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Influence of 5-HT_{1A} Receptor Antagonism on Plus-Maze Behaviour in Mice. I. Pindolol Enantiomers and Pindobind 5-HT_{1A}

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CAO, B. -J., AND R. J. RODGERS. *Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice. I. Pindolol enantiomers and pindobind 5-HT_{1A}.* PHARMACOL BIOCHEM BEHAV **58**(2) 583–591, 1997.—Studies on the behavioural effects of 5-hydroxytryptamine receptor subtype 1A (5-HT_{1A}) antagonists may provide important clues to the precise role of 5-HT_{1A} receptor mechanisms in anxiety. In the first of a series of experiments designed to address this issue, the effects of mixed 5-HT_{1A} and β-adrenergic receptor antagonists pindolol enantiomers and pindobind 5-HT_{1A} and of metoprolol and ICI 118,551 (selective β_1- and β_2-adrenoceptor antagonists, respectively) were assessed in the mouse elevated plus-maze using ethological techniques. Results showed that, at lower doses, (−)pindolol (0.1–1.6 mg/kg) and pindobind 5-HT_{1A} (0.1–0.5 mg/kg) produced changes in both conventional and ethological measures (increased percentage of open arm time and reduced risk assessment) indicative of anxiety reduction. However, these anxiolyticlike actions were less evident at higher doses. In contrast, (+)pindolol (0.1–6.4 mg/kg), metoprolol (2.0–18.0 mg/kg) and ICI 118,551 (1.0–9.0 mg/kg) were behaviourally inert under present test conditions. These data suggest that antagonist actions at 5-HT_{1A} receptors (but not β-adrenoceptors) are involved in the anxiolyticlike effects of (−)pindolol and pindobind 5-HT_{1A} in the murine elevated plus-maze test. © 1997 Elsevier Science Inc.

Anxiety Elevated plus-maze 5-HT_{1A} receptors β-Adrenoceptors (−)Pindolol (+)Pindolol Pindobind 5-HT_{1A} Metoprolol ICI 118,551 Mice

SEROTONIN (5-hydroxytryptamine, 5-HT) receptor subtypes are a major target for the development of novel psychotherapeutic agents. With respect to anxiety disorders, a potential link between 5-HT_{1A} receptor function and anxiety mechanisms was highlighted by the discovery that buspirone, a clinically effective nonbenzodiazepine anxiolytic, binds preferentially to 5-HT_{1A} receptors (59). Although a number of selective 5-HT_{1A} receptor agonists and partial agonists have been available for some time, the development of selective 5-HT_{1A} receptor ligands that function purely as antagonists has been frustratingly slow (12,22,51). However, the ability of some β-adrenoceptor antagonists (e.g., pindolol and propranolol) to interact with 5-HT receptors was discovered some 20 years ago (24,38). Since then, many studies have demonstrated that 5-HT_{1A} and 5-HT_{1B} receptor subtypes are specifically involved in the effects of these agents on 5-HT function (26–28,33). For this reason, and despite their lack of selectivity, these mixed 5-$HT_{1A/1B}$ receptor and β-adrenoceptor antagonists (especially pindolol) have been used frequently to demonstrate the involvement of 5-HT_{1A} receptors in behavioural and physiological processes.

One of the potential problems inherent in interaction studies employing these compounds concerns intrinsic behavioural effects. In the mouse (19) and hamster (20), black/white transitions tests and the mouse defensive burying task (18), racemic pindolol has been shown to antagonise the anxiolyticlike actions of ipsapirone and indorenate. However, this compound also blocks anxiogeniclike effects of 8-OH-DPAT in the rat elevated plus-maze (16,41) and actually potentiates the anxiolyticlike action of indorenate in the rat defensive burying behaviour paradigm (36). Furthermore, investigations concerning effects of pindolol per se on anxiety-related behaviour have produced inconsistent results. Thus, although anxiolyticlike profiles have been reported with pindolol in a

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variety of models, including the Vogel test (44,45), the mouse black/white box (53) and social interaction (4) paradigms and the rat defensive burying (36) and ultrasonic vocalisation (52) procedures, negative findings have also been obtained in most of these models (1,18–20,37,43) and in the rat Geller–Seifter (31) and social interaction (30) tests. In addition, in the rat elevated X-maze, pindolol increases the open/total arm entry ratio from 0.1 to 0.25 mg/kg, switching to a significant decrease at 1.0 mg/kg and no effect at 2.0 mg/kg (15,16,40).

In the search of selective 5-HT_{1A} receptor antagonists, a series of pindolol derivatives were screened for their ability to interact with 5-HT_{1A} binding sites. Among these compounds, N^1-bromoacetyl-N^8-[3′-(4-indolyloxy)-2′-hydroxy-propyl]-[Z]-1,8-diamino-*p*-menthane (pindobind 5-HT_{1A}) displays high affinity for [^{3}H] 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-labelled sites (Ki = 0.7 nM) and is 9- and 1800-fold selective for 5-HT_{1A} receptors relative to β-adrenoceptors and 5-HT_{1B} receptors, respectively (35). Functionally, this ligand appears to act as a 5-HT_{1A} receptor antagonist by reversing 8-OH-DPAT-induced inhibition of forskolin-stimulated adenylate cyclase activity but lacking effects on baseline activity (35). However, little is currently known about the behavioural pharmacology of this ligand. In the absence of intrinsic effects, pindobind 5-HT_{1A} inhibits the 5-HT syndrome induced by 8-OH-DPAT in the rat (35). Although this compound also prevents 5-carboxamidotryptamine-elicited hindlimb scratching in rats, this response may not be mediated centrally but rather by a neuronal 5-HT_{1A} receptor localised outside the blood–brain barrier (17). Of more direct relevance to the present work is a report that pindobind 5-HT_{1A} induces a decrease in one element (evasion) of murine defensive behaviour, an effect that may reflect anxiolysis (3).

Although buspirone has been used in the treatment of generalised anxiety disorder for more than 10 years and the mechanisms responsible for its anxiolytic efficacy are often attributed to actions at 5-HT_{1A} receptors, it is still unclear whether its anxiolytic effects are exerted via an agonist action at presynaptic sites, an antagonist action at postsynaptic sites or both (12,51). In this context, 5-HT_{1A} receptor antagonists may be useful tools in clarifing the role of 5-HT_{1A} receptors in anxiety and may even be effective therapeutic agents for anxiety disorders (22). In the first of a series of experiments designed to test this hypothesis, we assessed the influence of (−)pindolol and pindobind 5-HT_{1A} on plus-maze behaviour in mice, one of the most widely used animal models of anxiety. A detailed ethological technique was employed to provide comprehensive behavioural profiles (46,50). In view of the stereoselective actions of pindolol at 5-HT_{1A} receptors (27,28), the effects of its enantiomers were compared. In addition, to control the potential contribution of β-adrenoceptor antagonism to any observed effects with pindolol isomers and pindobind 5-HT_{1A}, the selective β_1- and β_2-adrenoceptor antagonists metoprolol (33,54) and ICI 118,551 (26,54) were included in the present studies. A companion paper (9) reports on the effects of WAY 100635 and SDZ 216-525 under identical test conditions.

METHODS

Animals

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

Drugs

(+)Pindolol (Sandoz, Berne, Switzerland) and (−)pindolol (RBI, Natick, MA, USA) were dispersed ultrasonically in normal saline to which Tween 80 (2 drops/10 ml) had been added; a corresponding saline–Tween 80 mixture was used for control injections. (±)Metoprolol tartrate (Sigma, Poole, UK), ICI 118,551 hydrochloride (RBI) and pindobind 5-HT_{1A} (RBI) were dissolved in saline, which served for control injections. With the exception of pindobind 5-HT_{1A} (subcutaneous route), all compounds were administered intraperitoneally in a volume of 10 ml/kg 30 min before testing. Doses cited refer to salts where applicable.

Apparatus and Procedure

The elevated plus-maze, test procedure and scoring methodology have been described in detail elsewhere (50). In brief, the maze (Plexiglas: black floor, clear walls) consisted of two open (30 × 5 × 0.25 cm) and two enclosed (30 × 5 × 15 cm) arms linked by a common central platform (5 × 5 cm) and elevated 60 cm above floor level. Testing was conducted during the dark phase of the light cycle in a dimly illuminated (4 × 60 W red, indirect) laboratory. In each experiment, mice were randomly allocated to treatment conditions (n = 10–15) and tested in 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 by using ethological analysis software (Hindsight, version 1.4; developed by Dr. Scott Weiss). Both conventional and ethological parameters (50) were recorded; intr-rater reliability was ⩾0.9.

Statistics

Data were subjected to single- or two-factor analysis of variance (ANOVA), and further comparisons were performed with 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 Kruskal–Wallis ANOVA, followed by Mann–Whitney U-test.

RESULTS

Experiment 1: (−)Pindolol

Data are summarised in Figs. 1–3 (left panels). The ANOVA indicated significant effects of drug treatment on total entries [$F(4,65) = 2.67$, $p < 0.05$] and percentage of open arm entries [$F(4,65) = 2.79$, $p < 0.05$]. Further comparisons showed significant increases in total entries at 6.4 mg/kg and percentage of open entries at 0.4 mg/kg ($p < 0.05$ in both cases). (−)Pindolol did not alter open entries [$F(4,65) = 1.96$, NS] or closed entries [$F(4,65) = 1.71$, NS].

Significant changes were also observed in time spent on different sections of the maze: percentage of open time [$F(4,65) = 4.31$, $p < 0.01$] and percentage of centre time [$F(4,65) = 4.20$, $p < 0.01$]. Follow-up tests revealed that (−)pindolol significantly reduced percentage of centre platform time at 0.1–1.6 mg/kg, an effect that was paralleled by in-

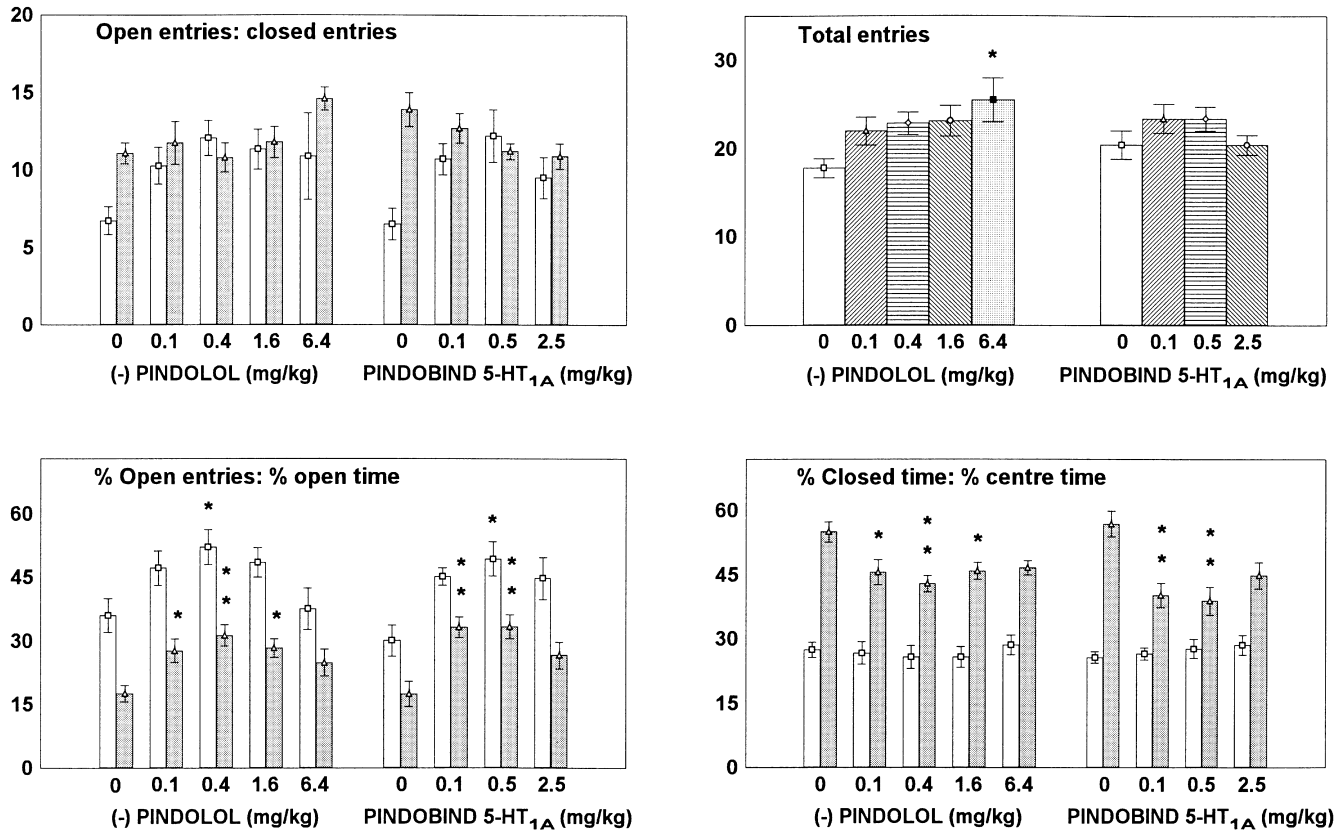

FIG. 1. Effects of (−)pindolol (0.1–6.4 mg/kg) and pindobind 5-HT_{1A} (0.1–2.5 mg/kg) on open, closed and total arm entries and on percentage of time spent on open, closed and centre parts of the elevated plus-maze in male Swiss Webster mice. Open entries: closed entries chart—open bars = open entries, 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 = 10–15). *$p < 0.05$, **$p < 0.01$ vs. vehicle control.

creases in percentage of open time ($p < 0.05$ to $p < 0.01$). No significant effects were observed in percentage of time spent in closed arms [$F(4,65) = 0.20$, NS]. Mice generally had a clear preference for different sections of the maze [$F(2,130) = 77.61$, $p < 0.01$], with vehicle-treated subjects showing a profile of centre > closed > open. This pattern was also significantly altered by (−)pindolol [$F(8, 130) = 2.91$, $p < 0.01$], with drugged groups failing to differentiate between closed and open arms.

On the ethological measures, (−)pindolol significantly altered protected head dips [$F(4,65) = 5.72$, $p < 0.01$], stretched attend postures [total and the protected form, $F(4,65) = 15.06$ and 20.74, respectively; $p < 0.01$ in both cases], closed arm returns ($H = 10.80$, $p < 0.05$), sniffing [$F(4,65) = 3.03$, $p < 0.01$] and percentage of protected flat-back approach [$F(4,65) = 3.80$, $p < 0.01$]. Further tests showed that (−)pindolol significantly ($p < 0.05$ to $p < 0.01$) reduced total and protected stretched attend postures (0.1–6.4 mg/kg), sniffing (0.1, 0.4 and 6.4 mg/kg), percentage of protected head dipping (0.1–1.6 mg/kg) and percentage of protected flat-back approach (0.1–0.4 mg/kg). Animals treated with 0.1–1.6 mg/kg showed a trend towards a decrease in closed arm returns (control group: 0.9 ± 0.4) that approached but just failed to reach significance at 0.1 and 1.6 mg/kg (0.1 ± 0.1; $p = 0.07$). No significant changes were seen in total rears [$F(4,65) = 0.17$, NS], rearing time [$F(4,65) = 0.16$, NS] or grooming duration [$F(4,65) = 0.61$, NS]. Although Kruskal–Wallis ANOVA indicated an influence of drug on immobility duration ($H = 9.91$, $p < 0.05$), post hoc comparisons failed to reveal any significant difference between control and (−)pindolol groups (data not shown).

Experiment 2: Pindobind 5-HT_{1A}

Data are summarised in Figs. 1–3 (right panels). Pindobind 5-HT_{1A} failed to alter open entries [$F(3,36) = 2.30$, NS], closed entries [$F(3,36) = 1.70$, NS] and total entries [$F(3,36) = 0.95$, NS]. However, a significant effect on percentage of open entries was observed [$F(3,36) = 3.23$, $p < 0.05$], with further comparisons revealing a significant increase in this measure at 0.5 mg/kg ($p < 0.05$). On the percentage time measures, the rank-order preference of centre platform > closed arms = open arms in saline-treated subjects was significantly altered by drug treatment [$F(6,72) = 3.99$, $p < 0.01$]. Thus, this preference pattern was changed to centre = open, centre > closed at 0.1 mg/kg and centre = closed = open at 0.5 mg/kg. Further analyses indicated that pindobind 5-HT_{1A} markedly altered percentage of open time and percentage of centre time [$F(3,36) = 4.61$ and 4.83, respectively; $p < 0.01$], with increases in the former and decreases in the latter at 0.1–0.5 mg/kg ($p < 0.01$ in all cases). No changes in percentage of closed time were observed [$F(3,36) = 0.31$, NS].

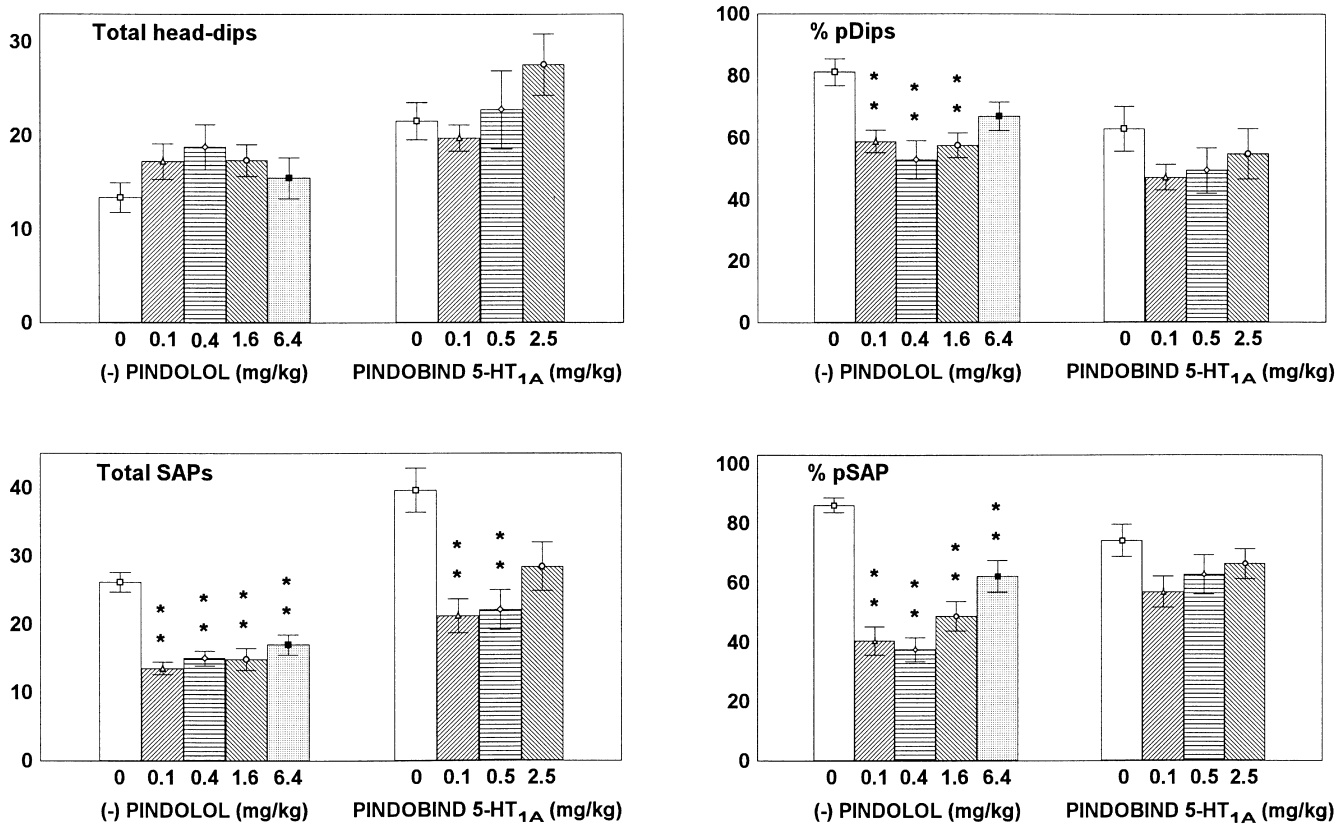

FIG. 2. Effects of (−)pindolol (0.1–6.4 mg/kg) and pindobind 5-HT_{1A} (0.1–2.5 mg/kg) on total head dips, percentage of protected head dips (%pDips), total stretched attend postures (SAPs) and percentage of protected stretched attend postures (%pSAP) in male Swiss Webster mice tested in the elevated plus-maze. Data are expressed as mean values ± SEM (n = 10–15). *$p < 0.05$, **$p < 0.01$ vs. vehicle control.

Pindobind 5-HT_{1A} did not significantly affect rearing [frequency: $F(3,36) = 0.24$, NS; duration: $F(3,36) = 0.19$, NS], total head dips [$F(3,36) = 0.89$, NS], percentage of protected dips [$F(3,36) = 0.68$, NS], percentage of protected stretched attend postures [$F(3, 36) = 1.11$, NS], flat-back approach [$F(3,36) = 1.55$, NS], total sniffing [$F(3,36) = 1.99$, NS], grooming [$F(3,36) = 2.60$, NS] or immobility ($H = 3.00$, NS). However, several other ethological measures were profoundly affected: total stretched attend postures [$F(3,36) = 5.09$, $p < 0.01$], percentage of protected flat-back approach [$F(3,36) = 8.05$, $p < 0.01$], percentage of protected sniffing [$F(3, 36) = 4.01$, $p < 0.05$] and closed arm returns (control: 0.7 ± 0.3; 0.1 mg/kg: 0.1 ± 0.1; 0.5 mg/kg: 0; $H = 8.62$, $p < 0.05$). Follow-up tests confirmed decreases in these measures at 0.1 and 0.5 mg/kg ($p < 0.05$ to $p < 0.01$).

Experiments 3–5: (+)Pindolol, Metoprolol and ICI 118,551

Data and corresponding ANOVA statistics are presented in Tables 1–3. (+)Pindolol failed to produce any significant behavioural effects over the dose range tested. Similarly, neither metoprolol (2.0–18.0 mg/kg) nor ICI 118,551 (1.0–9.0 mg/kg) was active under present test conditions.

DISCUSSION

The primary indices of anxiety in the elevated plus-maze test relate to open arm avoidance and are usually recorded as the proportion of open entries (relative to total entries) and the proportion of time spent on these aversive pairs of the maze (relative to test duration). The incorporation of a cluster of ethological parameters, identified as particicularly sensitive to anxiety-related manipulations (46,50), may enhance the utility of this paradigm. Consistent with profiles obtained with other antianxiety agents (46), increases in percentage of open entries and/or of open time were observed with (−)pindolol (0.1–1.6 mg/kg) and pindobind 5-HT_{1A} (0.1–0.5 mg/kg), two nonselective 5-HT_{1A} receptor antagonists. Anxiolyticlike effects were also apparent on ethological measures, including reductions in stretched attend postures (both drugs), sniffing [(−)pindolol] and closed arm returns (pindobind 5-HT_{1A}) and in the protected forms of stretched attend [(−)pindolol], sniffing [(−)pindolol)] and flat-back approach (both drugs). Importantly, we found that the route of administration had a significant influence on the effects of pindobind 5-HT_{1A} in the plus-maze: intraperitoneal injection of the same doses of pindobind 5-HT_{1A} as those used in the present study did not modify the majority of the recorded behaviours (unpublished observation). The pharmacokinetic data, which are not as yet available in literature, may explain this difference. The present findings are consistent with previously reported anxiolyticlike actions of (±) pindolol (4,36,44,45,52,53), pindobind 5-HT_{1A} (3) and more selective 5-HT_{1A} receptor antagonists (6,8,9,23,39,47) in a variety of animal procedures. Furthermore, when injected into ventral hippocampus, another mixed 5-$HT_{1A/1B}$ and β-adrenoceptor antagonist, tertatolol (34), reduces plus-maze anxiety in both experienced and unexperi-

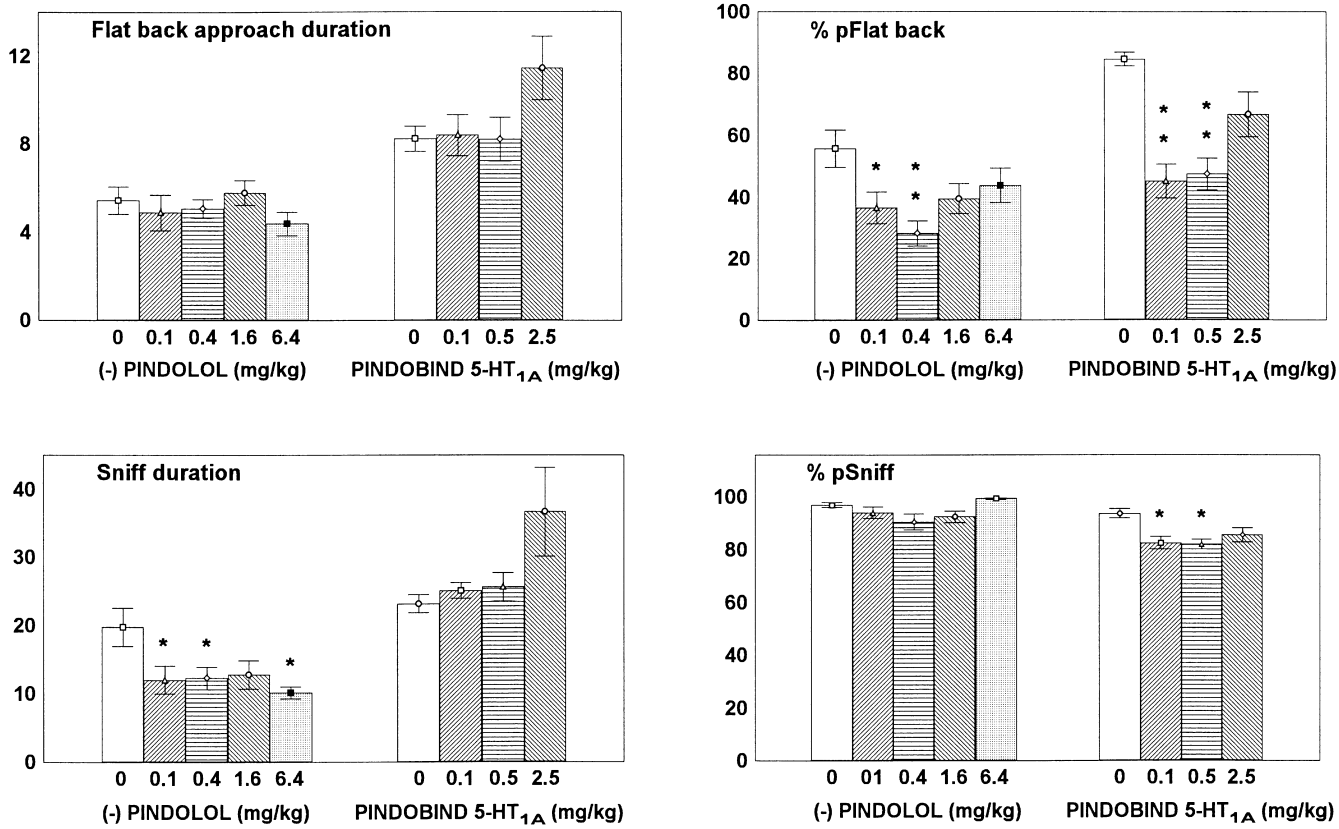

FIG. 3. Effects of (−)pindolol (0.1–6.4 mg/kg) and pindobind 5-HT_{1A} (0.1–2.5 mg/kg) on flat-back approach duration (s), percentage of protected flat-back approach (%pFlat back), sniff duration (s) and percentage of protected sniff (%pSniff) in male Swiss Webster mice tested in the elevated plus-maze. Data are expressed as mean values ± SEM (n = 10–15). *$p < 0.05$, **$p < 0.01$ vs. vehicle control.

TABLE 1

EFFECTS OF (+) PINDOLOL (0.1–6.4 mg/kg) ON PLUS-MAZE BEHAVIOUR IN MALE SWISS WEBSTER MICE

		(+) Pindolol (mg/kg)				
Behaviour	Vehicle	0.1	0.4	1.6	6.4	F(4, 45)
Open arm entries	7.2 ± 0.9	5.5 ± 1.0	8.3 ± 1.6	9.2 ± 1.6	8.1 ± 1.3	1.18, NS
Closed arm entries	10.5 ± 1.2	10.0 ± 0.8	11.6 ± 1.3	12.4 ± 1.5	8.6 ± 1.7	1.24, NS
Total arm entries	17.7 ± 1.2	15.5 ± 1.5	19.9 ± 2.1	21.6 ± 2.0	16.7 ± 2.1	1.87, NS
% Open arm entries	41.1 ± 4.8	33.2 ± 4.1	40.8 ± 6.2	41.8 ± 5.1	50.8 ± 6.1	1.42, NS
% Open arm time	21.6 ± 3.7	17.6 ± 2.5	23.1 ± 3.7	26.1 ± 4.1	26.9 ± 4.7	0.90, NS
% Closed arm time	26.7 ± 2.4	29.4 ± 1.6	26.3 ± 2.2	25.5 ± 2.3	24.8 ± 3.3	0.53, NS
% Centre platform time	51.7 ± 3.5	53.0 ± 2.7	50.6 ± 3.3	48.4 ± 3.1	48.3 ± 6.2	0.27, NS
Total head-dips	9.7 ± 1.3	7.8 ± 1.9	11.6 ± 2.9	12.5 ± 2.3	10.5 ± 1.9	0.72, NS
% Protected head-dips	76.1 ± 6.8	77.1 ± 10.5	84.3 ± 4.1	78.6 ± 7.4	69.2 ± 9.5	0.48, NS
Total stretched attend postures	17.5 ± 1.0	19.0 ± 1.7	18.4 ± 3.1	20.1 ± 2.7	12.0 ± 1.7	2.10, NS
% Protected stretched attend postures	79.7 ± 4.1	88.5 ± 2.1	82.1 ± 4.1	76.1 ± 6.3	79.0 ± 6.7	0.89, NS
Flat back approach duration (s)	9.3 ± 2.2	9.2 ± 1.1	7.3 ± 1.3	7.3 ± 0.7	7.6 ± 0.9	0.57, NS
% Protected flat back approach	58.1 ± 9.2	72.3 ± 5.6	61.5 ± 5.3	64.5 ± 6.6	62.3 ± 4.4	0.68, NS
Sniff duration (s)	13.8 ± 2.5	13.1 ± 1.5	13.7 ± 2.4	15.6 ± 1.7	13.2 ± 1.7	0.25, NS
% Protected sniff	99.3 ± 0.5	99.4 ± 0.6	98.1 ± 1.9	99.6 ± 0.4	98.7 ± 0.8	0.41, NS
Closed arm returns	0.3 ± 0.2	0.3 ± 0.2	0.7 ± 0.5	0.6 ± 0.3	0.0 ± 0.0	H = 3.85, NS
Total rears	9.3 ± 1.0	9.4 ± 2.0	12.6 ± 3.0	11.6 ± 2.3	6.4 ± 1.9	1.26, NS
Rear duration (s)	5.9 ± 0.6	5.8 ± 1.2	7.8 ± 1.7	8.3 ± 1.8	4.8 ± 1.6	1.04, NS
Groom (s)	6.4 ± 1.9	8.8 ± 2.2	5.2 ± 1.0	5.8 ± 1.7	10.7 ± 4.8	0.75, NS
Immobility (s)	0.2 ± 0.2	0.3 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	3.9 ± 3.9	H = 0.48, NS

Data are presented as mean values ± SEM (n = 10).

TABLE 2

EFFECTS OF METOPROLOL (2.0–18.0 mg/kg) ON PLUS-MAZE BEHAVIOUR IN MALE SWISS WEBSTER MICE

		Metoprolol (mg/kg)			
Behaviour	Vehicle	2.0	6.0	18.0	F(3, 36)
Open arm entries	8.5 ± 1.0	9.2 ± 1.6	9.8 ± 1.2	8.9 ± 1.4	0.17, NS
Closed arm entries	10.7 ± 0.7	11.8 ± 1.4	12.8 ± 1.1	9.3 ± 0.9	2.06, NS
Total arm entries	19.2 ± 1.2	21.0 ± 1.9	22.6 ± 1.7	18.2 ± 1.3	1.57, NS
% Open arm entries	43.3 ± 3.8	43.1 ± 5.6	42.7 ± 3.7	46.8 ± 5.6	0.16, NS
% Open arm time	23.4 ± 2.3	24.8 ± 4.2	24.8 ± 2.1	25.3 ± 4.7	0.05, NS
% Closed arm time	25.0 ± 1.7	24.3 ± 2.1	25.2 ± 1.8	25.1 ± 2.6	0.04, NS
% Centre platform time	51.6 ± 2.6	50.9 ± 3.7	50.0 ± 2.2	49.6 ± 4.4	0.07, NS
Total head-dips	22.3 ± 2.8	24.8 ± 3.1	15.0 ± 2.8	19.3 ± 2.3	2.35, NS
% Protected head-dips	63.8 ± 5.8	59.2 ± 6.4	55.6 ± 4.1	63.0 ± 7.0	0.41, NS
Total stretched attend postures	22.8 ± 2.7	18.0 ± 2.4	17.9 ± 2.2	15.8 ± 2.4	1.50, NS
% Protected stretched attend postures	65.7 ± 4.1	59.9 ± 8.8	56.2 ± 5.2	57.8 ± 9.1	0.33, NS
Flat back approach duration (s)	12.9 ± 1.1	10.9 ± 0.8	11.2 ± 1.0	11.5 ± 1.1	0.80, NS
% Protected flat back approach	63.5 ± 3.2	63.6 ± 6.6	62.2 ± 2.9	64.9 ± 3.7	0.07, NS
Sniff duration (s)	22.1 ± 0.9	21.6 ± 1.3	21.4 ± 1.5	22.4 ± 1.4	0.12, NS
% Protected sniff	88.0 ± 2.3	89.3 ± 3.9	91.5 ± 1.5	89.2 ± 2.5	0.20, NS
Closed arm returns	0.0 ± 0.0	0.6 ± 0.5	0.2 ± 0.1	0.2 ± 0.1	H = 2.30, NS
Total rears	12.2 ± 1.9	11.9 ± 2.3	14.7 ± 2.6	8.8 ± 2.2	1.17, NS
Rear duration (s)	8.3 ± 1.8	7.1 ± 1.7	10.5 ± 2.6	5.4 ± 1.5	1.21, NS
Groom (s)	5.7 ± 1.0	4.4 ± 0.9	5.3 ± 1.3	8.6 ± 2.5	1.34, NS
Immobility (s)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	H = 3.72, NS

Data are presented as mean values ± SEM (n = 10).

enced rats (21). Although the authors attributed this result to 5-HT_{1B} or β-adrenergic actions, the data are consistent with the possibility that postsynaptic 5-HT_{1A} receptor antagonism induces anxiolysis in the plus-maze model.

Although consistent with an anxiolyticlike action of 5-HT_{1A} receptor antagonists, other interpretations of the present data should be considered. For example, (−)pindolol displays some agonist activity at presynaptic 5-HT_{1A} receptors (12,22),

TABLE 3

EFFECTS OF ICI 118,551 (1.0–9.0 mg/kg) ON PLUS-MAZE BEHAVIOUR IN MALE SWISS WEBSTER MICE

		ICI 118,551 (mg/kg)			
Behaviour	Vehicle	1.0	3.0	9.0	F(3, 36)
Open arm entries	11.2 ± 1.2	7.9 ± 1.3	8.0 ± 1.0	8.0 ± 1.3	1.82, NS
Closed arm entries	9.8 ± 0.9	10.6 ± 1.0	10.0 ± 0.9	10.8 ± 0.9	0.26, NS
Total arm entries	21.0 ± 1.5	18.5 ± 1.9	18.0 ± 1.5	18.8 ± 1.5	0.68, NS
% Open arm entries	52.6 ± 4.1	41.4 ± 3.9	44.2 ± 3.6	41.3 ± 4.5	1.73, NS
% Open arm time	27.4 ± 3.3	19.9 ± 2.9	20.8 ± 1.7	21.6 ± 3.2	1.46, NS
% Closed arm time	2.1 ± 1.6	26.2 ± 2.7	25.6 ± 3.1	28.3 ± 1.9	1.36, NS
% Centre platform time	51.0 ± 4.0	53.9 ± 3.2	53.6 ± 3.6	50.1 ± 2.5	0.32, NS
Total head-dips	19.4 ± 2.4	17.4 ± 2.2	19.6 ± 2.4	18.7 ± 3.6	0.13, NS
% Protected head-dips	59.0 ± 5.5	68.6 ± 5.4	72.2 ± 3.9	62.6 ± 4.3	1.52, NS
Total stretched attend postures	23.9 ± 1.9	20.2 ± 1.9	21.5 ± 1.7	22.2 ± 2.0	0.67, NS
% Protected stretched attend postures	57.8 ± 6.1	71.4 ± 3.2	70.0 ± 5.0	64.6 ± 4.8	1.61, NS
Flat back approach duration (s)	8.0 ± 1.4	11.0 ± 1.3	9.2 ± 1.1	8.1 ± 1.0	1.38, NS
% Protected flat back approach	53.1 ± 6.7	59.4 ± 6.5	61.7 ± 3.5	60.4 ± 6.2	0.42, NS
Sniff duration (s)	23.1 ± 1.5	21.9 ± 1.8	23.9 ± 1.6	24.7 ± 2.0	0.47, NS
% Protected sniff	87.4 ± 2.3	89.9 ± 2.5	88.7 ± 2.1	92.3 ± 2.2	0.84, NS
Closed arm returns	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.5	0.1 ± 0.1	H = 2.52, NS
Total rears	11.6 ± 1.7	12.2 ± 2.0	11.1 ± 2.2	12.2 ± 1.7	0.08, NS
Rear duration (s)	5.8 ± 0.9	6.2 ± 1.2	6.8 ± 1.3	8.1 ± 1.6	0.63, NS
Groom (s)	6.1 ± 1.2	7.7 ± 1.4	11.6 ± 2.3	8.4 ± 1.4	1.99, NS
Immobility (s)	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	H = 6.59, NS

Data are presented as mean values ± SEM (n = 10).

and this action could account for the behavioural effects of (−)pindolol in the plus-maze. However, this explanation receives little support from recent findings showing that 5-HT_{1A} receptor agonists and pindolol produce opposite neurochemical changes. Thus, although 8-OH-DPAT decreases 5-HT release in dorsal (5) and ventral (2) hippocampus, a higher dose (10 mg/kg) of pindolol (5) and its (−)-enantiomer (2) significantly increases extracellular hippocampal 5-HT levels in rats. Furthermore, data from this laboratory have revealed important differences in the behavioural effects of (−)pindolol and 5-HT_{1A} receptor full or partial agonists: 8-OH-DPAT (48) and its R(+)-isomer (7), flesinoxan (49) and buspirone (13) all failed to alter conventional open entries/time measures at lower doses, whereas higher doses of these compounds were associated with gross behavioural suppression, including marked reductions in arm entries and rearing. Because these 5-HT_{1A} agonists inhibit 5-HT release (2,5) and, at high doses, decrease locomotor activity, increased 5-HT levels in some brain regions may be related to the locomotor stimulating effects of higher doses of (−)pindolol. Furthermore, most of the behavioural effects of (−)pindolol and pindobind 5-HT_{1A} in the elevated plus-maze are very similar to those reported for more selective 5-HT_{1A} receptor antagonists (57) in this model (8,9,47).

The majority of previous investigations of the influence of pindolol on anxiety have employed the racemic form of this compound. However, as a 5-HT_{1A} receptor antagonist, the (−)-enantiomer is the much more active form (27,28). The lack of any significant behavioural effects of (+)pindolol over an identical dose range suggests that the activity of pindolol on plus-maze anxiety resides primarily in the (−)-isomer. Similarly, the selective β-adrenoceptor antagonists metoprolol (β_1) and ICI 118,551 (β_2) were also without effect under present test conditions, further supporting the conclusion that an antagonist action at 5-HT_{1A} receptors, but not at β-adrenoceptors, is involved in the antianxiety effects of (−)pindolol in the elevated plus-maze. Both clinical (14,32) and preclinical [(16,41,44,45,52,53; present study] investigations have demonstrated consistently that ICI 118,551 is devoid of anxiolyticlike activity. Although clinical reports have demonstrated the effectiveness of metoprolol in the management of somatic anxiety (10,29,55,60,61), most animal studies have produced negative results [(15,16,52,56,58); present study].

The anxiolyticlike effects of (−)pindolol and pindobind 5-HT_{1A} in the present study cannot be easily attributed to behavioural nonspecificity because no significant changes in locomotion (closed arm entries and total arm entries) were seen at the active doses. However, at the top dose tested (6.4 mg/kg), (−)pindolol produced locomotor stimulation, i.e., a significant increase in total entries, an effect mainly due to increased closed arm entries (which approached but failed to reach significance). As such, decreases in stretched attend postures and sniffing observed at this dose might simply be a consequence of response competition rather than a reflection of changes in anxiety. The absence of alterations in any other anxiety measures supports this interpretation and further indicates loss of anxiolyticlike activity at the highest dose tested. Interestingly, although not accompanied by locomotor stimulation, the highest dose of pindobind 5-HT_{1A} was also associated with a loss of anxiolyticlike activity.

Although present and previous (41) comparisons of a variety of β-adrenoceptor antagonists suggest that only those with high affinity for 5-HT_{1A} receptors affect anxiety-related behaviours displayed by rodents on the elevated plus-maze, a potential interaction between the serotonergic and β-adrenergic systems and the modification of this interaction by (−)pindolol cannot be excluded. Indeed, a recent report has suggested that the 5-HT_{1A} receptor antagonistic potency of (−)penbutolol in aggressive mice can be attenuated by β-adrenoceptor-induced facilitation of serotonergic neurotransmission (54). (−)Pindolol also has high affinity for 5-HT_{1B} receptors in addition to its actions at 5-HT_{1A} receptors (2,26,27) and, as such, an action at 5-HT_{1B} sites may contribute to its effects in the murine elevated plus-maze. However, because pindobind 5-HT_{1A} has negligible affinity for 5-HT_{1B} receptors (35) and produces similar changes to (−)pindolol on anxiety (but not locomotion) measures, 5-HT_{1B} receptors may be involved in the motoric (but not anxiolyticlike) effects of (−)pindolol. In this context, CGS 12066B, a relatively selective 5-HT_{1B} ligand, produces dose-dependent stimulation of closed and total arm entries in the murine plus-maze (48), a finding that agrees well with the reported hyperlocomotion with this 5-HT_{1B} compound in rodents (11). Although CGS 12068B is generally considered as a 5-HT_{1B} agonist, its agonistic efficacy is actually relatively weak (40) and, because it can antagonise the locomotor stimulant effects of RU 24969 [another 5-HT_{1B} agonist (11)], it should perhaps be more accurately defined as a partial rather than as a full agonist at 5-HT_{1B} receptors. Similarly, both biochemical (25,42) and behavioural (62) data indicate that (−)pindolol possesses partial agonist activity at 5-HT_{1B} receptors. In view of the interpretative difficulties involved in the use of relatively nonselective ligands, further studies employing more selective 5-HT_{1A} receptor antagonists are required to characterise more fully the influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour. In this context, a companion paper (9) reports on the effects of WAY 100635 and SDZ 216-525 under identical test conditions to those employed in the presetn study.

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