiazepoxide (10.0 mg/kg), provide little support for the suggestion that clozapine possesses anxiolytic-like properties and further indicate that selective dopamine D_4 receptor antagonists are ineffective in the modulation of anxiety-related behaviours in the plus-maze.

Keywords: Anxiety; Elevated plus-maze; Clozapine; L-745,870 (3-[{4-(4-chlorophenyl)piperazin-1-yl)]methyl}-1*H*-pyrrolo[2,3b]pyridine); L-741,742 (5-(4-chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole); Dopamine D_4 receptor; Chlordiazepoxide; Mouse

1. Introduction

Dopamine receptor subtypes have been classified into two major families, D_1-like (i.e., D_1 and D_5) and D_2-like (i.e., D_2, D_3 and D_4) (review: Jaber et al., 1996), with recent evidence that clozapine binds preferentially to the dopamine D_4 receptor (Van Tol et al., 1991) leading to speculation that an action at this site may be responsible for its therapeutic effects (Seeman et al., 1993). The 'atypical' antipsychotic profile of clozapine includes a lower incidence of extrapyramidal side-effects as well as effectiveness against both the positive and negative symptoms of schizophrenia (Fitton and Heel, 1990). Of particular relevance is the suggestion that its efficacy against negative symptoms may be related to anxiolytic-like activity (Benvenga and Leander, 1995). In support of this proposal, preclinical reports indicate that clozapine increases punished responding in rodents (Spealman and Katz, 1980; Moore et al., 1992; Wiley et al., 1993; Moore et al., 1994), pigeons (Mansbach et al., 1988; Benvenga and Leander, 1995; Rigdon et al., 1996) and squirrel monkeys (Spealman and Katz, 1980; Spealman, 1985), suppresses shock-induced ultrasonic vocalizations (Bartoszyk et al., 1996) and conditioned freezing (Inoue et al., 1996; Ishidatokuda et al., 1996) in rats and reduces indices of anxiety in the rat elevated plus-maze (Szewczak et al., 1995), social interaction (Corbett et al., 1993; Szewczak et al., 1995) and open field (Bruhwyler et al., 1990) paradigms. However, not only are the mechanisms involved in these effects at present unknown, but their reliability has been challenged by negative findings in other laboratories (e.g. De Vry et al., 1993; Costall and Naylor, 1995).

Although the absence of selective ligands has thus far precluded detailed studies on the physiological and behavioural significance of the dopamine D_4 receptor, several selective dopamine D_4 receptor antagonists have now

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been characterized (Boyfield et al., 1996a,b; Hidaka et al., 1996; Kulagowski et al., 1996; Rowley et al., 1996; Ten-Brink et al., 1996). Among these novel agents, L-745,870 (3-[{4-(4-chlorophenyl)piperazin-1-yl)]methyl}-1*H*-pyrrolo[2,3b]pyridine) has more than a 2000-fold selectivity for dopamine D_4 over D_2 and D_3 receptors, penetrates mouse brain following peripheral administration (Patel et al., 1996) and is currently the most selective dopamine D_4 ligand currently available. Another compound, L-741,742 (5-(4-chlorophenyl)-4-methyl-3-(1-(2-phenylethyl) piperidin-4-yl)isoxazole), has >500 fold selectivity for dopamine D_4 over D_2 binding sites (Rowley et al., 1996). Functional studies have demonstrated that both ligands are full antagonists which, in the absence of intrinsic activity, potently attenuate dopamine-induced inhibition of forskolin-stimulated cAMP levels in cells stably expressing human dopamine D_4 receptors (Kulagowski et al., 1996; Rowley et al., 1996; Patel et al., 1996).

In view of the inconsistent literature on the effects of clozapine in animal models of anxiety, and the proposed role of the dopamine D_4 receptor in the behavioural effects of this compound, the present study employed ethological techniques (Rodgers and Cole, 1994; Rodgers and Johnson, 1995) to examine in detail the effects of clozapine, L-745,870 and L-741,742 on the behaviour of mice in a well-validated animal model of anxiolytic activity, the elevated plus-maze. For comparative purposes, chlordiazepoxide was included as a reference anxiolytic.

2. Materials and methods

2.1. Drugs

The compounds used were clozapine (Sigma, Poole, UK), L-745,870 trihydrochloride and L-741,742 hydrochloride (Tocris Cookson, Bristol, UK) and chlordiazepoxide hydrochloride (Sigma). Clozapine and L-745,870 were dissolved in distilled water to which a minimal amount of dilute hydrochloric acid was added, while L-741,742 and chlordiazepoxide were dissolved in 10% (v/v) dimethyl sulfoxide (DMSO) and physiological saline, respectively. Control groups received injections of the appropriate vehicles. All compounds were prepared freshly on test days and administered (10 ml/kg, i.p.) 30 min before testing. Doses cited refer to salts, where applicable.

2.2. Animals

Experimentally-naive male Swiss–Webster mice (Bantin and Kingman, Hull, UK), 8–10 weeks old at testing, were used. Subjects were housed in groups of 10 (cage size: $45 \times 28 \times 13$ cm) and maintained under a 12 h reversed light/dark cycle (lights off 07.00 h) in a temperature (21 ± 1°C)- and humidity (50 ± 5%)-controlled environment for at least 3 weeks before testing. Food and drinking water were available ad libitum except during the brief test sessions. Sample sizes of $n = 10$ were used throughout.

2.3. Apparatus and procedure

The plus-maze comprised 2 open ($30 \times 5 \times 0.25$ cm) and 2 enclosed ($30 \times 5 \times 15$ cm) arms, linked by a common central platform (5×5 cm) and elevated 60 cm above floor level. The maze floor was made of black Plexiglas and the walls of the enclosed arms of clear Plexiglas. All testing was conducted during the dark phase of the light cycle (10.00–16.00 h) in a dimly illuminated (4×60 W red) laboratory and commenced with the placement of a mouse on the central platform of the maze facing an open arm. A standard 5 min test duration was employed and the maze was thoroughly cleaned between subjects. Behaviours, scored off videotape by a trained observer using ethological analysis software ('Hindsight' v1.4; developed by Dr. Scott Weiss), are described in detail elsewhere (Rodgers and Johnson, 1995). In brief, they included both conventional (open/closed/total arm entries, % open arm entries and % time spent in centre, open and closed parts of the maze) and ethological (rearing, stretched attend postures, head-dipping, flatback approach, sniffing, grooming and immobility) measures. In view of the importance of thigmotactic cues to patterns of maze exploration (Treit et al., 1993), '% protected forms' (i.e., proportion of responses shown from closed arms and/or centre platform) of head-dipping, stretched attend postures, flatback approach and sniffing were also computed.

2.4. Statistical analysis

Data were subjected to single- or two-factor analysis of variance (ANOVA), with further comparisons performed using the appropriate error variance terms from the ANOVA summary tables (Dunnett's or Duncan multiple range tests). The only exceptions were data for closed arm returns, grooming and immobility which, due to their non-parametric nature, were analysed by Kruskal–Wallis and Mann–Whitney procedures. Statistical significance was accepted if $P < 0.05$.

2.5. Ethics

The experiments described in this manuscript were licensed by the UK Home Office under the Animals (Scientific Procedures) Act 1986.

3. Results

3.1. Chlordiazepoxide

Data are summarized in Table 1. ANOVA confirmed the robust anxiolytic-like effects of chlordiazepoxide (10.0

Table 1
Effects of chlordiazepoxide (10.0 mg/kg, i.p.) on behavioural measures in the mouse elevated plus-maze test

Behaviour	Saline	Chlordiazepoxide 10.0 mg/kg	F value
Open arm entries	5.9 ± 0.6	11.8 ± 0.8 [b]	35.40
Closed arm entries	12.7 ± 0.8	11.5 ± 0.8	1.19
Total arm entries	18.6 ± 0.5	22.8 ± 1.1 [b]	12.60
% open arm entries	31.9 ± 3.5	52.0 ± 2.8 [b]	19.98
% open arm time	22.5 ± 2.7	41.7 ± 3.0 [b]	22.22
% closed arm time	23.3 ± 2.4	17.4 ± 1.3 [a]	4.70
% central platform time	54.2 ± 2.4	40.9 ± 3.0 [b]	12.05
Total head-dips	28.7 ± 1.7	36.9 ± 2.1 [b]	9.32
% protected head-dips	74.0 ± 2.4	32.7 ± 1.6 [b]	205.70
Total stretched attend postures	27.3 ± 1.8	18.3 ± 2.2 [b]	10.12
% protected stretched attend postures	78.1 ± 3.5	48.5 ± 6.3 [b]	16.72
Flat back approach (s)	3.8 ± 0.4	3.9 ± 0.6	0.02
% protected flat back	71.7 ± 7.6	41.1 ± 6.7 [b]	9.03
Sniff duration (s)	59.5 ± 2.0	60.9 ± 2.4	0.21
% protected sniff	71.8 ± 1.6	54.4 ± 3.1 [b]	25.17
Closed arm returns	0.4 ± 0.2	0.4 ± 0.2	$H = 0$
Total rears	16.6 ± 2.0	14.8 ± 1.7	0.47
Rearing duration (s)	5.8 ± 0.8	7.8 ± 1.3	1.68
Grooming (s)	1.0 ± 0.3	0.5 ± 0.3	$H = 2.95$
Immobility (s)	0.1 ± 0.04	0.1 ± 0.04	$H = 0.002$

Data are expressed as mean ± SEM ($n = 10$).
[a] $P < 0.05$.
[b] $P < 0.01$ compared with saline control.

mg/kg) under the present test conditions. Significant increases were observed in total and open arm entries, % open entries, % open time and head-dipping ($P < 0.01$), together with reductions in % closed arm time, % centre time, stretched attend postures and the % protected forms of head-dipping, stretched attend, flatback approach and sniffing ($P < 0.05$–0.01). Control animals displayed a rank order preference for time spent on different sections of the

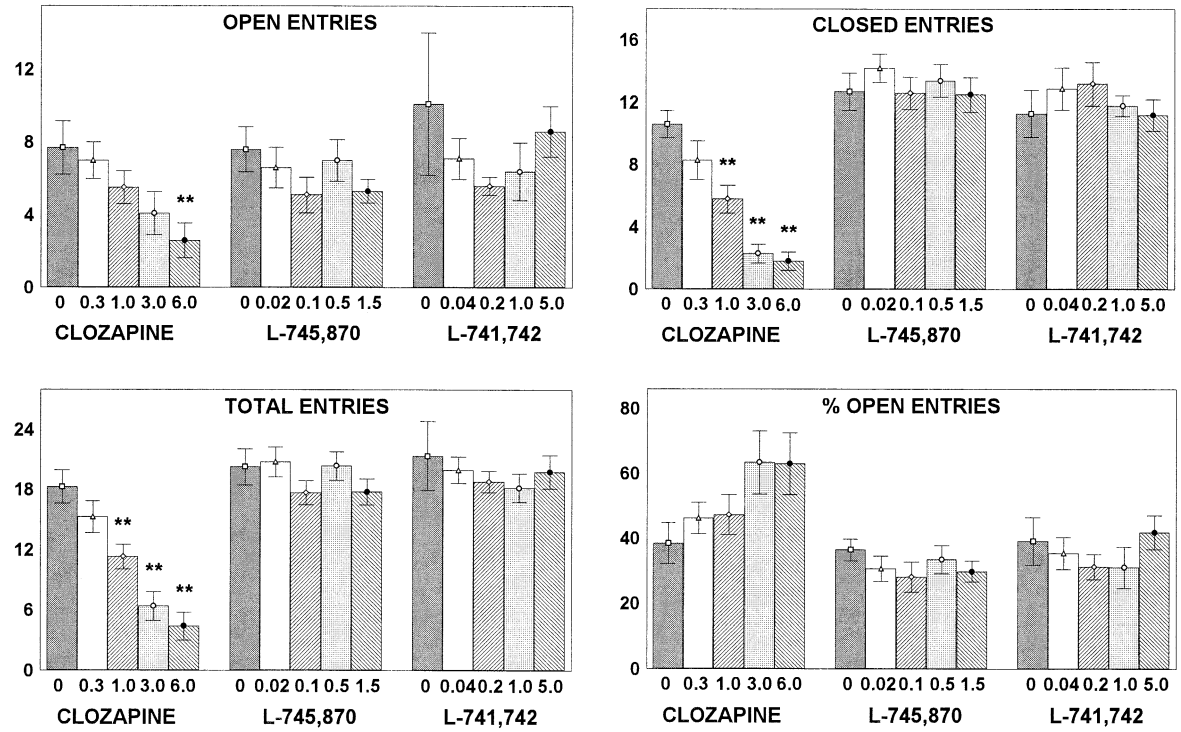

Fig. 1. Effects of clozapine (0.3–6.0 mg/kg), L-745,870 (0.02–1.5 mg/kg), L-741,742 (0.04–5.0 mg/kg) on the behaviour of mice in the elevated plus-maze: open, closed and total arm entries and percent open entries. Data are expressed as means ± S.E.M. ($n = 10$). * $P < 0.05$, ** $P < 0.01$ versus vehicle.

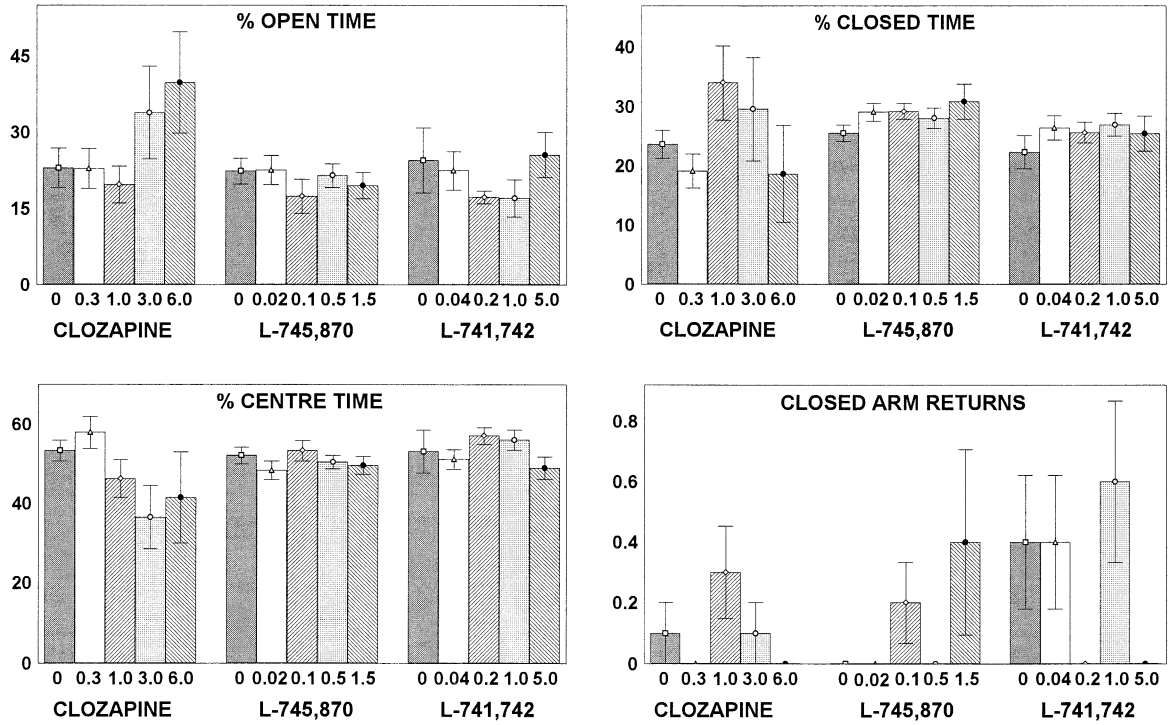

Fig. 2. Effects of clozapine (0.3–6.0 mg/kg), L-745,870 (0.02–1.5 mg/kg), L-741,742 (0.04–5.0 mg/kg) on the behaviour of mice in the elevated plus-maze: percent time spent on open arms, closed arms and centre platform, and closed arm returns. Data are expressed as means ± S.E.M. ($n = 10$).

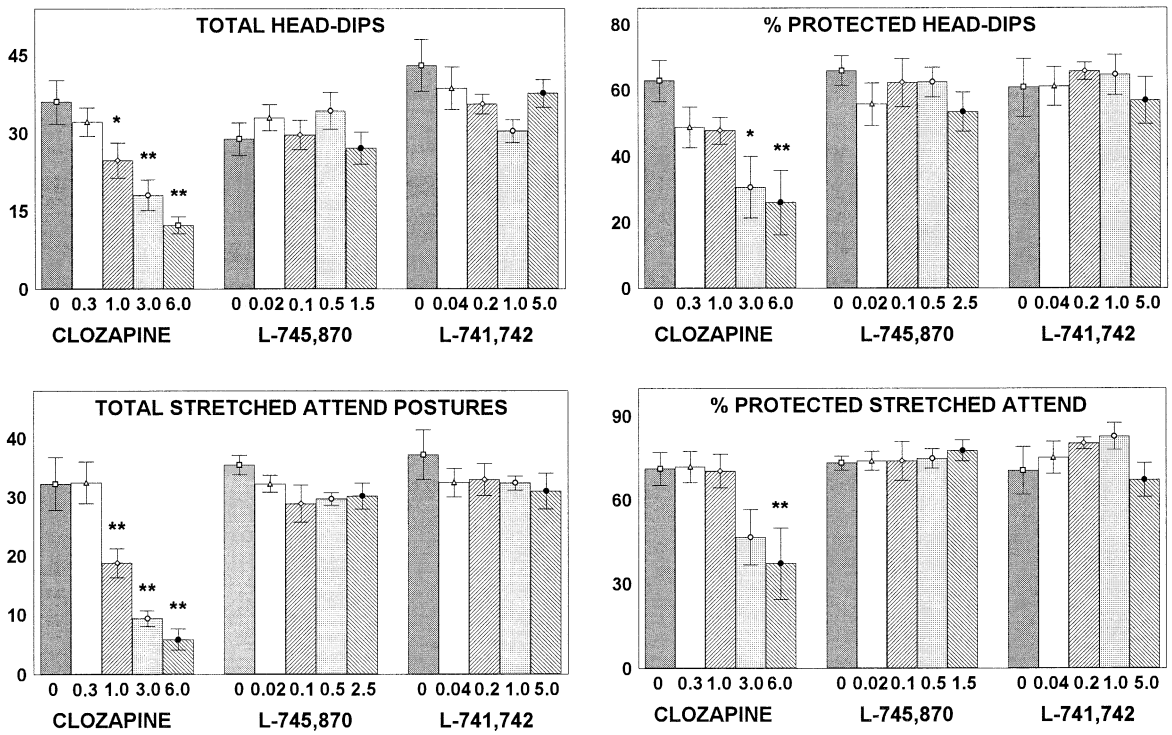

Fig. 3. Effects of clozapine (0.3–6.0 mg/kg), L-745,870 (0.02–1.5 mg/kg), L-741,742 (0.04–5.0 mg/kg) on the behaviour of mice in the elevated plus-maze: total head-dips, percent protected head-dips, total stretched attend postures and percent protected stretched attend. Data are expressed as means ± S.E.M. ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle.

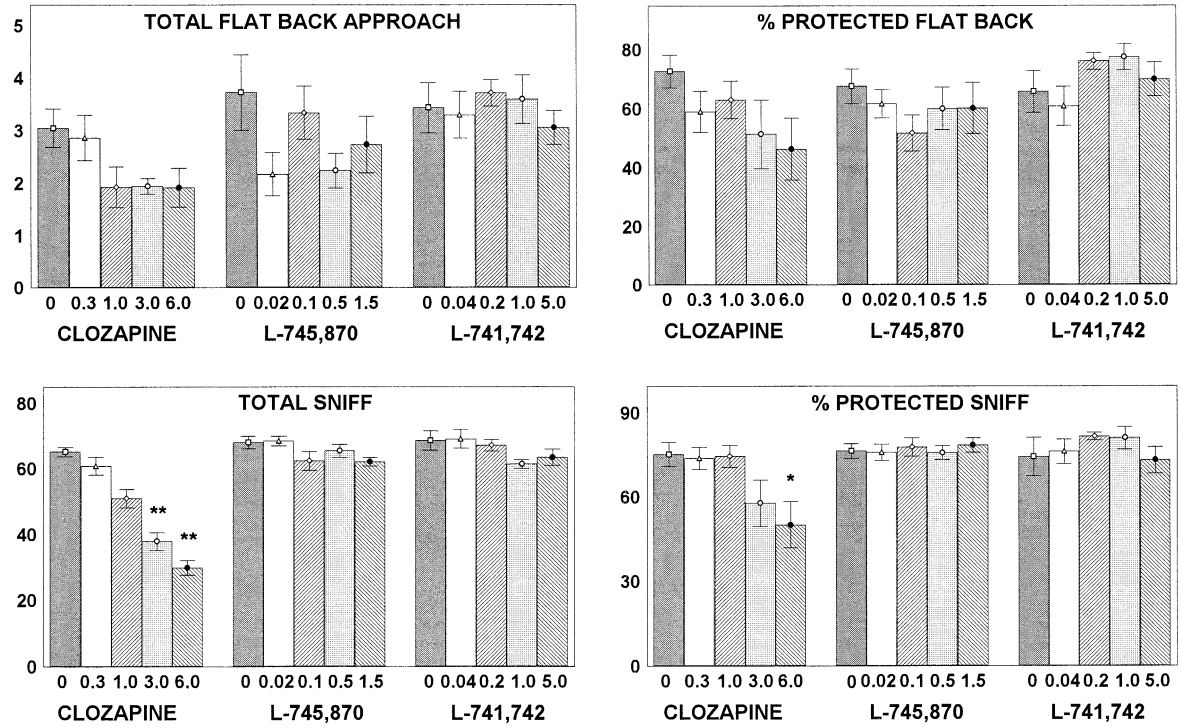

Fig. 4. Effects of clozapine (0.3–6.0 mg/kg), L-745,870 (0.02–1.5 mg/kg), L-741,742 (0.04–5.0 mg/kg) on the behaviour of mice in the elevated plus-maze: total flat back approach (s), percent protected flat back, total sniff (s) and percent protected sniff. Data are expressed as means ± S.E.M. ($n = 10$). * $P < 0.05$, ** $P < 0.01$ versus vehicle.

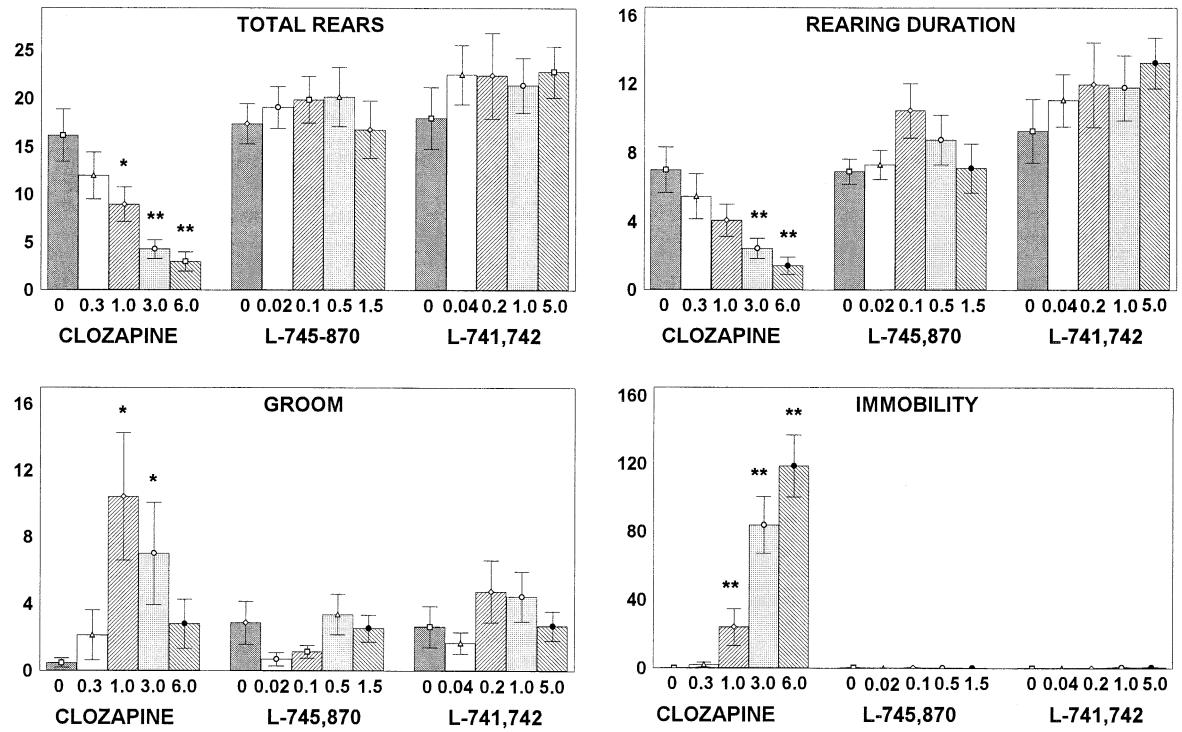

Fig. 5. Effects of clozapine (0.3–6.0 mg/kg), L-745,870 (0.02–1.5 mg/kg), L-741,742 (0.04–5.0 mg/kg) on the behaviour of mice in the elevated plus-maze: total rears, rearing duration (s), groom (s) and immobility (s). Data are expressed as means ± S.E.M. ($n = 10$). * $P < 0.05$, ** $P < 0.01$ versus vehicle.

maze with centre > closed = open, $F(2,36) = 38.43$, $P < 0.01$. This profile was altered by chlordiazepoxide, $F(2,36) = 15.01$, $P < 0.01$. Thus, drugged mice no longer differentiated between the central platform and open arms but preferred each of these areas to the closed arms. However, no significant treatment effects were observed for closed arm entries, rearing (frequency or duration), total flatback approach, total sniffing, closed arm returns, grooming or immobility.

3.2. Clozapine

Data are presented in Figs. 1–5 (left panels). Significant treatment effects were observed for open arm entries, $F(4,45) = 3.43$, $P < 0.01$, closed arm entries, $F(4,45) = 18.83$, $P < 0.01$, and total arm entries, $F(4,45) = 15.65$, $P < 0.01$. Further analysis showed that whereas both closed and total arm entries were suppressed at 1.0–6.0 mg/kg, open entries were reduced only at 6.0 mg/kg ($P < 0.01$). However, clozapine did not alter % open entries, $F(4,45) = 2.12$, NS, % open time, $F(4,45) = 1.62$, NS, % closed time, $F(4,45) = 1.13$, NS, or % centre time, $F(4,45) = 1.56$, NS. Furthermore, despite a strong overall temporal preference for different sections of the maze, $F(2,90) = 10.95$, $P < 0.01$, the pattern displayed by vehicle-treated mice (centre > closed = open) was unaffected by clozapine treatment, $F(8,90) = 1.45$, NS.

In addition to changes in arm entry scores, clozapine produced significant effects on total head-dips, $F(4,45) = 9.93$, $P < 0.01$, % protected head-dips, $F(4,45) = 4.04$, $P < 0.01$, stretched attend postures, $F(4,45) = 17.77$, $P < 0.01$, % protected stretched attend, $F(4,45) = 3.59$, $P < 0.05$, total sniff, $F(4,45) = 37.67$, $P < 0.01$, % protected sniff, $F(4,45) = 3.64$, $P < 0.05$, total rears, $F(4,45) = 7.94$, $P < 0.01$, rearing duration, $F(4,45) = 5.15$, $P < 0.01$, grooming, $H = 9.52$, $P < 0.05$, and immobility, $H = 35.71$, $P < 0.01$. Most of these alterations reached statistical significance at 1.0–6.0 mg/kg, with grooming and immobility increased and all other measures decreased. The total flatback approach, $F(4,45) = 2.55$, NS, the % protected form of this behaviour, $F(4,45) = 1.45$, NS, and closed arm returns, $H = 6.53$, NS, were not significantly influenced.

3.3. L-745,870

Data are summarized in Figs. 1–5 (centre panels). ANOVA indicated that, over the dose range tested, L-745870 did not significantly affect the behaviour of mice in the plus-maze (values in parentheses are F values with df = 4,45 and $F_{crit0.05} = 2.61$): open arm entries (1.06), closed arm entries (0.46), total arm entries (1.08), % open entries (0.72), % open time (0.62), % closed time (1.08), % centre time (0.78), total head-dips (0.95), % protected head-dips (0.78), total stretched attend postures (1.68), % protected stretched attend (0.17), flatback approach (1.72), % protected flatback (0.72), total sniff (2.28), % protected sniffing (0.72), total rears (0.35) and rearing duration (1.43). The H values for closed arm returns (6.39) grooming (5.65) and immobility (1.74) confirmed that these measures too were unaffected by L-745,870. Although a strong overall temporal preference for different maze sections was observed (vehicle group: centre > closed = open), $F(2,90) = 151.95$, $P < 0.01$, this pattern was similarly unaltered by drug treatment, $F(8,90) = 0.77$, NS.

3.4. L-741,742

As shown in Figs. 1–5 (right panels), L-741,742 also failed to modify any of the behavioural measures recorded (values in parentheses are F values with df = 4,45 and $F_{crit0.05} = 2.61$): open entries (1.06), closed entries (0.46), total entries (1.08), % open entries (0.70), % open time (0.62), % closed time (1.08), % centre time (0.78), total head-dips (0.95), % protected head dip (0.78), total stretched attend postures (1.68), % protected stretched attend (0.17), total flatback approach (1.72), % protected flatback (0.72), sniff (2.28), % protected sniff (0.18), total rears (0.35), rearing time (1.43). Similarly, the H values for closed arm returns (8.59), grooming (2.84) and immobility (7.22) did not reach significance. Although analysis of % time spent on different maze sections revealed a clear preference of centre > closed = open, $F(2,90) = 87.93$, $P < 0.01$, L-741742 did not alter this profile, $F(8,90) = 0.88$, NS.

4. Discussion

The present results confirm previous findings from this laboratory and elsewhere (review: Rodgers and Cole, 1994) that the murine plus-maze paradigm is sensitive to the anxiolytic-like effects of the benzodiazepine receptor full agonist, chlordiazepoxide (10.0 mg/kg). This reference anxiolytic increased open arm activity (entries, % open entries and % open time) and exploratory head-dipping, while reducing time spent on the centre platform and in the closed arms, risk assessment (stretched attend postures) and all 'protected' measures. Importantly, these effects were observed in the absence of significant changes in measures of general activity (i.e., closed arm entries, rearing, sniffing, grooming and immobility) and are entirely consistent with previous findings for chlordiazepoxide, bretazenil and diazepam in this model (e.g., Cole and Rodgers, 1993, 1995; Johnson and Rodgers, 1996).

In contrast, clozapine failed to alter the primary indices of plus-maze anxiety, i.e., percent open arm entries and percent open arm time. Although reductions in the percent protected forms of head-dipping, stretched attend postures and sniffing at higher doses may suggest a weak anxiolytic action, such an interpretation is negated by (a) the suppression of all active behaviours (arm entries, rearing, head-di-

pping, stretched attend postures, sniffing) and (b) marked increases in non-exploratory behaviours (grooming and immobility). Indeed, mice treated with 3.0 and 6.0 mg/kg clozapine spent approximately one third of the test session in non-exploratory behaviours (mainly immobility). Therefore, the alterations in the spatial distribution of some behaviours may most parsimoniously be interpreted as a consequence of profound behavioural depression rather than weak anxiolysis.

The behaviourally non-selective effects of clozapine in the mouse elevated plus-maze are at variance with previous studies which report acute anxiolytic-like activity of this compound in other animal models (Spealman and Katz, 1980; Spealman, 1985; Mansbach et al., 1988; Bruhwyler et al., 1990; Moore et al., 1992; Wiley et al., 1993; Moore et al., 1994; Benvenga and Leander, 1995; Szewczak et al., 1995; Bartoszyk et al., 1996; Inoue et al., 1996; Ishidatokuda et al., 1996; Rigdon et al., 1996). However, the latter effects often occurred over a narrow dose range and, in magnitude, were frequently much smaller than those produced by benzodiazepines (e.g. Spealman and Katz, 1980; Mansbach et al., 1988; Pollard and Howard, 1989; Wiley et al., 1993; Moore et al., 1994; Benvenga and Leander, 1995). As the present study employed a 20-fold dose range (0.3–6.0 mg/kg), with no behavioural effects noted at the lowest dose tested and motoric disruption at higher doses, the failure of clozapine to modify plus- maze anxiety cannot be attributed to use of inappropriate or inadequate doses. In this context, it is pertinent to note that clozapine has also been found to be inactive in the rat shock-induced ultrasonic vocalisation (De Vry et al., 1993) and social interaction (Costall and Naylor, 1995) tests as well as the mouse light/dark exploration paradigm (Costall and Naylor, 1995). Although the reasons underlying these discrepancies are unknown, present data provide little support for the suggestion that anxiolytic-like effects of clozapine (and other atypical antipsychotics) contribute to their therapeutic advantage over traditional neuroleptics in controlling the negative symptoms of schizophrenia (Benvenga and Leander, 1995; Szewczak et al., 1995).

The mechanism of action of clozapine is complex. Although the compound has high affinity for the dopamine D_4 receptor, there are substantial variations (7.4 to 28 times) in its reported selectivity for dopamine D_4 over D_2 receptors (Seeman and Van Tol, 1995; Patel et al., 1996). As two selective dopamine D_4 receptor antagonists were behaviourally inactive in the present study (see below), it is unlikely that the marked behavioural suppression observed with clozapine relates to an action at dopamine D_4 receptor populations. Furthermore, it is unlikely that its effects are due to dopamine D_2 receptor antagonism. Thus, while the dopamine D_2 receptor antagonist, haloperidol (Cole and Rodgers, 1994) and the mixed serotonin 5-HT_{1A} partial agonist-dopamine D_2 antagonist, buspirone (Cole and Rodgers, 1994; Cao and Rodgers, 1997), have both been found to have behavioural depressant actions in the current procedure, the more selective dopamine D_2/D_3 receptor antagonist, sulpiride, actually produces a very convincing anxioselective profile in the mouse plus-maze (Rodgers et al., 1994). However, it is important to note that clozapine binds to a number of other neurotransmitter receptors (e.g. serotonin 5-HT_{2A}) and also possesses anticholinergic activity (Coward et al., 1989; Fitton and Heel, 1990). In this context, although the hypolocomotor effects of subcutaneously administered clozapine in mice (minimum effective dose = 3.0 mg/kg) have recently been attributed to serotonin 5-HT_{2A} receptor antagonism (Gleason and Shannon, 1997), the serotonin 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, is devoid of behavioural activity in the mouse plus-maze (Rodgers et al., 1995). Furthermore, the antimuscarininc agent, scopolamine, has been reported to produce a plus-maze profile in mice more consistent with behavioural excitation than suppression (Rodgers and Cole, 1995). Since clozapine also has substantial affinity for serotonin 5-HT_{1A} receptors, where it appears to act as an agonist (Mason and Reynolds, 1992; Newman-Tancredi et al., 1996), attention is specifically drawn to strong parallels in the behavioural profiles of clozapine and the serotonin 5-HT_{1A} receptor full agonist, *R*(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), under identical test conditions (Cao and Rodgers, 1996).

Recently, significant progress has been made in the development of selective dopamine D_4 receptor antagonists (Boyfield et al., 1996a,b; Hidaka et al., 1996; Kulagowski et al., 1996; Patel et al., 1996; Rowley et al., 1996; TenBrink et al., 1996). However, despite extensive interest from the pharmaceutical industry, published behavioural research on these compounds is as yet very limited and, to our knowledge, effects in animal procedures used to screen anti-anxiety agents have not been reported. Despite the detailed behavioural profiles obtained by ethological analysis, present results show that L-745,870 (0.02–1.5 mg/kg) and L-741,742 (0.04–5.0 mg/kg), two novel and selective dopamine D_4 receptor antagonists (Kulagowski et al., 1996; Rowley et al., 1996; Patel et al., 1996), failed to alter any aspect of plus-maze behaviour in mice. This lack of effect stands in marked contrast to the pronounced behavioural depression seen with clozapine and cannot be attributed to inadequate dose ranges. Thus, L-745,870 easily penetrates the central nervous system and the doses currently used are higher than those required (0.1–1.5 mg/kg) to occupy 50% of central dopamine D_4 receptors in mice (Patel et al., 1996). As such, our inability to detect any behavioural effects of these two compounds strongly suggests that the dopamine D_4 receptor is not involved in the modulation of plus-maze anxiety. These negative findings are in agreement with recent behavioural studies showing selective dopamine D_4 receptor antagonists to be without effect in altering spontaneous locomotion or blocking amphetamine-induced hyperactivity in rodents (Bristow et al., 1996; Merchant et al., 1996).

In summary, the present results show that, in contrast to

chlordiazepoxide, clozapine dose-dependently suppresses all active behaviours in the mouse elevated plus-maze test without altering anxiety-related measures, while two selective dopamine D_4 receptor antagonists (L-745,870 and L-741,742) are behaviourally inert in this test. However, attention is drawn to the acute nature of the present work and the possibility that anxiolytic effects may be revealed following a period of chronic treatment with these agents. Furthermore, in view of growing evidence that different animal models of anxiolytic activity may actually be measuring different facets of anxiety (e.g. Rodgers et al., 1997), it will be important to determine the generality of present findings to other test situations.

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