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The Mouse Defense Test Battery: evaluation of the effects of non-selective and BZ-1 (ω1) selective, benzodiazepine receptor ligands

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The behavioral effects of several benzodiazepine (BZ) (ω) receptor ligands were compared using the Mouse Defense Test Battery which has been designed to assess defensive reactions of Swiss mice confronted with a natural threat (a rat) and situations associated with this threat. Primary measures taken before, during and after rat confrontation were escape attempts, flight, risk assessment and defensive threat and attack. The drugs used included non-selective BZ (ω) full (clonazepam, clorazepate, chlordiazepoxide and diazepam) and partial (bretazenil and imidazenil) agonists, and BZ-1 (ω1) selective (abecarnil, CL 218,872 and zolpidem) receptor ligands. With the exception of clonazepam, non-selective BZ (ω) receptor compounds only partially affected flight behaviors. The drugs reduced some but not all flight measures in response to the approaching rat, whereas clonazepam attenuated all flight reactions. In contrast to their mild and inconsistent actions on flight, the non-selective BZ (ω) receptor agonists displayed clear effects on risk assessment when subjects were chased by the rat. When contact was forced between the subject and the rat, the non-selective BZ (ω) receptor full agonists reduced defensive threat and attack reactions, while the partial agonists imidazenil and bretazenil only weakly attenuated defensive attack behavior. Similarly, after the rat had been removed from the test area, the non-selective BZ (ω) receptor full agonists displayed greater efficacy than the partial agonists in reducing escape attempts. Overall, results obtained with the selective BZ-1 (ω1) receptor ligands demonstrated either no clear effects or no specific action on defensive reactions. Taken together, these data demonstrate that: (1) non-selective BZ (ω) agonists displaying high intrinsic activity affect a wider range of defensive behaviors than non-selective BZ (ω) receptor partial agonists; (2) the defense system does not involve primarily BZ (ω) receptors containing the α1-subunit.

Keywords: Anxiety – Benzodiazepines – BZ (ω) receptor – Defensive behaviors – Flight – Mouse Defense Test Battery – Risk assessment – Swiss mice

INTRODUCTION

It is now widely acknowledged that benzodiazepines (BZs) produce their effects through an action at two distinct binding sites both associated with the $GABA_A$ receptor and called BZ-1 and BZ-2 (Squires *et al.*, 1979; Sieghart and Schuster, 1984). These receptors were subsequently designated as ω1 and ω2, respectively (Langer and Arbilla, 1988) and it is now clear that the BZ-1 (ω1) subtype corresponds to receptors containing the α1-subunit, while the BZ-2 (ω2) subtype represents a heterogeneous population of sites possessing α2-, α3- or α5-subunits (Pritchett *et al.*, 1989; Sieghart, 1995).

$GABA_A$ receptor subtypes provide the potential for different affinities or efficacies to be displayed by ligands of BZ (ω) sites. Although there are individual variations, BZs, such as diazepam or bretazenil act with high affinity at all receptor subtypes. However, diazepam exhibits high intrinsic efficacy, while bretazenil displays reduced efficacy and is therefore described as a partial agonist (Haefely *et al.*, 1990; Puia *et al.*, 1992; Wafford *et al.*, 1993). In addition, subtype-selective compounds have been characterized. This is exemplified by the imidazopyridine zolpidem which exhibits high binding affinity to receptors containing the α1-subunit while displaying lower affinity to receptors containing the α2- and α3-subunits and no affinity for receptors containing the α5-subunit (Pritchett and Seeburg, 1990; Faure-Halley *et al.*, 1993). As a result, it was suggested that a particular pharmacological profile may be related to a restricted pattern of receptor interactions (for review, see Haefely *et al.*, 1990; Perrault *et al.*, 1990; Sanger *et al.*, 1994). Thus far, a few studies using tests

based on conditioned (i.e. punished drinking paradigm and operant responding) (Depoortere *et al.*, 1986; Sanger, 1995) or on spontaneous (i.e. elevated plus—maze and light/dark choice task) (Griebel *et al.*, 1996a,b) responses have demonstrated that drugs selective for the BZ1 (ω1) receptor subtype have a weaker anxiety-reducing potential than non-selective BZ (ω) receptor agonists. Moreover, it was shown that when an anxiolytic-like action was detected with the selective BZ-1 (ω1) compounds, it was accompanied by motor impairment, suggesting that their effects may have been non-specific (Sanger and Zivkovic, 1988; Jones *et al.*, 1994; Griebel *et al.*, 1996a,b).

In order to explore further the idea that the behavioral profile of BZ (ω) receptor ligands may be associated with actions at a defined receptor subtype and/or their level of intrinsic activity, the present study compared the effects of a wide range of BZ (ω) receptor ligands in a recently developed animal model of anxiety, the Mouse Defense Test Battery (MDTB) (Griebel *et al.* 1995a), which is based on the work of Blanchard and colleagues on antipredator defense in rats (Blanchard *et al.*, 1993). These authors developed two test batteries, a Fear/Defense Test Battery (F/DTB) measuring defensive behaviors to present, approaching predators, and an Anxiety/Defense Test Battery (A/DTB) measuring reactions to potential threat. Both batteries have been used in conjunction with administration of potentially anxiolytic drugs (for more details see Blanchard *et al.*, 1993). In mice, the test battery combines many of the features of the F/DTB and the A/DTB into a single procedure, eliciting and measuring reactions to both present (i.e. a rat) and anticipated threat (Griebel *et al.*, 1995a). In a mouse-scaled oval runway Swiss mice show a precise delineation of defensive behaviors including flight, avoidance, escape attempts, vocalization, and defensive threat/attack, with each behavior controlled by specifiable characteristics of the threat stimulus and situation (Griebel *et al.*, 1995a). Preliminary findings have shown that the non-selective BZ (ω) receptor full agonists chlordiazepoxide and the non-selective BZ (ω) receptor partial agonist Ro 1908022 attenuated the intensity of several defensive reactions including defensive threat and attack responses, but only chlordiazepoxide reduced escape attempts after the rat was removed from the test apparatus. Moreover, Ro 19-8022 reduced flight behaviors, whereas chlordiazepoxide failed to affect these responses in a specific manner (Griebel *et al.*, 1995a). This suggested that BZ (ω) receptor agonists affect a different spectrum of defense responses depending on their intrinsic activity. The drugs used in the present study are listed in Table I. They included non-selective BZ (ω) receptor full and partial agonists, and selective BZ-1 (ω1) receptor ligands.

METHODS

Subjects

Subjects were naive Swiss mice aged 9 weeks at the time of testing, and male Long Evans rats (400–500 g). They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed singly in standard cages (mice: 30 × 20 × 14 cm; rats: 44 × 30 × 20 cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (22–23°C) and kept on a 12 h light/dark cycle with light onset at 06.00 h.

Apparatus

The test was conducted in an oval runway, 0.4 m wide, 0.3 m high and 4.4 m in total length, consisting of two 2 m straight segments joined to two 0.4 m curved segments and separated by a median wall (2.0 × 0.3 × 0.06 m). The apparatus was elevated to a height of 0.8 m from the floor to enable the experimenter to hold the rat easily, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. In addition, the apparatus was equipped with

TABLE I. The BZ (ω) receptor ligands used in the present study

Drug	Pharmacological profile	Doses (mg/kg)	*n*=
Chlordiazepoxide	Non-selective BZ (ω) receptor full agonist	2.5–25	11 or 12
Diazepam	Non-selective BZ (ω) receptor full agonist	0.3–10	11
Clorazepate	Non-selective BZ (ω) receptor full agonist	0.3–10	11 or 12
Clonazepam	Non-selective BZ (ω) receptor full agonist	0.03–1	12
Bretazenil	Non-selective BZ (ω) receptor partial agonist	1–30	12
Imidazenil	Non-selective BZ (ω) receptor partial agonist	0.3–10	12
Zolpidem	Selective BZ (ω) receptor full agonist	0.1–3	12
Abecarnil	Selective BZ (ω) receptor full agonist	0.1–3	11 or 12
CL 218,872	Selective BZ (ω) receptor partial agonist	0.3–10	12

n = number of animals per group

infrared beams and sensors capable of measuring the velocity of the animal during the chase/flight test. Experiments were performed under red light between 09.00 and 14.00 h.

Procedure

Pre-test (min 1 to 3). Subjects were placed into the runway for a 3-min familiarization period during which line crossings, wall rears, wall climbs, and jump escapes were recorded.

Rat avoidance test (min 4 to 6). Immediately after the 3-min familiarization period, a hand-held dead rat (killed by CO_2 inhalation) was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated five times. Mean avoidance distance (cm) was calculated for each subject. The results were expressed as mean avoidance distance and mean number of avoidances.

Chase/flight test (min 7 to 8). The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. The following parameters were recorded: flight speed (measured when the subject is running straight), number of stops (pause in movement) and orientations (subject stops, the orients the head towards the rat). The two latter responses are described as risk assessment activities (Griebel *et al.*, 1995a).

Straight alley (min 9 to 11). The runway was then converted to a straight alley by closing a door at one end. During 30 s, the hand-held rat remained at a constant distance of 40 cm from the subject. Measures taken included closest distance between the subject and the rat and the number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it).

Forced contact (min 12 to 13). Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites, vocalizations and upright postures by the subjects were noted. This was repeated three times. The results were expressed as mean number of bites, mean number of vocalizations and mean number of upright postures.

Post-test: contextual defense (min 14 to 16). Immediately after the straight alley test, the rat was removed and the door opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session. See Griebel *et al.* (1996d) for additional details regarding this test battery.

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing one or two drops of Tween 80. The drugs used were clorazepate, diazepam, clonazepam, chlordiazepoxide hydrochloride, zolpidem (synthesized by the chemistry department, Synthélabo Recherche), bretazenil (courtesy of Drs Q. Branca and P. Weber, F. Hoffman-La Roche Ltd), abecarnil (courtesy of Schering), imidazenil (courtesy of Dr A. Guidotti) and CL 218,872 (courtesy of Dr B Beer). Dose-ranges are listed in Table I. All doses are expressed as the bases and were chosen on the basis of previous results in an actimeter (Griebel *et al.*, 1996b). Drugs were administered i.p. in a constant volume of 20 ml/kg 30 min before experiments were carried out.

Statistical analysis

Data from the pre-test, rat avoidance, chase/flight, straight alley and forced contact tests were analyzed by one-way analysis of variance (ANOVA). Subsequent comparisons were carried out using Dunnett's *t*-test. Pre- versus post-exposure comparisons were made with a two-way ANOVA (drug x test) followed by a Newman–Keuls *post-hoc* comparison.

RESULTS

Data from the straight alley test are not presented a statistical analysis did not reveal any significant drug effects during this phase.

Non-selective BZ (ω) receptor full agonists

Pre-test: motor activity before exposure to the rat (Table II). Statistical analyzes revealed that chlordiazepoxide [$F(4,54) = 5.85$, $p < 0.001$], diazepam [$F(4,50) = 7.97$, $p < 0.001$], clorazepate [$F(4,54) = 9.64$, $p < 0.001$] but not clonazepam significantly decreased the number of line crossings at the highest doses.

Rat avoidance test (Fig. 1). All the drugs significantly reduced the number of avoidances [chlordiazepoxide: $F(4,54) = 5.27$, $p < 0.01$; diazepam: $F(4,50) = 11.24$, $p < 0.001$; clorazepate: $F(4,54) = 6.28$, $p < 0.001$; clonazepam: $F(4,55) = 7.49$, $p < 0.001$]. Additionally, chlordiazepoxide [$F(4,38) = 3.63$, $p < 0.05$], clorazepate

TABLE II. Effects of several BZ (ω) receptor ligands on spontaneous locomotor activity of swiss mice exposed to the oval runway cage before the introduction of the rat.

Non-selective BZ (ω) full agonists			Non-selective BZ (ω) partial agonists			Selective BZ (ω) ligands		
Drug	mg/kg	Line crossings	Drug	mg/kg	Line crossings	Drug	mg/kg	Line crossings
Chlordiazepoxide	0	116.92 ± 9.55	Imidazenil	0	110.67 ± 11.88	Zolpidem	0	150.67 ± 8.13
	2.5	124.55 ± 8.32		0.3	141.58 ± 9.23		0.1	137.58 ± 12.49
	5	138.67 ± 15.88		1	135.17 ± 6.24		0.3	144.00 ± 9.68
	10	130.00 ± 10.88		3	144.75 ± 9.84		1	145.00 ± 12.22
	25	72.33 ± 6.59*		10	135.50 ± 7.08		3	104.75 ± 10.23*
Diazepam	0	127.00 ± 6.62	Bretazenil	0	144.08 ± 6.33	Abecarnil	0	150.25 ± 11.04
	0.3	150.64 ± 8.25		1	170.83 ± 11.83		0.1	152.67 ± 14.26
	1	153.36 ± 10.99		3	167.92 ± 10.35*		0.3	70.33 ± 10.98*
	3	116.64 ± 8.45		10	203.83 ± 8.23*		1	59.45 ± 9.12*
	10	86.45 ± 12.93*		30	190.33 ± 10.98		3	66.64 ± 9.13*
Clorazepate	0	106.60 ± 11.20				CL 218,872	0	147.83 ± 14.19
	0.3	124.80 ± 14.60					0.3	128.92 ± 14.90
	1	162.10 ± 11.60					1	134.62 ± 7.84
	3	127.10 ± 8.40					3	139.83 ± 8.20
	10	69.30 ± 7.00*					10	131.58 ± 10.08
Clonazepam	0	109.17 ± 13.08						
	0.03	129.67 ± 12.49						
	0.1	132.17 ± 11.12						
	0.3	124.50 ± 7.16						
	1	111.08 ± 11.28						

Animals were observed during a 3-min period. Drugs were administered i.p. 30 min before experiments were carried out. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

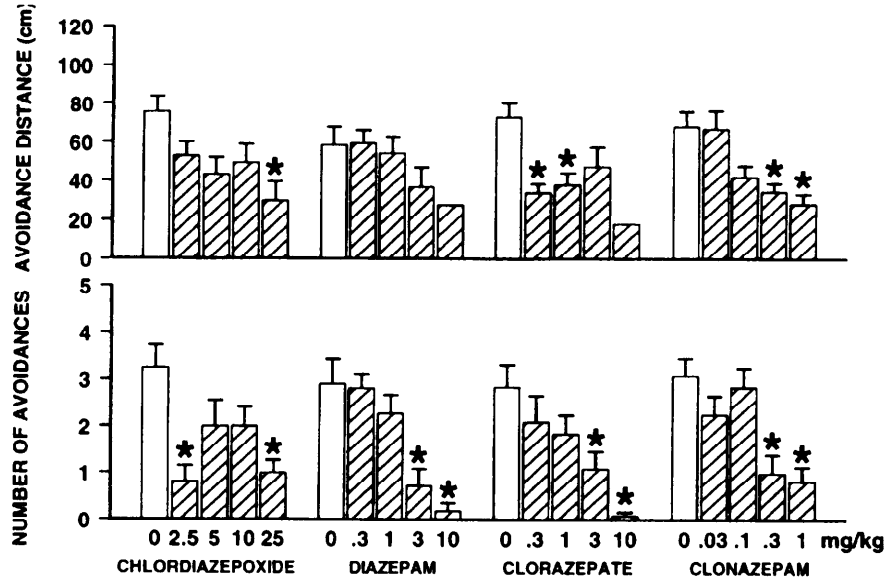

FIG. 1. Runway measures of avoidance to an approaching Long Evans rat for swiss mice administered four non-selective BZ (ω) receptor full agonists. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

TABLE III. Effects of non-selective BZ (ω) receptor full agonists on the behaviors of mice chased by a rat

Drug	mg/kg	Speed (m/s)	Orientations	Stops
Chlordiazepoxide	0	0.89 ± 0.08	3.42 ± 0.54	4.92 ± 0.79
	2.5	0.71 ± 0.06	2.27 ± 0.49	3.00 ± 0.52
	5	0.72 ± 0.08	2.25 ± 0.37	3.00 ± 0.59
	10	0.57 ± 0.06*	1.42 ± 0.47*	2.08 ± 0.63*
	25	0.44 ± 0.03*	0.92 ± 0.50*	1.25 ± 0.65*
Diazepam	0	0.64 ± 0.06	3.45 ± 0.67	4.45 ± 0.81
	0.3	0.81 ± 0.09	1.82 ± 0.46	2.64 ± 0.51
	1	0.56 ± 0.06	1.00 ± 0.36*	1.36 ± 0.43*
	3	0.47 ± 0.04	0.91 ± 0.55*	1.27 ± 0.51*
	10	0.46 ± 0.03	0.64 ± 0.31	0.73 ± 0.33*
Clorazepate	0	0.74 ± 0.06	3.83 ± 0.56	5.50 ± 0.99
	0.3	0.79 ± 0.16	4.75 ± 0.83	6.50 ± 1.24
	1	0.74 ± 0.09	2.50 ± 0.61	3.00 ± 0.56
	3	0.53 ± 0.03	1.55 ± 0.69	1.91 ± 0.87*
	10	0.44 ± 0.04	1.25 ± 0.43*	1.50 ± 0.44*
Clonazepam	0	1.04 ± 0.11	5.58 ± 0.67	10.00 ± 1.16
	0.03	0.60 ± 0.07*	5.64 ± 0.88	9.64 ± 1.23
	0.1	0.66 ± 0.06*	2.25 ± 0.55*	4.75 ± 0.74*
	0.3	0.59 ± 0.05*	1.58 ± 0.51*	3.08 ± 0.79*
	1	0.50 ± 0.07*	0.75 ± 0.30*	1.83 ± 0.53*

Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

[$F(4,54) = 5.26$, $p < 0.01$], clonazepam [$F(4,45) = 7.09$, $p < 0.001$] but not diazepam decreased the avoidance distance.

Chase/flight test (Table III). Flight speed was significantly reduced by chlordiazepoxide [$F(4,54) = 7.11$, $p < 0.001$] and clonazepam [$F(4,53) = 7.32$, $p < 0.001$] but not by clorazepate and diazepam. By contrast the risk assessment measures were decreased by all compounds [chlordiazepoxide: orientations: $F(4,54) = 4.06$, $p < 0.01$; stops: $F(4,54) = 4,51$, $p < 0.01$; diazepam: orientations: $F(4,50) = 5.56$, $p < 0.001$; stops: $F(4,50) = 7.58$, $p < 0.01$; clorazepate: orientations: $F(4,54) = 5.53$, $p < 0.001$; stops: $F(4,54) = 6.5$, $p < 0.001$; clonazepam: orientations: $F(4,53) = 13.24$, $p < 0.001$; stops: $F(4,53) = 15.33$, $p < 0.001$].

Forced contact test (Fig. 2). All the drugs significantly reduced the number of bites [chlordiazepoxide: $F(4,54) = 4.48$, $p < 0.01$; diazepam $F(4,50) = 3.05$, $p < 0.01$; clorazepate: $F(4,54) = 4.63$, $p < 0.01$; clonazepam: $F(4,55) = 22.65$, $p < 0.001$;] and the number of upright postures: [chlordiazepoxide: $F(4,54) = 4.39$, $p < 0.01$; diazepam $F(4,50) = 8.22$, $p < 0.001$; clorazepate: $F(4,54) = 14.68$, $p < 0.001$; clonazepam: $F(4,55) = 26.63$, $p < 0.001$;]. Diazepam [$F(4,50) = 7.86$, $p < 0.001$] clorazepate [$F(4,54) = 23.61$, $p < 0.001$], clonazepam [$F(4,55) = 6.2$, $p < 0.001$] but not chlordiazepoxide significantly decreased vocalizations.

Post-test escape attempts (Fig. 3) A two-way ANOVA indicated a significant drug × test interaction in all groups [chlordiazepoxide: $F(4,88) = 12.22$, $p < 0.001$; diazepam: $F(4,80) = 4.8$, $p < 0.001$; clorazepate: $F(4,88) = 24.44$, $p < 0.001$; clonazepam: $F(4,88) = 23.96$, $p < 0.001$]. *Post-hoc* analysis revealed that escape attempts increased in all control groups and that the drugs were able to inhibit this effect but only at the highest doses.

Non-selective BZ (ω) receptor partial agonists

Pre-test: motor activity before exposure to the rat (Table II). Bretazenil [$F(4,55) = 5.49$, $p < 0.001$] but not imidazenil significantly affected the number of line crossings, increasing motor activity at 3 and 10 mg/kg.

Rat avoidance test (Fig. 4). Only bretazenil reduced the number of avoidances [$F(4,55) = 4.61$, $p < 0.01$], whereas neither compound significantly affected the avoidance distance.

Chase/flight test (Table IV). Imidazenil significantly decreased all behavioral measures [flight speed:

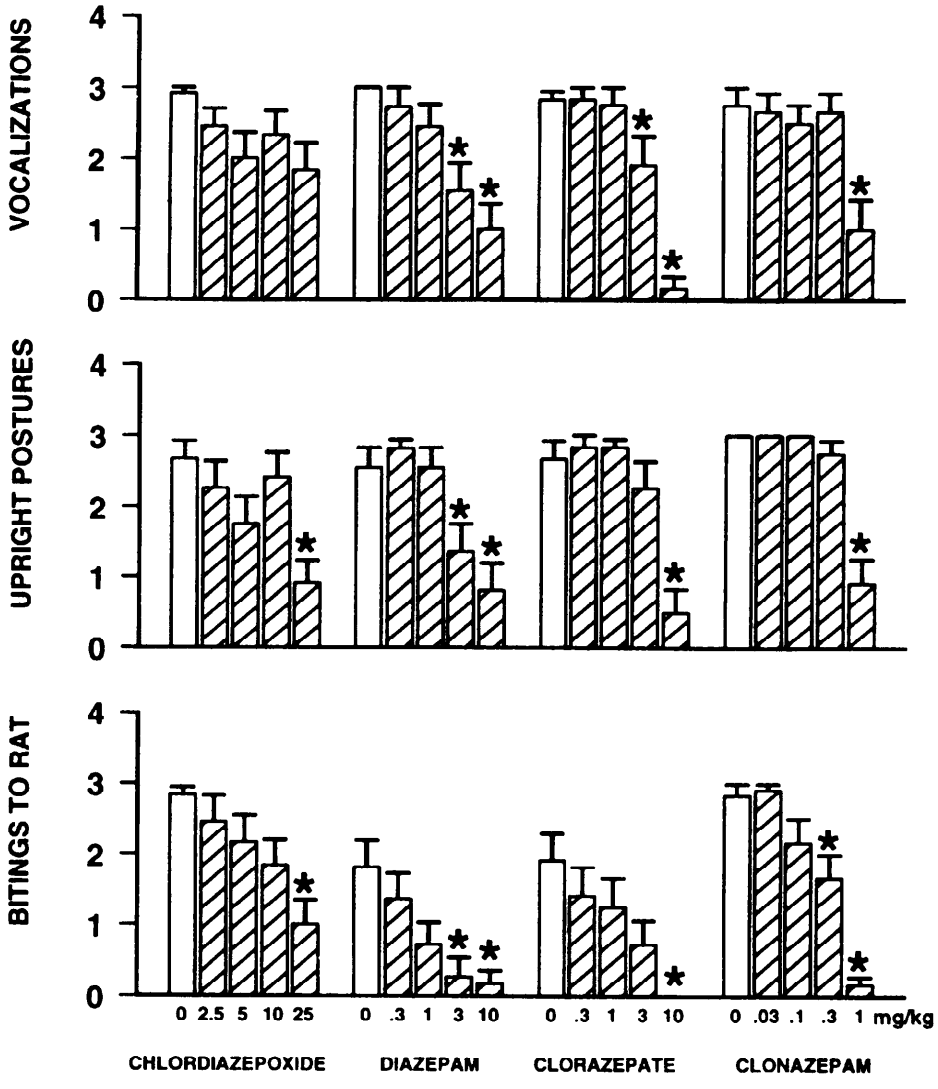

FIG. 2. Number of bites, defensive threat vocalizations and upright postures to forced contact with a Long Evans rat for subjects administered four non-selective BZ (ω) receptor full agonists. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett-s t-test).

$F(4,55) = 6.21$, $p < 0.001$; orientations: $F(4,55) = 15.78$, $p < 0.001$; stops: $F(4,55) = 34.7$, $p < 0.001$], while bretazenil only reduced the number of stops [$F(4,55) = 2.6$, $p < 0.05$].

Forced contact test (Fig. 5). The compounds significantly reduced the number of bites [imidazenil: $F(4,55) = 3.04$, $p < 0.05$; bretazenil: $F(4,55) = 2.74$, $p < 0.05$], but vocalizations and upright postures remained unchanged.

Post-test escape attempts (Fig. 6). Escape attempts were increased in all groups [imidazenil: $F(4,88) = 3.11$, $p < 0.05$; bretazenil: $F(4,88) = 9.06$, $p < 0.001$], but this effect was not significant at 1 mg/kg of imidazenil.

Selective BZ-1 (ω1) receptor full agonists

Pre-test: motor activity before exposure to the rat (Table II). With the exception of CL 218,872, the drugs significantly decreased the number of line crossing [zolpidem: $F(4,55) = 2.94$, $p < 0.05$; abecarnil: $F(4,53) = 17.92$, $p < 0.001$].

Rat avoidance test (Fig. 7). Zolpidem [$F(4,55) = 4.11$, $p < 0.01$], and abecarnil [$F(4,53) = 8.62$, $p < 0.0001$], but not CL 218,872 significantly reduced the number of avoidances. Avoidance distances remained unchanged in all groups.

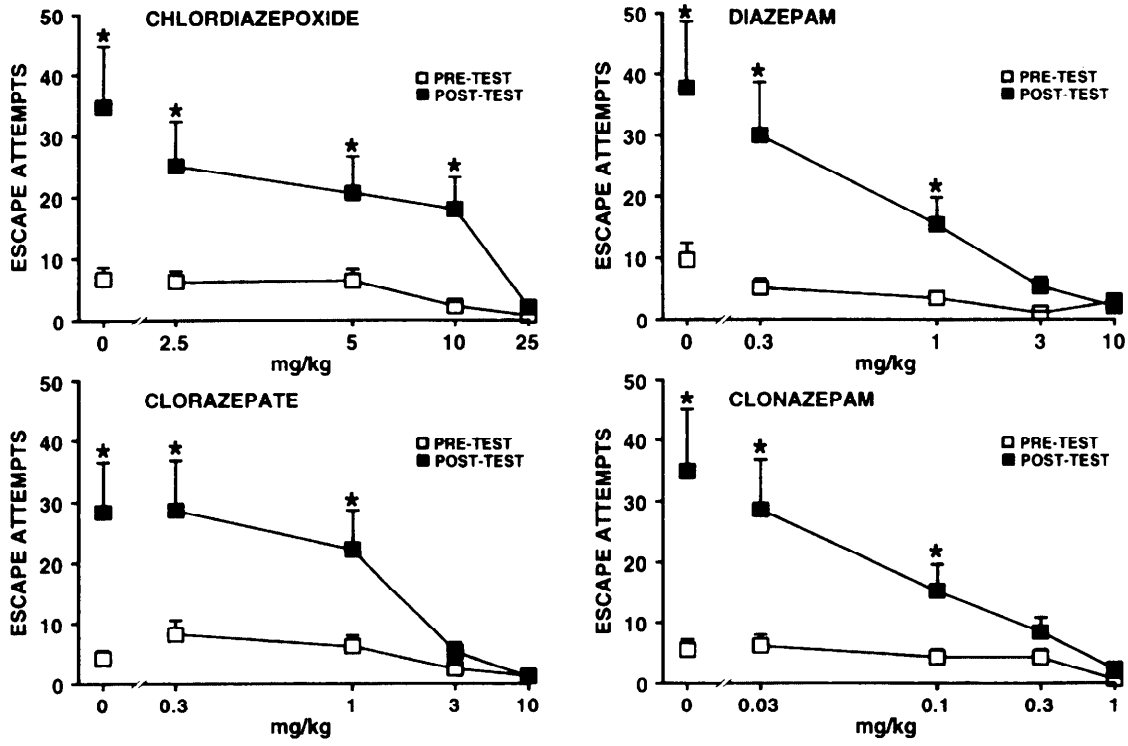

FIG. 3. Effects of four non-selective BZ (ω) receptor full agonists on escape attempts from the runway cage before (pre-test) and after (post-test) the exposure of a Long Evans rat. Data represent mean ± S.E.M. * $p < 0.05$ (versus pre-test) (Newman–Keuls).

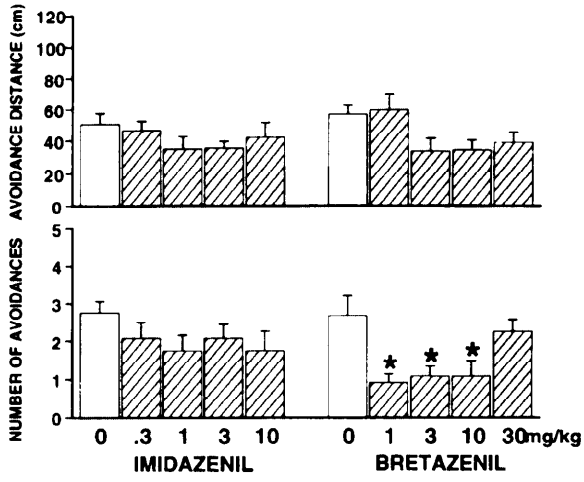

FIG. 4. Runway measures of avoidance to an approaching Long Evans rat for Swiss mice administered two non-selective BZ (ω) receptor partial agonists. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett-s *t*-test).

Chase/flight test (Table V). Abecarnil significantly decreased all behavioral measures [flight speed: $F(4,53) = 13.27$, $p < 0.001$; orientations: $F(4,53) = 7.5$, $p < 0.001$; stops: $F(4,53) = 17.7$, $p < 0.001$]. CL 218,872 reduced flight speed [$F(4,55) = 3.09$, $p < 0.05$] and the number of stops [$F(4,55) = 6.66$, $p < 0.001$], while zolpidem did not effect the behavior of mice in this phase.

Forced contact test (Fig. 8). Zolpidem, abecarnil but not CL 218,872 significantly reduced the number of bites [$F(4,55) = 5.41$, $p < 0.001$; abecarnil: $F(4,53) = 5.22$, $p < 0.001$] and upright postures [zolpidem: $F(4,55) = 3.89$, $p < 0.01$; abecarnil: $F(4,53) = 5.22$, $p < 0.01$]. Vocalizations remained unchanged by these drug treatments.

Post-test escape attempts (Fig. 9). Following the removal of the rat, escape attempts were increased in all groups [zolpidem: $F(4,88) = 7.25$, $p < 0.001$; abecarnil: $F(4,88) = 47.31$, $p < 0.001$; CL 218,872 and from the dose of 0.3 mg/kg of abecarnil.

DISCUSSION

The present data are in agreement with previous studies showing that systemic administration of BZ (ω) receptor ligands produced a number of changes in defense which may be related to modulation of fear or anxiety (Griebel *et al.*, 1995a,b). In addition, these results demonstrate that non-selective BZ (ω) receptor full agonists produce complex, but relative similar, patterns of behavioral

TABLE IV. Effects of non-selective BZ (ω) receptor partial agonists on the behaviors of mice chased by a rat

Drug	mg/kg	Speed (m/s)	Orientations	Stops
Imidazenil	0	0.82 ± 0.06	8.25 ± 0.95	15.33 ± 1.50
	0.3	0.67 ± 0.06	2.83 ± 0.78*	4.92 ± 0.79*
	1	0.55 ± 0.02*	2.55 ± 0.57*	3.25 ± 0.45*
	3	0.59 ± 0.02*	2.08 ± 0.36*	4.25 ± 0.63*
	10	0.58 ± 0.04*	1.83 ± 0.60*	3.17 ± 0.61*
Bretazenil	0	0.96 ± 0.08	7.50 ± 0.92	13.58 ± 1.29
	1	0.69 ± 0.05	5.33 ± 1.33	8.17 ± 1.52*
	3	0.85 ± 0.09	5.33 ± 0.98	9.08 ± 1.06
	10	0.75 ± 0.06	4.25 ± 0.92	8.25 + 1.62*
	30	0.81 ± 0.08	4.75 ± 1.06	8.17 ± 1.69*

Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

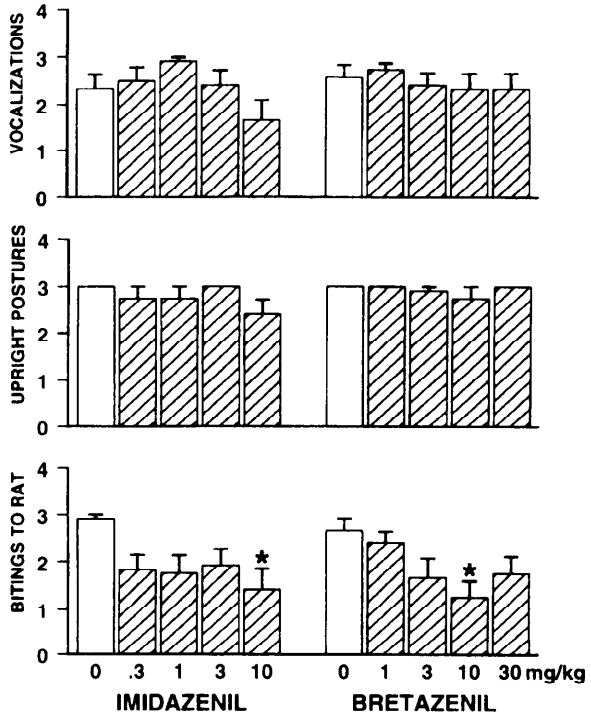

FIG. 5. Number of bites, defensive threat vocalizations and upright postures to forced contact with a Long Evans rat for subjects administered to non-selective BZ (ω) receptor partial agonists. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett-s t-test).

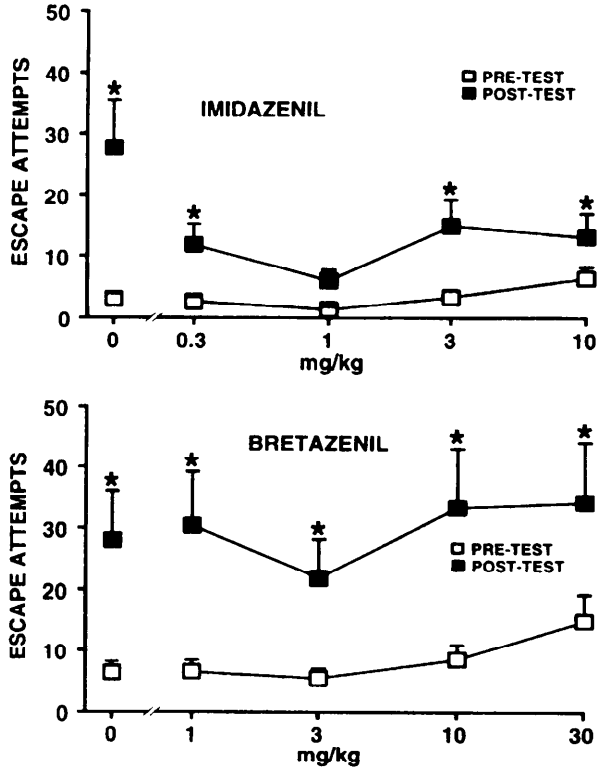

FIG. 6. Effects of two non-selective BZ (ω) receptor partial agonists on escape attempts from the runway cage before (pre-test) and after (post-test) the exposure of a Long Evans rat. Data represent mean ± S.E.M. *$p < 0.05$ (versus pre-test) (Newman–Keuls)

changes and, as is discussed below, that these changes differ somewhat from those seen with non-selective BZ (ω) receptor partial agonists and selective BZ-1 (ω1) receptor ligands.

Effects preceding and following rat exposure: 'contextual defense'

Diazepam, clorazepate and clonazepam counteracted the potentiation of escape attempts after the removal of the rat, at doses which did not affect motor activity, as revealed by the findings from the pre-test. By contrast, chlordiazepoxide decreased escape attempts during the post-test period only at the highest and motor-impairing dose of 25 mg/kg, suggesting that the effect may have been non-specific. The reason for this difference in behavioral profile between chlordiazepoxide and the other

TABLE V. Effects of selective BZ-1 (ω1) receptor ligands on the behaviors of mice chased by a rat

Drug	mg/kg	Speed (m/s)	Orientations	Stops
Zolpidem	0	0.79 ± 0.08	6.33 ± 0.78	11.75 ± 1.43
	0.1	0.89 ± 0.08	6.67 ± 1.07	11.83 ± 1.79
	0.3	0.90 ± 0.13	7.17 ± 1.04	13/75 ± 1.16
	1	0.74 ± 0.06	5.42 ± 0.91	10.92 ± 1.35
	3	0.56 ± 0.05	4.92 ± 0.80	8.25 ± 1.14
Abecarnil	0	1.00 ± 0.12	6.75 ± 0.81	10.75 ± 0.90
	0.1	0.72 ± 0.08*	4.58 ± 0.70	9.17 ± 0.98
	0.3	0.46 ± 0.04*	3.25 ± 0.35*	5.33 ± 0.76*
	1	0.40 ± 0.03*	3.18 ± 0.85*	4.27 ± 0.95*
	3	0.37 ± 0.03*	1.82 ± 0.58*	2.91 ± 0.71*
CL 218,872	0	1.24 ± 0.21	5.17 ± 0.6	8.75 ± 0.76
	0.3	1.10 ± 0.23	4.83 ± 0.79	7.92 ± 0.67
	1	1.00 ± 0.13	4.75 ± 0.99	7.67 ± 0.95
	3	0.71 ± 0.06	3.75 ± 0.66	8.00 ± 0.83
	10	0.59 ± 0.05*	2.83 ± 0.63	3.75 ± 0.57*

Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

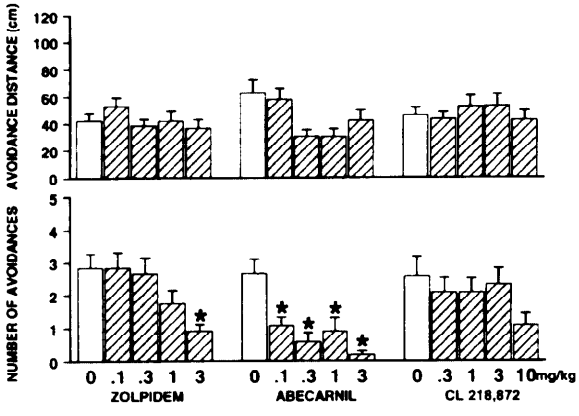

FIG. 7. Runway measures of avoidance to an approaching Long Evans rat for Swiss mice administered three selective BZ-1 (ω1) receptor ligands. Data represent mean ± S.E.M. *$p < 0.05$ (Dunnett's t-test).

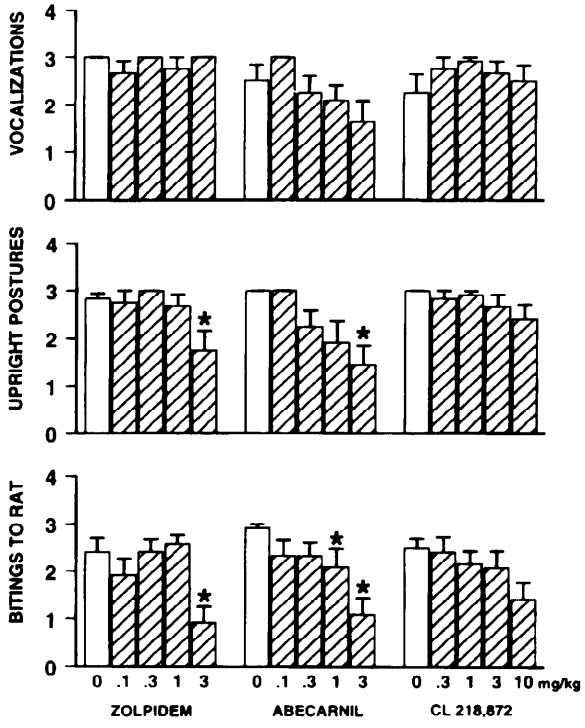

FIG. 8. Number of bites, defensive threat vocalizations and upright postures to forced contact with a Long Evans rat for subjects administered three selective BZ-1 (ω1) receptor ligands. Data represent mean ± S.E.M.. * $p < 0.05$ (Dunnett-s t-test).

non-selective BZ (ω) receptor full agonists is unclear. However, there is suggestive clinical evidence that at least diazepam seemed to exert greater efficacy than chlordiazepoxide in the treatment of generalized anxiety disorders (Lapierre, 1983). The two non-selective BZ (ω) receptor partial agonists bretazenil and imidazenil did not display clear effects on post-predator escape attempts. These results are consistent with the recent observation of a lack of effect on post-rat escape attempts of Ro 19-8022, another non-selective BZ (ω) receptor partial agonist (Griebel *et al.*, 1995a). Together, these findings suggest that only BZ (ω) ligands which display high intrinsic activity are able to reverse the strong potentiation of escape attempts following the removal of the rat.

Results obtained with the selective BZ-1 (ω1) receptor ligands demonstrated either no clear effects or no specific action on contextual defensiveness. Thus, abecarnil clearly prevented the increase in escape attempts

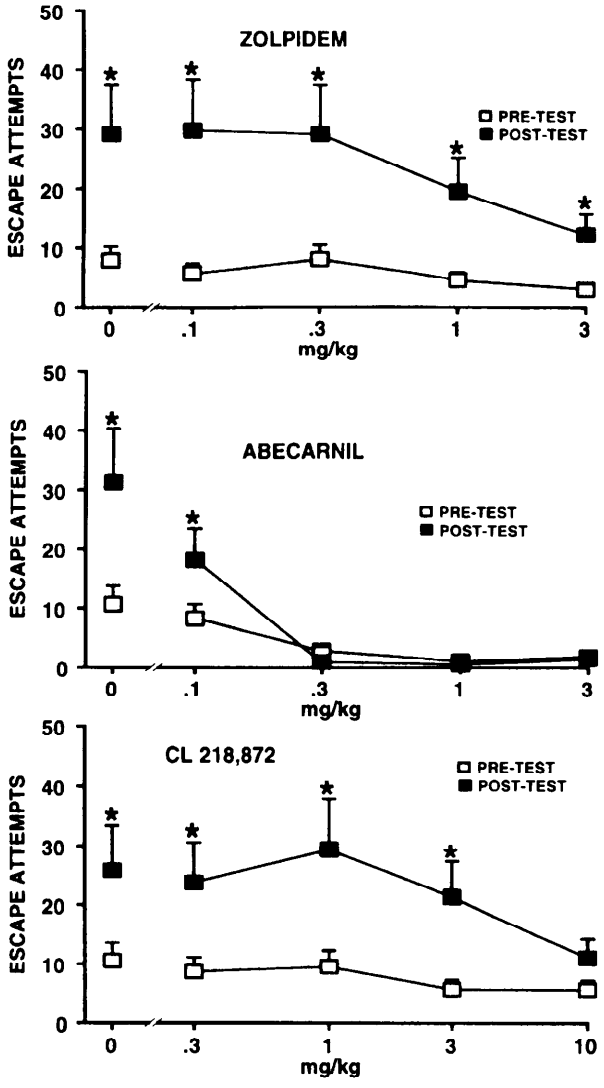

FIG. 9. Effects of three selective BZ-1 (ω1) receptor ligands on escape attempts from the runway cage before (pre-test) and after (post-test) the exposure to a Long Evans rat. Data represent mean ± S.E.M. $^*p < 0.05$ (versus pre-test) (Newman–Keuls).

at doses which reduced line crossing, indicating that depressant properties interfered with the drug's action on contextual defensiveness. In contrast, CL 218,872 significantly reduced the behavior at the highest dose (10 mg/kg) in the absence of effects on line crossings, whereas zolpidem failed to prevent the post—exposure potentiation of escape attempts. Together, these data suggest that BZ-1 (ω1) receptors do not mediate an effect on contextual defensiveness.

Effects during exposure to the rat

The present results indicate that with the exception of clonazepam, non-selective BZ (ω) receptor agonists only partially affected flight measures (i.e. avoidance distance and number, and flight speed). Thus, chlordiazepoxide, diazepam, clorazepate and bretazenil specifically reduced the number of avoidances, at doses where the avoidance distance remained unchanged. In the chase/flight test, diazepam, clorazepate and bretazenil failed to affect the flight speed, whereas chlordiazepoxide at a single dose, and imidazenil from 1 to 10 mg/kg reduced the measure in a specific manner. The effects of clonazepam on both avoidance responses at 0.3 and 1 mg/kg and on speed at all doses indicate that the drug exhibited a clear flight-reducing action. Flight behaviors in the MDTB have been shown to be particularly sensitive to panic-modulating drug treatments (Griebel *et al.*, 1996c). In view of the well documented clinical efficacy of clonazepam in the management of panic disorder (e.g. Beaudry *et al.*, 1985; Svebak *et al.*, 1990; Beauclair *et al.*, 1994), the flight-reducing action of the drug provides further evidence that this defense response may be of particular interest in the study of neural mechanisms underlying panic attacks.

At non-motor-impairing doses, the BZ-1 (ω1) receptor ligands either failed to affect flight (e.g. zolpidem), or weakly (e.g. abecarnil and CL 218,872) reduced some but not all flight measures. These results on flight indicate that the BZ-1 (ω1) receptor subtype cannot be considered as the primary target mediating the flight-reducing action of drugs interacting with the $GABA_A$/BZ receptor complex, and subsequently suggest that panic responses may not involve BZ-1 (ω1) receptors.

In contrast to their mild and inconsistent action on flight, non-selective BZ (ω) receptor agonists displayed clear effects on risk assessment during the chase/flight test. All compounds reduced the number of stops when the subject was chased by the rat and some but not all (i.e. chlordiazepoxide, diazepam, clonazepam and imidazenil) decreased the number of orientations to the danger source. Together, these results are in agreement with earlier findings with the rat and the mouse defense test batteries, indicating that risk assessment is particularly responsive to anxiolytic drugs (Blanchard *et al.*, 1993; Griebel *et al.*, 1995a,b). The reduced effectiveness of clorazepate and bretazenil in decreasing risk assessment compared to the other non-selective BZ (ω) receptor agonists is unclear, but it is noteworthy that in two other recent studies with bretazenil, the drug also failed to reduce significantly risk assessment (i.e. attempts at entry into the open arms of an elevated plus-maze and into a brightly illuminated box) (Griebel *et al.*, 1996a,b), suggesting that there may be differences in anxiolytic activity between the non-selective BZ (ω) receptor agonists regardless of their intrinsic activities. With regard to the two partial agonists, although they share a number of similarities, bretazenil but not imidazenil potentiates the GABA response at BZ (ω) receptors containing the

α4-and the α6-subunits (Mohler *et al.*, 1995). Thus, it is conceivable that these biological specificities might be involved in the different behavioral profiles displayed by the two compounds in this test.

Among the BZ-1 (ω1) receptor ligands, abecarnil markedly decreased risk assessment activities, but only at motor-impairing doses, as revealed by the pre-test, indicating that the inhibition of these responses may reflect a relatively non-specific reduction in these defense responses. In addition, CL 218,872 weakly affect risk assessment at it reduced only stops at the highest dose (i.e. 10 mg/kg). Finally, the selective BZ-1 (ω1) receptor full agonist zolpidem failed to alter both risk assessment measures, suggesting that the BZ-1 (ω1) receptor subtype does not mediate an effect on this particular defense response.

Studies using wild rats (*Rattus rattus*) have demonstrated that the most consistent influence of BZs (e.g. chlordiazepoxide, diazepam, midazolam) on defensive behaviors was a marked reduction in defensive threat (e.g. vocalizations, upright postures) and attack (e.g. bites), effects seen even at the lowest doses (Blanchard *et al.*, 1989, 1993). Consistent with previous data in mice (Griebel *et al.*, 1995a,c), the present results with the non-selective BZ (ω) receptor full agonist differ somewhat from the rat findings, as defensive threat and attack responses were reduced at high and mostly motor-impairing doses. Compared to the full compounds, the two non-selective BZ (ω) receptor partial agonists imidazenil and bretazenil displayed reduced efficacy in decreasing these defense reactions, as they reduced bites only. This latter finding contrasts with that recently observed in the MDTB with Ro 19-8022, another non-selective partial agonist at the BZ (ω) receptors, which was found to decrease in a dose-dependent manner all the defensive threat and attack measures (Griebel *et al.*, 1995a). Ro 19-8022 was reported to display a higher intrinsic activity than bretazenil (Facklam *et al.*, 1992). Thus, it is conceivable that at least in the case of bretazenil this difference may account for its lower efficacy in reducing defensive threat and attack responses.

Results obtained with the BZ-1 (ω1) receptor ligands during the forced contact test are consonant with the findings from the previous test situations, as the compounds either reduced some defensive reactions in a non-specific manner (e.g. zolpidem and abecarnil) or were devoid of any effects on defensive threat and attack responses (e.g. CL 218,872).

In conclusion, the results of the present study confirmed that classical and novel BZ (ω) receptor agonists reduce some but not all defensive reactions of mice confronted with a natural threat or a situation associated with the threat (Griebel *et al.*, 1995a,b). Moreover, these results revealed that the partial agonists bretazenil and imidazenil generally displayed lower efficacy in reducing defensive behaviors than non-selective BZ (ω) receptor full agonists. These data are in agreement with recent studies showing mild anxiolytic-like effects of non-selective BZ (ω) receptor partial agonists including bretazenil and imidazenil, in murine models of anxiety (Cole and Rodgers, 1993; Jones *et al.*, 1994; Sanger *et al.*, 1995; Griebel *et al.*, 1996b). However, these mice findings contrast with those obtained in rat studies, where partial agonists displayed the same or even greater efficacy than full agonists in reducing anxiety-related behaviors (Jenck *et al.*, 1992; Giusti *et al.*, 1993; Martin *et al.*, 1993; Costa *et al.*, 1995; Dazzi *et al.*, 1995; Sanger, 1995; Witkin *et al.*, 1996). The reasons for these species differences are unclear, but may question the relevance of murine models in the evaluation of the anxiety-reducing potential of partial agonists. Clearly, clinical trials are needed to decide which of the animal models are the best predictors. However, on a clinical level, only few trials with such agents have been carried out so far and little is known of their efficacy in the treatment of anxiety disorders (Potokar and Nutt, 1994). Finally, the present findings are consonant with recent studies using classical experimental models of anxiety (i.e. the elevated plus-maze, the light dark and the free-exploration tests), showing that the anxiolytic-like effects of selective BZ-1 (ω1) receptors ligands are weaker than those observed with non-selective BZ (ω) receptor agonists and are confounded by decreases in locomotor activity (Griebel *et al.*, 1996a,b). Interestingly, the selective BZ-1 (ω1) receptor ligands displayed different behavioral profiles in the MTDB. While zolpidem and CL 218,872 generally failed to modulate defensiveness, abecarnil markedly, albeit mostly at motor-impairing doses, decreased some defensive responses. It has been shown that, in addition to its selectivity for $GABA_A$ receptors containing the α1-subunit on which it acts as a full agonist, abecarnil also acts as a full agonist on receptors containing the α3-subunit, but as a partial agonist at receptors containing the α2- or α5-subunits (Knoflach *et al.*, 1993; Pribilla *et al.*, 1993). Thus, it is conceivable that the different behavioral profile of this β-carboline in the MDTB might be due to an interaction with specific receptor subtypes and/or its different intrinsic activities at these sties. Together, the finding of a lack of specific effect of the BZ-1 (ω1) receptor ligands suggests that the defense system does not involve primarily BZ (ω) receptors containing the α1-subunit.

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