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5-HT_{1A} Agonists Modulate Mouse Antipredator Defensive Behavior Differently From the 5-HT_{2A} Antagonist Pirenperone

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GRIEBEL, G., D. C. BLANCHARD, A. JUNG, C. K. MASUDA AND R. J. BLANCHARD. *5-HT_{1A} Agonists modulate mouse antipredator defensive behavior differently from the 5-HT_{2A} antagonist pirenperone*. PHARMACOL BIOCHEM BEHAV 51(2/3) 235-244, 1995. — The mouse defense test battery (MDTB) has been designed to investigate defensive reactions in Swiss-Webster mice to situations associated with a natural predator, the rat, such as flight, avoidance, defensive threat, defensive attack, and risk assessment activities. The present study evaluated the ability of 8-OH-DPAT (0.05–10 mg/kg, SC, 5) and gepirone (2.5–10 mg/kg, IP, 30), a full- and a partial agonist at 5-HT_{1A} sites, as well as pirenperone (0.25–1 mg/kg, IP, 30), a preferential 5-HT_{2A} receptor antagonist, to exert an anxiolytic-like action in the MDTB. The most consistent effect of both 5-HT_{1A} receptor agonists across tests was a marked reduction in predator assessment activity and defensive attack behavior. In contrast, neither of the two ligands was able to reduce flight responses to the approaching predator, and both failed to reduce in a specific manner contextual defense behaviors after the predator was removed. The 5-HT_{2A} receptor antagonist pirenperone did not provide significant indication of an anxiolytic effect on predator assessment activity and postpredator potentiation of contextual defense responses, and had negligible influence on antipredator defensive behavior. The most interesting exception to this profile was a dose-related reduction in flight-related measures. In view of previous results indicating that the panic-promoting drug yohimbine increases flight/escape reactions and that the panicolytic compound alprazolam reduces these responses, we tentatively suggest that the preferential 5-HT_{2A} receptor antagonist pirenperone may have some efficacy in improving panic attacks. In addition, the lack of effect of the 5-HT_{1A} receptor agonists on these flight responses is consistent with clinical findings indicating that these agents are of limited use in the treatment of panic disorder. These findings suggest that the MDTB provides behavioural measures capable of differentiating between various classes of antianxiety drugs.

5-HT _{1A} agonists	5-HT _{2A} antagonist	8-OH-DPAT	Gepirone	Pirenperone	Serotonin	Flight
Antipredator defense	Fear	Anxiety	Panic	Swiss-Webster mouse	Risk assessment	

CONSIDERABLE evidence has accrued in the last 2 decades to support the hypothesis that serotonergic (5-HT) processes may be involved in anxiety and in the action of anxiolytic drugs [see (31) for a recent review]. Multiple 5-HT binding sites have been identified (27,54), several of which have been implicated in the modulation of emotional responses through the study of selective 5-HT receptor ligands. Among these, 5-HT_{1A} receptor ligands and 5-HT_{2A} receptor antagonists have been of particular interest because clinical studies revealed

that these drugs are effective in the treatment of anxiety disorders. In particular, treatment with 5-HT_{1A} receptor agonists, such as buspirone, gepirone, and ipsapirone, have been demonstrated to be effective in generalized anxiety disorder (GAD) (9,15,26,33,50,52,64), and buspirone also improved phobic anxiety (10,44,53). Furthermore, ritanserin a 5-HT_{2A} receptor antagonist with nearly equal affinity for 5-HT_{2C} sites was reported to be effective in several small studies of patients with GAD (1,11,16).

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Despite the clinical efficacy of these drugs, highly variable effects have been reported for these compounds, both in classical and novel animal models of anxiety. Systemic administration of such agents in animals has been reported to produce anxiolytic-like effects in some studies and no specific action in others, or has even elicited anxiogenic-like responses (31). For example, several studies reported a lack of efficacy of gepirone in inhibiting anxious responses of rats in the elevated plus-maze and social interaction tests (14,34,43), whereas other data revealed that the drug increased entries per time spent in the open arms in the elevated plus-maze (23,57) and the occurrence of social interactions (17,34). In addition, some studies in rats revealed an anxiogenic-like profile of this compound in the elevated plus-maze test (43), the Vogel conflict procedure (13), or the open-field paradigm (41). Studies with ritanserin in animal models of anxiety have provided a similar profile of inconsistency (including anxiolysis, no effect, and anxiogenesis). Moreover, when anxiolytic-like effects were detected with both class of drugs, the 5-HT effects were frequently smaller in magnitude than those of benzodiazepines (BZPs).

By contrast, at least in the case of 5-HT_{1A} compounds, studies that have focussed directly on aspects of the rodent defensive responses have provided more consistent support for fear and anxiety reduction. For example, buspirone and gepirone markedly inhibit specific aspects of defensive reactions to human intrusion in monkeys (59) and wild rats (5). Furthermore, defensive fighting in rats and mice is dose dependently inhibited by buspirone, gepirone, ipsapirone, and 8-OH-DPAT (7,45,46,58,60).

In this context, we recently developed a new experimental procedure designed to assess the defensive reactions of Swiss-Webster mice to a natural predator, the rat. This mouse defense test battery (MDTB) involves confrontation of the subject with an unconditioned threat stimulus (rat). The primary measures taken before, during, and after rat presentation involve a full range of mouse antipredator defensive behaviors, including escape attempts, flight, risk assessment, immobility, and defensive attack. These behaviors are generally very similar to those seen in rats in parallel tests in which buspirone and gepirone, and also BZP anxiolytics, have been shown to produce specific profiles of behavior change (3,5,4,7). The MDTB has provided specific profiles of drug effects on a variety of defensive behaviors for chlordiazepoxide, alprazolam (30), and the panic-promoting drug yohimbine (8). For example, chlordiazepoxide reduced contextual defenses to the situation associated with the predator, and it also inhibited predator assessment behaviors, whereas neither acute or chronic alprazolam altered these behaviors. Furthermore, only chronic treatment with the panicolytic drug alprazolam reduced the prey-predator distance at which flight responses occurred.

In the present study, we used the MDTB to examine potential specific effects of the 5-HT_{1A} receptor full agonist 8-OH-DPAT, the 5-HT_{1A} receptor partial agonist gepirone, and the preferential 5-HT_{2A} receptor antagonist pirenperone on antipredator defense in Swiss-Webster mice.

METHODS

Animals

Subjects were 195 naive male Swiss-Webster mice obtained from Simonsen Laboratories (CA), 60–75 days old at the beginning of the experiment. They were housed singly in poly-

carbonate cages in a room maintained under a 12 L : 12 D cycle with light onset at 0600 h.

Drugs and Treatment Groups

(±)-8-OH-DPAT [8-hydroxy-2-(DI-*n*-propylamino)tetralin] Hbr, gepirone, and pirenperone (Research Biochemicals Inc., Natick, MA) were dissolved in an isotonic saline vehicle to various concentrations such that injections were always at a constant volume of 10.0 ml/kg. Mice were randomly assigned to the following three experiments: a) 8-OH-DPAT: control group and drug treatment groups (0.05, 0.5, 1, and 10 mg/kg): *n* = 15; b) gepirone: control group and drug treatment groups (2.5, 5, and 10 mg/kg): *n* = 15; and c) pirenperone: control group and drug treatment groups (0.25, 0.5, and 1 mg/kg): *n* = 15. Mice received a single injection of either saline, 8-OH-DPAT, gepirone, or pirenperone. Except for 8-OH-DPAT, which was injected subcutaneously 5 min before the test, the drugs were administered intraperitoneally (IP) 30 min before the experiment was carried out.

Apparatus

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 6.0 m in total length, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall (2.0 × 0.30 × 0.06). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with it. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with videocameras mounted above the apparatus. Experiments were performed under red light between 1300 and 1700 h.

Procedure

Contextual defense.

Evaluation of the impact of predator exposure on motor responses. Subjects were placed into the runway for a 3-min familiarization period, in which line crossings, wall rears, wall climbs, and jump escapes were recorded (min 1–3). The same behavioral parameters were also recorded during an equivalent period following tests involving exposure to a predator (posttest) (min 12–14). Changes in the latter three (escape) measures during the postpredator period provide an index of contextual defense.

Reactions to the predator.

Predator avoidance test (min 4–6). Immediately after the 3-min familiarization period, a deeply anesthetized handheld rat (Long-Evans male) was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. The experimenter stood adjacent to the runway while holding the anesthetized rat. Approach was terminated when contact was made with the subject or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated five times.

Chase/flight test (min 7–8). The handheld rat was brought up to the subject at a speed of approximately 2.0 m/s. The time it took to chase the subject a distance of 15 m was recorded. Overall flight speed (meters per second) and maximum flight speed (measured when the subject ran straight over a 1-m segment) were subsequently calculated from these measures. In addition, the following parameters were recorded: number of

stops (pause in movement), orientations (subject stopped, then orientated the head toward the rat), and reversals (subject stopped, then ran in the opposite direction).

Straight alley (min 9–11). The runway was then converted to a straight alley by the closing of doors at both ends. Three approaches, 15 s each at 1.20, 0.80, and 0.40 m, respectively, were made by a handheld rat toward the subject in this inescapable runway. Measures taken included immobility time, closest distance between the subject and the rat, and the number of approaches or withdrawals (subject had to move > 0.2 m forward from the closed door, then return to it). Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites, vocalizations, upright postures, and jump attacks by the subjects were noted.

Ledge test (min 15). Subjects were then placed on the median wall of the runway for 30 s and the number of falls was recorded. This additional control measure provided an indication of potential myorelaxant effects of the drugs.

Statistics

Data were analysed by a one-way analysis of variance (ANOVA) or the nonparametric Kruskal-Wallis ANOVA for some infrequently occurring or highly variable behaviors. Subsequent comparisons between treatment groups and control were carried out using Newman-Keuls procedures or the nonparametric Mann-Whitney *U*-test. In the contextual defense test, line crossing and wall rearing data were assessed by a combined repeated-measures ANOVA followed by a Newman-Keuls posthoc comparison. Furthermore, Kruskal-Wallis ANOVA followed by the Mann-Whitney *U*-test were used to evaluate the drugs' effects on wall climbing and jump escape. In addition, pre- or postexposure-to-predator differences for these latter responses were analyzed by the Wilcoxon matched pair test, and dose-related pre- vs. posttest comparisons were evaluated by a combined Friedman nonparametric ANOVA, followed by a Wilcoxon matched pair test analysis. In those cases where multiple comparisons of a single dependent variable were required, an approaching adjustment of α were made (40). Data from the ledge test were analysed by a χ^2 procedure.

RESULTS

Contextual Defense: Motor Activity Before and After Exposure to the Predator (Fig. 1)

8-OH-DPAT.

Drug effect. Comparisons (ANOVA) of 8-OH-DPAT treatment and saline control group indicated that the drug had a significant overall effect on all behavioral responses [frequency of line crossings: $F(4, 70) = 87.93, p < 0.00001$; frequency of wall rearings: $F(4, 70) = 28.77, p < 0.00001$; frequency of wall climbings: Kruskal-Wallis ANOVA: $H(4, 75) = 42.58, p < 0.00001$; frequency of jump escapes: Kruskal-Wallis ANOVA: $H(4, 75) = 34.78, p < 0.00001$]. Newman-Keuls comparisons indicated a reliable decrease in the number of line crossings and wall rearings at 0.5, 1, and 10 mg/kg ($p < 0.0002$ vs. control for all comparisons), and Mann-Whitney *U*-test revealed a similar effect on the occurrence of wall climbs and jump escapes at the same doses ($p < 0.0003$ vs. control).

Pre- and postexposure-to-predator differences. Except for wall rearing [$F(1, 70) = 0.15$], all behavioral measures increased significantly in the posttest period following presentation and removal of the predator [line crossing: $F(1, 70) =$

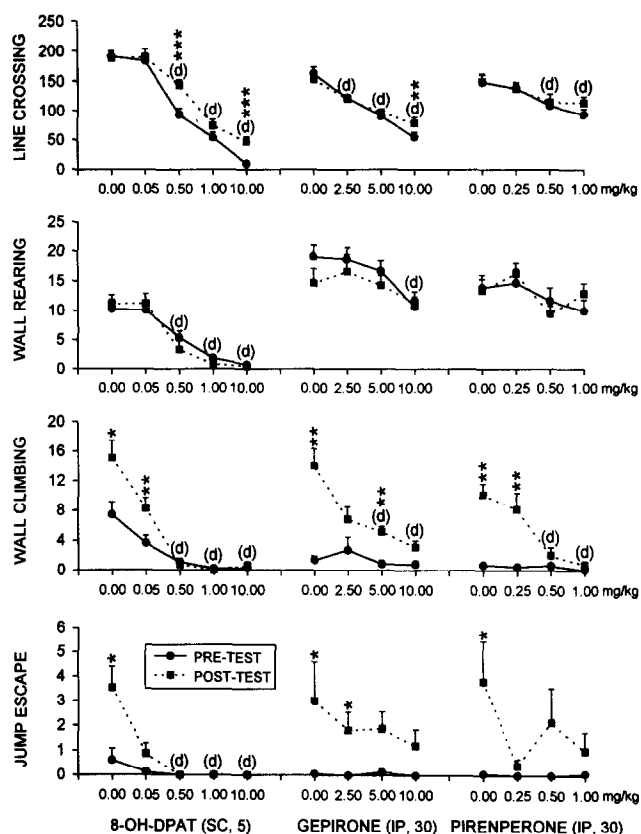


FIG. 1. Effects of a single acute dose of 8-OH-DPAT, gepirone, and pirenperone on the frequency of four response measures before (PRE-TEST) and after (POST-TEST) exposure to the predator. Data points and vertical bars represent means and SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$ (vs. pretest); (d), vs. vehicle.

21.25, $p < 0.00001$; wall climbing: Wilcoxon pair test: $p < 0.0004$; jump escape: Wilcoxon pair test: $p < 0.002$].

Dose-related pre- vs. posttest comparisons. For dose \times pre- or posttest, 4×2 ANOVA indicated a reliable interaction for line crossings [$F(4, 70) = 4.09, p < 0.005$], which subsequent Newman-Keuls analysis showed to be due to a significant posttest increase in the groups receiving 0.5 and 10 mg/kg of 8-OH-DPAT. This interaction was not reliable for wall rearing [$F(4, 70) = 0.76$]. Friedman ANOVA indicated reliable effects on wall climbing [$N(1, 75) = 4.9, p < 0.03$] and jump escape [$N(1, 75) = 11.27, p < 0.0008$], and subsequent analyses (Wilcoxon pair test) showed a posttest increase in both measures for vehicle control group and in wall climbs at 0.05 mg/kg.

Gepirone.

Drug effect. ANOVA revealed a reliable treatment effect for line crossing [$F(3, 56) = 24.64, p < 0.00001$], frequencies of wall rearing [$F(3, 56) = 3.72, p < 0.02$], and wall climbing [$H(3, 60) = 16.95, p < 0.0007$] but not for jump escape [$H(3, 60) = 2.49$]. Posthoc analyses indicated that gepirone significantly decreased frequencies of line crossings at all doses tested (Newman-Keuls: $p < 0.002$ vs. control), wall rearings at 10 mg/kg (Newman-Keuls: $p < 0.03$ vs. control), and wall climbs at 5 and 10 mg/kg (Mann-Whitney: $p < 0.002$ vs. control).

Pre- and postexposure-to-predator differences. For the gep-

irone group's line crossing [$F(1, 56) = 1.98$] and wall rearing [$F(1, 56) = 2.31$], responses were not changed in the posttest period when compared to the initial 3-min free-running session. By contrast, wall climbs (Wilcoxon pair test: $p < 0.000001$) and jump escapes (Wilcoxon pair test: $p < 0.000004$) increased significantly in the posttest period.

Dose-related pre- vs. posttest comparisons. ANOVA revealed a reliable interaction effect for line crossing [$F(3, 56) = 2.87$, $p < 0.05$], wall climbing [Friedman: $N(1, 60) = 40.16$, $p < 0.0001$], jump escape [Friedman: $N(1, 60) = 28$, $p < 0.0001$] but not for wall rearing [$F(3, 56) = 0.65$]. Subsequent Newman-Keuls analyses indicated that predator exposure significantly increased posttest line crossings in mice treated with the highest dose (10 mg/kg). Wilcoxon pair test analyses indicated that wall climbing increased in the saline-treated group at 5 mg/kg and jump escape in the control group at 2.5 mg/kg of gepirone.

Pirenperone.

Drug effect. Pirenperone produced a reliable effect on frequency of line crossing [$F(3, 56) = 4.72$, $p < 0.003$], wall climbing [$H(3, 60) = 27.58$, $p < 0.00001$], and jump escape [$H(3, 60) = 8.27$, $p < 0.04$] but failed to alter frequency of wall rearing [$F(3, 56) = 2.29$]. Posthoc analyses indicated reliably fewer line crossings (Newman-Keuls: $p < 0.03$ vs. control) and wall climbs (Mann-Whitney: $p < 0.0002$ vs. control) at 0.5 and 1 mg/kg.

Pre- and postexposure-to-predator differences. Neither line crossing [$F(1, 56) = 1.62$] nor wall rearing [$F(1, 56) = 0.13$] measures were significantly affected by the predator exposure. By contrast, both wall climbing (Wilcoxon pair test: $p < 0.000001$) and jump-escape responses (Wilcoxon pair test: $p < 0.00006$) increased significantly in the posttest period following presentation and removal of the predator.

Dose-related pre- vs. posttest comparisons. Dose \times pre- and posttest 4×2 two-way ANOVA failed to indicate a reliable interaction effect for line crossing [$F(3, 56) = 0.83$] and wall rearing [$F(3, 56) = 0.76$], but this interaction was reliable for wall climbing [$N(1, 60) = 34.11$, $p < 0.00001$] and jump escape [$N(1, 60) = 20$, $p < 0.000001$]. Subsequent Wilcoxon pair test analysis revealed a posttest increase in wall climbing for the saline and 0.25 mg/kg pirenperone groups. Posttest jump escape was also higher in the control group.

Reactions to the Predator

Predator avoidance test (Fig. 2).

8-OH-DPAT. ANOVA failed to reveal any reliable main effect for the number of avoidances [$H(4, 75) = 6.74$] and the avoidance distance [$F(4, 63) = 1.58$].

Gepirone. None of the behavioral measures was significantly affected by the drug treatment [number of avoidances: $H(3, 60) = 5.1$; avoidance distance: $F(3, 49) = 1.87$].

Pirenperone. ANOVA indicated a reliable drug effect on avoidance frequency [$H(3, 60) = 9.22$, $p < 0.03$] and the predator-subject distance at which avoidance occurred [$F(3, 46) = 11.27$, $p < 0.00002$]. The latter measure was reliably decreased at all doses tested, but the former measure was reduced only at 1 mg/kg.

Flight/predator orientation test (Table 1).

8-OH-DPAT. ANOVA failed to reveal a reliable drug effect on any behavioral measures: overall flight speed [$F(4, 64) = 2.27$], maximum flight speed [$F(4, 64) = 0.63$], arrests in movement [$F(4, 64) = 1.12$], orientation to the predator [Kruskal-Wallis: $H(3, 69) = 5.04$], and reversals [Kruskal-Wallis: $H(3, 69) = 5.28$].

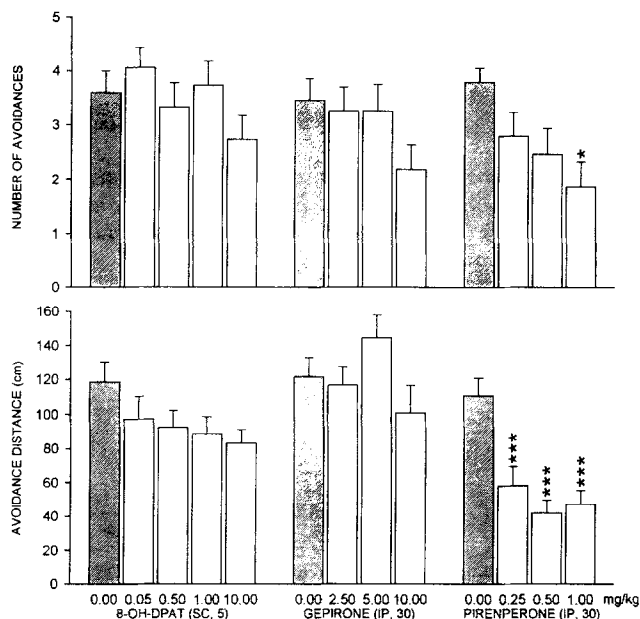


FIG. 2. Runway measures of avoidance to an approaching predator for mice administered 8-OH-DPAT, gepirone, and pirenperone. Columns and vertical bars represent means and SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

Gepirone. None of the behavioral responses was significantly modified by any of the doses: overall flight speed [$F(3, 46) = 1.5$], maximum flight speed [$F(3, 46) = 1.38$], arrests in movement [$F(3, 46) = 0.8$], orientation to the predator [Kruskal-Wallis: $H(3, 50) = 1.67$], and reversals [Kruskal-Wallis: $H(3, 50) = 3.1$].

Pirenperone. ANOVA indicated a reliable effect of drug treatment on maximum flight speed [$F(3, 52) = 4.37$, $p < 0.009$], but not for the other behavioral parameters: overall flight speed [$F(3, 52) = 2.93$], arrests in movement [$F(3, 52) = 0.46$], orientation to the predator [Kruskal-Wallis: $H(3, 56) = 1.67$], and reversals [Kruskal-Wallis: $H(3, 56) = 0.67$]. Subsequent analyses revealed that pirenperone significantly reduced flight speed at all doses tested (0.25–1 mg/kg).

Predator approach: Straight alley (Fig. 3).

8-OH-DPAT. ANOVA revealed a reliable main effect of treatment with 8-OH-DPAT for all behavioral measures: closest distance between animals [$F(4, 70) = 7.78$, $p < 0.00003$], immobility time [$F(4, 70) = 5.97$, $p < 0.0003$], and number of approaches and withdrawals [$H(4, 69) = 33.78$, $p < 0.00001$]. Subsequent posthoc analysis indicated that 8-OH-DPAT markedly increased the closest prey-predator distance from 0.5 to 10 mg/kg and the immobility time at 1 and 10 mg/kg. In addition, the drug reliably decreased the occurrence of approach/withdrawal responses toward the predator at 0.5, 1, and 10 mg/kg.

Gepirone. ANOVA indicated a reliable overall effects of treatment on the closest distance between subject and predator [$F(3, 56) = 4.07$, $p < 0.02$], immobility time [$F(3, 56) = 5.06$, $p < 0.004$], and number of approaches and withdrawals [$H(3, 60) = 12.57$, $p < 0.006$]. Newman-Keuls posthoc analysis showed that gepirone significantly increased the former at 5 and 10 mg/kg and the immobility time at only 10 mg/kg. Furthermore, comparison with the Mann-Whitney test indicated that the drug markedly reduced the subject's approaches/withdrawals toward the predator at 5 and 10 mg/kg.

TABLE 1
EFFECTS OF 8-OH-DPAT, GEPIRONE AND PIRENPERONE ON BEHAVIORAL RESPONSES OF MICE
CHASED BY A PREDATOR

	Overall Speed (m/s)	Maximum Speed (m/s)	Frequency of Stops	Frequency of Orientations	Frequency of Reversals
8-OH-DPAT (SC, 5) (mg/kg)					
0	0.60 (0.07)	0.78 (0.07)	11.93 (1.36)	1.60 (0.48)	1.33 (0.52)
0.05	0.54 (0.05)	0.72 (0.06)	12.40 (1.51)	2.67 (0.52)	1.60 (0.58)
0.5	0.43 (0.04)	0.79 (0.07)	13.87 (1.52)	3.13 (0.64)	0.67 (0.30)
1	0.42 (0.04)	0.82 (0.07)	15.36 (1.24)	3.57 (0.84)	0.57 (0.31)
10	0.46 (0.05)	0.87 (0.08)	11.70 (1.48)	2.20 (0.94)	0.70 (0.30)
Gepirone (IP, 30) (mg/kg)					
0	0.52 (0.05)	1.24 (0.04)	9.87 (1.11)	5.40 (0.84)	1.53 (0.48)
2.5	0.44 (0.04)	1.16 (0.07)	9.86 (1.31)	5.14 (1.24)	0.79 (0.46)
5	0.45 (0.04)	1.41 (0.13)	10.50 (1.14)	5.67 (0.77)	1.92 (0.79)
10	0.38 (0.05)	1.24 (0.13)	12.33 (0.94)	5.78 (1.05)	1.00 (0.37)
Pirenperone (IP, 30) (mg/kg)					
0	0.57 (0.04)	1.09 (0.06)	8.67 (0.83)	4.93 (0.69)	1.07 (0.50)
0.25	0.49 (0.05)	0.89 (0.06)*	10.60 (1.28)	4.13 (0.87)	0.67 (0.29)
0.5	0.42 (0.03)	0.82 (0.04)*	9.67 (1.46)	4.20 (0.60)	0.67 (0.23)
10	0.40 (0.05)	0.86 (0.08)*	10.45 (1.79)	4.73 (1.27)	0.55 (0.37)

Data are presented as means (\pm SEM). * $p < 0.05$.

Pirenperone. ANOVA failed to indicate any reliable effects of pirenperone for the closest distance between animals [$F(3, 56)$

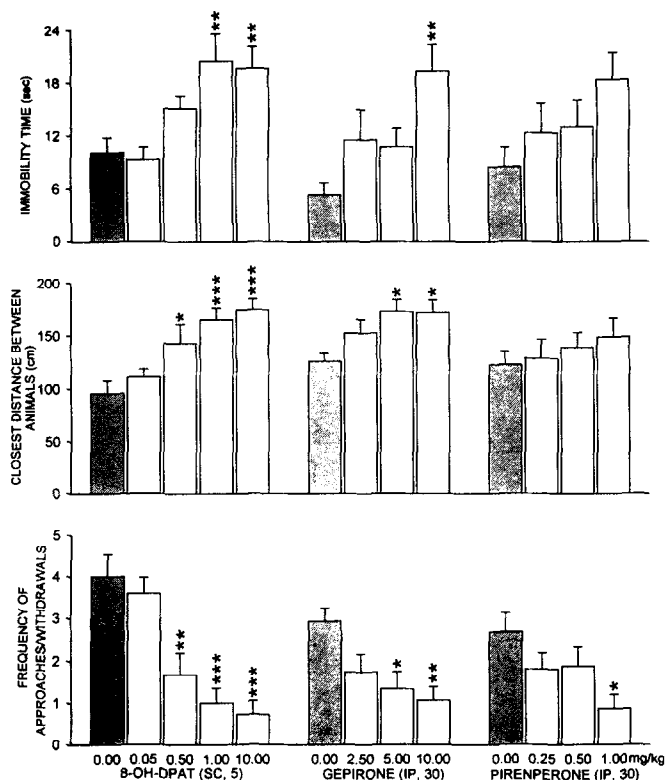


FIG. 3. Effects of 8-OH-DPAT, gepirone, and pirenperone in the straight alley on behavioral reactions to a predator that remained at constant distance from the subject. Columns and vertical bars represent means and SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

= 0.54] and immobility time [$F(3, 56) = 1.95$], but revealed a reliable main effect of the drug treatment for the frequency of approaches and withdrawals [$H(3, 60) = 8.59, p < 0.04$]. Mann-Whitney posthoc analysis showed that pirenperone reliably decreased the occurrence of the latter response at 1 mg/kg.

Forced contact with the predator (Fig. 4).

8-OH-DPAT. ANOVA indicated a reliable effect for frequency of biting to the rat [Kruskal-Wallis: $H(4, 72) = 22.09, p < 0.0002$] and the occurrence of upright posture [Kruskal-Wallis: $H(4, 72) = 15.04, p < 0.005$], but not for the frequencies of vocalization [Kruskal-Wallis: $H(4, 72) = 5.85$] and jump attacks toward the predator [Kruskal-Wallis: $H(4, 72) = 0.17$]. Subsequent Mann-Whitney U -tests revealed significant reductions in biting between 0.5 and 10 mg/kg and in upright posture at 0.5 and 10 mg/kg.

Gepirone. ANOVA indicated a reliable effect for frequency of biting [Kruskal-Wallis: $H(3, 60) = 16.89, p < 0.0007$], which a subsequent Mann-Whitney test showed to be due to a marked reduction of this response at all doses (2.5–10 mg/kg). In addition, ANOVA failed to reveal a reliable main effect for any of the other behavioral measures: upright posture [$H(3, 60) = 7.35$], vocalization [$H(3, 60) = 0.42$], and jump attack [$H(3, 60) = 6.1$].

Pirenperone. ANOVA indicated a reliable main effect for the frequency of biting [Kruskal-Wallis: $H(3, 60) = 10.01, p < 0.02$], but not for the occurrence of upright posture [$H(3, 60) = 4.9$], vocalization [$H(3, 60) = 6.12$], or jump attack [$H(3, 60) = 7.75$]. Mann-Whitney U -tests indicated a reliable decrease in biting at the highest dose of pirenperone (1 mg/kg).

Ledge Test (Table 2)

8-OH-DPAT. χ^2 analysis indicated a reliable increase in the frequency of falls at 10 mg/kg.

Gepirone. Frequency of falls was not significantly increased by any of the doses of gepirone.

Pirenperone. χ^2 analysis failed to indicate any significant

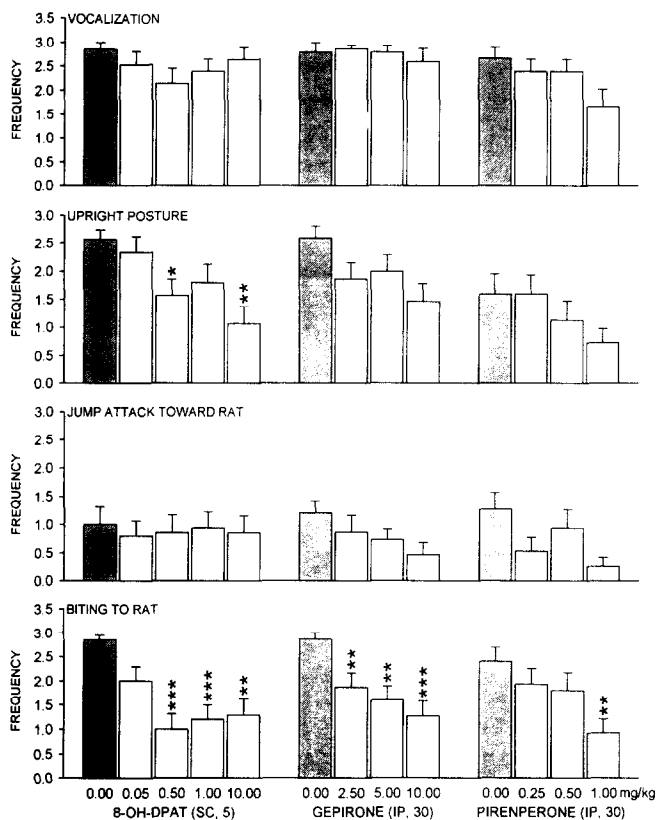


FIG. 4. Mean frequency of biting, defensive threat vocalization, upright posture, and jump attacks to forced contact with a deeply anesthetized rat for subjects under varying doses of 8-OH-DPAT, gepirone, and pirenperone. Columns and vertical bars represent means and SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

increase in the frequency of falls after the administration of pirenperone.

DISCUSSION

The behavioral responses displayed in response to predatory stimuli in the MDTB provide overall confirmation of previous findings in this laboratory (30). Thus, in the contextual defense situation, escape attempts (wall climbing and jump escape) were markedly increased during the postpredator period, compared to an equivalent period before the introduction of the predator. Similarly, in response to an approaching predator, saline-treated mice invariably showed active flight behavior with a consistent (prey-predator) avoidance distance of about 1.20 m in all control groups. When saline-treated subjects ran to escape the chasing predator, they frequently showed predator assessment consisting of an abrupt movement arrest often followed by orientation to the oncoming predator and sometimes a reversal of movement to approach the predator. It is striking that the maximum flight speed in the 8-OH-DPAT vehicle-treated group was a bit lower than the one measured in the two other groups. The reason for this difference is unclear, but might be attributable to the short interval between the injection and the test initiation and/or the administration route (SC vs. IP). In the straight-alley test, when mice were constrained in one part of the runway, they often displayed a pattern, apparently related to active predator assessment, consisting of approaches to the

predator followed by withdrawals. Defensive threat and attack to the rat occurred almost invariably upon forced contact.

Motoric Effects: Measures of Sedation and Myorelaxation

In a previous study using the MDTB (30), drug effects on measures of myorelaxation (maximum flight speed and falls from the wall) were very similar, with a good relationship between those drug doses at which flight speed decreased and falls increased. However, line crossings, taken as a measure of sedative effects, were little changed at these same doses, suggesting a clear dissociation of the dose levels at which myorelaxant and sedative effects occurred for the BZPs used. In the present study, evidence for myorelaxant effects was minimal, with no compound producing both a reliable decrease in flight speed and an increase in falls. A pattern of differences for alterations of line crossings, as opposed to the two measures of myorelaxation, was apparent. However, here the line-crossings measure was reduced at much lower doses than was required to produce the (minimal) obtained evidence of myorelaxation. As discussed subsequently, this reduction in line crossings may reflect one component of a 5-HT syndrome of motor activity rather than the sedation seen at higher doses of BZPs. The present results nonetheless support earlier findings of a differentiation of line-crossing changes as opposed to myorelaxant effects, and also indicate that the relationship between the doses at which these effects occur may be quite different for different classes of compounds.

Drug Effects Preceding and Following Predator Exposure: "Contextual Defense"

In the postpredator, compared to the prepredator, situation 8-OH-DPAT produced a number of behavioral changes consistent with contextual fear and anxiety reduction, including inhibition of the postpredator potentiation of escape attempts (0.5–10 mg/kg). Gepirone produced a somewhat lesser

TABLE 2

NUMBER OF SUBJECTS SHOWING FALL FROM THE LEDGE AFTER THE ADMINISTRATION OF VARIOUS DOSES OF 8-OH-DPAT, GEPİRONE AND PIRENPERONE

	No. of Falls	n
8-OH-DPAT (SC, 5) (mg/kg)		
0	0	15
0.05	2	15
0.5	3	15
1	3	15
10	5*	15
Gepirone (IP, 30) (mg/kg)		
0	0	15
2.5	0	15
5	1	15
10	1	15
Pirenperone (IP, 30) (mg/kg)		
0	1	15
0.25	1	15
0.5	2	15
10	2	15

* $p < 0.05$.

reduction in the postrat inhibition of these escape responses, counteracting only the postrat increase in wall climbs, and only at the two highest doses. As indexed in the line-crossing measure discussed earlier, as well as in reduced wall rearing, both drugs markedly interfered with horizontal and vertical spontaneous motor activity during pretest and postrat periods. These findings are somewhat in variance with our previous data in wild or laboratory rats showing that these same compounds increased locomotor activity at anxiolytic doses (5,7). In fact, the effects of 8-OH-DPAT and gepirone on spontaneous locomotor responses may be a result of stereotyped uncoordinated "ambulation" that forms a part of the 5-HT syndrome. Widely described in rats (2,6,22,24,61), it has also been observed in mice (24,65,66). It is interesting that the specific behavioral components of the 5-HT syndrome induced by 8-OH-DPAT may differ between species. Thus, hyperlocomotor effects of the drug have been mostly described in rats (6,24,61), whereas authors using mice invariably reported decreased locomotor response (24,42,65). However, whereas the overall effect of both 5-HT_{1A} agonists was clearly to reduce line crossings, it is notable that drugged animals often showed reliably more line crossings during the postrat test than in the pretest, suggesting that even under relatively high doses of these compounds, subjects are capable of more activity in stressful situations.

Because of these strong effects on line crossing and wall rearing, and despite the relative lack of myorelaxant effects of 8-OH-DPAT (i.e., increased falls only at the highest dose) and gepirone in the ledge test, our results suggest that the postrat inhibition of escape attempts at certain doses of 8-OH-DPAT (0.5–10.0 mg/kg) and gepirone (10.0 mg/kg) was not solely due to a specific anxiety/fear reduction action of the drugs, but may also involve some components of the mouse 5-HT motor syndrome. These effects may not be entirely independent: Jacobs and Fornal (37) recently hypothesized that the primary function of 5-HT systems is to modulate motor output and concurrently inhibit sensory information processing, a view that suggests both emotional and motoric effects from activation of the same mechanisms.

The administration of the preferential 5-HT_{2A} receptor antagonist pirenperone only partially suppressed postrat potentiation of escape attempts, with reduced wall climbing at 0.5 and 1.0 mg/kg. Line crossings were also reduced at the two highest doses (0.5 and 1.0 mg/kg), suggesting that motoric changes may have been a factor in these effects. Nonetheless, pirenperone may have some specific as well as nonspecific (i.e., mediated through motoric changes) effects on the post-encounter contextual defense responses, although the evidence for this is less than for 8-OH-DPAT.

Drug Effects During Exposure to the Predator

Flight. When the predator approached and chased the subject, neither 8-OH-DPAT nor gepirone impaired performance (number of avoidances, avoidance distance, flight speed, and predator assessment). It is extremely interesting to note that for neither compound did a dose that strongly affected locomotion in the pre- and posttest interfere with animals' responses to the predator. This suggests that responses to highly threatening stimuli (i.e., an approaching predator) may involve central mechanisms that can override the strong hypolocomotor effect seen in the contextual defense situation, in which there is no discrete threat stimulus, and levels of defensiveness are undoubtedly lower. This view is supported by the finding, described earlier, of an enhancement of activity by

5-HT_{1A} agonists during the postpredator test, compared to the less stressful pretest period. These data indicate that flight-related behaviors remain intact at dose levels of 5-HT_{1A} agents that are highly effective in modulating behavior in novel situations or situations associated with previous exposure to a predator.

In contrast to the 5-HT_{1A} receptor agonists, pirenperone markedly reduced both avoidance measures in the predator avoidance test and maximum flight speed during the chase/flight paradigm. These results, in the absence of myorelaxant effects, and given the lack of relationship between reduced line crossings and flight/avoidance of the predator for the 5-HT_{1A} receptor agonists, suggest that pirenperone effects on flight to an approaching predator were rather specific.

Several authors have suggested that the spontaneous activation of neuronal systems mediating the flight component of defense reactions may underlie human panic disorder (18, 19,28). We recently addressed this issue by showing that the panic-promoting drug yohimbine potentiated flight behavior (8), whereas chronic treatment with the panicolytic agent alprazolam reduced these responses in the MDTB (30). In this context, and in view of the present results, one can suppose that: a) 5-HT_{1A} receptor ligands are ineffective in reducing panic; and b) the preferential 5-HT_{2A} receptor antagonist pirenperone may have some efficacy in the treatment of panic disorder.

With regard to the first statement, not only preclinical data but also human studies provide undisputed evidence of a lack of efficacy of 5-HT_{1A} receptor ligands in flight/panic reactions. For instance, in Graeff's (29) procedure, in which the activation of the rat dorsal periaqueductal grey (DPAG) leads to behavioral manifestations identified as panic-like, 8-OH-DPAT and ipsapirone were ineffective (38,39). Clinical data almost invariably failed to report an antipanic efficacy of 5-HT_{1A} agents (49,51,52,55,56). Indeed, panic may even be exacerbated by buspirone (12,25). The only exception recently emerged from an open-label trial showing that gepirone reduced the frequency of panic attacks (47).

The situation is much more promising in the case of the preferential 5-HT_{2A} receptor antagonist pirenperone. The recognition that some antidepressants exert beneficial effects in panic, and the finding that chronic treatment with most, but not all, antidepressants results in a downregulation of the postsynaptic 5-HT_{2A} receptors (48), have led to the suggestion that selective 5-HT_{2A} receptor antagonists might have some efficacy in the treatment of panic (63). However, so far, no clinical study has reported conclusively a reduction in panic symptoms with such agents. Although the pilot study by Humble and colleagues (36) and an open trial study (32) found that ritanserin reduced panic attacks, three double-blind placebo-controlled studies clearly reported that the drug does not improve or even aggravates this condition (20,21,62). Nevertheless, these findings should be interpreted with certain caution as the studies were performed with a pharmacologic agent that is extremely nonselective. Indeed, ritanserin shows a nearly equal affinity for both 5-HT_{2A} and 5-HT_{2C} sites (35). It is obvious that clinical trials with more selective antagonists at the 5-HT_{2A} receptor would allow us more accurately to evaluate the possible involvement of these receptors in the pathogenesis of panic, but none of these agents has yet been tested against panic. Preclinical data tend to argue for the hypothesis of a 5-HT_{2A} mediation in panic. Jenck and co-workers (38,39) showed that the preferential 5-HT_{2A} receptor blockers ketanserin and pirenperone dose-dependently increased the aversive threshold of DPAG stimulation, whereas mixed 5-HT_{2A/2C}

receptor antagonists, such as ritanserin, cyproheptadine, and mianserin, had no such effect.

Finally, comparisons of drug effects on the flight/avoidance data from situations in which the subject was actually exposed to a predator, and on contextual defense measures such as wall-climbing and jump-escape responses, are in agreement with previous findings suggesting that the former, but not the latter, respond to panic-altering drugs. Although the panicogenic agent yohimbine increased some wall-climbing measures in a postpredator test, it did so against a background in which these measures were not increased by exposure to a predator. In fact, in the inescapable and confined situation used in that study, cat exposure increased immobility, and yohimbine decreased it. Thus, drug treatment might have released wall climbing by its diminution of crouching and freezing. However, in the same study, yohimbine also potentiated flight to a predator and reduced the defensive (prey-predator) distance of subjects in a runway situation (8). Moreover, a known panicolytic agent, alprazolam, administered on a repeated basis, failed to decrease such contextual defense or escape responses in tests and situations identical to those of the present study, but did reduce flight responses to an approaching predator (30).

Predator assessment. In the present straight-alley test, when the subject was constrained in one part of the runway and faced the predator, which remained at a set distance, both 5-HT_{1A} receptor ligands tended to suppress predator assessment responses. This effect was revealed by a dramatic decrease in the occurrence of approach and withdrawal movements toward the predator and a dose-dependent increase in the prey-predator distance, with both effects occurring between 0.5 and 10.0 mg/kg for 8-OH-DPAT and 5 and 10 mg/kg for gepirone. Because this reduction in active responses occurred in the same test in which active flight measures were not changed, it is difficult to attribute them to the same mechanism responsible for the reduced line crossings obtained in the pre- and posttests. Although immobility times did increase in this test, for both 8-OH-DPAT and gepirone, reliable predator assessment changes occurred at dose levels at which no increased immobility was obtained: In addition, because these animals often showed an immobile stance as the predator approached, a sharp decrease in approaches and withdrawals without any compensating active behavior would be expected to produce an increase in immobility. Thus, the inhibition of predator assessment, at least in the case of gepirone, appears

to reflect a relatively specific reduction in predator assessment aspects of defensiveness. Pirenperone failed to significantly alter predator assessment in the straight alley, except at the highest dose used, again providing less indication of anxiolytic activity on this measure than was obtained with the 5-HT_{1A} receptor ligands.

When contact was forced between the predator and the subject, both 5-HT_{1A} ligands had a clear impact on defensive attack, reducing defensive biting at all doses tested. Such an effect is consistent with previous findings in rats showing that the 5-HT_{1A} agonists gepirone and buspirone (and also BZPs such as diazepam, midazolam, chlordiazepoxide, and, in mice, alprazolam) all reduced defensive threat or attack toward a predator (3,5,4,30). Pirenperone produced a much less dramatic effect on this measure, with a significant reduction only at the highest (10 mg/kg) dose.

In summary, the present data in mice support previous rat findings that 5-HT_{1A} receptor agonists selectively alter predator- or risk-assessment activities and also reduce defensive attack. Although motoric effects of these compounds are apparent, these are most notable in novel situations and in situations associated with predator exposure, where they may be a factor in drug-induced alterations of contextual defense reactions. Such motoric effects cannot account for the predator assessment and defensive attack changes, which appear to be relatively specific. These results are quite different from those of the preferential 5-HT_{2A} receptor antagonist pirenperone, which decreased flight and increased the prey-predator distance at which flight occurred, in a specific manner, while showing much less potent effects on other defensive behaviors. These findings thus provide further evidence of the existence of multiple 5-HT mechanisms in the regulation of emotional behavior (31). These results, taken together with previous findings with BZPs in the MDTB, provide strong support for the use of the present multiparameter test paradigm which, unlike traditional animal models of anxiety, provides behavioural measures capable of differentiating between various classes of antianxiety drugs.

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