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ORIGINAL INVESTIGATION

Guy Griebel · R. John Rodgers · Ghislaine Perrault
David J. Sanger

Behavioural profiles in the mouse defence test battery suggest anxiolytic potential of 5-HT$_{1A}$ receptor antagonists

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Abstract *Rationale:* Compounds varying in selectivity as 5-HT$_{1A}$ receptor antagonists have recently been reported to produce anxiolytic-like effects comparable to those of benzodiazepines in the mouse elevated plus-maze procedure. *Objective:* In view of the potential clinical significance of these findings, the present experiments compared the behavioural effects of diazepam (0.5–3.0 mg/kg) with those of several non-selective 5-HT$_{1A}$ receptor antagonists [NAN-190, 0.1–3.0 mg/kg, MM-77, 0.03–1.0 mg/kg, (*S*)-UH-301, 0.3–3.0 mg/kg and pindobind-5-HT$_{1A}$, 0.03–1.0 mg/kg], and three selective 5-HT$_{1A}$ receptor antagonists (WAY100635, 0.01–3.0 mg/kg, *p*-MPPI, 0.1–3.0 mg/kg and SL88.0338, 0.3–3.0 mg/kg) in the mouse defence test battery (MDTB). *Methods*: In this well-validated anxiolytic screening test, Swiss mice are directly confronted with a natural threat (a rat) as well as situations associated with this threat. Primary measures taken during and after rat confrontation were flight, risk assessment (RA), defensive threat/attack and escape attempts. *Results:* Diazepam significantly decreased flight reactions after the rat was introduced into the runway, reduced RA activities of mice chased by the rat, increased RA responses displayed when subjects were constrained in a straight alley and reduced defensive upright postures and biting upon forced contact. All the selective 5-HT$_{1A}$ receptor antagonists and NAN-190 also reduced flight, RA in the chase test, and defensive threat and attack behaviours. (*S*)-UH-301 and pindobind-5-HT$_{1A}$ reduced RA in the chase test, but only partially modified defensive threat and attack. Unlike the other drugs tested, MM-77 produced significant effects only at doses which also markedly reduced spontaneous locomotor activity, suggesting a behaviourally non-specific action. In contrast to diazepam, the 5-HT$_{1A}$ receptor ligands failed to affect RA in the straight alley test. Following removal of the rat from the test area, only diazepam and (*S*)-UH-301 reduced escape behaviour (contextual defence) at doses which did not decrease locomotion. Overall, the present findings indicate that except for one RA behaviour and escape responses, the 5-HT$_{1A}$ receptor ligands studied modified the same defensive behaviours as diazepam, suggesting potential therapeutic efficacy in the management of anxiety disorders. However, the magnitude of the effects of the 5-HT$_{1A}$ compounds on defence was generally smaller than that of the benzodiazepine. *Conclusion:* As all of the 5-HT$_{1A}$ compounds tested in this series share antagonistic activity in models of postsynaptic 5-HT$_{1A}$ receptor function, it is proposed that this action accounts for their effects on defence.

Key words Anxiety · Defensive behaviour · 5-HT$_{1A}$ receptor antagonist · Diazepam · Flight · Risk assessment · Swiss mice

G. Griebel (✉) · G. Perrault · D.J. Sanger
Synthélabo Recherche, 31 avenue Paul Vaillant-Couturier,
F-92220 Bagneux, France
e-mail: ggriebel@compuserve.com,
Fax: +33-1-45-36-20-70

R.J. Rodgers
School of Psychology, University of Leeds, Leeds LS2 9JT, UK

Introduction

Since the discovery that the non-benzodiazepine anxiolytic buspirone modifies 5-hydroxytryptamine (5-HT) neurotransmission (Hjorth and Carlsson 1982) via an action at 5-HT$_{1A}$ receptors (Gozlan et al. 1983), much research has focused on understanding the role of this receptor in anxiety disorders. Although the development of selective agonists for 5-HT$_{1A}$ receptors has facilitated investigations of their functional role in these pathophysiological states, full characterization of this involvement has been hampered by the lack of selective antagonists for these sites. In addition to their utility as research tools, it has been suggested that selective 5-HT$_{1A}$ receptor antagonists may themselves have anxiolytic potential (Fletcher et al. 1993b). The rationale for this proposal is based on the assumption that a general decrease in 5-HT release has an anxiolytic effect (Gardner 1986). As 5-HT$_{1A}$ receptors are located in high densities in forebrain areas which are be-

lieved to be involved in emotional processes (Pazos et al. 1987), it would be predicted that 5-HT_{1A} receptor antagonists should have anxiolytic activity.

Until recently, only non-selective agents have been used as 5-HT_{1A} receptor antagonists. These include (–)-pindolol and (–)-propranolol, which have greater affinity for β-adrenoceptors than for 5-HT_{1A} receptors (Hoyer 1988; Pierson et al. 1990; Liau et al. 1991), and spiperone, which displays high affinity for 5-HT_2 and dopamine D_2 receptors (Leysen et al. 1981; Hoyer 1988). A number of compounds were initially designated as selective 5-HT_{1A} receptor antagonists, e.g. BMY7378 (Yocca et al. 1987), NAN-190 (Glennon et al. 1988) and MM-77 (Mokrosz et al. 1994) but, while demonstrating antagonistic-like activity in postsynaptic 5-HT_{1A} receptor models, these compounds showed agonist-like activity at presynaptic somatodendritic 5-HT_{1A} receptors (Hjorth and Sharp 1990; Sharp et al. 1990; Claustre et al. 1991; Mokrosz et al. 1994). The first ligands which displayed consistent 5-HT_{1A} receptor antagonist properties were the (*S*)-enantiomer of 5-fluoro-8-OH-DPAT, (*S*)-UH-301 (Hillver et al. 1990), the phenyl-piperazine derivative, WAY100135 (Fletcher et al. 1993a), and the pindolol derivative, pindobind-5-HT_{1A} (Liau et al. 1991). However, (*S*)-UH-301 and pindobind-5-HT_{1A} have only 8- and 9-fold selectivity for 5-HT_{1A} relative to D_2 receptors and α_1-adrenoceptors, respectively (Hillver et al. 1990; Liau et al. 1991). Furthermore, (*S*)-UH-301 was found to display D_2 agonist-like activity (Arborelius et al. 1993), while WAY100135 has demonstrated both 5-HT_{1A} receptor partial agonist activity (Millan et al. 1993; Assie and Koek 1995) and α_1-adrenoceptor antagonism (Routledge 1995). It is only within the last few years that selective 5-HT_{1A} receptor antagonists have become available. These include the phenyl-piperazine derivative WAY100635 and its close structural analogues, *p*-MPPI and the amino-methyl-piperidine SL88.0338. WAY100635, *p*-MPPI and SL88.0338 display high affinities for 5-HT_{1A} receptors (K_i=4.5, 1 and 2 nM, respectively) but only low to moderate affinities for α_1, D_2 and β receptors, and have demonstrated antagonistic-like activity at both somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors (Kung et al. 1994, 1995; Zhuang et al. 1994; Fletcher et al. 1995; Forster et al. 1995; Assie and Koek 1996; Thielen et al. 1996; Cohen et al. 1998).

Studies of the effects of selective and non-selective 5-HT_{1A} receptor antagonists on anxiety-related behaviours have produced variable results (for reviews, see Griebel 1995; Cao and Rodgers 1997a,b). For example, although anxiolytic-like effects have been reported with WAY100635 in a variety of models, including the mouse light/dark (Sanchez 1996) and elevated plus-maze (Cao and Rodgers 1997b) tests, and the rat fear-potentiated startle model (Joordens et al. 1997), negative findings have been obtained in rat and pigeon conflict (Overshiner et al. 1995; Samanin et al. 1996; King et al. 1997; Millan et al. 1997), ultrasonic vocalization (Bartoszyk et al. 1996; Brocco et al. 1996; Remy et al. 1996; Xu et al. 1997; Schreiber et al. 1998), conditioned emotional response (Overshiner et al. 1995; Stanhope and Dourish 1996), stress-induced hyperthermia (Olivier et al. 1998), social interaction (File et al. 1996) and rat elevated plus-maze (Bickerdike et al. 1995; File et al. 1996; Collinson and Dawson 1997; Millan et al. 1997) tests. Furthermore, at certain doses, WAY100635 was found to display anxiogenic-like activity in the shock-induced ultrasonic vocalization (Groenink et al. 1995) and light/dark (Sanchez 1996) tests in rats. While some of these negative findings may be due to the use of limited dose ranges, the reasons for these discrepancies are not yet fully understood. It has been suggested that a more detailed analysis of behaviour may yield a clearer picture of the profile displayed by 5-HT_{1A} receptor antagonists in anxiety models (Cao and Rodgers 1997a,b,c). These authors used a detailed ethological technique to examine the effects of several selective and non-selective 5-HT_{1A} receptor antagonists (WAY100135, WAY100635, *p*-MPPI, pindobind-5-HT_{1A}) on plus-maze behaviour in mice. Results showed that these compounds produced clear anxiolytic-like effects on both conventional (open arm activity) and ethological (risk assessment) measures.

Following the suggestion that the defensive behaviours of lower mammals may be relevant to understanding human emotional disorders (Blanchard and Blanchard 1984, 1988), several studies have clearly shown that rodent defence reactions are bidirectionally sensitive to pharmacological manipulations designed to modulate anxiety-related responses (for reviews, see Griebel et al. 1996a,b; Blanchard et al. 1997, 1998). Thus, recent experiments using the mouse defence test battery (MDTB) have confirmed that the defensive repertoire of this species may be particularly useful in studying potential anxiety-modulating properties of psychoactive drugs. In this test, mice show a precise delineation of defensive behaviours including flight, risk assessment, escape attempts and defensive threat/attack, with each element of the repertoire controlled by specifiable characteristics of the threat stimulus and situation. Pharmacological studies have demonstrated that whereas chronic treatment with compounds used in the clinical management of panic (e.g. imipramine, fluoxetine, phenelzine, befloxatone, alprazolam and clonazepam) specifically reduce flight responses, those used in the treatment of generalized anxiety disorder (GAD) (e.g. chlordiazepoxide, clorazepate) consistently reduce risk assessment, defensive threat/attack responses and escape attempts (Griebel et al. 1996b,c, 1997a, 1998a). On the basis of these findings, it has been suggested that the MDTB may be useful for the screening of both anti–panic and anti-GAD drugs and that the specific focus on defensive behaviours improves test validity (for reviews, see Griebel 1996a,b; Blanchard et al. 1997, 1998; Rodgers 1997).

The present study used the MDTB to examine the action of several non-specific (MM-77, NAN-190, (*S*)-UH-301, pindobind-5-HT_{1A}) and selective (WAY100635, *p*-MPPI, SL88.0338) 5-HT_{1A} receptor antagonists. Effects were directly compared to those of the prototypical anxiolytic diazepam, which was used throughout as a positive control.

Materials and methods

Ethics

All procedures described here comply fully with French legislation covering animal experimentation.

Animals

Subjects were naive male Swiss mice aged 10 weeks at the time of testing, and male Long Evans rats (400–500 g). They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed singly in standard cages (mice: 30×20×14 cm; rats: 44×30×20 cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (22–24°C; relative humidity: 35–58%) and kept on a 12-h light/dark cycle with light onset at 6 a.m. Nine or ten animals per group were used in each experiment.

Compounds

Diazepam, WAY100635 (*N*-{2-[4-(2-methoxyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride), NAN-190 (1-(2-methoxyphenyl)-4-(4-(2-phthalimido)butyl)piperazine), SL88.0338 (4-((3,4-dihydro-5,8-dimethoxy-2(1*H*)-isoquinolinyl)methyl)-1-(3-ethoxybenzoyl)-piperidine) (synthesized by the chemistry department, Synthélabo Recherche), (*S*)-UH-301 (5-fluoro-8-hydroxy-2-(dipropylamino)tetralin), Pindobind-5-HT_{1A} (N^1-(bromoacetyl)-N^8-[3-(4-indolyloxy)-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane), *p*-MPPI (4-(2'-methoxyphenyl)-1-[2'-[*N*-(2"-pyridinyl)-p-iopobenzamido]-ethyl]piperazine) (RBI, Natick, Mass., USA) and MM-77 (1-(2-methoxyphenyl)-4-[(4-succinimido)butyl]-piperazine) (Tocris Cookson, Bristol, UK) were dissolved or prepared as suspensions in physiological saline containing 1 or 2 drops of Tween 80. Diazepam and NAN-190 were administered intraperitoneally (IP), and WAY100635 was injected subcutaneously (SC) 30 min before experiments were carried out. The other drugs were given SC 15 min before the test. All doses are expressed as the bases and, except for MM-77, were chosen on the basis of previously published behavioural studies (Bell and Hobson 1993; Griebel et al. 1996c; Cao and Rodgers 1997a,b,c; Cohen et al. 1998).

Apparatus

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by a central wall (2.0×0.30×0.06 m). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. The room illumination was provided by one red neon tube fixed on the ceiling and two desk lamps with red bulbs placed respectively on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lux. Experiments were performed under red light between 9.30 a.m. and 3 p.m. The experimenter was unaware of treatment conditions.

Procedure

Effects on spontaneous locomotor activity: the pre-test

Subjects were placed into the runway for a 3-min. familiarization period during which line crossings were recorded (min 1–3).

Effects on flight responses: the rat avoidance test (min 4–6)

This test was run immediately after the 3-min familiarization period and, to ensure an initial separation of 2 m between the threatening stimulus and subject, commenced only when the mouse was at one end of the apparatus. At this point, a hand-held dead rat (killed by CO_2 inhalation) was introduced at the opposite end of the apparatus and brought up to the subject at an approximate speed of 0.5 m/s. Approach was initiated only if the subject was at a standstill with its head oriented towards the hand-held rat. Consequently, intervals between trials were variable but never exceeded 15 s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. The rat was removed from the apparatus between each trial so that there was no visual contact between the predatory stimulus and the subject. This procedure was repeated five times, with mean avoidance distance (cm) and avoidance frequency calculated for each subject.

Effects on risk assessment: the chase (min 7–8) and the straight alley (min 9–11) tests

The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. As was the case in the rat avoidance test, a constant distance of 2 m separated the rat and the subject when the former was introduced in the runway. Chase was initiated only when the subject was at a standstill with its head oriented toward the hand-held rat, and was completed when the subject had travelled a distance of 15 m. During the chase, a constant distance of 20 cm was maintained between the two animals. Consequently, if the subject stopped fleeing before travelling the full 15 m, the chase was stopped in order to avoid actual stimulus contact. The experimenter then moved the hand-held rat quickly from left to right in front of the subject to elicit flight. During the chase, the number of stops (pause in movement) and orientations (subject stops, then orients the head toward the rat) were recorded. The rat was removed after the chase was completed. By the closing of two doors (60 cm distant from each other), the runway was then converted to a straight alley in which the subject was constrained. The rat was introduced in one end of the straight alley. This phase was initiated only when the subject faced the rat and at a stimulus-subject distance of 40 cm. During 30 s, the number of approaches/withdrawals (subject must move more than 20 cm forward from the closed door, then return to it) was recorded. The hand-held rat remained at the place it was introduced for the full 30 s, after which it was removed from the straight alley.

Effects on defensive threat and attack responses: the forced contact test (min 12–13)

In this test, the experimenter brought the rat up to contact the subject in the straight alley. Approaches were directed quickly (within 1 s) to the subject's head. For each such contact, bites and upright postures by the subjects were noted. If no defensive threat and/or attack responses were elicited within 15 s, the rat was removed from the apparatus. This was repeated three times. The time interval between each trial was approximately 5±1 s. The results were expressed as mean number of upright postures and bites.

Effects on contextual defence: the post-test (min 14–16)

Immediately after the forced contact test, the rat was removed and the door opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session. See Griebel et al. (1997b) for additional details on this test battery.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA). Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Results

Effects on spontaneous locomotor activity: the pre-test

Table 1 shows that prior to confrontation with the rat, WAY100635 [$F(4,45)=6.62$, $P<0.001$], (*S*)-UH-301 [$F(4,45)=13.4$, $P<0.001$], *p*-MPPI [$F(4,55)=5.8$, $P<0.001$] and MM-77 [$F(4,40)=40.3$, $P<0.001$], but not the other drugs, significantly modified the number of line crossings. Whereas WAY100635, (*S*)-UH-301 and *p*-MPPI decreased locomotor activity at the highest dose only (3 mg/kg), MM-77 reduced it from 0.1 mg/kg. With NAN-190, ANOVA just failed to reach statistical significance ($P=0.06$), but the drug tended to decrease line crossings at 3 mg/kg.

Effects on flight responses: the rat avoidance test

Table 2 shows that, except for pindobind-5-HT_{1A}, all drugs significantly modified avoidance distance and frequency [distance: diazepam: $F(3,30)=11.6$, $P<0.001$; WAY100635: $F(4,37)=4.9$, $P<0.01$; NAN-190: $F(4,42)=5.2$, $P<0.01$; (*S*)-UH-301: $F(4,43)=5.2$, $P<0.01$; *p*-MPPI: $F(4,48)=5.6$, $P<0.001$; MM-77: $F(4,35)=9.8$, $P<0.001$; SL88.0338: $F(3,37)=2.6$, $P<0.05$; frequency:

Table 1 Pre-test: locomotor activity in the runway cage before the confrontation with the rat. Diazepam, WAY100635 and NAN-190 were administered 30 min before the beginning of the test. The other drugs were injected 15 min before the test. Data represent mean±SEM

	Dose (mg/kg)	Line crossings
Diazepam	0	125.0±11.3
	0.5	140.7±7.5
	1	150.5±7.8
	3	118.7±12.0
WAY100635	0	124.4±7.0
	0.01	115.4±9.6
	0.1	141.0±9.8
	1	108.1±9.1
	3	77.2±11.4*
p-MPPI	0	171.8±10.3
	0.1	139.8±8.3
	0.3	144.6±5.9
	1	143.6±12.4
	3	96.3±16.2*
SL88.0338	0	136.4±12.6
	0.3	110.6±7.5
	1	103.5±14.7
	3	100.5±10.4
NAN-190	0	123.4±9.8
	0.1	107.2±10.6
	0.3	99.3±7.0
	1	90.9±11.1
	3	78.8±14.5
MM-77	0	149.4±4.7
	0.03	131.0±7.2
	0.1	93.9±7.0*
	0.3	86.3±5.8*
	1	38.3±8.7*
(*S*)-UH-301	0	133.4±9.6
	0.3	101.8±8.2
	1	113.4±7.1
	2	116.2±6.1
	3	60.1±6.9*
Pindobind-5-HT_{1A}	0	143.6±13.5
	0.03	150.6±13.1
	0.1	138.5±15.3
	0.3	148.4±10.0
	1	150.7±6.1

*$P<0.05$ (Dunnett's *t*-test)

Table 2 Rat avoidance test: effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on flight behaviour when the rat was first placed in the test apparatus. Data represent mean±SEM

	Dose (mg/kg)	Avoidance distance (cm)	Number of avoidance
Diazepam	0	135.6±9.4	4.4±0.2
	0.5	131.7±9.1	3.5±0.5
	1	98.0±6.7*	2.3±0.4*
	3	55.8±10.2*	0.8±0.3*
WAY100635	0	149.5±8.9	4.4±0.2
	0.01	132.6±10.8	3.6±0.3
	0.1	130.0±8.7	2.6±0.5*
	1	107.9±14.5*	2.6±0.4*
	3	68.6±15.6*	0.6±0.3*
p-MPPI	0	141.3±6.8	3.9±0.3
	0.1	155.2±7.1	3.3±0.3
	0.3	129.2±7.9	3.8±0.3
	1	97.9±8.8*	2.4±0.5*
	3	128.8±15.1	1.3±0.5*
SL88.0338	0	149.9±7.2	3.9±0.2
	0.3	140.9±7.8	3.0±0.2
	1	122.5±8.5*	2.7±0.4*
	3	122.3±12.5*	1.9±0.4*
NAN-190	0	171.5±8.2	4.3±0.7
	0.1	154.8±8.7	3.9±0.4
	0.3	143.5±12.7	3.3±0.4
	1	114.0±10.0*	2.6±0.4*
	3	116.0±10.8*	1.4±0.4*
MM-77	0	159.8±11.7	3.6±0.4
	0.03	145.5±7.5.0	3.9±0.4
	0.1	143.0±11.5	2.8±0.5
	0.3	140.5±9.2	2.9±0.5
	1	67.0±12.5*	0.8±0.4*
(*S*)-UH-301	0	154.0±6.9	3.6±0.3
	0.3	144.0±9.0	3.5±0.3
	1	135.1±10.2	3.0±0.2
	2	101.1±12.7*	2.3±0.5*
	3	100.5±13.3*	2.1±0.4*
Pindobind-5-HT_{1A}	0	152.7±16.5	3.7±0.4
	0.03	147.2±11.8	3.1±0.4
	0.1	155.0±8.4	3.1±0.3
	0.3	158.4±9.4	2.9±0.3
	1	142.6±4.6	3.6±0.4

*$P<0.05$ (Dunnett's *t*-test)

diazepam: $F(3,36)=18$, $P<0.001$; WAY100635: $F(4,45)=17.6$, $P<0.001$; NAN-190: $F(4,45)=9.6$, $P<0.001$; (*S*)-UH-301: $F(4,45)=3.7$, $P<0.05$, *p*-MPPI: $F(4,55)=7.8$, $P<0.001$; MM-77: $F(4,40)=7.4$, $P<0.001$; SL88.0338: $F(3,39)=7$, $P<0.001$]. Post-hoc analysis showed that diazepam, WAY100635, NAN-190 and SL88.0338 significantly reduced both measures at 1 and 3 mg/kg, and (*S*)-UH-301 at 2 and 3 mg/kg. *p*-MPPI significantly decreased avoidance distance at 1 mg/kg and the number of avoidances at 1 and 3 mg/kg. MM-77 reduced both parameters in a significant manner at 1 mg/kg only.

Effects on RA

Chase test

Figure 1 shows that the drugs significantly decreased the number of orientations [diazepam: $F(3,36)=3.54$, $P<0.05$; WAY100635: $F(4,45)=3.7$, $P<0.05$; NAN-190: $F(4,45)=2.9$, $P<0.05$; (*S*)-UH-301: $F(4,45)=4.4$, $P<0.01$; pindobind-5-HT_{1A}: $F(4,45)=2.9$, $P<0.05$; *p*-MPPI: $F(4,55)=5.9$, $P<0.001$; MM-77: $F(4,40)=4.4$, $P<0.01$; SL88.0338: $F(3,39)=4.2$, $P<0.05$] and stops [diazepam: $F(3,36)=23.6$, $P<0.001$; WAY100635: $F(4,45)=14.5$, $P<0.001$; NAN-190: $F(4,45)=10.3$, $P<0.001$; (*S*)-UH-301: $F(4,45)=17.1$, $P<0.001$; pindobind-5-HT_{1A}: $F(4,45)=9.2$, $P<0.001$; *p*-MPPI: $F(4,55)=13.3$, $P<0.001$; MM-77: $F(4,40)=6.3$, $P<0.01$; SL88.0338: $F(3,39)=13.2$, $P<0.001$]. Dunnett comparisons indicated that orientations were significantly reduced by diazepam and SL88.0338 from 1 mg/kg, by WAY100635 at 3 mg/kg, by NAN-190 and *p*-MPPI at 0.3 and 3 mg/kg, by (*S*)-UH-301 at all doses (0.3–3 mg/kg), by pindobind-5-HT_{1A} at 1 mg/kg and by MM-77 from 0.3 mg/kg. Stops were significantly decreased by diazepam, WAY100635, (*S*)-UH-301, pindobind-5-HT_{1A} and SL88.0338 over the entire dose-range. NAN-190, MM-77 and SL88.0338 reduced this response from 0.3 mg/kg, while *p*-MPPI decreased it from 1 mg/kg.

Straight alley test

Diazepam [$F(3,36)=4.01$, $P<0.05$] and pindobind-5-HT_{1A} [$F(4,45)=2.5$, $P<0.05$] significantly increased the number of approaches followed by withdrawal responses at 1 and 3 mg/kg, and at 0.1 mg/kg, respectively. Similar though non-significant trends were apparent with WAY100635, SL88.0338 and (*S*)-UH-301, but not *p*-MPPI, NAN-190 and MM-77.

Effects on defensive threat and attack responses: the forced contact test

Figure 2 shows that diazepam [$F(3,36)=22.1$, $P<0.001$], WAY100635 [$F(4,45)=17.6$, $P<0.001$], NAN-190 [$F(4,45)=15.4$, $P<0.001$], *p*-MPPI [$F(4,55)=5.5$, $P<0.001$], MM-77 [$F(4,40)=25$, $P<0.001$], SL88.0338 [$F(3,39)=3.9$, $P<0.05$] but not (*S*)-UH-301 or pindobind-5-HT_{1A}, significantly affected upright defensive postures. Further comparisons indicated that diazepam, WAY100635 and NAN-190 significantly decreased this defence reaction at 1 and 3 mg/kg, whereas *p*-MPPI and

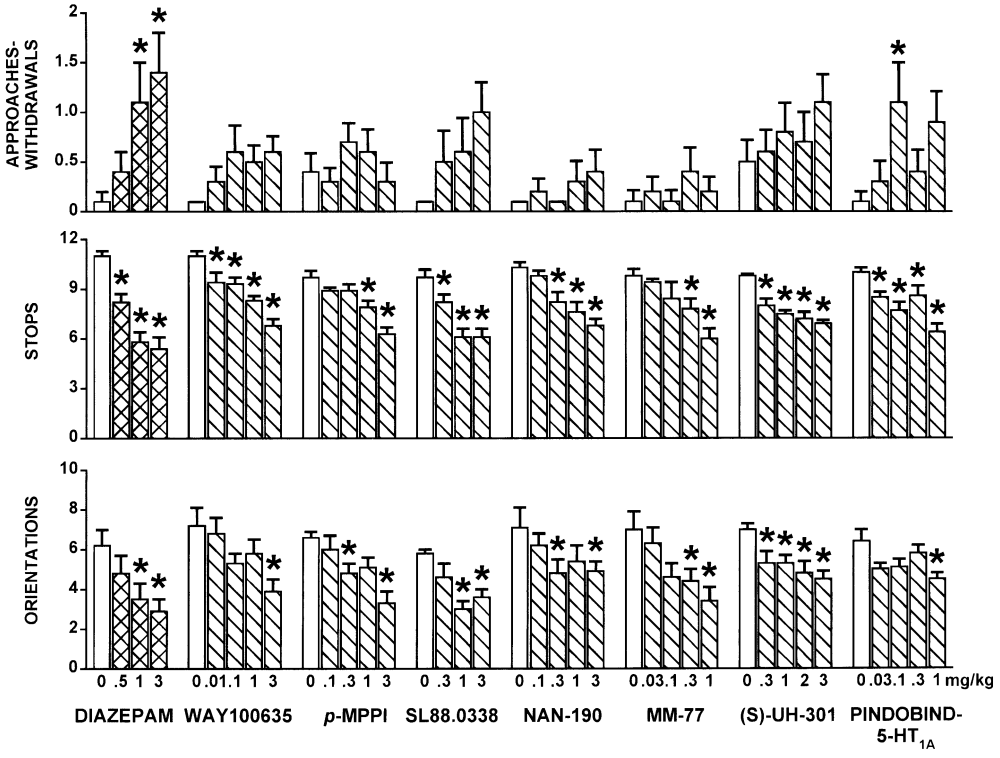

Fig. 1 Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on risk assessment responses measured during the chase test (stops and orientations) and in the straight alley situation (approaches/withdrawals). Data represent mean±SEM. **P*<0.05 (Dunnett's *t*-test)

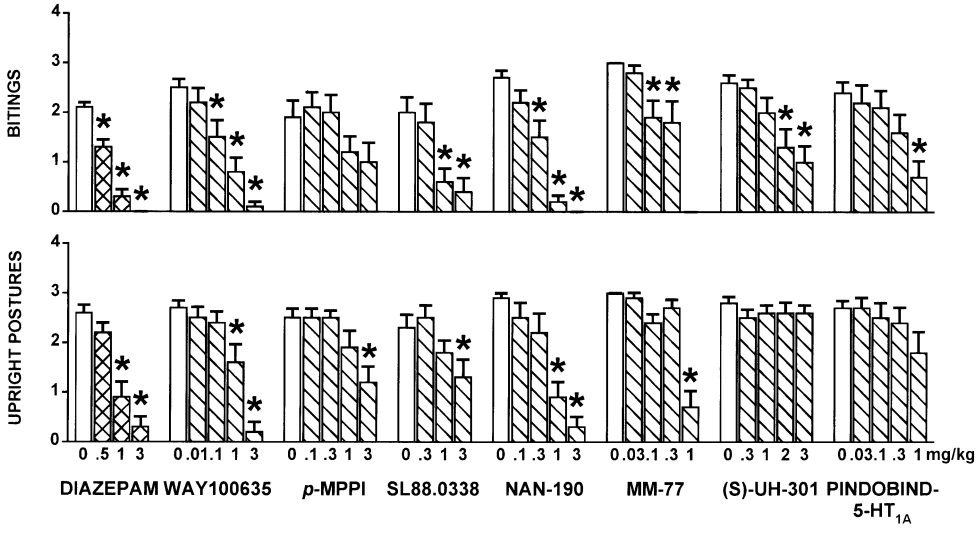

Fig. 2 Forced contact test: effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on defensive threat and attack behaviours upon forced contact with a Long-Evans rat. Data represent mean±SEM. *P<0.05 (Dunnett's t-test)

Table 3 Post-test: effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on escape attempts from the runway apparatus after the removal of the rat in the mouse defence test battery. Data represent mean±SEM

	Dose (mg/kg)	Escape attempts
Diazepam	0	40.7±12.9
	0.5	44.0±13.9
	1	32.7±10.3
	3	18.0±5.7*
WAY100635	0	39.2±12.4
	0.01	37.8±12.0
	0.1	38.5±12.2
	1	29.7±9.4
	3	19.7±6.2*
p-MPPI	0	39.9±11.5
	0.1	34.3±9.9
	0.3	29.5±8.5
	1	34.4±9.9
	3	17.0±4.9*
SL88.0338	0	35.6±10.7
	0.3	34.6±10.4
	1	37.8±12.0
	3	29.9±9.0
NAN-190	0	36.6±11.6
	0.1	31.5±10.0
	0.3	36.1±11.4
	1	30.3±9.6
	3	21.8±6.9*
MM-77	0	41.2±13.7
	0.03	31.8±10.6
	0.1	28.7±9.6*
	0.3	20.8±6.9*
	1	11.8±3.9*
(*S*)-UH-301	0	32.6±10.3
	0.3	27.2±8.6
	1	24.0±7.6*
	2	20.9±6.6*
	3	13.2±4.2*
Pindobind-5-HT_{1A}	0	39.4±12.5
	0.03	34.9±11.0
	0.1	37.0±11.7
	0.3	32.0±10.1
	1	35.3±11.2

*P<0.05 (Dunnett's t-test)

SL88.0338 reduced it at 3 mg/kg, and MM-77 at 1 mg/kg. Defensive biting was also significantly altered by diazepam [F(3,36)=65.1, P<0.001], WAY100635 [F(4,45)=15.1, P<0.001], NAN-190 [F(4,45)=32.4, P<0.001], (*S*)-UH-301 [F(4,45)=6.5, P<0.001], pindobind-5-HT_{1A} [F(4,45)=4.2, P<0.01], MM-77 [F(4,40)=21, P<0.001] and SL88.0338 [F(3,39)=7.2, P<0.001]. Further analyses showed that this behaviour was reduced by diazepam at all doses, by WAY100635 and MM77 from 0.1 mg/kg, by NAN-190 from 0.3 mg/kg, by (*S*)-UH-301 from 2 mg/kg, by pindobind-5-HT_{1A} at 1 mg/kg and by SL88.0338 from 1 mg/kg. Animals treated with *p*-MPPI showed a trend towards a decrease in biting but ANOVA just failed to reach significance.

Effects on contextual defence: the post-test

Data are summarized in Table 3. ANOVA indicated that diazepam [F(3,36)=5.69, P<0.01], WAY100635 [F(4,45)=5.1, P<0.01], NAN-190 [F(4,45)=2.7, P<0.05], (*S*)-UH-301 [F(4,45)=10, P<0.001], *p*-MPPI [F(4,55)=4.8, P<0.01] and MM-77 [F(4,40)=13.3, P<0.001] modified escape attempts from the runway cage following the removal of the rat. Post-hoc comparisons revealed that diazepam, WAY100635, NAN-190 and MM-77 decreased this behaviour at the highest dose only (3 mg/kg), whereas (*S*)-UH-301 and MM-77 reduced it from 1 and 0.1 mg/kg, respectively. No significant changes were seen in escape attempts after the administration of SL88.0338 or pindobind-5-HT_{1A}.

Discussion

The present results show that compounds with selective and, to a lesser extent, non-selective 5-HT_{1A} receptor antagonist activity attenuate defensive behaviours of Swiss mice confronted with a rat-stimulus, thereby indicating that these compounds possess anxiolytic-like properties.

In the pre-test, locomotor activity was not significantly affected by diazepam, SL88.0338 or pindobind-5-HT_{1A} but was reduced by WAY 100635, *p*-MPPI and (*S*)-UH-301 at the highest doses tested (3.0 mg/kg), and by MM-77 from 0.1 to 1 mg/kg. Although the effects of NAN-190 on line crossings just failed to reach statistical significance, the drug tended to reduce them at 3 mg/kg. Clearly, these findings have a direct bearing on the issue of the behavioural selectivity of any changes observed in defensive responding.

In the rat avoidance test, diazepam, WAY100635, *p*-MPPI, SL88.0338, NAN-190, MM-77 and (*S*)-UH-301 decreased flight reactions after the rat was introduced into the runway, although the magnitude of the effects of the 5-HT_{1A} compounds was generally less than that of the benzodiazepine. This was in contrast to pindobind-5-HT_{1A}, which failed to produce a significant decrease in either avoidance measure. Importantly, the effects of diazepam, WAY100635, *p*-MPPI, SL88.0338, NAN-190 and (*S*)-UH-301 on avoidance were unrelated to motor impairment. Thus, data from the pre-test indicated that diazepam was without effect on locomotor activity while the 5-HT_{1A} receptor ligands reduced avoidance measures at doses below the level required to decrease activity (3.0 mg/kg). In contrast, MM-77 reduced avoidance distance at a dose (1 mg/kg) which also impaired line crossings, suggesting that these effects may have been contaminated by behavioural suppression. Although pindobind-5-HT_{1A} did not significantly influence flight in the present study, it is possible that doses higher than 1 mg/kg may have been more effective. In line with this idea is a report that pindobind-5-HT_{1A} decreased evade (i.e. flight) behaviour in a mouse resident-intruder paradigm at 2.5 but not at 0.5 mg/kg (Bell and Hobson 1993). Nevertheless, it is pertinent to note that in a recent study using the elevated plus-maze test in mice (Cao and Rodgers 1997c), pindobind-5-HT_{1A} produced clear anxiolytic-like effects from 0.1 to 0.5 mg/kg, indicating that, under certain test conditions, this compound can modify anxiety-related behaviours at doses lower than 1 mg/kg. Extensive pharmacological evaluation of the MDTB has demonstrated that panic-modulating compounds specifically affect flight responses (most notably, avoidance distance) in the MDTB, with increases produced by panicogenics (e.g. yohimbine) and decreases by panicolytics (e.g. clonazepam, alprazolam, imipramine, fluoxetine, moclobemide, phenelzine) (Blanchard et al. 1993a; Griebel et al. 1996b,c, 1997a, 1998a). Although nothing is currently known about the clinical effects of selective 5-HT_{1A} receptor antagonists, the present data fit well with theoretical suggestions that 5-HT_{1A} binding sites may be involved in the pathogenesis of panic disorder (Norman and Judd 1989) and that 5-HT_{1A} receptor antagonists in particular may have anti-panic potential (Fletcher et al. 1993b).

In the chase test, diazepam and all 5-HT_{1A} receptor ligands reduced risk assessment activities (i.e. stops and orientations) whereas, in the straight alley situation, only diazepam (and, to a lesser extent, pindobind-5-HT_{1A}) increased risk assessment responses displayed when subjects were constrained in one part of the runway (i.e. approaches followed by withdrawals). As was the case in the avoidance test, MM-77 produced significant effects at motor-impairing doses only, suggesting a non-specific decrease in defensive behaviour. In addition, although the 5-HT_{1A} compounds decreased significantly risk assessment in the chase test over a wide dose-range, it is again important to note the magnitude of the effects was smaller than that of diazepam. Risk assessment consists of various information-gathering activities which occur primarily in the context of uncertainty concerning the threat characteristics of the stimulus (Blanchard et al. 1991). Because of a potential isomorphism between risk assessment activities and certain key features of GAD (e.g. hypervigilance, apprehensive expectation and scanning), it has been suggested that they may represent a pattern of responses particularly sensitive to anxiolytic drug challenge (Blanchard et al. 1991). As subsequent pharmacological investigations confirmed the sensitivity of these responses to benzodiazepines (Blanchard et al. 1993b; Griebel et al. 1995a, 1996c), the currently observed effects of 5-HT_{1A} receptor ligands on risk assessment in the chase test would be entirely consistent with an anxiolytic-like effect. However, their lack of clear or significant effects on risk assessment in the straight alley test indicates only partial efficacy in affecting this behaviour, thereby suggesting that these drugs may have somewhat weaker anxiolytic effects compared to benzodiazepines. It is relevant to note that a similar point has recently been made on the basis of direct profile comparisons of chlordiazepoxide and WAY 100635 in the mouse elevated plus-maze paradigm (Cao and Rodgers 1998).

When contact was forced between threat stimulus and subject (forced contact test), diazepam, the selective 5-HT_{1A} receptor antagonists, NAN-190 and MM-77 reduced defensive threat and attack reactions. Nevertheless, MM-77 decreased this behaviour at motor-impairing doses only, suggesting that the reduction in defensive reactions upon forced contact with the rat may have been confounded by behavioural suppression. Although responses tended to be lower than in vehicle-treated mice, *p*-MPPI failed to significantly modify biting. Furthermore, despite clear effects on biting, (*S*)-UH-301 and pindobind-5-HT_{1A} did not alter upright postures. These findings are somewhat surprising in view of a previous result from a factor analysis showing that defensive biting and upright postures load on the same factor (Griebel et al. 1996a). Overall, however, the profile of 5-HT_{1A} receptor ligands in the forced contact test indicates that these compounds display comparable efficacy to diazepam in reducing defensive threat and attack behaviours (present results, Griebel et al. 1995a, 1996c).

In the post-test, following removal of the rat from the runway, only diazepam and (*S*)-UH-301 specifically decreased escape attempts from the test apparatus. Although WAY100635, NAN-190, *p*-MPPI and MM-77 markedly reduced escape, such effects may be attributed

to behavioural non-specificity because significant changes in this behaviour were observed at high and motor-impairing doses only. Unlike all other compounds, SL88.0338 and pindobind-5-HT_{1A} failed to modify escape attempts over the dose-range tested. The reason for differences between (*S*)-UH-301 and the other 5-HT_{1A} receptor ligands is unclear, but may be explained by the involvement of mechanisms other than blockade of 5-HT_{1A} receptors. As mentioned above, (*S*)-UH-301 has only 8-fold selectivity for 5-HT_{1A} relative to D_2 receptors and may behave as a D_2 receptor agonist (Hillver et al. 1990; Arborelius et al. 1993). Thus, it can be speculated that the effects of this compound on contextual defence may involve an action at D_2 receptors. However, further investigations are needed to support this hypothesis.

The precise mechanisms underlying the effects of the 5-HT receptor ligands in this study remain to be determined. These compounds have all demonstrated antagonistic-like activity on postsynaptic 5-HT_{1A} receptors (Hillver et al. 1990; Hjorth and Sharp 1990; Sharp et al. 1990; Claustre et al. 1991; Liau et al. 1991; Kung et al. 1994; Mokrosz et al. 1994; Zhuang et al. 1994; Fletcher et al. 1995; Forster et al. 1995; Kung et al. 1995; Assie and Koek 1996; Thielen et al. 1996; Cohen et al. 1998). Although it has been reported that exposure to aversive stimuli (e.g. elevated plus-maze, social interaction) increases 5-HT release (e.g. Bickerdike et al. 1993; File et al. 1993), there is as yet no direct evidence that exposure to the MDTB increases 5-HT release. Thus it is not clear whether postsynaptic 5-HT_{1A} receptor blockade accounts for the effects of the compounds studied on defensive behaviours. As mentioned above, (*S*)-UH-301, pindobind-5-HT_{1A} and NAN-190 have high affinity for binding sites other than 5-HT_{1A} (D_2, α_1- or β-adrenergic), and as such, an action at these receptors may contribute to their effects in the MDTB. However, as WAY100635, *p*-MPPI and SL88.0338 have negligible or no affinity for these sites (Kung et al. 1994, 1995; Zhuang et al. 1994; Fletcher et al. 1995; Forster et al. 1995; Assie and Koek 1996; Thielen et al. 1996; Cohen et al. 1998) yet produce similar changes in the presence of the threat stimulus, it may be assumed that 5-HT_{1A} receptors are primarily involved in these effects. In this context, it is pertinent to note that control studies with D_2, α_1-, β_1- and β_2-receptor antagonists have shown that actions at these sites are not relevant to the anxiolytic-like effects of 5-HT_{1A} receptor antagonists in the mouse elevated plus-maze (Cao and Rodgers 1997b,c). It is also important to note that MM-77 displayed a profile in the MDTB which is somewhat different from that of the other compounds tested in this study as it reduced defensive behaviours in a non-specific manner. Nevertheless, it must be emphasized that MM-77 has only two-fold selectivity for 5-HT_{1A} receptors (K_i=6.4 nM) relative to α_1-adrenoceptors (K_i= 11.9 nM) and shows moderate affinity for D_2 receptors (K_i=490 nM) (Mokrosz et al. 1994). It is possible that an action at these receptors may contribute to its marked hypolocomotor effects.

It is notable that, in contrast to the bell-shaped dose-response functions observed with 5-HT_{1A} receptor antagonists in the mouse plus-maze (Cao and Rodgers 1997a,b,c,), present findings almost invariably demonstrate linear dose-dependency. However, it should be noted that the dose ranges currently employed were generally lower than those used by Cao and Rodgers in their plus-maze studies. Furthermore, it may be speculated that differences in threat intensity posed in these two paradigms (i.e. predator cues versus a potentially dangerous environment) would result in differential 5-HT activation and, hence, differential sensitivity to 5-HT_{1A} receptor blockade. This suggestion would not be inconsistent with recent findings in rats where much lower doses of WAY 100635 are required to produce anxiolytic-like effects in the light/dark exploration test (Sanchez 1996) than in the potentiated startle paradigm (Joordens et al. 1997).

In conclusion, the profiles displayed by selective and, to a lesser extent, non-selective 5-HT_{1A} receptor antagonists in the MDTB are comparable to that of diazepam, although the magnitude of the effects of the 5-HT_{1A} compounds was generally smaller than that of the benzodiazepine. With the exception of one risk assessment measure (i.e. approach/withdrawal behaviour in the straight alley test), selective 5-HT_{1A} receptor antagonists affected all other defensive responses in the presence of the threat stimulus. However, unlike diazepam, these compounds attenuated contextual defence only at high and mostly motor-impairing doses. Despite this latter result, the present findings demonstrate that selective 5-HT_{1A} receptor antagonists produced clear anxiolytic-like effects in the MDTB. Interestingly, comparisons with 5-HT_{1A} receptor ligands previously assessed in this test battery indicate substantial differences. Thus, the full agonist 8-OH-DPAT and the partial agonists buspirone and gepirone have all been found to affect the behaviour of mice in the MDTB. However, while producing some reductions in defensive threat and attack responses and post-test escape attempts, they failed to modify flight and risk assessment behaviours (Griebel et al. 1995b; 1998b). Together with recent findings in the mouse plus-maze (Cao and Rodgers, 1997a,b,c), our results suggest that the anxiety-reducing potential of 5-HT_{1A} receptor antagonists may be superior to that of full or partial agonists for this receptor.

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