Pharmacology Biochemistry and Behavior, Vol. 58, No. 2, pp. 593–603, 1997
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0091-3057/97 $17.00 + .00

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PII S0091-3057(97)00279-7

Influence of 5-HT_{1A} Receptor Antagonism on Plus-Maze Behaviour in Mice. II. WAY 100635, SDZ 216-525 and NAN-190

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Received 13 September 1996; Revised 20 December 1996; Accepted 20 December 1996

CAO, B. -J. AND R. J. RODGERS. *Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice. II. WAY 100635, SDZ 216-525 and NAN-190.* PHARMACOL BIOCHEM BEHAV **58**(2) 593–603, 1997.—To understand further the role of 5-hydroxytryptamine receptor subtype 1A (5-HT_{1A}) mechanisms in anxiety, the behavioural effects of 5-HT_{1A} receptor antagonists with different selectivity and intrinsic activity were examined using an ethological version of the murine elevated plus-maze test. WAY 100635 (0.03–9.0 mg/kg) produced a behavioural profile indicative of an anxiolyticlike effect, with an apparent bell-shaped dose–response relationship and increases in nonexploratory behaviours at the largest dose tested. SDZ 216-525 exerted a dose-dependent antianxiety action at doses of 0.05–0.8 mg/kg, with some loss of activity at 3.2 mg/kg. In contrast, smaller doses of NAN-190 had a significant effect, whereas higher doses (2.5–10.0 mg/kg) decreased locomotor activity and other active behaviours, a profile similar to that produced by the α_1-adrenoceptor antagonist prazosin (2.5 mg/kg), which also inhibited open arm activity. Findings are discussed in relation to 5-HT_{1A} receptor and α_1-adrenoceptor antagonism and corresponding neurochemical changes. The results of the present series support the view that 5-HT_{1A} receptor antagonists have therapeutic potential in the management of anxiety. © 1997 Elsevier Science Inc.

Anxiety Elevated plus-maze 5-HT_{1A} receptors α_1-Adrenoceptors WAY 100635 SDZ 216-525 NAN-190 Prazosin Mice

AMONG the serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes, 5-HT_{1A} receptors are of particular interest because of the therapeutic potential of drugs acting at these sites. Over the past few years, attempts have been made to develop selective 5-HT_{1A} receptor antagonists as both pharmacological tools and potential therapeutic agents. Some progress has been made in identifying such ligands, and several compounds are now available to faciliate research on the physiological and behavioural consequences of 5-HT_{1A} receptor antagonism. The first ligand described as an antagonist at both pre- and postsynaptic 5-HT receptors was the (S)-enantiomer of the 5-fluoro analogue of 8-OH-DPAT, (S)-UH-301 (9). However, a lack of selectivity for 5-HT_{1A} receptors limits the use of this compound as a research tool (53). SDZ 216-525 (57) and (S)-WAY 100135 (21,22) were originally thought to be selective and silent (devoid of agonist activity) 5-HT_{1A} receptor antagonists. However, both compounds have since been shown to be neither "selective" nor "silent": they decrease 5-HT release and dorsal raphe neuronal cell firing via an α_1-adrenoceptor antagonist action and/or partial agonist activity at 5-HT_{1A} autoreceptors (4,53,58). A significant development in the search for selective 5-HT_{1A} compounds that function purely as antagonists has been the characterisation of the phenylpiperazine WAY 100635, which is the first potent ligand that truly satisfies the requirement of selectivity and antagonist activity at both somatodendritic and postsynaptic 5-HT_{1A} receptors (4,23,24,53).

The clinical anxiolytic efficacy of buspirone, a 5-HT_{1A} receptor partial agonist, has focussed considerable attention on a potential link between 5-HT_{1A} receptor function and anxiety. In this context, extensive investigations employing full and partial agonists have produced inconsistent results, and many controversial issues remain to be solved (19,25). Stimulation of presynaptic 5-HT_{1A} receptors and/or blockade of postsynaptic 5-HT_{1A} sites may result in a decrease in 5-HT neurotransmission (7,17,22), either or both of which may account for the anxiolytic effect of buspirone. Following this line of reasoning, 5-HT_{1A} receptor antagonists should have

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anxiolytic potential (22). In this context, although the effects of antagonists have not been evaluated systematically in procedures used to detect changes in anxiety, initial findings have not been fully consistent with the classic 5-HT theory of anxiety. A number of compounds with 5-HT_{1A} receptor antagonist properties reduce anxiety-related responses in the rodent elevated plus-maze [(S) UH-301, (S) WAY 100135 and p-MPPI, a close structural analogue of WAY 100635 (62); (12,42,50)], mouse light/dark exploration [(S) UH-301, (S) WAY 100135, WAY 100635 (7,23,42)], rat potentiated startle [(S) WAY 100135 (21)], ferret territorial avoidance [WAY 100635 (59)] and mouse antipredator defense [S 21187 and S 21357 (26,27)] tests. However, negative results have also been reported with several of these agents in conflict procedures (14,15,42,46,48) and in models based on spontaneous behaviour, e.g., the rat social interaction (10) and elevated plus-maze (6,18,41) paradigms. Furthermore, WAY 100635 may exert anxiogeniclike effects in the shock-induced ultrasonic vocalisation test in rats (28). Because the reasons for these discrepancies are not clear at present, more research is needed to clarify the anxiolytic potential of 5-HT_{1A} receptor antagonists.

In the present study, a detailed ethological technique (49) was employed to examine the effects of 5-HT_{1A} receptor antagonists on plus-maze behaviour in mice. WAY 100635 was chosen because it is the most selective compound of this class currently available. For comparative purposes, SDZ 216-525 and NAN-190, which have partial agonist activity at presynaptic sites and behave as mixed 5-HT_{1A} receptor agonists–antagonists (16,17,22,37,53,58), were included in the series. To control for the α_1-adrenoceptor antagonist activity of these 5-HT_{1A} ligands, the effects of prazosin were also examined under the same test conditions.

METHODS

Animals

Male Swiss Webster mice (Bantin & Kingman, Hull, UK), aged 8–9 weeks at the time of testing, were group housed (n = 10) for at least 3 weeks prior to experimentation. They were maintained in a temperature- (20 ± 1°C) and humidity- (50 ± 5%) controlled environment in which a reversed light cycle was used (lights off: 0700–1900 h). Food and drinking water were freely available with the exception of the brief test periods. All mice were experimentally naive.

Drugs

WAY 100635, N-{2-[4-(2-methoxyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (Wyeth Research Ltd., Taplow, UK), NAN-190 hydrobromide (RBI, Natick, MA, USA) and prazosin hydrochloride (RBI) were dissolved in physiological saline, which served for control

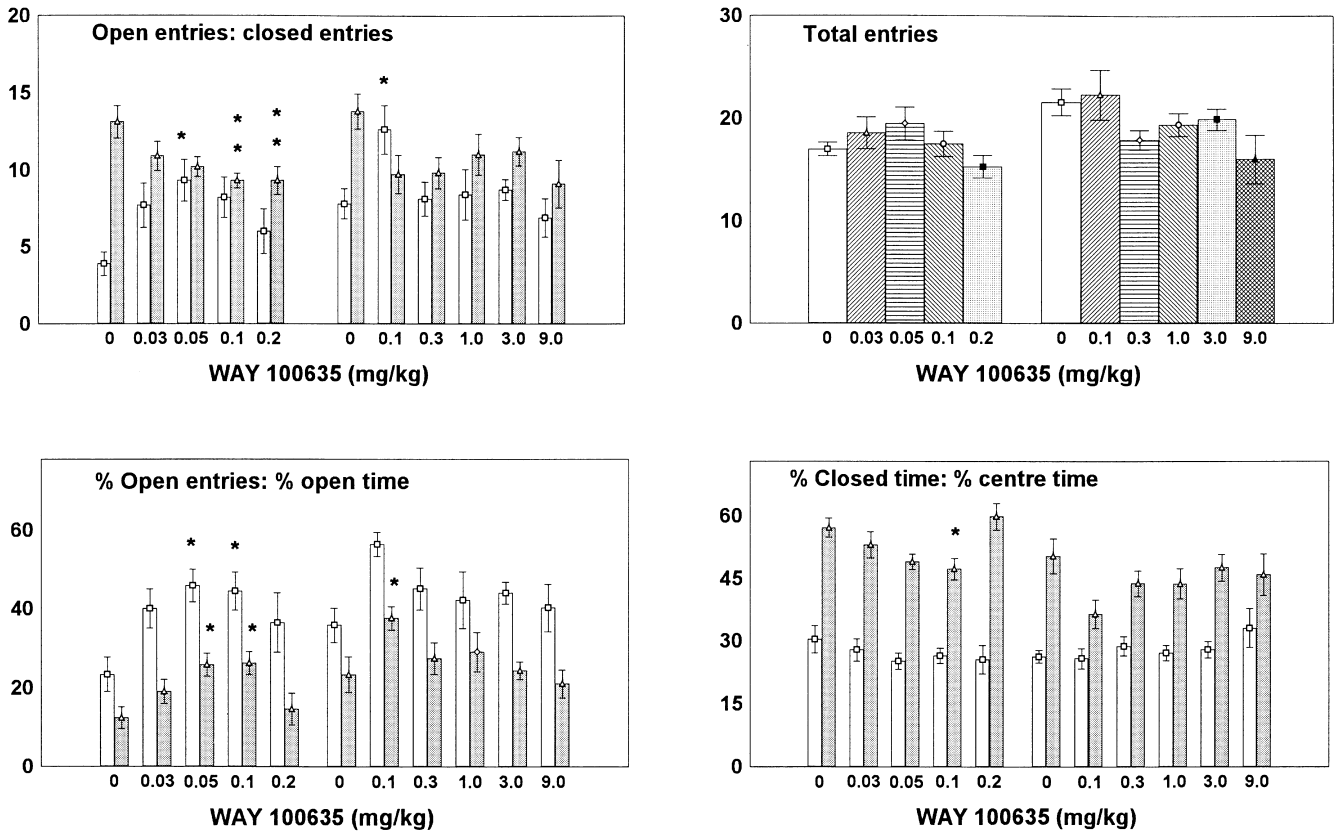

FIG. 1. Effects of WAY 100635 (0.03–0.2 mg/kg and 0.1–9.0 mg/kg) on open, closed and total arm entries and on percentage of time spent on open, closed and centre parts of the elevated plus-maze in male Swiss Webster mice. Open entries: closed entries chart—open bars = open entries, stippled bars = closed entries. %Open entries: % open time chart—open bars = %open entries, stippled bars = %open time. %Closed time: %centre time chart—open bars = %closed time, stippled bars = %centre time. Data are expressed as mean values ± SEM (n = 10). *p < 0.05, *p < 0.01 vs. vehicle control.

injections. SDZ 216-525, methyl 4-{4-[4-1,1,3-trioxo-2H-1,2-bernzoisothiazol-2-yl)butyl]-1-piperazinyl}1H-indole-2-carboxylate (Sandoz Pharma Ltd., Basel, Switzerland) was dissolved in lactic acid (5 drops in 10 ml 0.9% saline), and a corresponding lactic acid–saline solution was used for control injections. With the exception of SDZ 216-525, which was injected subcutaneously, all compounds were administered intraperitoneally (10 ml/kg) 30 min before testing. Doses cited refer to salts, where applicable.

Apparatus

The elevated plus-maze (Plexiglas: black floor, clear walls) was a modification of that validated for NIH Swiss mice by Lister (39) and consisted of two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) that radiated from a central platform (5 × 5 cm) to form a plus sign. The entire apparatus was raised to a height of 60 cm above floor level.

Procedure

The test procedure and scoring methodology have been described in detail elsewhere (52). In brief, testing was conducted during the dark phase of the light cycle in a dimly illuminated (4 × 60 W red, indirect) laboratory. To facilitate habituation, animals were transported to the laboratory and left undisturbed for at least 1 h before testing. In each experiment, mice were randomly allocated to treatment conditions (n = 10) and tested in a counterbalanced order. Testing commenced by placing a mouse on the central platform facing an open arm. A 5-min test duration was employed and, between subjects, the maze was cleaned thoroughly with damp and dry cloths. Test sessions were recorded on videotape and subsequently scored blind with ethological analysis software (Hindsight, version 1.4; developed by Dr. Scott Weiss). Both conventional and ethological parameters (49,52) were recorded; intrarater reliability was ⩾0.9.

Statistics

Data were subjected to single-factor (drug treatment) or two-factor (drug treatment and maze location; repeated measures on location) analyses of variance (ANOVA); further comparisons were performed by using the appropriate error variance terms from the ANOVA summary tables (Dunnett or Duncan tests). Due to their nonparametric nature, data for closed arm returns and immobility were analysed by the Kruskal–Wallis ANOVA, followed by the Mann–Whitney U-test.

RESULTS

Experiment 1: WAY 100635

Lower dose range (0.03–0.2 mg/kg). Data are presented in the left panels of Figs. 1–4. ANOVA indicated significant

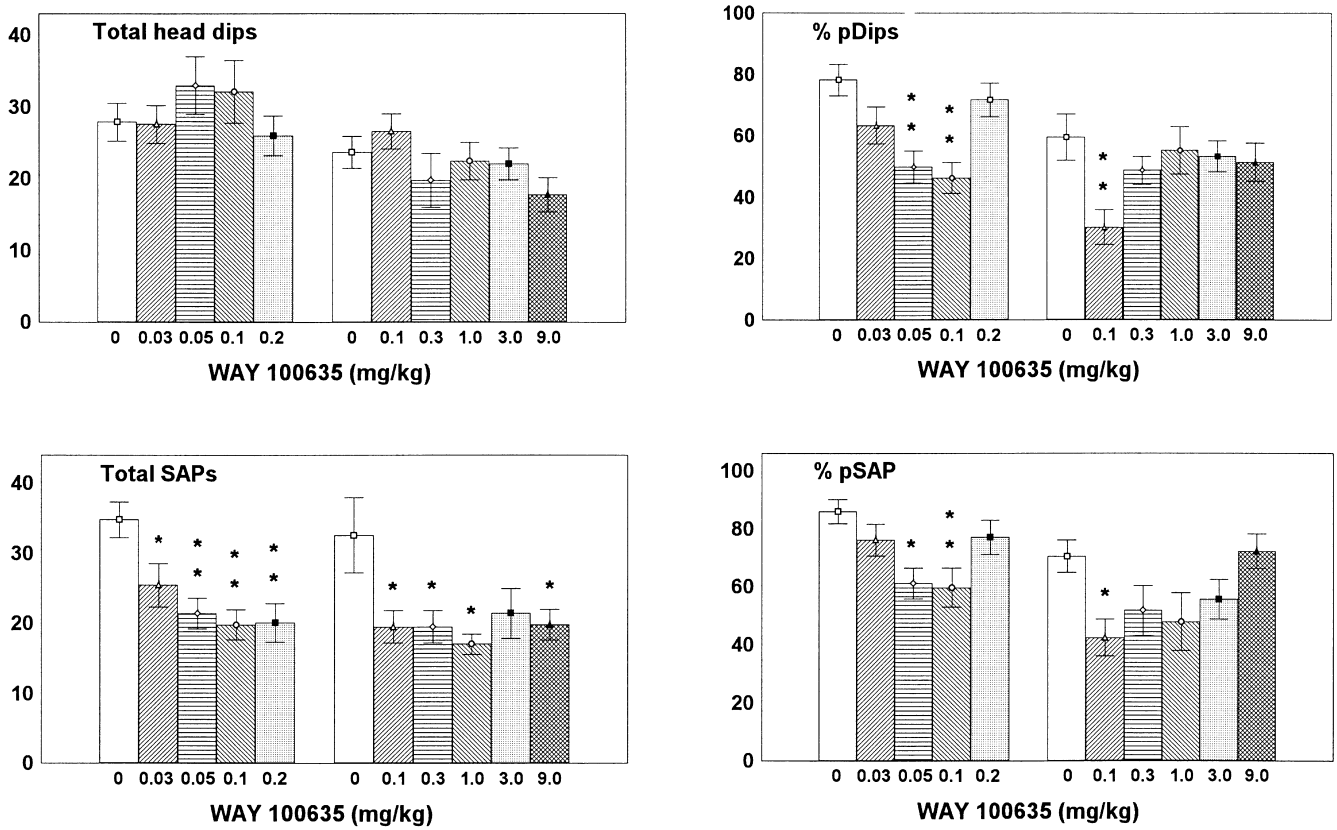

FIG. 2. Effects of WAY 100635 (0.03–0.2 mg/kg and 0.1–9.0 mg/kg) on total head dips, percentage of protected head dips (%pDips), total stretched attend postures (SAPs) and percentage of protected stretched attend postures (%pSAP) in male Swiss Webster mice tested in the elevated plus-maze. Data are expressed as mean values ± SEM (n = 10). *$p < 0.05$, *$p < 0.01$ vs. vehicle control.

main effects of drug treatment on open entries [$F(4,45) = 2.67$, $p < 0.05$], closed entries [$F(4,45) = 3.61$, $p < 0.05$] and percentage of open entries [$F(4,45) = 2.67$, $p < 0.05$]. WAY 100635 significantly increased open entries at 0.05 mg/kg ($p < 0.05$) and percentage of open entries at 0.05 and 0.1 mg/kg ($p < 0.05$). Decreases in closed entries were seen at 0.1 and 0.2 mg/kg ($p < 0.01$). This compound did not have an effect on total entries [$F(4,45) = 1.58$, NS]. Analysis of percentage of time measures revealed a highly significant preference for different sections of the maze [$F(2,90) = 127.61$, $p < 0.01$], with saline-treated mice showing a rank-order preference for centre platform > closed arms > open arms. This profile was altered by WAY 100635 [$F(8,90) = 2.96$, $p < 0.01$] such that mice treated with 0.03–0.1 mg/kg did not differentiate between closed and open arms. Time spent on open arms and centre platform was also affected by drug treatment [$F(4,45) = 3.98$, $p < 0.01$] in each case. Post hoc comparisons revealed increases in percentage of open time at 0.05 and 0.1 mg/kg and a decrease in percentage of centre time at 0.1 mg/kg ($p < 0.05$ in all cases). WAY 100635 lacked effects on percentage of closed time [$F(4,45) = 0.61$, NS].

Ethological measures were also sensitive to the effects of WAY 100635. Significant reductions in total stretched attend postures [$F(4,45) = 5.96$, $p < 0.01$], and the percentage of protected forms of head dips [$F(4,45) = 6.51$, $p < 0.01$], stretched attend [$F(4,45) = 4.12$, $p < 0.01$], flat-back approach [$F(4,45) = 9.45$, $p < 0.01$] and sniff [$F(4,45) = 3.19$, $p < 0.05$] were observed, with most of these alterations evident at 0.05 and 0.1 mg/kg ($p < 0.05$ to $p < 0.01$). Closed arm returns were also altered by drug treatment ($H = 11.14$, $p < 0.05$), with reductions at 0.05 and 0.1 mg/kg ($p < 0.05$ in both cases). Over the dose range tested, WAY 100635 did not have a significant effect on rearing frequency [$F(4,45) = 1.24$, NS], rearing duration [$F(4,45) = 0.88$, NS], total head dips [$F(4,45) = 0.82$, NS], flat-back approach [$F(4,45) = 0.10$, NS], sniffing [$F(4,45) = 1.67$, NS], grooming [$F(4,45) = 0.40$, NS] and immobility ($H = 3.06$, NS).

Upper dose range (0.1–9.0 mg/kg). Data are summarised in the right panels of Figs. 1–4. ANOVA indicated that WAY 100635 altered open arm entries [$F(5,54) = 2.56$, $p < 0.05$] and percentage of open time [$F(5,54) = 2.37$, $p = 0.05$], with increases in both measures at 0.1 mg/kg ($p < 0.05$). Further analyses confirmed the effect of WAY 100635 on percentage of open time: subjects displayed a distinct pattern of activity in the maze [$F(2,108) = 31.94$, $p < 0.01$], with control animals showing a rank-order preference for centre > closed = open. Although drug treatment failed to alter this profile significantly [$F(10,108) = 1.77$, $p = 0.07$], mice treated with 0.1 mg/kg WAY 100635 did not differentiate different sections of the maze (centre = closed = open). The F-values for other conventional spatiotemporal measures did not reach significance: total entries [$F(5,54) = 1.96$], closed entries [$F(5,54) = 1.94$], percentage of open entries [$F(5,54) = 1.89$], percentage of centre time [$F(5,54) = 0.19$] and percentage of closed time [$F(5,54) = 1.00$].

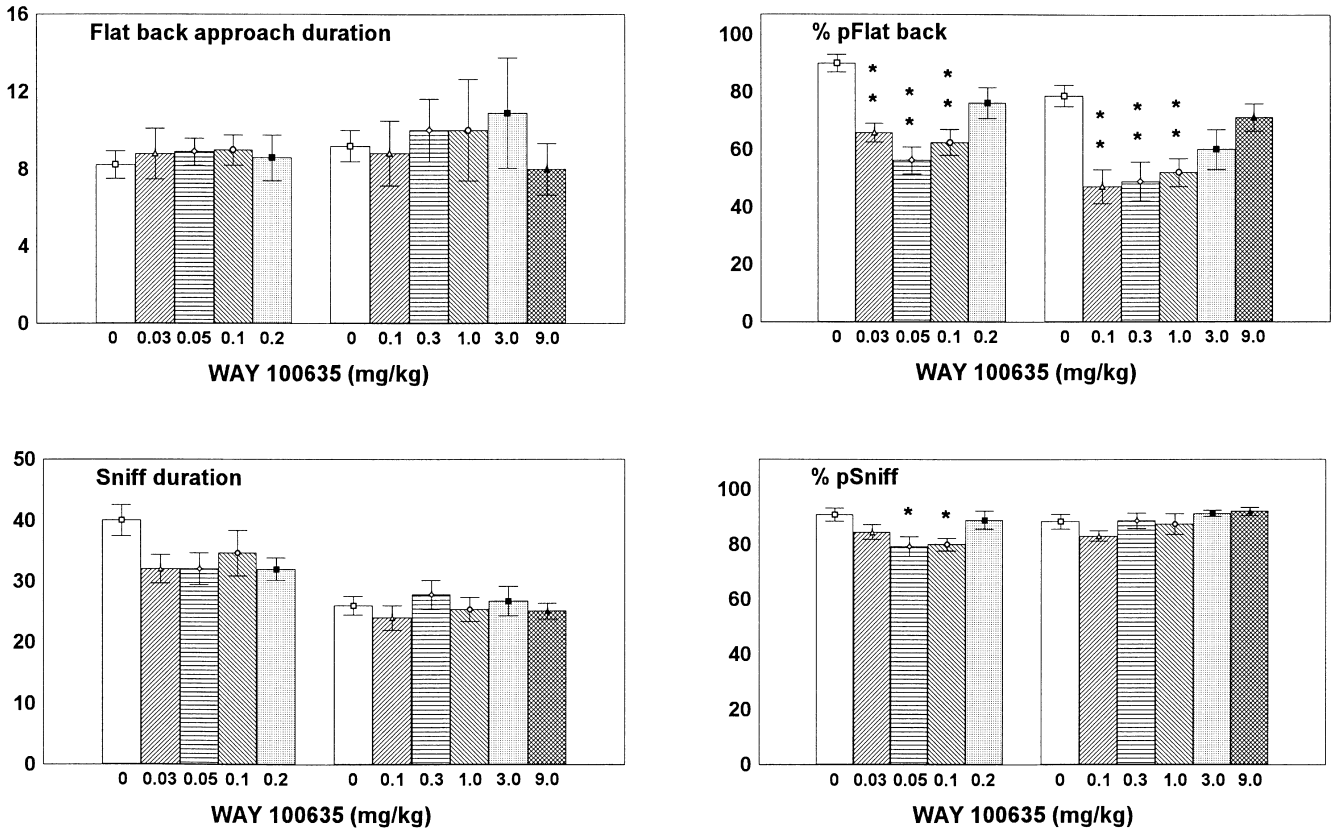

FIG. 3. Effects of WAY 100635 (0.03–0.2 mg/kg and 0.1–9.0 mg/kg) on flat-back approach duration (s), percentage of pretected flat-back approach (%pFlat back), sniff duration (s) and percentage of protected sniff (%pSniff) in male Swiss Webster mice tested in the elevated plus-maze. Data are expressed as mean values ± SEM ($n = 10$). *$p < 0.05$, *$p < 0.01$ vs. vehicle control.

WAY 100635 had significant effects on stretched attend postures [$F(5,54) = 3.10$, $p < 0.05$], closed arm returns ($H = 9.35$, $p < 0.01$), grooming [$F(5,54) = 9.87$, $p < 0.01$], immobility ($H = 36.05$, $p < 0.01$) and on the percentages of protected forms of head dipping [$F(5,54) = 2.71$, $p < 0.05$], stretched attend [$F(5,54) = 2.69$, $p < 0.05$] and flat-back approach [$F(5,54) = 5.22$, $p < 0.01$]. Follow-up comparisons confirmed that, whereas grooming and immobility were increased at the top dose tested ($p < 0.01$ in both cases), all other parameters were reduced at 0.1 mg/kg ($p < 0.05$ to $p < 0.01$), with reductions in total stretched attend postures and protected flat back also seen at higher doses. No significant alterations were noted in total head dips [$F(5,54) = 1.33$, NS], sniff [$F(5,54) = 0.45$, NS], flat-back approach [$F(5,54) = 0.28$, NS] and protected sniffing [$F(5,54) = 1.64$, NS].

Experiment 2: SDZ 216-525

Data are presented in Fig. 5. Of the conventional measures taken, total arm entries and percentage of centre time were not significantly altered by drug treatment [$F(4,45) = 0.69$ and 1.21, respectively; NS]. However, the F-value for percentage of closed arm time closely approached significance [$F(4,45) = 2.53$, $p = 0.053$]. Variables that showed significant treatment effects [$F(\text{crit},0.05) = 2.61$] were open entries (2.67, $p < 0.05$), closed entries (4.90, $p < 0.01$), percentage of open entries (4.71, $p < 0.01$) and percentage of open time (2.89, $p < 0.05$). Significant changes on these measures were evident at 0.2–0.8 mg/kg ($p < 0.05$ to $p < 0.01$), with increases in open entries, percentage of open entries and open time and decreases in closed entries and percentage of closed time. The significant reduction in closed entries remained apparent at 3.2 mg/kg. Mice displayed a very strong preference for different sections of the maze [$F(2,90) = 97.23$, $p < 0.01$], with vehicle-treated subjects showing a rank-order preference of centre > closed > open. This profile was also affected by SDZ 216-525 [$F(8,90) = 2.10$, $p < 0.05$] such that mice treated with SDZ 216-525 (0.05–3.2 mg/kg) did not differentiate open and closed arms.

Significant changes were also noted in total stretched attend postures [$F(4,45) = 5.16$, $p < 0.01$], closed arm returns ($H = 13.07$, $p < 0.05$; data not shown) and in the percentage of protected forms of head dipping [$F(4,45) = 3.68$, $p < 0.05$], stretched attend posture [$F(4,45) = 3.19$, $p < 0.05$] and flat-back approach [$F(4,45) = 4.85$, $p < 0.01$]. Post hoc tests confirmed reductions in all these measures, with most of the alterations apparent at 0.2–3.2 mg/kg. SDZ 216-525 had no significant effects on total head dips [$F(4,45) = 1.68$, NS], flat-back approach [$F(4,45) = 0.24$, NS], sniff [$F(4,45) = 0.29$, NS], percentage of protected sniff [$F(4,45) = 2.41$, NS], rearing [frequency: $F(4,45) = 2.09$, NS; duration: $F(4,45) = 2.31$, NS], grooming [$F(4,45) = 0.54$, NS] and immobility ($H = 8.16$, NS).

Experiment 3: NAN-190

The effects of NAN-190 are summarised in Table 1, which also presents associated F- and H-values. Over the dose range

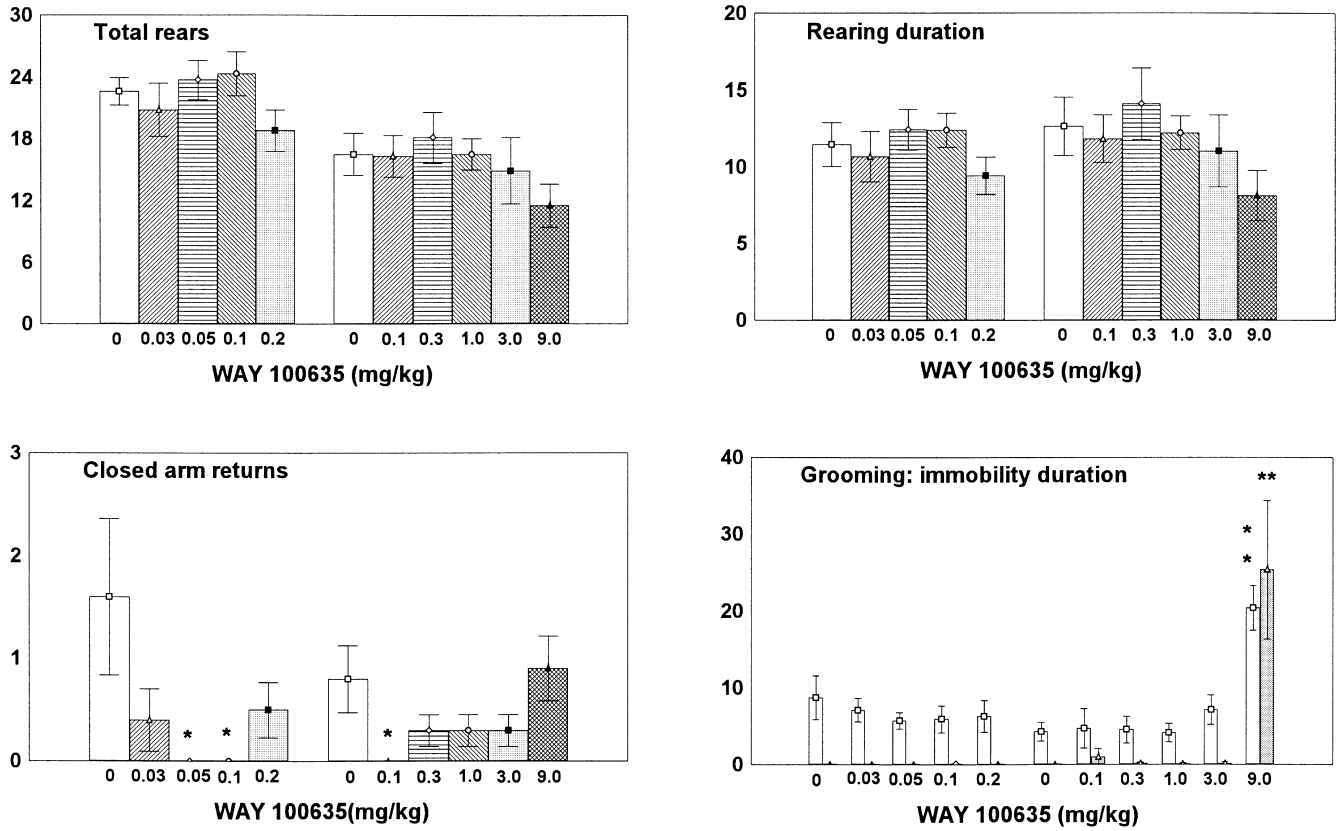

FIG. 4. Effects of WAY 100635 (0.03–0.2 mg/kg and 0.1–9.0 mg/kg) on total rears, rearing duration (s), closed arm returns, grooming duration (s) and immobility duration (s) in male Swiss Webster mice tested in the elevated plus-maze. Grooming: immobility duration chart—open bars = grooming, stippled bars = immobility. Data are expressed as mean values ± SEM ($n = 10$). *$p < 0.05$, *$p < 0.01$ vs. vehicle control.

tested, this compound had no significant effects on open entries, percentage of open entries/time, closed arm returns and percentage of protected forms of head dipping, stretched attend postures, flat-back approach and sniffing. However, significant reductions in rearing frequency, total head dipping, stretched attend postures and sniffing were observed at 2.5–10.0 mg/kg ($p < 0.05$ to $p < 0.01$), with an increase in immobility. In addition, reductions in closed entries, total entries, rearing duration and flat-back approach reached significance at 10.0 mg/kg ($p < 0.05$ to $p < 0.01$). For percentage of time, the rank-order preference of centre platform > closed arms = open arms in saline-treated mice [$F(2,90) = 24.99$, $p < 0.01$] was not altered by NAN-190 [$F(8,90) = 1.35$, NS].

Experiment 4: Prazosin

The effects of prazosin are summarised in Table 2, which also presents associated *F*- and *H*-values. A significant decrease in total head dips at 0.5–2.5 mg/kg ($p < 0.01$ in both cases) and an increase in immobility at 2.5 mg/kg ($p < 0.05$) were observed. Although *F*-values for other variables did not reach significance, animals treated with 2.5 mg/kg of prazosin showed reductions (or a trend towards decreases) in open arm entries, total entries, percentage of open entries, percentage of open time, total stretched attend postures, flat-back approach and sniffing duration. In addition, increases in percentage of closed time, percentage of protected stretched attend and protected sniffing were observed at the highest dose tested. Subjects displayed a very strong preference for different sections of the maze [$F(2,90) = 42.60$, $p < 0.01$]. Although ANOVA indicated that prazosin did not significantly influence percentage of time spent on the different maze sections [$F(8,90) = 0.99$, NS], mice treated with 2.5 mg/kg prazosin showed a rank-order preference for central square > closed arms > open arms, which was different from the preference of other groups (centre > closed = open).

DISCUSSION

It is only within the last few years that selective 5-HT_{1A} receptor antagonists have become available (53), and their effects in animal models of anxiety have been somewhat variable. Thus, although (S)-UH-301 (42), (S) WAY 100135 (50) and p-MPPI (12) have anxiolyticlike effects in the rodent plus-maze, other groups have reported that WAY 100635 (18), WAY 100135 (41) and another novel 5-HT_{1A} receptor antagonist, LY297996 (32), do not have significant effects in this model. Interestingly, although (S) WAY 100135 and WAY 100635 given alone failed to alter the behaviour of rats in this test, they did attenuate the anxiogeniclike profile of the unsulfated form of cholecystokinin-octapeptide (6). Although the reasons for these inconsistencies are not fully understood,

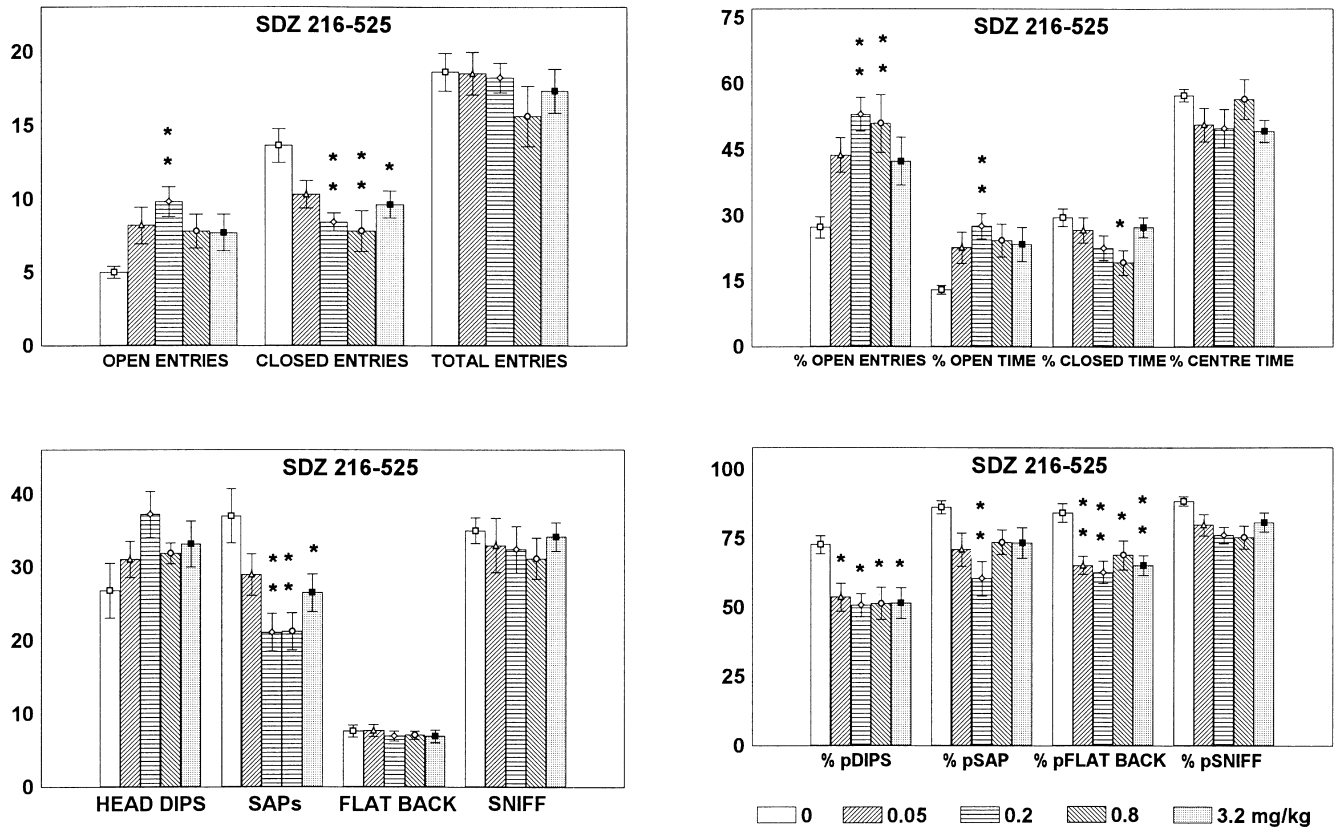

FIG. 5. Effects of SDZ 216-525 (0.05–3.2 mg/kg) on open, closed and total arm entries; percentage of time spent on open, closed and centre parts of the maze; total head dips, total stretched attend postures (SAPs), flat-back approach duration (flat back; s) and sniff duration (s); and percentages of protected head dips (%pDips), protected stretched attend postures (%pSAP), pretected flat-back approach (%pFlat back) and protected sniff (%pSniff) in male Swiss Webster mice tested in the elevated plus-maze. Data are expressed as mean values ± SEM ($n = 10$). *$p < 0.05$, *$p < 0.01$ vs. vehicle control.

TABLE 1

EFFECTS OF NAN-190 (0.1–10.0 MG/KG) ON PLUS-MAZE BEHAVIOUR IN MALE SWISS WEBSTER MICE

		NAN-190 (mg/kg)				
Behaviour	Vehicle	0.1	0.5	2.5	10.0	F(4, 45)
Open arm entries	5.5 ± 0.8	6.4 ± 1.4	7.2 ± 1.1	6.9 ± 1.6	3.3 ± 0.9	1.69, NS
Closed arm entries	12.0 ± 1.1	10.0 ± 1.1	9.2 ± 1.0	8.6 ± 1.0	6.0 ± 1.2**	3.94, $p < 0.01$
Total arm entries	17.5 ± 1.7	16.4 ± 1.8	16.4 ± 2.0	15.5 ± 1.7	9.3 ± 1.7**	3.39, $p < 0.05$
% Open arm entries	30.2 ± 3.0	35.1 ± 7.0	41.8 ± 3.6	40.9 ± 6.5	35.0 ± 9.7	0.55, NS
% Open arm time	17.4 ± 2.5	21.7 ± 5.5	26.4 ± 4.3	22.3 ± 5.3	21.1 ± 9.0	0.31, NS
% Closed arm time	25.5 ± 2.4	27.0 ± 3.2	23.7 ± 2.6	26.6 ± 2.8	41.3 ± 8.3	2.50, NS
% Centre platform time	57.1 ± 3.9	51.3 ± 4.1	49.9 ± 5.8	51.1 ± 5.3	37.7 ± 7.4	1.71, NS
Total head-dips	37.4 ± 2.6	30.2 ± 4.5	30.6 ± 1.1	23.1 ± 3.1**	12.1 ± 2.2**	10.81, $p < 0.01$
% Protected head-dips	68.7 ± 5.3	56.6 ± 8.0	55.5 ± 7.9	56.8 ± 7.6	50.6 ± 9.5	0.75, NS
Total stretched attend postures	33.9 ± 2.9	27.2 ± 2.9	27.4 ± 2.7	18.8 ± 1.6**	12.8 ± 2.4**	10.48, $p < 0.01$
% Protected stretched attend postures	83.5 ± 2.9	78.7 ± 7.1	76.5 ± 3.8	76.1 ± 8.0	80.2 ± 10.2	0.19, NS
Flat back approach duration(s)	5.7 ± 0.8	6.6 ± 0.7	5.0 ± 0.4	4.0 ± 0.4	3.1 ± 0.5**	5.08, $p < 0.01$
% Protected flat back approach	60.6 ± 4.8	72.0 ± 5.6	55.7 ± 6.7	58.7 ± 8.5	72.5 ± 9.3	1.19, NS
Sniff duration(s)	36.4 ± 0.7	31.2 ± 1.6	37.2 ± 1.6	28.2 ± 1.6**	24.0 ± 2.3*	11.58, $p < 0.01$
% Protected sniff	86.7 ± 2.1	81.1 ± 5.7	78.7 ± 4.0	79.5 ± 5.9	80.9 ± 8.7	0.30, NS
Closed arm returns	0.5 ± 0.3	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	H = 2.45, NS
Total rears	15.7 ± 2.2	13.2 ± 1.5	10.2 ± 2.3	6.6 ± 2.1**	5.3 ± 1.7**	4.79, $p < 0.01$
Rear duration(s)	7.0 ± 1.1	7.2 ± 1.1	4.1 ± 1.3	3.2 ± 1.0	2.7 ± 0.9*	3.86, $p < 0.01$
Groom(s)	2.3 ± 1.5	1.9 ± 1.2	6.6 ± 2.8	4.2 ± 1.6	8.4 ± 2.5	0.75, NS
Immobility(s)	0.1 ± 0.1	0.7 ± 0.5	0.2 ± 0.1	4.3 ± 2.1*	54.5 ± 14.5**	H = 28.29, $p < 0.01$

Data are presented as mean values ± SEM ($n = 10$). *$p < 0.05$, **$p < 0.01$ vs. vehicle.

at least some of the negative results may be due to the use of limited dose ranges. This consideration led us to employ wide dose ranges in the present investigation of the influence of WAY 100635, SDZ 216-525 and NAN-190 on plus-maze behaviour in mice.

WAY 100635 is the most selective, perhaps the only, silent (no intrinsic agonist activity at both pre- and postsynaptic sites) 5-HT$_{1A}$ antagonist currently available (4,23,24,53). The influence of this ligand on anxiety-related behaviour has been evaluated in several paradigms in addition to the elevated

TABLE 2

EFFECTS OF PRAZOSIN (0.02–2.5 MG/KG) ON PLUS-MAZE BEHAVIOUR IN MALE SWISS WEBSTER MICE

		Prazosin (mg/kg)				
Behaviour	Vehicle	0.02	0.1	0.5	2.5	F(4, 45)
Open arm entries	8.0 ± 1.7	5.5 ± 1.3	5.9 ± 1.4	4.6 ± 1.0	2.9 ± 1.2*	1.95, NS
Closed arm entries	7.6 ± 1.2	8.7 ± 0.9	8.0 ± 1.3	6.2 ± 1.0	6.9 ± 1.3	0.68, NS
Total arm entries	15.6 ± 2.1	14.2 ± 1.3	13.9 ± 2.0	10.8 ± 1.6	9.8 ± 2.2	1.73, NS
% Open arm entries	48.7 ± 7.1	36.5 ± 6.3	37.0 ± 8.3	40.9 ± 6.8	23.2 ± 6.6	1.64, NS
% Open arm time	27.4 ± 6.5	23.3 ± 4.9	22.0 ± 4.5	21.8 ± 4.4	10.2 ± 3.8	1.72, NS
% Closed arm time	18.2 ± 3.0	21.9 ± 2.2	20.9 ± 3.9	27.3 ± 4.6	32.9 ± 7.1	1.69, NS
% Centre platform time	54.4 ± 6.4	54.8 ± 4.4	57.1 ± 6.1	51.0 ± 5.7	56.9 ± 7.7	0.16, NS
Total head-dips	37.8 ± 3.2	29.1 ± 3.8	35.1 ± 3.6	19.6 ± 1.8**	15.3 ± 3.2**	9.22, $p < 0.01$
% Protected head-dips	59.7 ± 8.1	66.4 ± 8.0	61.1 ± 7.2	49.4 ± 10.0	76.7 ± 8.4	1.38, NS
Total stretched attend postures	24.0 ± 3.3	19.1 ± 2.9	18.2 ± 2.6	15.1 ± 2.2	14.5 ± 2.1	2.05, NS
% Protected stretched attend postures	68.9 ± 8.4	71.4 ± 6.4	76.3 ± 6.4	78.0 ± 6.3	91.2 ± 4.1	1.83, NS
Flat back approach duration(s)	3.8 ± 0.7	3.9 ± 0.5	2.2 ± 0.4	3.2 ± 0.5	2.4 ± 0.5	2.33, NS
% Protected flat back approach	76.3 ± 5.4	61.6 ± 7.9	73.4 ± 7.3	69.2 ± 4.6	77.8 ± 8.8	0.88, NS
Sniff duration(s)	40.3 ± 3.2	36.8 ± 2.0	34.7 ± 2.3	32.9 ± 2.4	29.6 ± 2.7	2.52, NS
% Protected sniff	75.8 ± 5.7	79.0 ± 4.9	80.0 ± 4.5	79.7 ± 4.6	92.4 ± 3.7	1.83, NS
Closed arm returns	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	H = 1.61, NS
Total rears	11.2 ± 3.1	11.2 ± 2.8	6.5 ± 1.5	5.9 ± 1.4	7.1 ± 2.4	1.25, NS
Rear duration(s)	4.8 ± 1.4	5.8 ± 1.6	3.0 ± 1.0	3.1 ± 0.8	4.2 ± 1.4	0.87, NS
Groom(s)	2.0 ± 1.0	1.1 ± 0.7	6.4 ± 3.0	8.5 ± 3.1	6.2 ± 1.8	2.11, NS
Immobility(s)	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 4.0	2.4 ± 1.7	32.8 ± 18.2**	H = 12.46, $p < 0.05$

Data are presented as mean values ± SEM ($n = 10$). *$p < 0.05$, **$p < 0.01$ vs. vehicle.

plus-maze, with findings ranging from anxiolysis [the mouse light/dark and ferret territorial avoidance tests (23,59)], through no effect [the rat shock-induced ultrasonic vocalisation and conditioned emotional response paradigms (48,61)] to anxiogenesis [the rat shock-induced ultrasonic vocalisation model (28)]. In the present study, WAY 100635 produced a behavioural profile indicative of anxiety reduction, with an apparent bell-shaped dose–response relationship. Significant anxiolyticlike effects were seen over a limited dose range (0.05–0.1 mg/kg) on both conventional and ethological indices of anxiety, including increased percentage of open entries and/or open arm time and decreases in several risk assessment behaviours (protected head dipping, stretched attend postures, protected flat-back approach, protected sniffing and closed arm returns). These effects were lost at higher doses, a profile accompanied (at the top dose tested, 9.0 mg/kg) by a significant increase in nonexploratory behaviours (grooming and immobility), a decrease in closed arm entries and a trend towards to reduction in total entries. Although WAY 100635 exhibits only moderate affinity for α_1-adrenoceptors (4), its metabolite, WAY 100634, has a high affinity for these binding sites in vitro (44). Thus, the behaviourally nonselective high dose effect of WAY 100635 may be mediated via an action at α_1-adrenoceptors. Although little previous work has been done with SDZ 216-525 in animal models of anxiety, this compound reduces anxiety and/or defensive behaviour in mice tested in the light/dark exploration (7) and social interaction (5) tests. Present data show that, at 0.05–0.8 mg/kg, SDZ 216-525 exerts a dose-dependent anxiolyticlike action. Although reductions in several ethological measures were maintained at a higher dose of 3.2 mg/kg, some loss of antianxiety activity was noted on the conventional percentage of open arm entries/time parameters.

Although prazosin increases punished responding in pigeons (35), abolishes foot shock-induced ultrasonic vocalisation in rats (55), facilitates exploratory behaviour of mice in the white compartment in a fully automated two-compartment black-and-white test box (56), inhibits stress-induced hyperthermia in mice (66) and reduces rodent defensive behaviours (40,65), this α_1-adrenoceptor antagonist is apparently inactive in the Vogel conflict test (36,45,47) and, in isolated male mice, may even enhance aspects of the defensive repertoire (2). An initial report by Handley and Mithani (30) showing that low doses of prazosin increase the percentage of open arm entries in the rat elevated plus-maze, suggesting an anxiolyticlike action, has not been replicated by other investigators (43,60). Present data show that, at 0.02–0.1 mg/kg, prazosin does not alter the behaviour of mice tested on the elevated plus-maze. At a higher dose (2.5 mg/kg), the majority of active behaviours were inhibited, whereas nonexploratory behaviours (grooming and immobility) were increased. Although caution is warranted in view of the reduction in locomotor activity, the observed decreases in open arm entries/time and trends towards increases in stretched attend postures and sniffing displayed from insecure areas of the maze (%pSAP and %pSniff) is consistent with a moderate enhancement in anxiety. Higher doses [0.5–1.0 mg/kg, (30); 0.1–10.0 mg/kg, (60)] of prazosin have also been reported to have anxiogeniclike and hypolocomotor effects in the rat plus-maze.

Contrary to the profiles of WAY 100635 and SDZ 216-525, lower doses (0.1–0.5 mg/kg) of NAN-190 had little influence on the recorded behavioural parameters, whereas the highest doses studied (10.0 mg/kg) produced a profound behavioural suppression consisting of reductions in virtually all active behaviours (including open/closed/total arm entries, exploratory head dips, stretched attend postures, flat-back approach and sniffing) and a corresponding increase in immobility. These results are in agreement with the reports that NAN-190 is devoid of antipunishment effects in both the rat and pigeon (1,31). Although several 5-HT_{1A} receptor antagonists produce significant anxiolyticlike activity in the mouse light/dark exploration (7,23) and elevated plus-maze [(12,50); this study] tests, NAN-190 failed to modify anxiety-related behaviour. Inactivity of NAN-190 in murine models of anxiety may be due to a lack of activity of this ligand in this species (7). Such an interpretation seems highly speculative, and other mechanisms should be considered. In fact, the affinity of NAN-190 for α_1-adrenoceptors is somewhat higher than that for 5-HT_{1A} receptors (63), and in rat cortical slices, the compound has been shown to be 330-fold more potent in blocking α_1-adrenoceptor-mediated effects than those mediated via 5-HT_{1A} receptors (16). In addition, NAN-190 is much more potent than prazosin in antagonising norepinephrine-induced stimulation of phosphoinositide turnover (16). Thus, a predominant α_1-adrenoceptor antagonist action of NAN-190, associated with mixed anxiogenic and behavioural depressive effects (prazosin profile), may counteract any antianxiety action resulting from 5-HT_{1A} receptor antagonism. This suggestion is supported by microdialysis studies in which NAN-190 decreased 5-HT release through blockade of α_1-adrenoceptors rather than through stimulation of 5-HT_{1A} autoreceptors (54). SDZ 216-525 also has α_1-adrenoceptor antagonist activity (53) but modifies plus-maze behaviour in a totally different manner. However, in the present study, the highest dose of this compound may have been too low to display α_1-adrenergic activity. Due to the limited solubility of this agent, we did not test higher doses, which may be the reason why weak anxiolyticlike effects of SDZ 216-525 were maintained at the dose of 3.2 mg/kg.

It is generally believed that decreased 5-HT release following the activation of presynaptic receptors is responsible for the anxiolyticlike effects of 5-HT_{1A} receptor agonists and partial agonists (19). At variance with this hypothesis are several lines of evidence that indicate that reduced 5-HT levels in terminal regions may be neither a necessary nor sufficient basis for the anxiolyticlike effects of 5-HT_{1A} ligands, at least in the elevated plus-maze test. Firstly, although both diazepam and ipsapirone inhibit the increase in extracellular 5-HT levels in ventral hippocampus following exposure to the elevated plus-maze, only diazepam (but not ipsapirone) increased open arm activity (64). Secondly, both selective [(12), present study] and nonselective (13) 5-HT_{1A} receptor antagonists, which do not alter 5-HT release at commonly used doses, produce unambiguous behavioural alterations indicative of anxiety reduction in the murine plus-maze, although the precise mechanisms and anatomical sites involved in these effects remain to be determined. However, the finding that the mixed 5-HT_{1A}/1B and β-adrenoceptor antagonist, tertatolol (38), injected into ventral hippocampus, produces anxiolyticlike effects in the rat elevated plus-maze (20) suggests an involvement of postsynaptic 5-HT_{1A} receptors. This explanation would not be inconsistent with reported anxiogenic effects of intrahippocampal 8-OH-DPAT in the rat social interaction test (3) and of intraamygdaloid 8-OH-DPAT in the Geller–Seifter conflict test (34). Thirdly, NAN-190 and prazosin can significantly decrease 5-HT release (33,54) but are without anxiolyticlike action in the murine elevated plus-maze. Finally, we have found that the prototypic 5-HT_{1A} agonist 8-OH-DPAT and its R(+)-isomer, which potently inhibit 5-HT release (33), produce a marked suppression of general activity (arm entries,

head dipping and rearing) without altering anxiety indices (11,51). Therefore, decreased 5-HT release is probably primarily associated with inhibition of locomotor activity (particularly at higher doses) and other active behaviours in the mouse plus-maze. Although significant decreases in closed arm entries [the major index of locomotion in the plus-maze; e.g., (52)] were seen at some doses of WAY 100635 and SDZ 216-525, these effects are unlikely to reflect motoric impairment because such changes were compensated for by corresponding increases in open arm entries, whereas total arm entries and rearing were not affected.

A loss of anxiolyticlike activity at higher doses seems to be a common feature of 5-HT_{1A} receptor antagonists [(12,13, 23,50); present study]. Although the mechanisms involved are unclear at this time, two possible explanations may be considered. Firstly, higher doses of WAY 100635 increase 5-HT release, probably due to a blockade of tonic 5-HT inhibition through somatodendritic 5-HT_{1A} receptors (29,53). The increased 5-HT release may counteract the antagonistic action of these compounds at postsynaptic 5-HT_{1A} sites. Secondly, at high doses, the α_1-adrenoceptor antagonist action of these 5-HT_{1A} ligands may become apparent, resulting in an opposing anxiogeniclike action (prazosin profile). However, because (−)pindolol and pindobind 5-HT_{1A} lack affinity for α_1-adrenoceptor binding sites but show a loss of anxiolyticlike activity at higher doses (13), α_1-adrenergic actions are unlikely to be a major contributing factor.

In summary, both selective and nonselective 5-HT_{1A} receptor antagonists display consistent anxiolyticlike profiles in the mouse elevated plus-maze, effects that are evident on both conventional and ethological measures and that show a wide dose separation from those producing clear motoric effects. Whether agents of this class ultimately prove to have utility in the management of clinical anxiety states is an open question, particularly in view of the fact that, despite the demonstrable clinical efficacy of buspirone and the enormous subsequent preclinical investment in other 5-HT-related compounds, no other direct-acting 5-HT agents have been marketed for the treatment of anxiety. In this context, although results from our laboratory suggest that the plus-maze may be more sensitive than other animal models to the anxiety-modulating effects of 5-HT_{1A} receptor ligands (partial agonists, antagonists), species may be the more important variable. More specifically, such drugs tend to produce more consistent effects in mouse vs. rat anxiety models [e.g., (11–13,25,49); present literature review], a pattern that may reflect reported species differences in 5-HT_{1A}-receptor-mediated processes. For example, although 8-OH-DPAT induces hypothermia in both rats and mice, this effect is postsynaptically mediated in the rats but presynaptically mediated in mice (8). These findings suggest that the mouse may be a more appropriate subject than the rat for studies on the anxiolytic potential of 5-HT_{1A} receptor ligands.

ACKNOWLEDGEMENTS

B.-J.C. is supported by the Wellcome Trust (045176/Z/95/Z/JMW/JPS). We thank Wyeth Research ltd. (UK) and Sandoz Pharma Ltd. (Basel, Switzerland) for the kind gifts of compounds used.

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