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The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard

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1 In addition to possessing anti-anxiety activity in man, triazolobenzodiazepines have been reported to have antidepressant and antipanic properties. In this they differ from classical 1,4-benzodiazepines that have only anti-anxiety activity. The purpose of the present study was to examine the effects of the triazolobenzodiazepines in two animal tests of anxiety and in the holeboard, to see whether clear differences could be observed between them and the 1,4-benzodiazepines.

2 After acute administration, U-43,465 (16 mg kg^{-1}) had a significant anxiolytic effect in the social interaction test. Neither adinazolam (1–3.5 mg kg^{-1}) nor alprazolam (0.125–2 mg kg^{-1}) had a significant effect. It is suggested that this is because, with adinazolam and alprazolam, doses at which anxiolytic effects can be observed are close to those at which sedative effects can be observed.

3 U-43,465 (8–16 mg kg^{-1}) and alprazolam (1–2 mg kg^{-1}) had significant anxiolytic effects in the elevated plus-maze test of anxiety.

4 U-43,465 (8–32 mg kg^{-1}), adinazolam (0.5–5 mg kg^{-1}) and alprazolam (0.2–2.0 mg kg^{-1}) caused dose-related reductions in exploratory head-dipping, locomotor activity and rearing in the holeboard.

5 In general the results seen in the three tests with the triazolobenzodiazepines alprazolam and adinazolam were similar to those seen with classical 1,4-benzodiazepines. With U-43,465, however, an anxiolytic effect was observed in the social interaction test after acute treatment; chronic treatment is required to see an effect with classical 1,4-benzodiazepines. In this U-43,465 resembles the effects of several novel non-benzodiazepine putative anxiolytic compounds that are believed to have less sedative potential than the benzodiazepines.

Introduction

Alprazolam, adinazolam and U-43,465 (8-chloro-1-(2-dimethyl-amino) ethyl)-6-phenyl-4H-s-triazolo (4,3-a) (1,4)benzodiazepine *p*-toluenesulphonate) are triazolobenzodiazepine derivatives that, in addition to possessing benzodiazepine-like activity at benzodiazepine receptors and in animal tests (Sethy & Hodges, 1982; Thiébot *et al.*, 1982; von Voigtlander & Puech, 1983), have additional antidepressant activity that may be conferred by the triazolo ring (Sethy & Hodges, 1982; von Voigtlander & Puech, 1983; Lahti *et al.*, 1983; O'Connor *et al.*, 1985). Alprazolam has been found in double-blind clinical trials to possess, in the same dose range (1.0–4.0 mg day^{-1}), potent anxiolytic (Cohn, 1981; Davison *et al.*, 1985) and antidepressant (Fabre & McLendon, 1980; Ansseau *et al.*, 1984) activity; at higher doses (3–9 mg day^{-1}) open studies (Sheehan, 1982; Chouinard *et al.*, 1982) and a double-blind study (Dunner *et al.*, 1984) have shown that alprazolam is also effective in the treatment of panic disorders. Alprazolam therefore differs markedly from classical 1,4-benzodiazepines, that are believed to possess only anxiolytic activity without antidepressant or antipanic properties (see Boulenger & Uhde, 1983; Johnson, 1985).

These drugs have not been widely studied in animal tests; the purpose of the present studies was to investigate the actions of the triazolobenzodiazepines in several tests of behaviour in the rat that are sensitive to the effects of 1,4-benzodiazepines and to see whether differences can be observed between the two kinds of compound that parallel their different profiles in man. The activity of these three triazolobenzodiazepines was investigated in two extensively validated animal tests of anxiety, both of which can detect the anxioytic effects of classical 1,4-benzodiazepines and novel putative anxioytics: (a) the social interaction test (File & Hyde, 1978; File, 1980, 1985a): in this test, the decrease in social interaction

between pairs of rats placed in a novel or brightly-lit enclosure is attenuated by anxiolytics: and (b) the elevated plus-maze (Pellow *et al.*, 1985; Pellow & File, 1985a): in this test, the aversion that rats show for the two open arms of an elevated plus-maze is attenuated by anxiolytics. In order to assess the sedative liability of these compounds, their effects were also studied in the holeboard, which provides independent measures of exploratory head-dipping and locomotor activity (File & Wardill, 1975) and which is sensitive to the depressant effects of a variety of compounds with different mechanisms of action (see File, 1985b for review).

Methods

Male hooded Lister rats (Olac Ltd., Bicester) weighing 150–200 g were used. For the social interaction test, rats had been singly-housed for 5 days before the experiment, and were allocated to pairs on the basis of weight. For the holeboard test, rats were housed in groups of 8. Animals were housed in a room with an 11 h light: 13 h dark cycle and allowed free access to food and water.

Apparatus and procedure

Social interaction test The test arena was a wooden box, 60 × 60 × 35 cm, with infrared photocells 4.5 cm from the floor to provide a measure of motor activity. On day one of testing, pairs of rats were placed in the test arena, with which they were unfamiliar, for 7.5 min, under low illumination. On day two, the rats were placed individually, undrugged, in the box for 7.5 min to familiarise them with the apparatus. On the third day, the same pairs of rats were once again placed in the test arena for 7.5 min. On test days, the duration of the following social behaviours was scored, from a video camera, by two independent observers sitting in a different room, and entered directly into a Control Universal microcomputer: sniffing, following, grooming, kicking, boxing, biting, wrestling, crawling under or over the partner. Passive body contact was scored separately. Rats were tested in an order randomised for drug-treatment between 0.8 h 00 min and 12 h 30 min. Pairs of rats were allocated to the following test groups: (a) vehicle control, adinazolam (1, 2 and 3.5 mg kg^{-1}), U-43,465 (16 and 32 mg kg^{-1}); (b) vehicle control, alprazolam (0.125, 0.5, 1 and 2 mg kg^{-1}), $n = 7-10$ pairs per group. Both members of the pair always received the same drug treatment, which was administered 30 min before testing.

Elevated plus-maze The plus-maze consisted of two open arms (50 × 10 cm), facing each other, and two closed arms (50 × 50 × 40 cm) with an open roof, and was elevated to a height of 50 cm. The following measures were taken by an observer during a 5 min test period after the rat had been placed on the centre of the maze: the number of entries into, and the time spent in, each of the two types of arm. The total number of arm entries provided a measure of general activity. Rats were tested in an order randomised for drug treatment between 14 h 00 min and 16 h 00 min, after random allocation to the following treatment groups: vehicle control ($n = 16$), alprazolam (1 and 2 mg kg^{-1}), adinazolam (2 and 4 mg kg^{-1}), U-43,465 (8 and 16 mg kg^{-1}), $n = 8$ per group.

Holeboard The holeboard was a wooden box, 60 × 60 × 35 cm, with four holes of 3.8 cm diameter equally spaced in the floor. Infrared photocells in the sides of the box, 4.5 and 11 cm from the floor, provided measures of locomotor activity and of the number of rears, respectively. Photocells below the surface of the holes provided measures of the number of head-dips and the time spent head-dipping. All measures were entered directly into a Control Universal microcomputer. Rats were placed singly in the centre of the holeboard, and during a 7.5 min trial the following measures were recorded by computer: the number of head-dips, the time spent head-dipping, the locomotor activity score, the number of rears. Rats were tested in an order randomised for drug treatment between 07 h 30 min and 12 h 30 min, after random allocation to the following groups: (a) vehicle control, alprazolam (0.2, 1 and 2 mg kg^{-1}), adinazolam (0.5, 1 and 5 mg kg^{-1}); (b) vehicle control, U-43,465 (8, 16 and 32 mg kg^{-1}), $n = 8$ per group.

Statistics

All data were analysed by analysis of variance: one for each drug treatment, with dose as the factor (for social interaction, with dose and familiarity as the two factors). Posthoc comparisons between individual treated groups and controls were made with Dunnett's t tests.

Drugs

Alprazolam, adinazolam and U-43,465 (Upjohn) were dissolved in distilled water with a drop of Tween 20, and injected i.p. 30 min before testing in concentrations to give an injection volume of 2 ml kg^{-1}. Control animals received the drug vehicle.

Results

Social interaction test

In experiments with alprazolam, adinazolam and U-43,465 there was a significant familiarity term

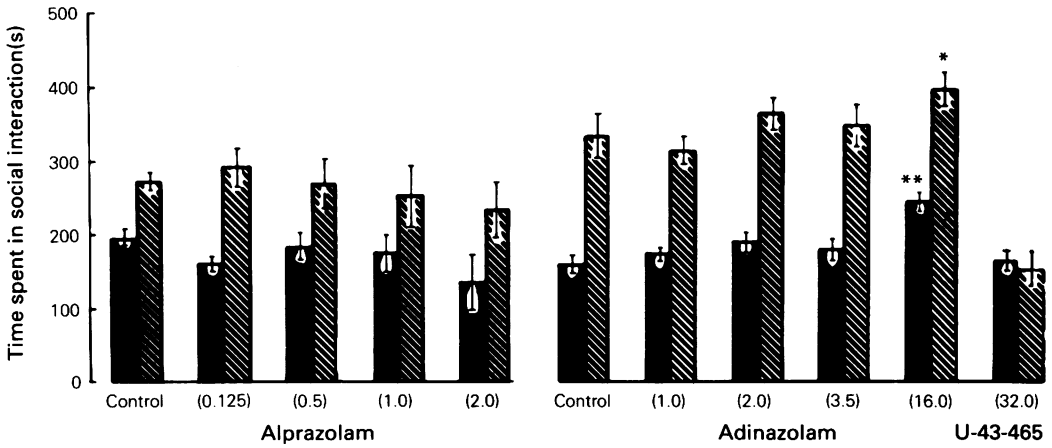

Figure 1 Mean time (s) spent in active social interaction for pairs of male rats given a 7.5 min trial, 30 min after drug injection; s.e.means shown by vertical lines. Drugs were: alprazolam, adinazolam and U-43,465 (mg kg^{-1} in parentheses), $n = 7-10$ pairs per group. Test conditions were low light, unfamiliar (filled columns) and low light, familiar (hatched columns). *$P < 0.05$, **$P < 0.01$, significantly different from controls, Dunnett's t test after analysis of variance.

on the ANOVA for social interaction in each case ($F(1,34) = 101.5$, $P < 0.0001$; $F(1,28) = 9.32$, $P < 0.005$; $F(1,26) = 46.79$, $P < 0.0001$, respectively), indicating that social interaction increased with increasing familiarity (Figure 1). Neither alprazolam (0.125–2.0 mg kg^{-1}) nor adinazolam (1.0–3.5 mg kg^{-1}) had a significant effect on social interaction ($F(4,34) = 0.9$ and $F(3,28) = 0.82$, respectively); the drug × familiarity interaction term similarly did not reach significance in either case ($F(4,34) = 1.33$ and $F(3,28) = 0.44$ respectively), see Figure 1. However, there was a significant drug effect on motor activity ($F(4,34) = 11.91$, $P < 0.0001$; $F(3,28) = 13.46$, $P < 0.0001$ respectively) and Table 1 shows that alprazolam (2 mg kg^{-1}) and adinazolam (3.5 mg kg^{-1}) significantly reduced motor activity in both unfamiliar and familiar test conditions.

In contrast to alprazolam and adinazolam, U-43,465 (16–32 mg kg^{-1}) caused a significant drug effect on social interaction ($F(2,26) = 27.25$, $P < 0.0001$). Subsequent analysis showed that there were differences between the effects of U-43,465 at 16 and at 32 mg kg^{-1}. At 16 mg kg^{-1}, U-43,465 significantly elevated the time spent in active social interaction in both unfamiliar and familiar test conditions (Figure 1). At this dose there was no significant effect on locomotor activity (Table 1). There was no significant effect on social interaction at 32 mg kg^{-1} (Figure 1). Analysis showed that at 32 mg kg^{-1}, U-43,465 significantly reduced motor activity scores in both test conditions (Table 1). There was a significant drug × familiarity interaction term on social interaction ($F(2,26) = 15.43$, $P < 0.0001$), suggesting that the drug effects differed in the two test conditions. No clear differences were observed at the 16 mg kg^{-1} dose between test conditions (there was a significant elevation of social interaction in each); this effect is therefore most likely to be explicable by the fact that the group of rats treated with U-43,465 (32 mg kg^{-1}) had not increased their scores with increasing familiarity as happened with the control groups and U-43,465 (16 mg kg^{-1}) (Figure 1). It is not clear why

Table 1 Mean (± s.e.mean) locomotor activity score for pairs of rats given a 7.5 min social interaction trial, 30 min after injection with alprazolam (0.125–2 mg kg^{-1}), adinazolam (1–3.5 mg kg^{-1}) or U-43,465 (16–32 mg kg^{-1})

Drug (mg kg^{-1})		*LU*	*LF*
Control		988.0 ± 37.98	1043.1 ± 29.38
Adinazolam	1.0	916.9 ± 26.74	997.9 ± 58.00
	2.0	841.4 ± 23.18	1164.5 ± 43.02
	3.5	698.4 ± 71.03*	796.2 ± 81.67*
U-43,465	16.0	918.6 ± 42.30	1046.7 ± 26.75
	32.0	718.5 ± 56.98*	248.1 ± 28.32*
Control		841.8 ± 31.28	832.0 ± 27.65
Alprazolam	0.125	715.5 ± 40.79	858.6 ± 32.36
	0.5	645.2 ± 44.20	845.8 ± 45.60
	1.0	646.8 ± 72.30	577.2 ± 44.50
	2.0	432.5 ± 87.40*	331.5 ± 58.64*

$n = 7-10$ pairs per group. Test conditions were low light, unfamiliar (LU) and low light, familiar (LF). *$P < 0.01$ significantly different from controls, Dunnett's t test after analysis of variance.

Table 2 Mean (± s.e.mean) total number of arm entries for rats given a 5 min test in the elevated plus-maze, 30 min after drug treatment

Drug (mg kg^{-1})		*Total*
Control		12.1 ± 0.97
Alprazolam	1	9.5 ± 0.88
	2	9.2 ± 1.19
Adinazolam	2	11.5 ± 1.50
	4	8.9 ± 0.61
U-43,465	8	8.4 ± 1.38
	16	6.4 ± 1.06*

$n = 8$ per group.
*$P < 0.05$, Dunnett's t test after analysis of variance.

this should be so; however, Table 1 shows that the effects of U-43,465 at this dose on the second test were considerably stronger than on the first test i.e. there was a much greater reduction in motor activity. We do not know why the effects of this drug at this dose were greater on the second treatment; one possible explanation is that U-43,465 may have an active metabolite with a long half-life.

Elevated plus-maze

Alprazolam (1–2 mg kg^{-1}) did not significantly alter the total number of arm entries made by rats ($F(2,30) = 2.32$, Table 2). However, alprazolam significantly elevated both the percentage of open arm entries ($F(2,30) = 15.67$, $P < 0.001$) and of time spent on the open arms ($F(2,30) = 22.9$, $P < 0.0001$); Dunnett's tests showed that at 2 mg kg^{-1} alprazolam significantly differed from controls (Figure 2). Adinazolam (2–4 mg kg^{-1}) did not significantly change the total number of arm entries made by rats ($F(2,29) = 2.08$, Table 2). However, adinazolam significantly elevated both the percentage of open arm entries ($F(2,29) = 15.66$, $P < 0.0001$) and the percentage of time spent on the open arms ($F(2,29) = 13.77$, $P < 0.0001$); Dunnett's tests showed that at 4 mg kg^{-1} adinazolam significantly differed from controls (Figure 2). U-43,465 (8–16 mg kg^{-1}) significantly reduced the total number of arm entries made by rats ($F(2,28) = 6.32$, $P < 0.01$); analysis showed that at 16 mg kg^{-1} rats made significantly fewer entries compared with controls (Table 2). Although there was no significant overall drug effect on the percentage of open arm entries ($F(2,28) = 2.15$), Dunnett's tests showed that at 16 mg kg^{-1} U-43,465 significantly elevated the percentage of open arm entries compared with controls (Figure 2). There were no significant effects on the percentage of time spent on the open arms ($F(2,30) = 0.52$).

Holeboard

Alprazolam had significant effects on the number of head-dips, locomotor activity and the number of rears ($F(3,28) = 3.41$, $P < 0.05$; $= 7.9$, $P < 0.001$; and $= 4.03$, $P < 0.05$, respectively). Analysis showed that at the highest dose (2 mg kg^{-1}) alprazolam significantly reduced the number of head-dips and the motor activity score; at 1 mg kg^{-1} there was also a significant reduction in motor activity (Table 3). None of the individual drug groups differed significantly from the

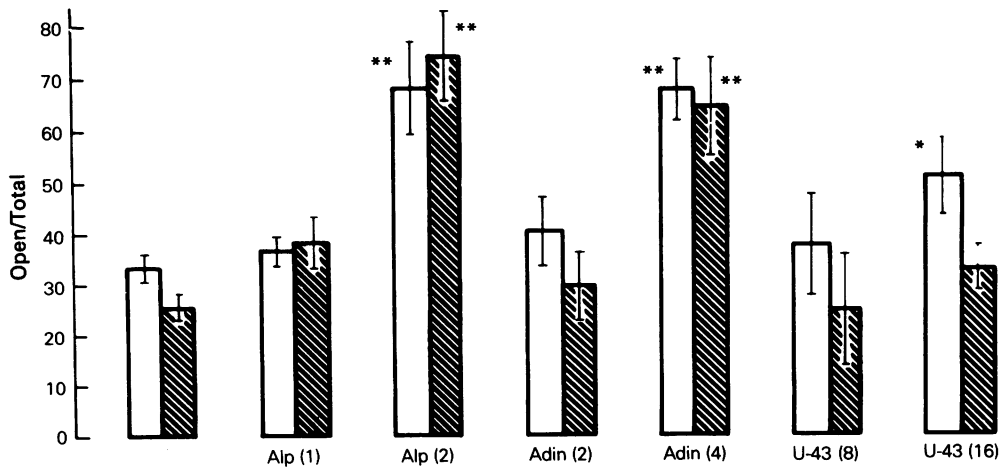

Figure 2 Mean total percentage of open arm entries (open columns) or of time spent in the open arms (hatched columns) of an elevated plus-maze, in rats given a 5 min test, 30 min after drug administration; s.e.means shown by vertical lines. Drugs were: alprazolam (Alp), adinazolam (Adin) and U-43,465 (mg kg^{-1} in parentheses), $n = 8$ rats per group. *$P < 0.05$, **$P < 0.01$, significantly different from controls, Dunnett's t test after analysis of variance.

Table 3 Mean (± s.e.mean) number of head-dips, time (s) spent head-dipping, locomotor activity score, and number of rears for rats given a 7.5 min holeboard trial, 30 min after drug injection

Drug (mg kg^{-1})		*Number*	*Time*	*Motor*	*Rears*
Control		47.0 ± 4.03	33.5 ± 3.22	392.5 ± 52.46	31.4 ± 7.13
Alprazolam					
	0.125	44.1 ± 3.42	36.7 ± 4.14	285.0 ± 21.12	44.9 ± 3.05
	1.0	34.4 ± 5.38	28.9 ± 4.69	190.0 ± 29.12**	22.0 ± 5.13
	2.0	25.4 ± 7.53*	35.4 ± 9.87	152.4 ± 42.31**	18.4 ± 7.22
Adinazolam					
	1.0	41.5 ± 5.25	48.4 ± 4.83	531.1 ± 24.04	36.6 ± 3.85
	2.0	44.6 ± 3.79	38.5 ± 3.91	477.2 ± 28.15	28.4 ± 3.60
	5.0	22.6 ± 6.70**	35.7 ± 10.97	242.4 ± 55.63**	8.4 ± 3.39**
Control		26.9 ± 2.67	25.2 ± 2.51	335.5 ± 30.62	34.1 ± 1.91
U-43,465					
	8	20.0 ± 3.60	19.8 ± 3.63	319.7 ± 25.23	38.7 ± 3.27
	16	19.1 ± 3.83	20.5 ± 3.29	330.1 ± 32.74	36.0 ± 4.62
	32	3.9 ± 0.87**	4.1 ± 0.92**	129.2 ± 24.94**	13.7 ± 4.23**

$n = 8$ per group.
*$P < 0.05$, **$P < 0.01$, significantly different from controls, Dunnett's t test after analysis of variance.

controls on the number of rears made (Table 3). There was no significant effect on the time spent head-dipping ($F(3,28) = 0.32$, Table 37). Adinazolam had significant effects on the number of head-dips, locomotor activity and the number of rears ($F(3,28) = 4.78$, $P < 0.01$; $= 8.79$, $P < 0.0005$; and $= 6.76$, $P < 0.005$, respectively). Analysis showed that at the highest dose (5 mg kg^{-1}) adinazolam significantly reduced all three measures (Table 3). There were no significant effects on the time spent head-dipping ($F(3,28) = 0.81$).

U-43,465 had significant effects on the number of head-dips, the time spent head-dipping, locomotor activity and the number of rears ($F(3,28) = 10.58$, $P < 0.0001$; $= 10.86$, $P < 0.0001$; $= 12.2$, $P < 0.0001$; and $= 9.75$, $P < 0.0001$, respectively). Analysis showed that the highest dose of U-43,465 (32 mg kg^{-1}) caused significant reductions in all four measures (Table 3).

Discussion

The results from the holeboard test show that, like classical 1,4-benzodiazepines, all three triazolobenzodiazepines reduced exploratory head-dipping and locomotor activity in the rat. The minimum depressant doses in the test were: for alprazolam, 2 mg kg^{-1}; for adinazolam, 5 mg kg^{-1}; for U-43,465, 32 mg kg^{-1}. All three triazolobenzodiazepines produced an anxiolytic-like effect in the elevated plus-maze test. Pellow *et al.* (1985) proposed that the preference shown by vehicle-treated rats for the closed arms of the maze reflects an aversion for the open arms, caused by fear of heights and open spaces. The present results suggest that, like 1,4-benzodiazepines (Pellow *et al.*, 1985) and non-benzodiazepine putative anxiolytics (Pellow & File, 1985a), alprazolam, adinazolam and U-43,465 reverse this aversion for the open arms. The effective doses were: for alprazolam, 2 mg kg^{-1}; for adinazolam, 4 mg kg^{-1}; for U-43,465, 16 mg kg^{-1}. We have previously found the effective doses for chlordiazepoxide and diazepam, classical 1,4-benzodiazepines, to be 7.5 and 2 mg kg^{-1}, respectively (Pellow *et al.*, 1985).

In the social interaction test, benzodiazepines (File, 1980) and novel putative anxiolytics (File, 1982; File & Pellow, 1985a,b; Pellow & File 1985b prevent the reduction in social interaction induced by an unfamiliar environment or high lighting conditions. In the case of 1,4-benzodiazepines, the sedative effects of these compounds lead to a depression of all spontaneous behaviour in this test; chronic treatment is therefore necessary so that an increase in social interaction can be seen when tolerance has developed to the sedative effects of the drugs (File, 1980). Since alprazolam and adinazolam also possess sedative properties, as measured in the present study by a depression of spontaneous exploratory and locomotor activity in the holeboard, this could explain why anxiolytic effects were not observed in the social interaction test. Doses below those causing sedation, as in the case of other benzodiazepines, were ineffective. However, an anxiolytic effect was observed with U-43,465 at a dose (16 mg kg^{-1}) that did not cause appreciable sedation either in the social interaction or the holeboard tests; this effect was no longer obtainable at a higher (sedative) dose (32 mg kg^{-1}). These

Table 4 A comparison of the minimal effective doses of the 1,4-benzodiazepine chlordiazepoxide and the triazolobenzodiazepines alprazolam, adinazolam and U-43,465 on measures of anxiety and sedation in the holeboard, the social interaction test, and the elevated plus-maze

Drug	*Holeboard*	*Social interaction*		*Plus-maze*	
	Sedation (mg kg)	*Sedation (mg kg)*	*Anxiety (mg kg)*	*Sedation (mg kg)*	*Anxiety (mg kg)*
Chlordiazepoxide	5–7.5	5	5 (chronic)	7.5	7.5
Alprazolam	1–2	2	—	—	2
Adinazolam	5	3.5	—	—	4
U-43,465	32	32	16	16	16

Drug administration (all mg kg^{-1}) was acute unless stated otherwise

results suggest that the effective anxiolytic dose-range of U-43,465 in the social interaction test is more distinctly separated from the sedative dose-range than is the case with the other two triazolobenzodiazepines or with 1,4-benzodiazepines. Similar results have been obtained with four other non-benzodiazepine putative anxiolytics: CL 218,872 (3-methyl-6-[3-(trifluoromethyl)-phenyl]-1,2,4-triazolo [4,3-b]pyridazine) (File, 1982), tracazolate (File & Pellow, 1985a), CGS 9896 (2-(4-chlorophenyl)-2,5-dihydropyrazolo [4,3-c]quinolin-3(3H)-one) (File & Pellow 1985b) and the β-carboline ZK 91296 (Pellow & File 1985b). Interestingly, the pattern of effects obtained with U-43,465 resembled that observed with barbiturates rather than 1,4-benzodiazepines. Barbiturates tend to cause an elevation in social interaction in both familiar and unfamiliar test conditions; in contrast, the benzodiazepines have a greater effect the more anxiogenic the test condition. Similar results have been obtained with other novel putative anxiolytics (File, 1982; File & Pellow 1985a,b; Pellow & File, 1985b).

Table 4 provides a comparison of the effective doses of the triazolobenzodiazepines on measures of sedation and anxiety in the test procedures used in the present study. The sedative doses of these compounds in the holeboard and in the social interaction test show considerable similarity; however, differences emerge in the elevated plus-maze, particularly with U-43,465. Whereas in the holeboard and the social interaction test, reductions in motor activity are observed only at a dose of 32 mg kg^{-1}, in the elevated plus-maze a reduction in the total number of arm entries is apparent at 16 mg kg^{-1}, i.e. the plus-maze is more sensitive to the behavioural depressant effects of U-43,465. The other two triazolobenzodiazepines, in contrast, do not reduce activity in the plus-maze even at doses that reduced activity in the other two tests, i.e. the plus-maze is less sensitive to their depressant effects. It is possible that the differences between the plus-maze and the social interaction and holeboard results are explicable by the differing methods for measuring activity. In the social interaction test and the holeboard, activity is measured by the breaking of photobeams as the rats move around the arena. In the plus-maze, activity is measured by the total number of entries into the arms of the maze. Whilst, particularly in the holeboard, motor activity is less contaminated by exploratory factors (since a measure of directed exploration is provided by head-dipping) this is not the case in the plus-maze, and the general activity is likely to reflect both motor activity and exploratory tendencies. It is therefore possible that the two types of measure are differentially sensitive to drug effects. In conclusion, apart from the anxiolytic effect of U-43,465 after acute treatment in the social interaction test, clear differences have not emerged between the triazolobenzodiazepines and the 1,4-benzodiazepines in the three test procedures considered in the present study.

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