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Anxiolytic and Anxiogenic Drug Effects on Exploratory Activity in an Elevated Plus-Maze: a Novel Test of Anxiety in the Rat

SHARON PELLOW[1] AND SANDRA E FILE

MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

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PELLOW, S AND S E FILE *Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze A novel test of anxiety in the rat* PHARMACOL BIOCHEM BEHAV **24**(3) 525–529, 1986 —The current studies further investigated the effects, in animal models of anxiety, of novel putative anxiolytic and anxiogenic compounds believed to induce their effects by actions at the GABA-benzodiazepine receptor complex It was expected that the results would also provide further validation for a novel test of anxiety based on the ratio of open to closed arm entries in an elevated plus maze in the rat The novel putative anxiolytics CL 218,872 (10–20 mg/kg) and tracazolate (5 mg/kg) significantly elevated the percentage of time spent on the open arms of an elevated plus-maze, consistent with their anxiolytic activity in several other animal tests Also consistent with results from other animal tests, no anxiolytic activity was observed for the phenylquinoline PK 8165 (10–25 mg/kg), the 3,4-benzodiazepine tofisopam (25–50 mg/kg), or buspirone (0 5–20 mg/kg) The benzodiazepine receptor inverse agonists FG 7142 (1–5 mg/kg) and CGS 8216 (3–10 mg/kg) had anxiogenic activity in this test, as did the atypical benzodiazepine Ro 5-4864 (1–5 mg/kg) Interestingly, however, the benzodiazepine receptor antagonists Ro 15-1788 (10–20 mg/kg) and ZK 93426 (5–10 mg/kg) had no anxiogenic activity in this test

Anxiolytic compound Anxiogenic compound Exploratory activity Anxiety Elevated plus-maze

RECENT evidence has shown that specific binding sites for benzodiazepines exist on a supramolecular chloride ionophore-associated complex, with receptors for the neurotransmitter GABA, and binding sites for convulsant and anticonvulsant compounds (see [20]) Several new compounds that act at the various sites on this receptor complex have been synthesised in the hope that new anxiolytic drugs will be discovered that possess clear advantages over the benzodiazepines Among the novel putative anxiolytics that have emerged are several compounds that have been reported to have behavioural profiles quite unlike that of the benzodiazepines For example, certain drugs have been claimed to be lacking in the anticonvulsant or sedative/ataxic effects of the benzodiazepines, others have even been shown to differ from the benzodiazepines in their actions in several animal tests of anxiety, being effective in certain tests but not in others (see Pellow [22] for review) It is therefore important to investigate the behavioural actions of these compounds in several different test procedures, an aim of the present study was to examine the effects of several putative anxiolytics in a new animal test of anxiety The putative anxiolytic compounds selected were the pyrazolopyridazine derivative tracazolate [19,21], the triazolopyridine CL 218,872 [18], tofisopam, a clinically effective 3,4-benzodiazepine (see [26]), the phenylquinoline PK 8165 [17] and buspirone (see [29])

Since the discovery of β-carboline compounds that were found to act at benzodiazepine receptors [1] but to induce anxiety, both in animals (see Pellow and File [24] for review) and in man [2], considerable interest has centered around the investigation of anxiogenic drug effects, and recent work has shown that at least two sites on the GABA-benzodiazepine receptor complex are capable of mediating anxiogenic drug effects [24] A problem with such research has been that most animal tests of anxiety were developed specifically to detect anxiolytic effects, the purpose of the present study was therefore to investigate the behavioural effects of these drugs in a test that is equally capable of detecting anxiolytic and anxiogenic drug effects The putative anxiogenic compounds that we selected were FG 7142, a β-carboline derivative found to be anxiogenic in man [2], CGS 8216, a pyrazoloquinoline [30], Ro 5-4864, an atypical 1,4-benzodiazepine (see Pellow and File [25]), the imidazodiazepine Ro 15-1788 (flumazepil, [14]), and the β-carboline derivative ZK 93426 [15]

The test procedure used in the present study used exploratory activity in an elevated plus-maze in the rat as a measure of anxiety The behavioural, physiological and

[1]Requests for reprints should be addressed to Sharon Pellow, Département de Pharmacologie, Faculté de Médecine Pitié-Salpétrière, Université Paris VI, 91, boulevard de l'Hôpital, 75634 Paris Cédex 13, France

pharmacological validation of this test has been described by Pellow *et al* [23] It was shown that exposure to the open arms of the plus-maze produced significantly more anxiety-related behaviour than exposure to the closed arms (freezing, defaecation, elevated plasma corticosterone concentrations) In this test, anxiolytic compounds (benzodiazepines and, to a lesser extent barbiturates) selectively elevated the percentage of time that rats would spend on the two open arms of the maze, compared to the time spent in the two enclosed arms, and in contrast, anxiogenic compounds (yohimbine, pentylenetetrazole, caffeine, amphetamine) selectively diminished the percentage of time spent on the open arms Antidepressants and major tranquilisers non-selectively reduced the total number of arm entries [23] Results from the present study will extend the pharmacological validation of this test Doses of the compounds chosen for investigation were selected on the basis of previous studies

METHODS

Animals

Animals were male hooded Lister rats (Olac Ltd , Bicester) weighing 250–350 g, housed in groups of 6–7 in a room with an 11 hr light 13 hr dark cycle, and allowed free access to food and water

Apparatus

The +-maze consisted of two open arms, 50×10 cm, and two enclosed arms, 50×10×40 cm with an open roof, arranged such that the two arms of each type were opposite each other (see Fig 1) The maze was elevated to a height of 50 cm The measures indicated were taken by an observer sitting in the same room The wooden test arena in which rats were placed before exposure to the maze was 60×60×35 cm

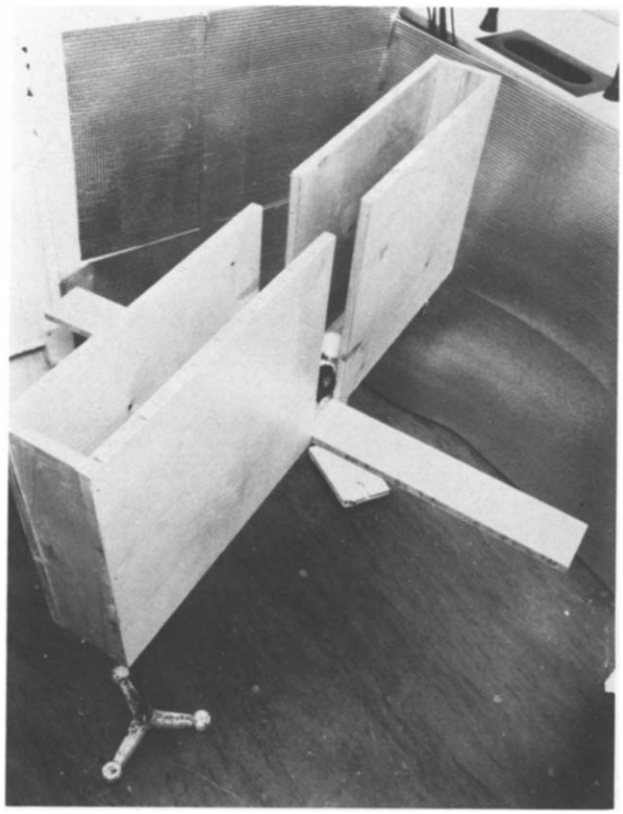

FIG 1 The elevated plus-maze apparatus

Drugs

Tracazolate (ICI), CL 218,872 (Lederle), tofisopam (Pierre Fabre), PK 8165 (Pharmuka), buspirone (Bristol-Myers), FG 7142 (Ferrosan), CGS 8216 (Ciba-Geigy), Ro 5-4864 (Hoffman-La Roche), Ro 15-1788 (flumazepil, Hoffman-La Roche) and ZK 93426 (Schering) were suspended by ultrasound in distilled water with a drop of Tween 20 All compounds were injected IP 30 min before testing (except for Ro 15-1788, which was given 20 min before) in concentrations to give an injection volume of 2 ml/kg

Procedure

Each rat received an injection and was then returned to his home cage After 25 min (15 min after Ro 15-1788) it was placed in a wooden arena for 5 min (previous studies had found that this procedure resulted in an elevation of the total arm entries on the maze) and was then immediately placed at the centre of the +-maze, facing one of the enclosed arms During a 5-min test period, the following measures were taken by an observer the number of entries into, and the time spent in, (a) open and (b) enclosed arms, the total number of arm entries

Rats were randomly allocated to the following groups (a) vehicle control (n=16), tracazolate (5 and 25 mg/kg), CL 218,872 (10 and 20 mg/kg), tofisopam (25 and 50 mg/kg), buspirone (0 5 and 20 mg/kg), PK 8165 (10 and 25 mg/kg), n=7–8 (b) vehicle control (n=17), FG 7142 (1 and 5 mg/kg), CGS 8216 (3 and 10 mg/kg), Ro 5-4864 (1 and 5 mg/kg), Ro 15-1788 (10 and 20 mg/kg), ZK 93426 (5 and 10 mg/kg), n=7–8

Statistics

Analysis of variance was performed on the percentage of open arm entries and of time spent in the open arms, with drug treatment as the factor Analysis of variance was also performed on the total number of arm entries Where a drug increased or decreased both total arm entries and the percentage of open arm entries, analysis of covariance was performed to determine to what extent the reduction in open arm entries was independent of any effect on closed arm entries Analysis was carried out with open arm entries or time spent on the open arms as the dependent variable and closed arm entries or time spent in the closed arms as the covariate A significant effect indicated that the reduction in open arm entries was independent of effects on closed arm entries Posthoc comparisons between individual treatment groups and controls were performed using Dunnett's *t*-tests

RESULTS

Putative Anxiolytic Compounds

Tracazolate (5–25 mg/kg) had a significant effect on the total number of arm entries, $F(2,29)=20\ 93$, $p<0\ 0001$, posthoc analysis showed that at 5 mg/kg there was a significant increase in the number of entries compared with controls ($p<0\ 01$, see Table 1) and at 25 mg/kg a significant decrease in this measure ($p<0\ 01$, see Table 1). Tracazolate (5–25 mg/kg) did not significantly alter the percentage of

TABLE 1

MEAN (±S E M) TOTAL NUMBER OF ARM ENTRIES MADE BY RATS DURING A 5-MIN TEST IN THE ELEVATED PLUS-MAZE, 30 MIN AFTER DRUG INJECTION (20 MIN AFTER Ro 15-1788)

Putative Anxiolytics (mg/kg)		Total	Putative Anxiogenics (mg/kg)		Total
Control		12 1 ± 0 97	Control		15 2 ± 1 09
Tracazolate	5 mg/kg	16 6 ± 0 77†	FG 7142	1 mg/kg	15 9 ± 1 49
	25 mg/kg	5 5 ± 1 24†		5 mg/kg	9 4 ± 2 03*
CL 218,872	10 mg/kg	8 6 ± 0 91	CGS 8216	3 mg/kg	7 6 ± 0 99†
	20 mg/kg	12 5 ± 2 08		10 mg/kg	9 6 ± 0 73†
PK 8165	10 mg/kg	12 1 ± 1 44	Ro 15-1788	10 mg/kg	15 0 ± 2 39
	25 mg/kg	8 9 ± 2 08		20 mg/kg	10 8 ± 2 52
Buspirone	0 5 mg/kg	13 0 ± 1 70	ZK 93426	5 mg/kg	13 6 ± 1 99
	20 mg/kg	1 2 ± 0 40†		10 mg/kg	14 4 ± 1 36
Tofisopam	25 mg/kg	13 7 ± 1 69	Ro 5-4864	1 mg/kg	12 0 ± 1 38
	50 mg/kg	9 9 ± 1 37		5 mg/kg	10 6 ± 0 61†

*$p<0$ 05, †$p<0$ 01, significantly different from controls, Dunnett's t-test after analysis of variance
n=7–8 per group

open arm entries, F(2,29)=0 81, however, there was a significant increase in the percentage of time spent on the open arms, F(2,29)=3 98, $p<0$ 05, and posthoc analysis showed that this effect was due to the 5 mg/kg dose that significantly differed from the controls ($p<0$ 01, see Fig 2)

CL 218,872 (10–20 mg/kg) had no significant effect on the total number of arm entries, F(2,29)=2 15, (see Table 1) There was a significant elevation in the percentage of entries into the open arms, F(2,29)=4 92, $p<0$ 05, and Dunnett's tests showed that there was a significant effect of both 10 mg/kg ($p<0$ 05) and 20 mg/kg ($p<0$ 01) doses compared with controls, (see Fig 2) CL 218,872 also significantly elevated the percentage of time spent in the open arms, F(2,29)=6 83, $p<0$ 005), Dunnett's tests showed that a significant effect was obtained with the 20 mg/kg dose compared with controls, $p<0$ 01 (see Fig 2)

PK 8165 (10–25 mg/kg) had no significant effect on the total number of arm entries, F(2,28)=1 46, (see Table 1) or the percentage of time spent on the open arms, F(2,28)=0 45, (see Fig 2) However, there was a significant effect on the percentage of open arm entries, F(2,28)=6 93, $p<0$ 005, and posthoc analysis showed that at 10 mg/kg PK 8165 significantly reduced this measure compared with controls, $p<0$ 01, (see Fig 2)

Tofisopam (25–50 mg/kg) had no significant effect on the total number of arm entries, F(2,29)=1 77, (see Table 1), the percentage of open arm entries, F(2,29)=0 64, (see Fig 2) or the percentage of time spent on the open arms, F(2,29)=0 37, (see Fig 2)

Buspirone (0 5–20 mg/kg) significantly reduced the total number of arm entries, F(2,29)=32 06, $p<0$ 0001 Posthoc analysis showed that this was due to a significant effect at 20 mg/kg to severely depress activity compared with controls, $p<0$ 01, (see Table 1) Buspirone also significantly reduced the percentage of open arm entries, F(2,29)=40 05, $p<0$ 0001, posthoc analysis again showed that this was an effect of the 20 mg/kg dose compared with controls, $p<0$ 01, (see Fig 2) There was similarly a significant reduction in the percentage of time spent on the open arms, F(2,29)=24 18, $p<0$ 0001, and posthoc analysis showed that both doses of

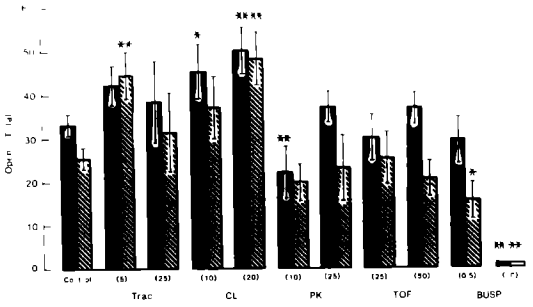

FIG 2 Mean (±S E M) percentage of open arm entries (closed bars) and percentage of time (sec) spent in the open arms (hatched bars) in rats given a 5-min test in the elevated plus-maze, 30 min after IP injection with CL 218,872 (10–20 mg/kg), tracazolate (5–25 mg/kg), PK 8165 (10–25 mg/kg), buspirone (0 5–20 mg/kg) and tofisopam (25–50 mg/kg) *$p<0$ 05, **$p<0$ 01, significantly different from controls, Dunnett's t-test after analysis of variance

buspirone significantly reduced this measure compared with controls (0 5 mg/kg, $p<0$ 05, 20 mg/kg, $p<0$ 01) (see Fig 2) ANCOVA on the number of arm entries did not reveal a significant effect of buspirone to reduce open arm entries when the concomitant reduction in closed arm entries was taken into account, F(2,28)=2 65 This suggests that the effects of buspirone in this test were primarily to reduce overall spontaneous activity

Putative Anxiogenic Compounds

FG 7142 (1–5 mg/kg) caused a significant reduction in the total number of arm entries, F(2,25)=5 25, $p<0$ 05, an effect that was mainly due to the 5 mg/kg dose, $p<0$ 05, (see Table 1) FG 7142 also caused a significant reduction in the percentage of open arm entries, F(2,25)=11 17, $p<0$ 0005, and posthoc analysis showed that both doses significantly reduced this measure compared with controls (1 mg/kg, $p<0$ 05, 5 mg/kg, $p<0$ 01) (see Fig 3) Analysis of

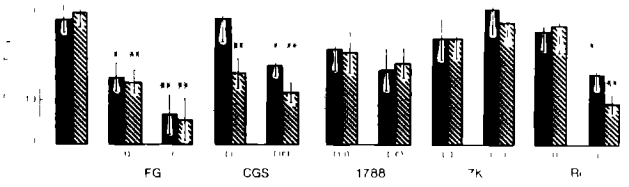

FIG 3 Mean (±S E M) percentage of open arm entries (closed bars) and percentage of time (sec) spent in the open arms (hatched bars) in rats given a 5-min test in the elevated plus-maze, 30 min after IP injection of FG 7142 (1–5 mg/kg), CGS 8216 (3–10 mg/kg), Ro 5-4864 (1–5 mg/kg), ZK 93426 (5–10 mg/kg) or 20 min after IP injection of Ro 15-1788 (10–20 mg/kg) *$p<0$ 05, **$p<0$ 01, significantly different from controls, Dunnett s t-test after analysis of variance

covariance showed that the reduction in open arm entries was quite independent of the reduction in closed arm entries, $F(2,24)=4$ 62, $p<0$ 05 FG 7142 caused a significant reduction in the percentage of time spent on the open arms, $F(2,25)=12$ 77, $p<0$ 0005, and posthoc analysis showed a significant effect at both doses compared with controls, $p<0$ 01, (see Fig 3)

CGS 8216 (3–10 mg/kg) caused a significant reduction in the total number of arm entries, $F(2,24)=15$ 91, $p<0$ 0001, an effect that reached significance at both doses tested compared with controls, $p<0$ 01, (see Table 1) There was an effect of borderline significance on the percentage of open arm entries, $F(2,24)=3$ 30, $p<0.06$, posthoc analysis showed that at 10 mg/kg, CGS 8216 significantly reduced this measure compared to controls, $p<0$ 05, (see Fig 3) Analysis of covariance showed that the reduction in open arm entries was quite independent of any reduction in closed arm entries, $F(2,23)=4$ 07, $p<0$ 05 CGS 8216 also significantly reduced the percentage of time spent on the open arms, $F(2,24)=11$ 59, $p<0$ 0005, posthoc analysis showed that both doses of CGS 8216 significantly reduced this measure compared with controls, $p<0$ 01, (see Fig 3)

Ro 15-1788 (10–20 mg/kg) had no significant effect on the total number of arm entries, $F(2,21)=0$ 31, the percentage of open arm entries, $F(2,21)=1$ 54, or the percentage of time spent on the open arms, $F(2,21)=0$ 29 (see Table 1 and Fig 3)

ZK 93426 (5–10 mg/kg) had no significant effect on the total number of arm entries, $F(2,25)=0$ 33, the percentage of open arm entries, $F(2,25)=0$ 52, or the percentage of time spent on the open arms, $F(2,25)=0$ 50 (see Table 1 and Fig 3)

Ro 5-4864 (1–5 mg/kg) significantly reduced the total number of arm entries, $F(2,23)=4$ 92, $p<0$ 05, posthoc analysis showed that at 5 mg/kg Ro 5-4864 significantly reduced entries compared with controls, $p<0$ 01, (see Table 1) Ro 5-4864 reduced the percentage of open arm entries, $F(2,25)=3$ 34, $p<0$ 06, an effect that reached significance at the 5 mg/kg dose compared with controls, $p<0$ 01, (see Fig 3) Analysis of covariance showed that the reduction in open arm entries was quite independent of any reduction in closed arm entries, $F(2,22)=3$ 07, $p<0$ 07 Ro 5-4864, similarly, reduced the percentage of time spent on the open arms, $F(2,23)=7$ 49, $p<0$ 005, an effect that reached significance at the 5 mg/kg does, $p<0$ 01, (see Fig 3)

DISCUSSION

The present study has shown that the elevated plus-maze can detect the anxiolytic activity, not only of benzodiazepine-like compounds, but of putative anxiolytics from other chemical classes It has been hypothesised [23] that the preference shown by vehicle-treated rats for the closed arms reflects an aversion toward the open arms, caused by fear or anxiety induced by high and open spaces Diazepam (2 mg/kg) significantly elevated the percentage of open arm entries (to 158% of control levels) and of time spent in the open arms (to 171% of control levels) and of time spent in the open arms (to 171% control levels), and also reduced the total number of arm entries at this dose (to 66% of control levels) Both tracazolate (5 but not 25 mg/kg) and CL 218,872 (10–20 mg/kg) elevated the percentage of open arm entries and the percentage of time spent in the open arms These results agree with those from other tests Tracazolate (5 but not 25 mg/kg) also had an anxiolytic-like profile in the social interaction test [9], and at higher doses (around 25 mg/kg) was active in the Vogel punished drinking test and a test of punished locomotor activity in the mouse [21] Interestingly, however, tracazolate has no effect in the Geller-Seifter conflict procedure [21] CL 218,872 (5–10 mg/kg) similarly had anxiolytic effects in the social interaction test [3] and is also active in the Vogel punished drinking test [18]

The elevated plus-maze, like most other animal tests of anxiety, failed to detect anxiolytic activity of PK 8165, buspirone or tofisopam The former compound was initially suggested to be a putative anxiolytic on the basis of results showing an increase in punished drinking in the Vogel conflict test [17] However, Pellow [22] showed that PK 8165 also increased unpunished drinking No effects have been found in other tests of anxiety [5,16] and indeed the effect that we did find in the plus-maze was in the *anxiogenic* rather than the anxiolytic direction Such a finding is consistent with the reported proconvulsant properties of PK 8165 [13] Although both tofisopam and buspirone have been found to be clinically effective anxiolytics animal tests have failed to consistently identify these compounds as anxiolytics (see Pellow and File [26,27], File [4]) The possibility that the anxiety reduction observed with these two compounds in the clinic differs qualitatively from that observed with classical anxiolytics, is discussed by Pellow and File [26]

The elevated plus-maze was also very sensitive to the effects of a number of anxiogenic compounds FG 7142, a β-carboline derivative shown to induce severe anxiety in man [2] and in animals [8,28], selectively reduced the percentage of open arm entries and of time spent on the open arms According to the hypothesis of Pellow *et al* [23], this would reflect increased aversion to the open arms due to increased fear or anxiety Similarly, anxiogenic effects were obtained with the pyrazoloquinoline CGS 8216, that has anxiogenic activity in several other test procedures [5 28] and the atypical benzodiazepine Ro 5-4864, that is anxiogenic in the social interaction test [16] and the Vogel test [25]

Interestingly, anxiogenic effects were not seen with the benzodiazepine receptor antagonists Ro 15-1788 and ZK 93426 Both compounds have anxiogenic activity in the social interaction test [7,12] and the Vogel test [10,15], however, certain other tests have failed to detect their anxiogenic actions [11,15] and the anxiogenic properties of Ro 15-1788 are less evident in man (see File and Pellow [11] for review) These results support our hypothesis [11] that the behavioural actions of benzodiazepine receptor antagonists are critically dependent on the test situation, probably reflecting the level of the endogenous tone within the system

In conclusion, the present study extends the investigations into the behavioural actions of putative anxiolytic and anxiogenic compounds in animal tests In addition, we have extended the pharmacological validation of the elevated plus-maze as a measure of anxiety in the rat, showing that it can detect the activity of non-benzodiazepine anxiolytics, and of several putative anxiogenic compounds

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