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The effects of compounds varying in selectivity as 5-HT_{1A} receptor antagonists in three rat models of anxiety

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Abstract

Compounds varying in selectivity as 5-HT_{1A} receptor antagonists have recently been reported to produce benzodiazepine-like antianxiety effects in mice. To assess the cross-species generality of these findings, the present experiments compared the effects of diazepam (0.625–5 mg/kg) with those of several non-selective (MM-77, 0.03–1 mg/kg and pindobind-5-HT_{1A}, 0.1–5 mg/kg) and selective (WAY100635, 0.01–10 mg/kg, *p*-MPPI, 0.01–3 mg/kg and SL88.0338, 0.3–10 mg/kg) 5-HT_{1A} receptor antagonists in three well-validated anxiolytic screening tests in rats: punished lever-pressing, punished drinking, and the elevated plus-maze. In the punished lever-pressing conflict test, none of the 5-HT_{1A} receptor antagonists modified rates of punished responding, whereas in the punished drinking test, WAY100635 (0.3–1 mg/kg), SL88.0338 (3–10 mg/kg), *p*-MPPI (1 mg/kg), MM-77 (0.03–0.3 mg/kg), but not pindobind-5-HT_{1A}, produced clear anticonflict activity. However, the increase in punished responding with the 5-HT_{1A} compounds was smaller than that produced by diazepam, indicating weaker anxiolytic-like activity. In the elevated plus-maze test, WAY100635 (0.1–0.3 mg/kg), SL88.0338 (0.3–10 mg/kg), MM-77 (0.01–3 mg/kg), pindobind-5-HT_{1A} (0.1–3 mg/kg), but not *p*-MPPI, showed anxiolytic-like activity on traditional behavioral indices, increasing the percentage of time spent in open arms and the percentage of open arm entries. As was the case in the punished drinking test, the magnitude of the positive effects of the 5-HT_{1A} compounds was generally smaller than that of diazepam. Of the ethological measures recorded in the plus-maze, all compounds markedly decreased risk assessment (i.e. attempts) over the entire dose-range, but only diazepam clearly increased directed exploration (i.e. head-dipping). Although the present results demonstrate that 5-HT_{1A} receptor antagonists elicit anxiolytic-like effects in rats, this action appears to be test-specific and, unlike previous findings in mice, smaller than that observed with benzodiazepines. The data are discussed in relation to the possible relevance of species differences in 5-HT_{1A} receptor function and the nature of the anxiety response studied.

Keywords: 5-HT_{1A} receptor antagonists; Anxiety; Conflict tests; Diazepam; Elevated plus-maze; Rats

1. Introduction

After more than three decades of preclinical research on the relationship between serotonin (5-HT) and anxiety, only one direct 5-HT-acting compound has been launched as an anxiolytic agent (i.e. buspirone) (Goa and Ward, 1986; Apter and Allen, 1999). Nevertheless, interest in this research area has not diminished and novel 5-HT-modulating agents are still being developed (for review, see Griebel, 1997). Despite increasing interest in drugs combining 5-HT_{1A}, 5-HT_2 and/or 5-HT reuptake inhibitor properties, it is not yet clear whether the therapeutic potential of these agents will prove superior to that of selective 5-HT compounds. As such, research attention remains firmly focused on selective 5-HT_{1A} receptor ligands and, in this context, recent animal studies suggest that selective blockade of 5-HT_{1A} receptors may yield anxiolytic-like activity comparable to that of benzodiazepines (Cao and Rodgers, 1997a,b,c; Cao and Rodgers, 1998a,b; Griebel et al., 1999). For example, in the mouse elevated plus-maze test, several selective and non-selective 5-HT_{1A} receptor antagonists (WAY100135, WAY100635, *p*-MPPI, pindobind-5-HT_{1A}) have been shown to produce robust anxiolytic-like effects on both conventional (open arm activity) and

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ethological (risk assessment) measures (Cao and Rodgers, 1997a,b,c; Cao and Rodgers, 1998a,b). Similarly, in a mouse defense test battery, where animals are directly confronted with a natural threat (i.e. a rat) as well as situations associated with this threat, selective 5-HT_{1A} receptor antagonists (WAY100635, SL88.0338) were found to modify defensive behaviors in much the same way as diazepam (Griebel et al., 1999). Furthermore, evidence for an anxiolytic-like action of 5-HT_{1A} receptor antagonists has also been reported in certain rat models of anxiety, such as the fear-potentiated startle (Joordens et al., 1998) and light/dark exploration (Sanchez, 1996) tests.

Despite these positive findings, however, there are a significant number of reports indicating that 5-HT_{1A} receptor antagonists are inactive in anxiety models. Thus, 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; Kennett et al., 1998), rat ultrasonic vocalization (Bartoszyk et al., 1996; Brocco et al., 1996; Remy et al., 1996; Xu et al., 1997; Schreiber et al., 1998), rat conditioned emotional response (Overshiner et al., 1995; Stanhope and Dourish, 1996), mouse stress-induced hyperthermia (Olivier et al., 1998), rat 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. While some of these negative data may be due to the use of limited dose ranges, the general pattern of inconsistency has yet to be adequately explained. Based on the finding that the selective 5-HT_{1A} receptor antagonist, LY297996, produces anxiolytic-like activity in the murine elevated plus-maze in the mid-dark, but not the mid-light, phase, it has been suggested that circadian factors may be important in the detection of 5-HT_{1A} receptor antagonist anxiolysis (Cao and Rodgers, 1998a; Rodgers et al., 1998). Alternatively, as positive effects have largely been obtained in mouse models, and negative findings in rat models, the inconsistent profiles of 5-HT_{1A} receptor antagonists might be attributed to a species difference in the role of this receptor in anxiety-related processes.

The aim of the present experiments was to investigate the effects of several compounds varying in selectivity as 5-HT_{1A} receptor antagonists (WAY100635, SL88.0338, *p*-MPPI, MM-77 and pindobind-5-HT_{1A}) under identical test conditions in three well-validated rat models of anxiety. The tests chosen were two conflict procedures (punished lever-pressing and punished drinking) and one exploratory model (elevated plus-maze). Effects were directly compared to those of the prototypical anxiolytic diazepam, which was used as a positive control. We used different test procedures since there is now growing evidence that the measures of anxiety from different tests may reflect different states of anxiety (File, 1992; Belzung and Le Pape, 1994; Beuzen and Belzung, 1995; Rodgers, 1997). This was shown by the application of factor analysis of the various behavioral parameters obtained in different anxiety models. For example, File (1992) and Lister (1987) and Lister (1987) revealed that parameters recorded in several anxiety models (e.g. elevated plus-maze, social interaction, holeboard, Vogel conflict) produced distinct anxiety factors, thereby indicating that they reflect different emotional states.

The phenyl–piperazine derivative, WAY100635, and its close structural analogs, *p*-MPPI and the amino–methyl–piperidine 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; Assie and Koek, 1996; Thielen et al., 1996; Cohen et al., 1998). Unlike the pindolol derivative, pindobind-5-HT_{1A}, which displays antagonistic-like activity in both pre- and postsynaptic 5-HT_{1A} receptor models (Liau et al., 1991), MM-77 shows agonist-like activity at presynaptic somatodendritic 5-HT_{1A} receptors yet antagonistic-like activity in postsynaptic 5-HT_{1A} receptor models (Mokrosz et al., 1994). In addition, pindobind-5-HT_{1A} and MM-77 have only nine and two-fold selectivity for 5-HT_{1A} relative to α_1-adrenoceptors, respectively (Liau et al., 1991; Mokrosz et al., 1994).

2. Methods

2.1. Ethics

All procedures described here fully comply with French legislation on research involving animal subjects.

2.2. Subjects

Male Wistar rats (Charles River France, Saint-Aubin-les-Elbeuf) were used in the punished lever-pressing procedure. They weighed 180–200 g at the beginning of training and 400–500 g at the time of testing. Male Sprague–Dawley rats (Iffa Credo, L'Arbresle and Charles River France), weighing 180–300 g at time of testing, were used in the punished drinking (Vogel) and elevated plus-maze tests. Rats used in the Vogel procedure and the elevated plus-maze test were housed in groups of eight, whereas those used in the punished lever-pressing procedure were housed singly. The latter subjects were restricted to the food obtained during sessions together with a daily ration of 15–20g of standard laboratory chow given at the end of each weekday and over the weekend. All animals were maintained under

standard laboratory conditions (22–23°C) and kept on a 12:12-h light–dark cycle with light onset at 7 a.m.

2.3. Drugs

Diazepam, WAY100635 (N-{2-[4-(2-methoxyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride), SL88.0338 (4-((3,4-dihydro-5,8-dimethoxy-2(1*H*)-isoquinolinyl)methyl)-1-(3-ethoxybenzoyl)-piperidine) (synthesized by the CNS Chemistry Department, Synthélabo Recherche), 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, 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 one or two drops of Tween 80. Diazepam was administered intraperitoneally (i.p.), and WAY100635 was injected subcutaneously (s.c.) 30 min before experiments were carried out. The other drugs were given s.c. 15 min before the test. All doses are expressed as the bases and were chosen on the basis of previously published behavioral studies in mice (Bell and Hobson, 1993; Cao and Rodgers, 1997a,b,c; Griebel et al., 1999) and in rats (Stanhope and Dourish, 1996). All compounds were injected in a constant volume of 2 ml/kg.

2.4. Procedure

2.4.1. Punished lever-pressing

The procedure was a modification of that described previously (Sanger et al., 1985). Animals were tested in standard rat operant test chambers (MED Associates, Inc., GA) placed in sound-attenuated boxes with ventilation fans. Each chamber was fitted with a stainless-steel grid floor through which electric shocks could be delivered (shock generator and scrambler: MED Associates, Inc.). A total of 11 rats were trained initially to press a lever for food reward (45 mg precision food pellets, PJ Noyes, Inc., Lancaster). As training progressed, schedule parameters were gradually changed to a variable interval (VI) schedule (VI 30 s) of food reinforcement during daily 15 min sessions. After several sessions of VI 30 s responding, five 60 s periods of a visual stimulus were presented during a 25 min session. Each visual stimulus consisted of three stimulus lights situated above the food pellet dispenser and to the right of the response lever, which flashed at a rate of 1 s on, 1 s off. In this component, a footshock punishment schedule consisting of two independent VI schedules (VI 30 s for food, VI 10 s for shock) was in operation. Footshock was initially set at 0.1 mA. The first stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 s after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3 to 0.65 mA) to obtain stable baselines of responding (i.e. an average lever pressing rate of 8 ± 2 presses in each 60 s punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were started.

Drug injections were given once or twice each week with at least two nondrug days intervening between two drug administrations. Vehicle was injected on all nondrug days. Drugs and doses were given in a mixed order, and treatment effects on punished and unpunished response rates assessed. The former corresponds to responses recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60 s periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the nondrug session preceding the drug sessions were used as control scores. Drug effects were analyzed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA.

2.4.2. Punished drinking

The procedure was a modification of the technique described by Vogel et al. (1971). At the beginning of the experiment, rats, deprived of water for 48 h prior to testing, were placed in cages (27 × 22 × 21 cm) with a stainless steel grid floor. Each cage contained a drinking tube connected to an external 50 ml burette filled with tap water. Trials commenced only after the animal's tongue contacted the drinking tube for the first time. An electric shock (0.06 mA) was delivered through the drinking spout after every twenty licks, and the number of shocks received was recorded automatically during a 3-min period. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

2.4.3. Elevated plus-maze

The test apparatus was based on that described by Pellow et al. (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). To prevent rats falling off, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. The light

intensity on the central platform was 10 lux. At the beginning of the experiment, rats were placed in the center of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries and number of closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via video-link by an observer located in an adjacent room. This permitted the recording of the more ethologically-orientated measures: (a) attempt: attempt at entry into open arms followed by avoidance responses. This includes stretched attend posture (the rat stretches forward and retracts to original position); (b) head-dipping: protruding the head over the edge of an open arm and down towards the floor (this response can occur while the animal's body is in a closed arm, central square or on an open arm). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of both closed and open arm entries, mean total number of closed arm entries, mean total number of attempts and mean total number of head-dips. Data were analyzed by one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

3. Results

3.1. Punished lever pressing

Fig. 1 shows that the rates of responding decreased by the punishment contingency were significantly increased by diazepam (χ^2=20.9, $p<0.001$), but not by the other compounds tested. The only compound to affect unpunished responding was WAY100635 which significantly decreased these response rates at 3 and 10 mg/kg (χ^2=24.3, $p<0.001$) (Table 1).

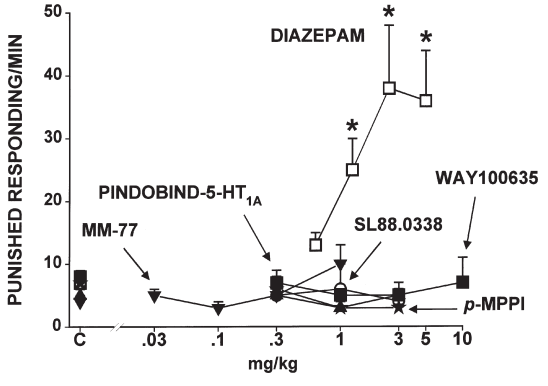

Fig. 1. Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on rates of punished lever pressing in rats. Data represent mean±SEM. n=6–8. *$p<0.05$ (Dunnett's *t*-test).

3.2. Punished drinking

Fig. 2 shows that, except for pindobind-5-HT_{1A}, all compounds significantly modified the number of shocks received [diazepam: $F(3,47)=10.4$, $p<0.001$; WAY100635: $F(3,39)=4.8$, $p<0.01$; SL88.0338: $F(3,79)=2.6$, $p<0.05$; *p*-MPPI: $F(4,49)=2.7$, $p<0.05$; MM-77: $F(4,53)=4.9$, $p<0.01$]. Post-hoc analysis indicated that while diazepam (2.5 and 5 mg/kg), WAY100635 (0.3 and 1 mg/kg), SL88.0338 (3 and 10 mg/kg) and MM-77 (0.03–0.3 mg/kg) significantly increased punished responding at several doses, *p*-MPPI produced a significant effect at one dose only (1 mg/kg).

3.3. Elevated plus-maze

Fig. 3 shows that, with the exception of *p*-MPPI, all drugs significantly modified both the percentage of open arm time [diazepam: $F(3,30)=7.6$, $p<0.001$; WAY100635: $F(4,65)=2.8$, $p<0.05$; SL88.0338: $F(4,33)=5.5$, $p<0.01$; MM-77: $F(4,65)=4.6$, $p<0.01$;

Table 1
Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on rates of unpunished responding in rats[a]

	Dose (mg/kg)	Unpunished responding/min
Diazepam	0	58±8
	0.625	74±9
	1.25	77±13
	2.5	76±10
	5	65±9
WAY100635	0	74±6
	0.3	65±8
	1	56±7
	3	49±8*
	10	23±7*
SL88.0338	0	70±8
	0.3	67±9
	1	64±9
	3	60±10
p-MPPI	0	67±5
	0.3	61±8
	1	67±5
	3	64±9
MM-77	0	51±5
	0.03	57±5
	0.1	48±6
	0.3	55±8
	1	41±7
Pindobind-5-HT_{1A}	0	62±10
	0.3	58±10
	1	54±15
	3	48±5

[a] Data represent mean±SEM. n=6–8. *$p<0.05$ (Friedman).

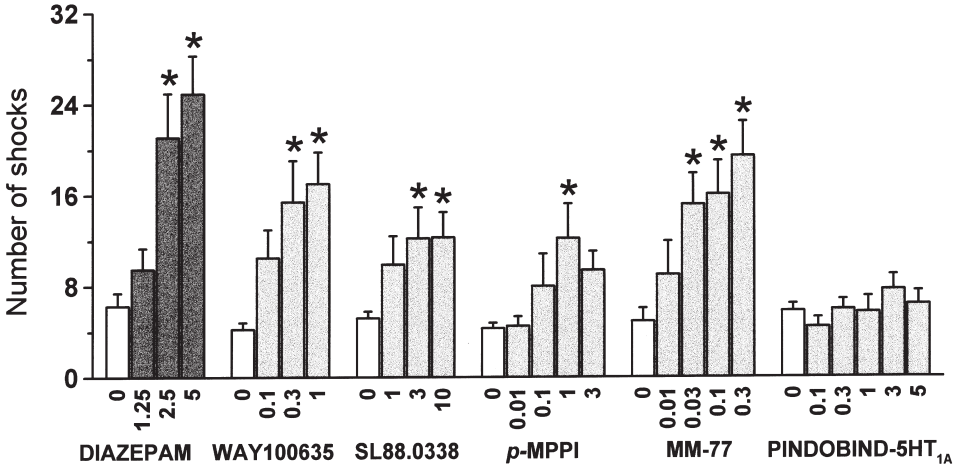

Fig. 2. Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists in the punished drinking conflict test in rats. Data represent mean±SEM. n=10–20. *$p<0.05$ (Dunnett's t-test).

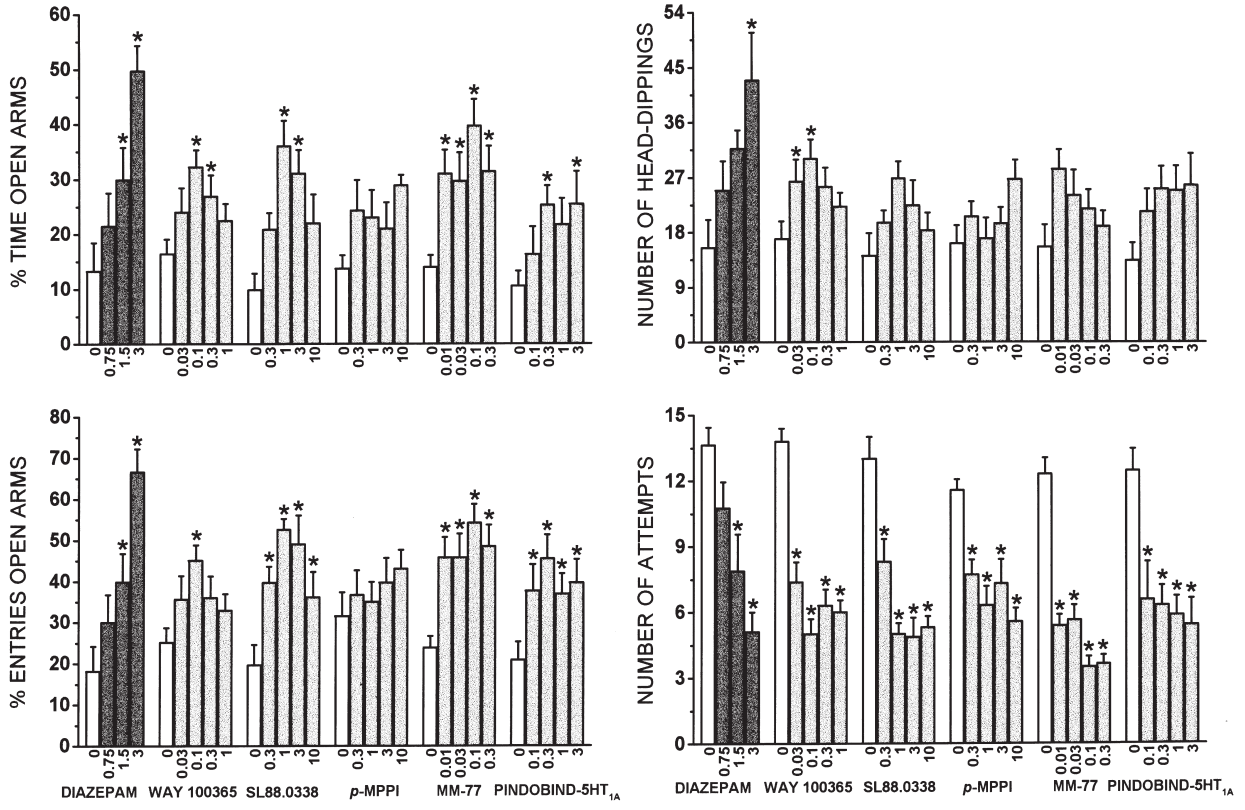

Fig. 3. Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on four anxiety-related measures in the elevated plus-maze test in rats. Data represent mean±SEM. n=6–14. *$p<0.05$ (Dunnett's t-test).

pindobind-5-HT_{1A}: $F(4,32)=2.7$, $p<0.05$] and the percentage of open arm entries [diazepam: $F(3,30)=9.8$, $p<0.001$; WAY100635: $F(4,65)=2.5$, $p<0.05$; SL88.0338: $F(4,33)=5.8$, $p<0.01$; MM-77: $F(4,65)=5.8$, $p<0.001$; pindobind-5-HT_{1A}: $F(4,32)=3.6$, $p<0.001$]. Post-hoc analysis indicated that each of these drugs significantly increased open arm activity at several doses. With respect to the ethologically-derivated measures, all compounds modified the number of attempts at entry into open arms followed by avoidance responses [diazepam: $F(3,30)=8.9$, $p<0.001$; WAY100635: $F(4,65)=24.5$, $p<0.001$; SL88.0338: $F(4,33)=17.2$, $p<0.001$; p-MPPI: $F(4,34)=8.8$, $p<0.001$; MM-77: $F(4,65)=39.3$, $p<0.001$; pindobind-5-HT_{1A}: $F(4,32)=7.7$, $p<0.001$]. Post-hoc analysis indicated that all compounds significantly reduced attempts over a

wide dose-range. In addition, diazepam [$F(3,30)=3.3$, $p<0.05$] and WAY100635 [$F(4,65)=2.8$, $p<0.05$] modified directed exploration (head-dippings). Post-hoc analysis revealed that this response was significantly increased by diazepam at 3 mg/kg and by WAY100635 at 0.03 and 0.1 mg/kg. Finally, Table 2 shows that only diazepam [$F(3,30)=7.7$, $p<0.001$] and MM-77 [$F(4,65)=5$, $p<0.01$] significantly decreased the number of closed arm entries, whereas none of the drugs significantly modified the total number of arm entries.

4. Discussion

The present study compared the behavioral profiles of compounds varying in selectivity as 5-HT_{1A} receptor antagonists with those of diazepam in three classical rat models of anxiety. As expected, diazepam was active in all three models, increasing rates of punished lever-pressing, punished drinking and open arm activity. However, the effects of the 5-HT_{1A} receptor antagonists varied according to the test employed.

Table 2
Effects of diazepam and compounds varying in selectivity as 5-HT_{1A} receptor antagonists on two measures of general activity in the elevated plus-maze test in rats[a]

	Dose (mg/kg)	Closed arm entries	Total arm entries
Diazepam	0	10.4±0.9	12.8±0.8
	0.75	10.1±0.7	14.8±0.6
	1.5	8.3±1	14.4±1.6
	3	4.6±1.1*	13.1±1.4
WAY100635	0	11.6±1.3	15.4±1.3
	0.03	8.8±0.7	14.1±0.5
	0.1	8.5±0.6	15.5±0.6
	0.3	9.8±0.8	15.6±0.9
	1	10.6±1.2	15.6±1.1
SL88.0338	0	9.5±0.9	12.3±1.8
	0.3	7±0.7	11.7±1
	1	5.7±0.4	12.1±1
	3	6.4±0.9	12.6±1
	10	6.9±1.1	10.9±1.4
p-MPPI	0	9.6±1.4	14.1±1.5
	0.3	8.1±0.6	13.1±1
	1	8.3±0.4	13±0.9
	3	8.3±1.3	13.6±1.3
	10	7.1±0.6	12.6±0.6
MM-77	0	10±0.9	13±1
	0.01	7.8±0.8	14.7±1
	0.03	7.5±0.9	14.3±1.2
	0.1	6.1±0.6*	13.1±1
	0.3	5.6±0.6*	11.4±0.9
Pindobind-5-HT_{1A}	0	10.2±0.9	13.2±1.2
	0.1	7.4±1.1	11.7±0.9
	0.3	8.1±0.4	15.6±1.3
	1	8.9±0.8	14.3±1.4
	3	9.2±1.7	15.2±2

[a] Data represent mean±SEM. n=6–14. *$p<0.05$ (Dunnett's t-test).

In the punished lever-pressing conflict test, none of the 5-HT_{1A} receptor antagonists modified rates of punished responding, whereas in the punished drinking test, WAY100635, SL88.0338, MM-77 and, to a lesser extent, *p*-MPPI (but not pindobind-5-HT_{1A}) produced anticonflict activity. In addition, the observation that WAY100635 decreased unpunished responding at 3 and 10 mg/kg is in agreement with a previous finding in mice that this compound induces immobility at a dose of 9 mg/kg (Cao and Rodgers, 1997c). The general absence of significant modifications in rates of unpunished responding in the lever-pressing procedure (at doses active in the punished drinking test) indicates that the anxiolytic-like effects on punished drinking were observed at doses which did not impair motor activity. However, it is important to note that the increase in punished responding with the 5-HT_{1A} compounds was somewhat smaller than that produced by diazepam, indicating weaker anxiolytic-like activity. It is unlikely that the positive effects of 5-HT_{1A} receptor antagonists in the punished drinking test are due to decreased sensitivity to electric shocks since these drugs have been reported to be inactive in reflexive tests of analgesia (i.e. the tail-flick an hot-plate), irrespective of stimulus quality or intensity (Millan, 1994). Although pindobind-5-HT_{1A} was inactive in both conflict tests, it is possible that doses higher than 5 mg/kg may have been more effective. However, in a previous study the drug was shown to elicit anxiolytic-like effects in mice from 0.1 to 0.5 mg/kg (Cao and Rodgers, 1997b) indicating that, under certain test conditions, this compound can modify anxiety-related behaviors at doses lower than 5 mg/kg.

The failure of 5-HT_{1A} receptor antagonists to modify punished lever-pressing, while entirely consistent with previous findings in rat and pigeon conflict tests (Overshiner et al., 1995; Samanin et al., 1996; King et al., 1997; Millan et al., 1997), is difficult to reconcile with the positive effects obtained in the punished drinking (Vogel) test. Moreover, our data contrast with those obtained by Kennett et al. (1998) in the Vogel conflict test. In this study WAY100635 was found inactive at 0.1 and 0.3 mg/kg, whereas we found effects with somewhat higher doses (0.3–1.0 mg/kg). Thus dose range could be the problem here as could differences in control levels of punished drinking (high in Kennett's study vs us: around 9 vs 4 shocks accepted). In addition, Kennett et al. used a very different test procedure. For example, their deprivation schedule was different. Further, in their study, a pre-test was performed one day prior to testing, suggesting that animals were less stressed than those used here, that didn't see the test apparatus before. Overall, it seems likely that these models may be tapping different facets or levels of anxiety. Thus, it is not unreasonable to assume that the level of stress in the punished drinking test is higher than that in the punished lever pressing procedure. In the former, the experimental

situation was novel to the rats and they had never experienced electric shock prior to testing. In the latter, however, animals had been handled daily and extensively trained (several months) in the same cage and were fully experienced with electric shock before drug testing. This distinction suggests that the increase in 5-HT release, generally produced by exposure to aversive stimuli (e.g. Blanchard et al., 1991; Bickerdike et al., 1993; File et al., 1993; Kawahara et al., 1993; Shekhar et al., 1994; Yoshioka et al., 1995), may well be lower in the lever-pressing procedure than in the punished drinking test. Thus, assuming that endogenous 5-HT tone contributes significantly to the emotional responses displayed by rats in the latter test, 5-HT_{1A} antagonists would be predicted to attenuate these reactions. Although there is as yet no direct evidence that these conflict tests differentially modify 5-HT release, it is notable that the α_2-adrenoceptor ligand, yohimbine, which exhibits marked activity at 5-HT_{1A} receptors (Winter and Rabin, 1992), has been found to exert anticonflict activity in the rat punished drinking test by decreasing 5-HT neurotransmission (Soderpalm et al., 1995a,b). Alternatively, the discrepancy between both conflict tests may be related to the different housing conditions used with these tests. While rats used in the lever pressing test were housed singly, those employed in the punished drinking test were housed in groups of eight. Based on the finding that housing conditions affect the 5-HT system (Crespi et al., 1992), it is possible that a different 5-HT regulation between these rats may lead to changes in the sensitivity to 5-HT_{1A} receptor antagonists.

In the elevated plus-maze, and fully consistent with previous findings in the mouse version of this test (Cao and Rodgers, 1997a,b,c; Cao and Rodgers, 1998a,b), all drugs showed anxiolytic-like activity on traditional behavioral indices i.e. increases in percentage open arm entries and time. However, despite trends in the appropriate direction, the effects of *p*-MPPI were not statistically significant. The reason for this is unclear, as previous work has reported robust anxiolytic-like effects with this compound in the mouse elevated plus-maze (Cao and Rodgers, 1997a). However, it is pertinent to note that, in the mouse defense test battery, *p*-MPPI also elicited weaker anxiolytic-like effects than either WAY100635 or SL88.0338 (Griebel et al., 1999). Importantly, effects of the 5-HT_{1A} ligands on spatiotemporal measures occurred at doses that did not decrease closed or total arm entries (reliable measures of locomotor activity), thereby suggesting that the anxiolytic-like activity was not contaminated by motor impairment. However, as for the punished drinking test, the magnitude of the effects observed with the 5-HT_{1A} compounds on conventional plus-maze measures was generally smaller than that of diazepam. A similar potency differential has recently been reported in a direct comparison of the effects produced by chlordiazepoxide and WAY100635 in the mouse elevated plus-maze test (Cao and Rodgers, 1998b). The behavioral profile of WAY100635 in this study contrasts with several previous findings in rats showing that the drug failed to modify open arm activity (Bickerdike et al., 1995; File et al., 1996; Collinson and Dawson, 1997; Millan et al., 1997). This variability cannot be attributed to dose range as doses currently used overlap with those employed in previous investigations. Similarly, the discrepancy cannot be explained by strain differences (Sprague–Dawley rats were used in two studies) or by differences in route of injection/injection-test intervals (similar in all studies). However, it is important to note that, in previous work, baseline levels of time spent on the open arms were above 20% whereas, in the present study, values ranged between 10 and 16% (i.e. slightly higher baseline anxiety). Assuming that basal release of 5-HT increases as a function of the degree of stress experienced, it could be predicted that current test conditions would be more likely (than those operating in previous research) to reveal behavioral activity for 5-HT_{1A} receptor antagonists (e.g. see Hogg, 1996).

Regarding the ethological plus-maze measures, all compounds markedly decreased risk assessment (i.e. attempts) over a wide dose-range, but only diazepam clearly increased directed exploration (i.e. head-dipping). Benzodiazepine-induced stimulation of head-dipping in exploratory models of anxiety has been widely reported in the literature (e.g. Cole and Rodgers, 1993; Shepherd et al., 1994; Griebel et al., 1996; Cao and Rodgers, 1998b). Although WAY100635 also significantly increased head-dipping, the magnitude of this effect was more comparable to the small increase seen with the other 5-HT_{1A} receptor ligands than to the robust effect of diazepam. The results for attempts further confirm that this risk assessment response is particularly sensitive to the action of 5-HT_{1A} receptor ligands (Rodgers et al. 1994, 1995; Griebel et al., 1997; Setem et al., 1999). Interestingly, comparisons with 5-HT_{1A} receptor agonists previously assessed in the elevated plus-maze in our laboratory indicate some potentially important differences. Thus, full agonists (8-OH-DPAT and flesinoxan) and partial agonists (buspirone and ipsapirone) have all been found to affect the behavior of rats in this test (Griebel et al. 1997, 1998). However, while producing clear reduction in attempts, they failed to modify the conventional open arm avoidance measures. Taken together with findings in the murine plus-maze (Cao and Rodgers, 1997a,b,c) and defense test battery (Griebel et al., 1999), present results suggest that the anxiety-reducing potential of 5-HT_{1A} receptor antagonists may be superior to those of either full or partial agonists for this receptor.

Overall, the results of the present series of experiments demonstrate that 5-HT_{1A} receptor antagonists can produce anxiolytic-like effects in rats. However, these

effects appear to be test-specific and, unlike results obtained in mice, generally weaker than those produced by the prototypical anxiolytic, diazepam. This profile, along with dose–response and rate-dependency considerations, may partially explain inconsistent findings with these agents in previous studies using rat models of anxiety. Furthermore, the apparently greater consistency of effect observed in mouse versus rat models may be related to a species difference in the molecular pharmacology of the 5-HT_{1A} receptor subtype. In this context, and while there is little evidence of a major species difference in the central distribution of 5-HT_{1A} receptors (Pazos and Palacios, 1985; Waeber et al., 1989), in vivo studies would suggest an important species difference in 5-HT_{1A} receptor function. For example, in mice, 8-OH-DPAT-induced hypothermia is mediated by presynaptic 5-HT_{1A} autoreceptors, whereas in rats, it might be mediated by postsynaptic 5-HT_{1A} receptors (Bill et al., 1991). It is therefore conceivable that distinct 5-HT_{1A}-mediated mechanisms may underlie the behavioral responses to 5-HT_{1A} receptor antagonists in rat and mouse models of anxiety. However, given the general similarity of effect observed in rat and mouse versions of the same test (i.e. plus-maze), an alternate interpretation is that the extent to which 5-HT_{1A} receptor mechanisms are involved in the regulation of anxiety may depend critically upon the precise nature of the response studied.

Several recent studies using gene-targeting technology to generate 5-HT_{1A} receptor knockout mice have shown that these animals display more anxious-like behaviors (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). These results differ from those obtained in the present study where 5-HT_{1A} receptor blockade leads to an opposite action. However, the "chronic" blockade of 5-HT_{1A} receptors in mutant mice can hardly be compared to an acute blockade of these receptors by an antagonist. The lack of 5-HT_{1A} receptors in mutant mice may have produced developmental compensations which compromise a direct comparison between both types of studies.

The precise mechanisms underlying the positive effects of 5-HT_{1A} receptor antagonists in anxiety models remain to be determined. The compounds used in this study have all demonstrated antagonistic-like activity on postsynaptic 5-HT_{1A} receptors. It it is therefore possible that this mechanism may underlie the anxiolytic-like effects of these compounds. In addition, based on the findings that exposure to aversive stimuli like those used in the above studies increases 5-HT release, we would expect a 5-HT_{1A} receptor antagonist to attenuate this effect and thus display anxiolytic activity. However, further studies are clearly warranted to determine why these compounds failed to be active in several models of anxiety.

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