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Further Evidence for Differences Between Non-selective and BZ-1 (ω1) Selective, Benzodiazepine Receptor Ligands in Murine Models of "State" and "Trait" Anxiety

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Summary—The behavioural effects of several BZ (ω) receptor ligands were compared in mice using the light/dark choice task, an animal model of "state" anxiety, and the free-exploration test, which has been proposed as an experimental model of "trait" anxiety. The drugs used included non-selective full (alprazolam, clorazepate, chlordiazepoxide and diazepam), partial agonists (bretazenil, imidazenil and Ro 19-8022) and BZ-1 (ω1) selective receptor ligands (abecarnil, CL 218,872 and zolpidem). In the light/dark choice task, non-selective full agonists elicited clear anxiolytic-like effects increasing time spent in the lit box and simultaneously reducing attempts at entry into the illuminated cage followed by withdrawal responses, a measure of risk assessment. With the exception of abecarnil, both non-selective partial agonists and BZ-1 (ω1) selective receptor ligands displayed reduced efficacy compared to the full agonists as they decreased risk assessment responses without altering time in the lit box. In addition, the weak anxiolytic-like actions displayed by selective BZ-1 (ω1) agents were evident only at doses which reduced locomotor activity, indicating that this effect may be non-specific. In the free-exploration test, non-selective BZ (ω) receptor agonists markedly increased the percentage of time spent in the novel compartment and reduced the number of attempts to enter, whereas selective BZ-1 (ω1) receptor ligands displayed a weaker neophobia-reducing effect as they reduced risk assessment responses only. As was the case in the light/dark choice task, this latter effect was observed at locomotor depressant doses. These findings indicate that while both full and partial BZ (ω) receptor agonists are equally effective against "trait" anxiety, full agonists may be superior in reducing "state" anxiety. In addition, the lack of specific effects of selective BZ-1 (ω1) receptor ligands in reducing both types of anxiety suggests that the BZ-1 (ω1) receptor subtype cannot be considered as the primary target mediating the anxiolytic action of drugs interacting with the $GABA_A$/benzodiazepine receptor complex. Copyright © 1996 Elsevier Science Ltd.

Keywords—Benzodiazepines, BZ (ω) receptor, light/dark choice task, free-exploration test, risk assessment, "trait" and "state" anxiety, BALB/c mice.

Despite their unwanted effects (e.g. sedation and risk of dependence with long-term use), benzodiazepines (BZs) remain the first choice drugs for the treatment of generalised anxiety disorder. It has been demonstrated that these compounds produce their effects by acting at two distinct binding sites both associated with the $GABA_A$ receptor complex 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). Experiments with recombinant and photolabelled receptors indicated that the BZ-1 (ω1) subtype corresponds to $GABA_A$ receptors containing the α1 subunit, while the BZ-2 (ω2) subtype represents a heterogeneous population of receptors containing α2, α3 or α5 subunits (Pritchett *et al*., 1989; Sieghart, 1995).

Extensive research in medicinal chemistry has led to the synthesis of a large variety of agents interacting with ω receptors. Classical BZs, such as diazepam, exhibit high intrinsic efficacy and act with high affinity at all receptor subtypes, while partial agonists, typified by bretazenil, show reduced efficacy compared to diazepam but display similar high affinity at BZ (ω) receptors (Haefely *et al*., 1990; Puia *et al*., 1992; Wafford *et al*.,

Table 1. The BZ (ω) receptor ligands used in the present study. Doses for each test were expressed as mg/kg

		Light/dark test	Free-exploration test	Actimeter
Chlordiazepoxide	Non-selective BZ (ω) receptor full agonist	0–20	0–10	0–40
Diazepam	Non-selective BZ (ω) receptor full agonist	0–4	0–2	0–8
Clorazepate	Non-selective BZ (ω) receptor full agonist	0–4	0–2	0–8
Alprazolam	Non-selective BZ (ω) receptor full agonist	0–1	0–1	0–10
Ro 19-8022	Non-selective BZ (ω) receptor partial agonist	0–30	0–30	0–60
Bretazenil	Non-selective BZ (ω) receptor partial agonist	0–40	0–30	0–60
Imidazenil	Non-selective BZ (ω) receptor partial agonist	0–3	0–1	0–30
Zolpidem	Selective BZ-1 (ω1) receptor full agonist	0–3	0–3	0–30
Abecarnil	Selective BZ-1 (ω1) receptor full agonist	0–3	0–1	0–10
CL 218,872	Selective BZ-1 (ω1) receptor partial agonist	0–30	0–10	0–60

1993). In addition, agents selective for subpopulations of receptors have been characterized. For example, the imidazopyridine zolpidem and the triazolopyridazine CL 218,872 exhibit high binding affinity for α1-containing receptors, whereas they show low to no affinity for α2, α3 and α5 containing receptors (Pritchett and Seeburg, 1990; Faure-Halley *et al*., 1993). On the basis of such results, it was hypothesized that particular pharmacological profiles might be related to certain receptor subtypes (for review, see Sanger *et al*., 1994). Several findings from this laboratory support this idea. For instance, it was shown that although zolpidem displayed anticonflict activity in a punished drinking paradigm as do non-selective BZ (ω) receptor agonists, it showed a lower efficacy in terms of the maximum effect (Depoortere *et al*., 1986) and did not increase rates of operant responding suppressed by punishment (Sanger and Zivkovic, 1988). Furthermore, it was shown recently that several selective BZ-1 (ω1) receptor ligands displayed low efficacy in increasing punished operant responding (Sanger, 1995) and in reducing avoidance of the open arms of an elevated plus-maze in rats (Griebel *et al*., 1996), while non-selective BZ (ω) receptor agonists produced clear effects in both procedures. Together, these findings indicate that selective activation of BZ-1 (ω1) receptors is associated with a reduced potential to produce anxiety compared to non-selective BZ (ω) receptor agonists.

The present study addressed this issue with two experimental procedures designed to investigate anxiety-modulating agents in mice, namely the light/dark choice task (LDT) and the free-exploration test (FET). The LDT, initially designed by Crawley and colleagues (Crawley and Goodwin, 1980), has been modified by several authors (Costall *et al*., 1989; Misslin *et al*., 1989). Misslin and colleagues claimed that the LDT is based on the innate tendency of mice to seek refuge in a dark box and their propensity to escape novel places in which they have been constrained (Misslin *et al*., 1989). It has proven useful for the investigation of both classical (BZs) and novel or potential (e.g. 5-HT_{1A} receptor ligands, CCK_B receptor antagonists) anxiolytic drugs (Belzung *et al*., 1987, 1989, 1994; Belzung, 1988; Misslin *et al*., 1990; Griebel *et al*., 1992). The FET is based on the strong neophobic reactions exhibited by BALB/c mice when confronted simultaneously with a familiar and a novel compartment (Griebel *et al*., 1993). Preliminary pharmacological investigations indicated that the FET, unlike the LDT, is exclusively sensitive to BZ (ω) receptor ligands (Griebel *et al*., 1993; Belzung *et al*., 1994), thereby suggesting that the procedures do not evaluate the same emotional state. The FET has been proposed as an experimental model of "trait" anxiety and the LDT as a model of "state" anxiety (Griebel *et al*., 1993; Belzung and Le Pape, 1994; Beuzen and Belzung, 1995). "State" anxiety was defined by Lister (1990) as anxiety that the subject experiences at a particular moment in time and that is increased by the presence of anxiogenic stimulus (e.g. a brightly illuminated box). In contrast, "trait" anxiety does not vary from moment to moment and is considered to be an "enduring feature of an individual". As Lister pointed out, the anxiety tests used most widely assess "state" anxiety and a recent study by Belzung and colleagues showed that parameters recorded in the LDT and in the FET do not reflect the same psychological state (Belzung and Le Pape, 1994). Based on the finding that no neurovegetative changes were apparent in mice that had free access to novelty when compared to the modifications induced by situations in which these animals were forced, the FET can be considered to be devoid of clear anxiogenic stimuli (Misslin and Cigrang, 1986). Consequently, the observation that BALB/c mice display strong neophobic reactions in the FET indicates that neophobia represents a constant feature of their behaviour. Together, these findings led to the idea that the FET with BALB/c mice can be considered as a model of "trait" anxiety.

The drugs used in the present study included non selective BZ (ω) receptor full (chlordiazepoxide, diazepam, clorazepate, alprazolam) and partial agonists (Ro 19-8022, bretazenil, imidazenil), and selective BZ-1 (ω1) receptor ligands (zolpidem, abecarnil, CL 218,872) (Table 1). Among these latter compounds, zolpidem is a full agonist at α1-containing receptors (Wafford *et al*., 1993), CL 218,872 is also α1-selective but is a partial agonist at many receptor subtypes (α1, α2, α3 and α5) (Wafford *et al*., 1993) and abecarnil is a full agonist at α1- and α3-containing receptors, and a partial agonist at α2-

and α5-containing $GABA_A$ receptors (Knoflach *et al*., 1993). The specificity of drug response was examined by recording general activity in both tests and by measuring spontaneous locomotion in activity cages in separate groups of animals.

MATERIALS AND METHODS

Animals

Male BALB/c mice used were nine weeks old at the time of testing. All animals were housed in groups of five and maintained under standard laboratory conditions (21–22°C, relative humidity 40–55%) with free access to food and water. They were kept on 12:12 h light–dark cycle with light onset at 06:00. Animals were bred and supplied by Iffa Credo (L'Arbresle, France).

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, alprazolam, chlordiazepoxide hydrochloride, zolpidem (synthesized by the chemistry department, Synthélabo Recherche), bretazenil and Ro 19-8022 (both courtesy of Drs Q. Branca and P. Weber, F. Hoffman-La Roche Ltd), abecarnil (courtesy of Schering), imidazenil (courtesy of Dr A. Guidotti, Fidia), CL 218,872 (courtesy of Dr B. Beer, American Cyanamid). All doses are expressed as the bases. Drugs were administered intraperitoneally (i.p.) in a constant volume of 20 ml/kg 30 min before experiments were carried out.

Behaviour in the light/dark choice task

The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with plexiglas. One of the boxes was darkened. A light from a desk lamp (100 W), approximately 10 cm above the other box, and one neon tube fixed on the ceiling provided the room illumination. Light intensity on the bottom of the illuminated box was approximately 4000 lux. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the illuminated one. At the beginning of the experiment, a mouse was placed in the centre of the illuminated box, facing the tunnel, and observed for 4 min. Recording started when the mouse entered the tunnel for the first time. Subjects were observed via a video camera by an observer located in an adjacent room. The floor of each box was cleaned between test sessions. The following parameters were recorded: (a) time spent in the lit box; (b) attempts at entry into the lit box followed by avoidance responses. This includes stretch attend posture (the mouse stretches forward and retracts to original position in the tunnel). These behaviours are collectively referred to as "risk assessment". This latter concept has emerged from the work on antipredator defense in rodents. It typically refers to a pattern of responses invariably observed in potentially dangerous situations and which is particularly sensitive to anxiety-modulating drug treatment (Blanchard *et al*., 1991); and (c) total number of tunnel crossings. Although this parameter may be contaminated by anxiety, it was recorded in order to evaluate general motor activity in the same context as the anxiety measures. The results were expressed as mean time spent in the lit box (sec), mean total number of attempts and mean total number of tunnel crossings. Testing was performed between 08.30 and 13:00.

Behaviour in the free-exploration test

The apparatus consisted of a polyvinylchloride box (30 × 20 × 20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small entries. It could be divided in half lengthwise by closing three temporary partitions. Approximately 20 h before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with fresh sawdust and the animal was given unlimited access to food and water. On the following day, the subject was exposed to both familiar and novel compartments by removal of the temporary partitions. It was then observed under red light, for 5 min via a closed circuit TV camera by an observer located in an adjacent room. The following parameters were recorded: (a) time spent in the novel compartment; (b) attempts at entry into the novel compartment followed by avoidance responses. This included stretch attend posture; and (c) total number of unit changes. This parameter was recorded in order to evaluate general motor activity in the same context as the anxiety measures. It must be emphasized however that total number of unit changes may also be an element of anxiety. The results were expressed as mean percentage of time spent in the novel compartment, mean total number of attempts and mean total number of unit changes. Testing was performed between 08:30 and 13:00. Previous experiments with the FET revealed that this test is particularly sensitive to sedative/myorelaxant drug action. Thus, to attempt to avoid contamination by the depressant properties of the drugs, doses chosen were somewhat lower than those used in the LDT.

Effects on spontaneous locomotor activity: the actimeter

Testing was conducted in square, clear Plexiglas boxes (22 × 27 × 10 cm) equipped with infrared beams and sensors. They were placed in sound attenuated cupboards. Horizontal locomotor activity was quantified as total number of beams crossed during a 5 min period. Thirty minutes after injection, a mouse was placed in the centre of the apparatus. Testing was performed between 08:30 and 13:00.

Statistical analysis

Each dose–response curve was assessed by a one-way analysis of variance (ANOVA) or the nonparametric Kruskal–Wallis ANOVA (time spent in the lit box).

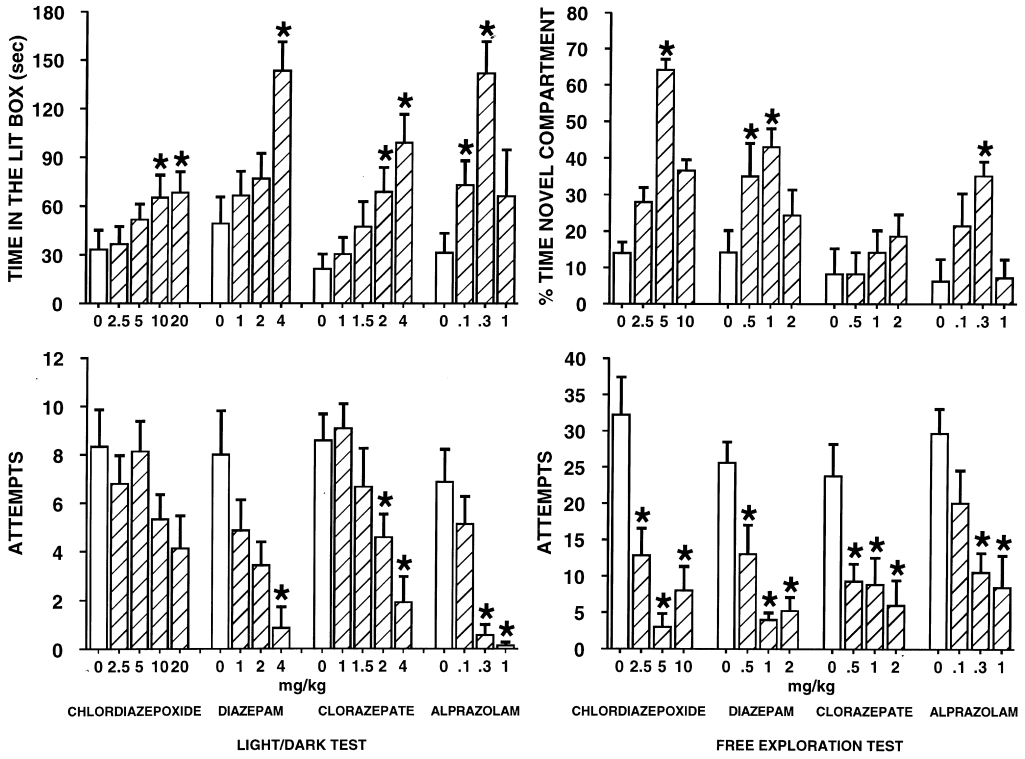

Fig. 1. Effects of four non-selective BZ (ω) receptor full agonists on the behavior of BALB/c mice exposed to the light/dark choice task and to the free-exploration test. Drugs were administered intraperitoneally 30 min before testing. Data represented means $\pm$ SEM. *$P < 0.05$.

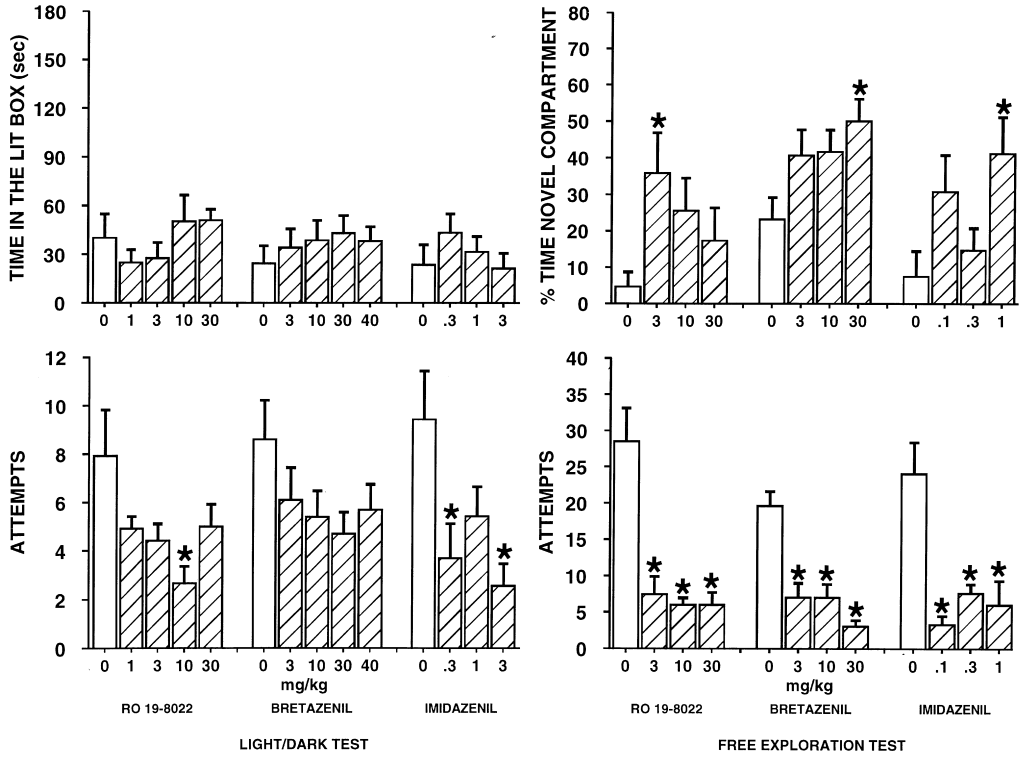

Fig. 2. Effects of three non-selective BZ (ω) receptor partial agonists on the behavior of BALB/c mice exposed to the light/dark choice task and to the free-exploration test. Drugs were administered intraperitoneally 30 min before testing. Data represented means $\pm$ SEM. *$P < 0.05$.

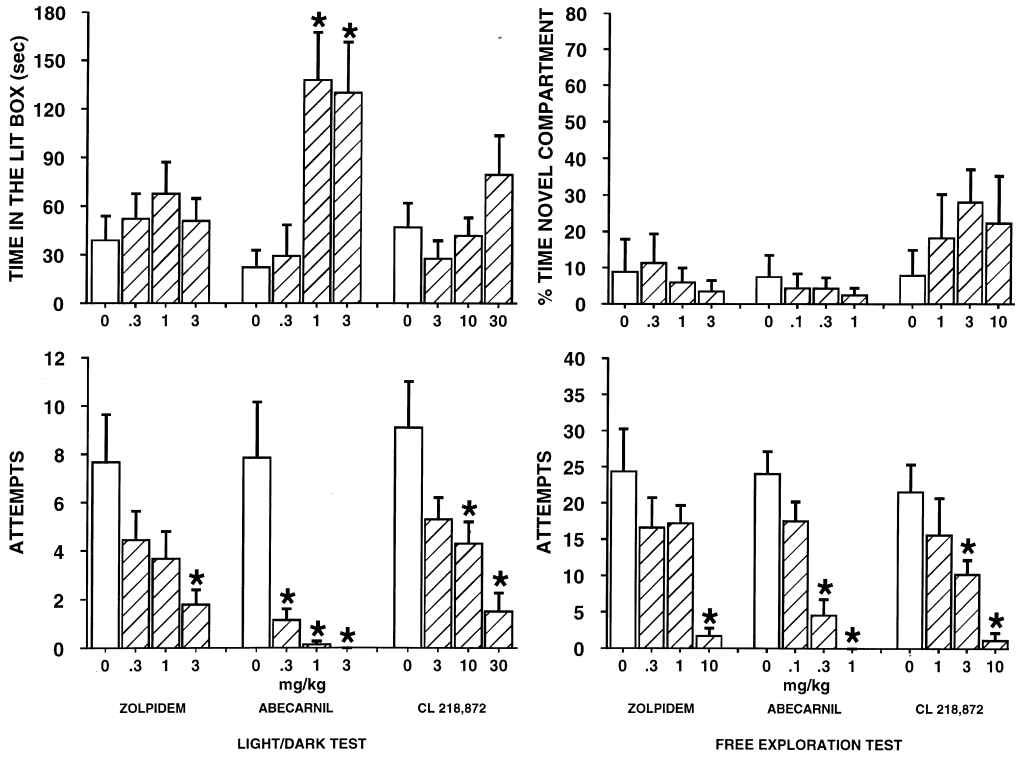

Fig. 3. Effects of three selective BZ-1 (ω1) receptor ligands on the behavior of BALB/c mice exposed to the light/dark choice task and to the free-exploration test. Drugs were administered intraperitoneally 30 min before testing. Data represented means ± SEM. *$P < 0.05$.

Subsequent comparisons between treatment groups and control were carried out using Dunnett's *a posteriori* T-test or the nonparametric Mann–Whitney *U*-test.

RESULTS

The measures of behaviour in the LDT and the FET are shown in Figs 1–3. For purposes of comparing drug effects the total number of tunnel crossings and the total number of unit changes are displayed with the locomotor activity data in Figs 4–6.

Light/dark choice task

Non selective BZ (ω) receptor full agonists. Figure 1 shows that all four compounds significantly increased time spent by mice in the lit box [chlordiazepoxide: $H = 9.27$, $P < 0.05$; diazepam: $H = 11.64$, $P < 0.01$; clorazepate: $H = 14.64$, $P < 0.01$ and alprazolam: $H = 11.23$, $P < 0.05$]. The number of attempts at entry in the lit box was significantly reduced by diazepam [$F(3,24) = 5.37$, $P < 0.01$], clorazepate [$F(4,55) = 9.17$, $P < 0.001$] and alprazolam [$F(3,24) = 6.4$, $P < 0.01$]. Although the effect of chlordiazepoxide did not reach statistical significance for this measure, a tendency to decrease was observed. Chlordiazepoxide [$F(4,70) = 4.63$, $P < 0.01$] and alprazolam [$F(3,24) = 7.23$, $P < 0.01$] increased the total number of tunnel crossings (Fig. 4).

Non selective BZ (ω) receptor partial agonists. Figure 2 shows that none of the drugs significantly affected the time spent in the lit box. Ro 19-8022 and imidazenil, but not bretazenil, significantly decreased the number of attempts at entry into the illuminated box [Ro 19-8022: $F(4,55) = 3.31$, $P < 0.05$ and imidazenil: $F(3,24) = 4.32$, $P < 0.05$]. All three compounds failed to modify the total number of tunnel crossings (Fig. 5).

Selective BZ-1 (ω1) receptor agonists. Figure 3 shows that only abecarnil significantly increased the time spent in the lit box [$H = 11.61$, $P < 0.01$]. As was the case with the non-selective BZ (ω) receptor agonists, these agents reduced the number of attempts at entry into the illuminated box [zolpidem: $F(3,32) = 3.45$, $P < 0.05$; abecarnil: $F(3,24) = 10.15$, $P < 0.001$ and CL 218,872: $F(3,36) = 6.31$, $P < 0.01$]. The total number of tunnel crossings was not significantly affected by any of the drugs (Fig. 6).

Free-exploration test

Non selective BZ (ω) receptor full agonists. Figure 1 shows that with the exception of clorazepate, the drugs significantly increased the proportion of time spent in the novel compartment [chlordiazepoxide: $F(3,16) = 4.63$, $P < 0.05$; diazepam: $F(3,36) = 3.55$, $P < 0.05$ and alprazolam: $F(3,26) = 4.89$, $P < 0.01$]. Moreover, all drugs decreased the number of attempts [chlordiazepoxide: $F(3,16) = 11.88$, $P < 0.001$; diazepam: $F(3,36) = 14.01$,

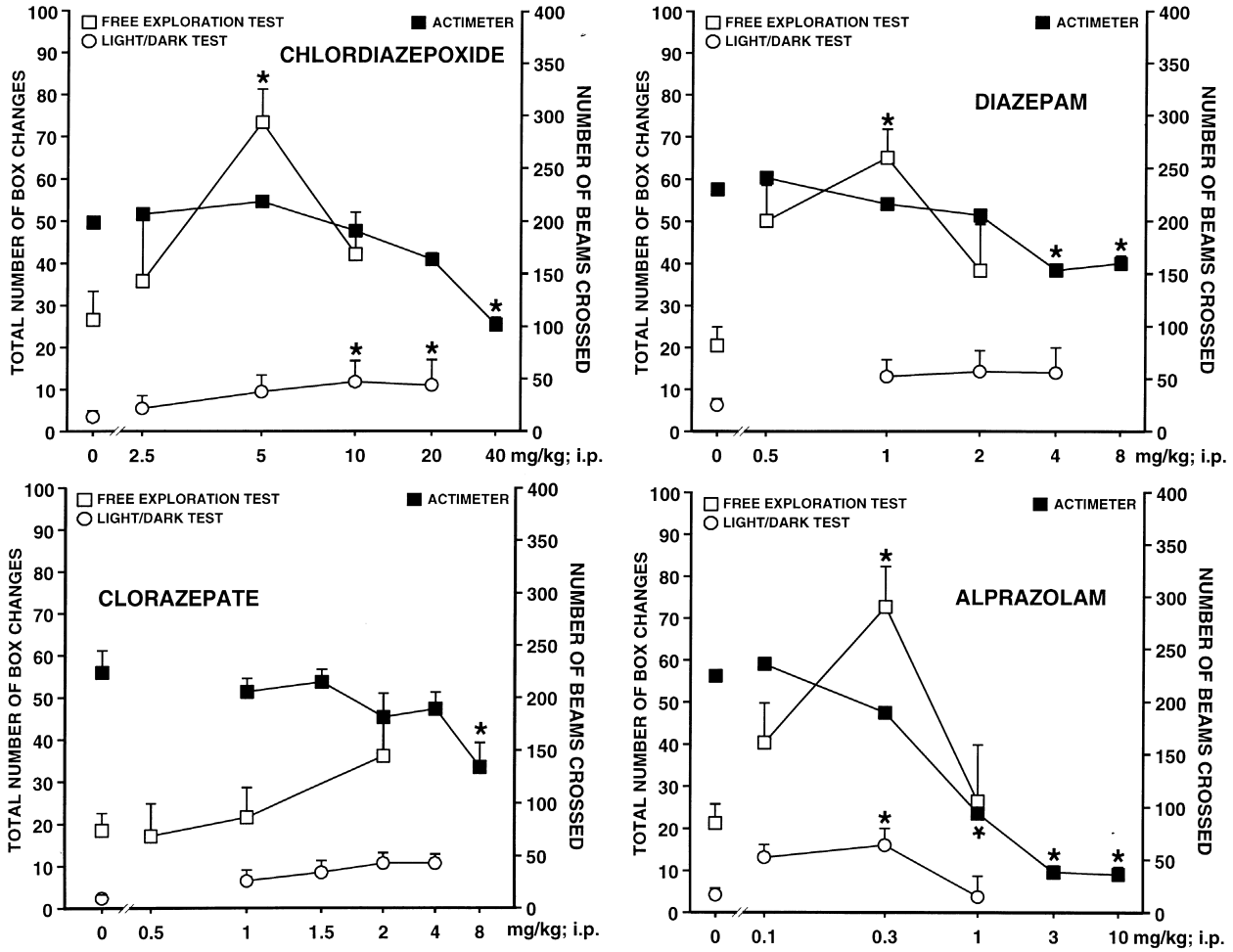

Fig. 4. Effects of four non-selective BZ (ω) receptor full agonists on horizontal locomotor activity in an actimeter (solid symbols), on total number of tunnel crossings in the light/dark choice task (open symbols) and on total number of unit changes in the free-exploration test (open symbols). Drugs were administered intraperitoneally 30 min before testing. Data represented means $\pm$ SEM. *$P < 0.05$.

$P < 0.001$; clorazepate: $F(3,26) = 5.12$, $P < 0.01$ and alprazolam: $F(3,26) = 7.08$, $P < 0.001$] and increased the total number of unit entries [chlordiazepoxide: $F(3,16) = 3.48$, $P < 0.05$; diazepam: $F(3,36) = 6.08$, $P < 0.01$ and alprazolam: $F(3,26) = 6.22$, $P < 0.01$]. The effect of the doses of clorazepate studied was not statistically significant on this latter measure (Fig. 4).

Non selective BZ (ω) receptor partial agonists. Figure 2 shows that all three compounds significantly increased percentage of time spent by mice in the novel compartment [Ro 19-8022: $F(3,24) = 3.28$, $P < 0.05$; bretazenil: $F(3,56) = 3.74$, $P < 0.05$ and imidazenil: $F(3,26) = 3.41$, $P < 0.05$] and reduced the number of attempts at entry into this area [Ro 19-8022: $F(3,24) = 26$, $P < 0.001$; bretazenil: $F(3,56) = 14.68$, $P < 0.001$ and imidazenil: $F(3,26) = 10.07$, $P < 0.001$]. Only imidazenil significantly increased the total number of unit changes [$F(3,26) = 4.52$, $P < 0.05$] (Fig. 5).

Selective BZ-1 (ω1) receptor agonists. Figure 3 shows that these compounds did not change significantly the proportion of time spent in the novel compartment. By contrast, zolpidem [$F(3,21) = 5.75$, $P < 0.01$], abecarnil [$F(4,25) = 22.23$, $P > 0.001$] and CL 218,872 [$F(3,26) = 6.68$, $P < 0.05$] significantly reduced attempts at entry in the unknown area. Although all drugs decreased the total number of unit entries, ANOVA revealed that only abecarnil significantly affected this measure [$F(4,25) = 7.34$, $P < 0.001$]. Great inter-individual variability may account for the lack of significant effect of the other drugs (Fig. 6).

Spontaneous locomotor activity

Non selective BZ (ω) receptor full agonists (Fig. 4). Spontaneous locomotor activity was significantly affected by all compounds tested: chlordiazepoxide: $F(5,42) = 5.45$, $P < 0.001$; diazepam: $F(5,54) = 5.58$, $P < 0.001$; clorazepate: $F(5,42) = 3.1$, $P < 0.05$ and alprazolam: $F(5,54) = 45.02$, $P < 0.001$. Dunnett comparisons indicated a significant decrease in the number of beams crossed at the highest dose of chlordiazepoxide

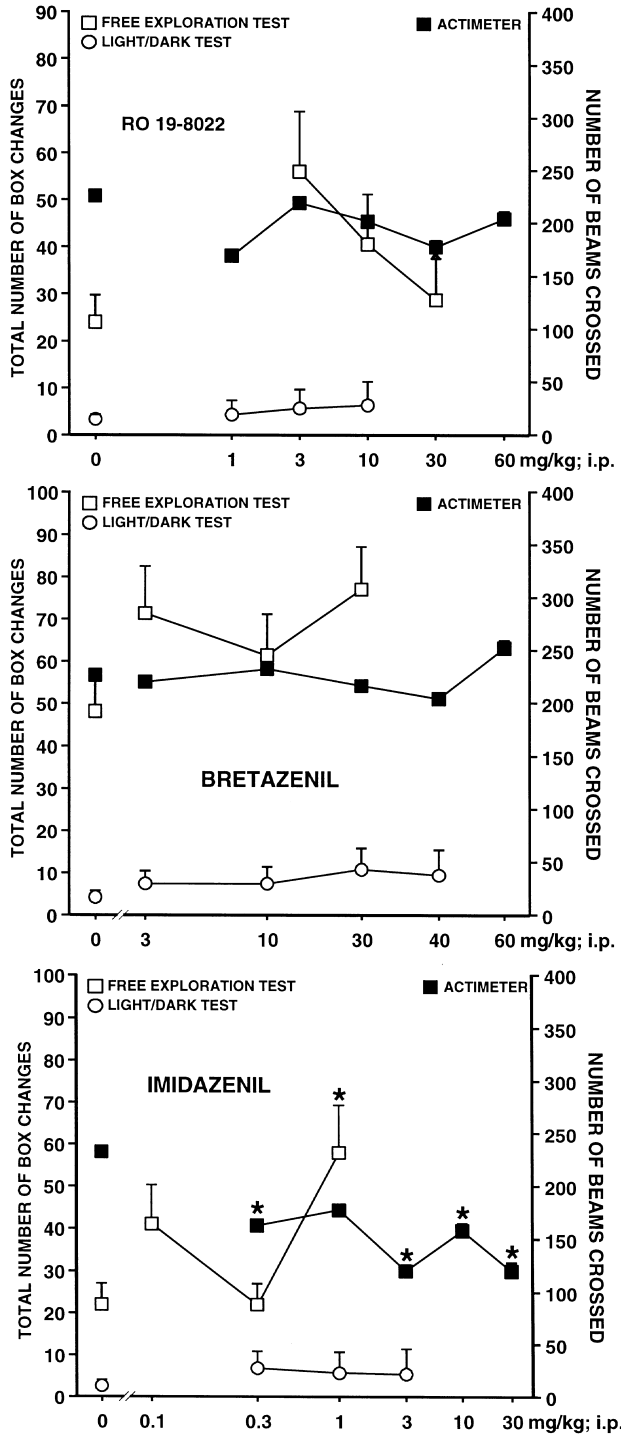

Fig. 5. Effects of three non-selective BZ (ω) receptor partial agonists on horizontal locomotor activity in an actimeter (solid symbols), on total number of tunnel crossings in the light/dark choice task (open symbols) and on total number of unit changes in the free-exploration test (open symbols). Drugs were administered intraperitoneally 30 min before testing. Data represented means ± SEM. *$P < 0.05$.

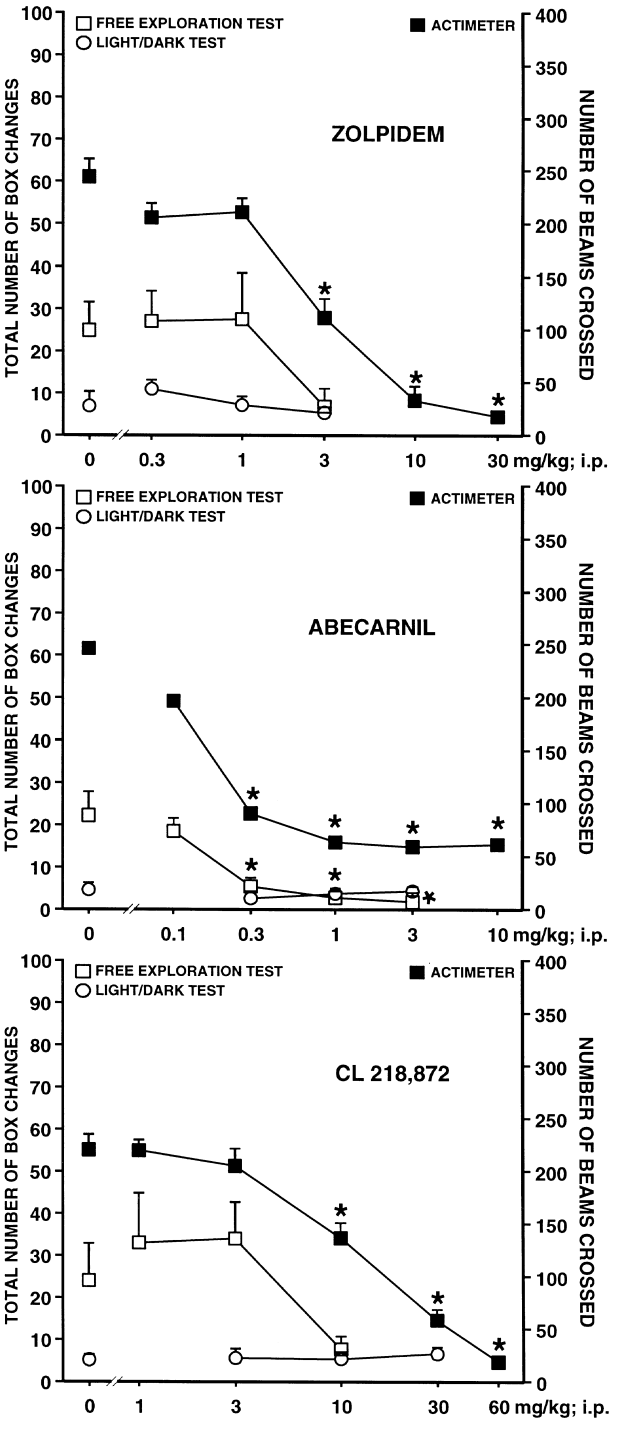

Fig. 6. Effects of three selective BZ-1 (ω1) receptor ligands on horizontal locomotor activity in an actimeter (solid symbols), on total number of tunnel crossings in the light/dark choice task (open symbols) and on total number of unit changes in the free-exploration test (open symbols). Drugs were administered intraperitoneally 30 min before testing. Data represented means ± SEM. *$P < 0.05$.

and clorazepate (40 and 8 mg/kg, respectively). Diazepam significantly depressed this response at 4 and 8 mg/kg and alprazolam at 1, 3 and 10 mg/kg.

Non selective ω receptor partial agonists (Fig. 5). Only imidazenil significantly affected locomotor activity [$F(5,42) = 5.89$, $P < 0.001$]. Subsequent post-test comparisons revealed that the drug decreased this response in a significant manner at doses of 0.3, 3 and 10 mg/kg.

Selective BZ-1 (ω1) receptor agonists (Fig. 6). All four drugs belonging to this group significantly decreased locomotor responses: zolpidem: $F(5,42) = 48.81$, $P < 0.001$; abecarnil: $F(5,41) = 26.95$, $P < 0.001$; CL 218,872: $F(5,42) = 39.51$, $P < 0.001$. Post-test comparisons indicated that zolpidem significantly depressed this behaviour from the dose of 3 mg/kg, while abecarnil produced a significant effect from 0.3 mg/kg.

DISCUSSION

The findings of the present study revealed that both non-selective BZ (ω) receptor agonists and selective BZ-1 (ω1) receptor ligands elicited anxiolytic-like effects in the mouse LDT and FET. However, differences in terms of the efficacy and the specificity of the effects observed were noted between these agents.

Previous studies with the LDT demonstrated that the administration of non-selective ω receptor full agonists (e.g. chlordiazepoxide and clorazepate) increased time spent by mice in the illuminated part of the apparatus (Belzung *et al*., 1987, 1988). The results obtained in the present study generally agree with these data as all compounds belonging to this category produced a similar increase in this measure. However, the lack of effect of the partial agonists on the time spent in the lit box contrasts with that observed by Belzung *et al*. (1989) in the LDT who showed that bretazenil produced a specific and clear increase in this parameter over a wide dose-range (1.5–48 mg/kg). However, these authors used Swiss rather than BALB/c mice. These latter are described as particularly "emotional" (Robertson, 1979) and they generally display a higher level of emotional arousal than other strains in stressful situations (Makino *et al*., 1991; Trullas and Skolnick, 1993; Beuzen and Belzung, 1995). In Belzung's study (1989), control Swiss mice spent about 20% of the total time in the lit area, whereas in the present situation, baseline levels with BALB/c mice barely reached 10%.

Regarding the ethologically derived measure, the present results with the non-selective BZ (ω) receptor full and partial agonists indicated that attempts at entry into the lit box may be viewed as a valid index of anxiety. All compounds decreased attempts, although this effect was not statistically significant for chlordiazepoxide and bretazenil. The reason for this is unclear, but it is noteworthy that in a recent study with these compounds, chlordiazepoxide and bretazenil also failed to reduce significantly a similar risk assessment response (i.e. attempts at entry into the open arms of an elevated plus-maze) (Griebel *et al*., 1996), suggesting that there may be differences in terms of anxiolysis between non-selective BZ (ω) receptor agonists regardless of their intrinsic activities. In addition, the effect of diazepam on both measures and imidazenil on attempts may be non-specific as active doses also decreased locomotor activity in the actimeter.

The present effects with the partial agonists somewhat differ from those reported in previous studies in which bretazenil, imidazenil or Ro 19-8022 produce clear anxiolytic-like actions in several rodent models of anxiety, including conflict procedures and exploration tests (Martin *et al*., 1988, 1993; Deacon *et al*., 1991; Rijnders *et al*., 1991; Jenck *et al*., 1992; Giusti *et al*., 1993; Sanger, 1995; Sanger *et al*., 1995). The use of a particularly "emotional" mouse strain in the present study might account for this discrepancy. Given the clear effect of the non-selective BZ (ω) receptor full agonists in the LDT, one can assume that only BZ (ω) ligands which display high intrinsic activity are able to reverse the strong behavioural inhibition of BALB/c mice exposed to the LDT. The two selective BZ-1 (ω1) receptor ligands zolpidem and CL 218,872 produced results which closely resemble those obtained with the non-selective BZ (ω) receptor partial agonists as they decreased attempts without affecting the time spent in the lit area. However, data with zolpidem and CL 218,872 revealed that the effects on attempts appeared at doses which also produced locomotor depression in the actimeter, indicating that the action of these selective BZ-1 (ω1) receptor ligands may be non-specific. Interestingly, the motor activity parameter recorded in the LDT (i.e. total number of tunnel crossings) was not decreased by any of the compounds, suggesting that it may be contaminated by anxiety. In the LDT, responses to aversive stimuli (i.e. novelty combined with bright light) may involve central mechanisms that can override the effect on locomotion seen in the actimeter, a less aversive situation in which the level of arousal is probably lower. However, it must be emphasized that baseline levels of tunnel crossings were low (less than five crossings in most cases), thereby questioning the possibility of a further decrease in this measure.

Abecarnil displayed a behavioural profile which is very similar to that observed with the non-selective BZ (ω) full agonists. It increased the time spent in the lit box and decreased the number of aborted attempts. However, unlike the non-selective agents abecarnil reduced motor activity in the actimeter at the effective doses. It is noteworthy that although all three selective BZ-1 (ω1) receptor ligands produced a behavioural impairment in the actimeter at the doses which also affected the behaviour in the LDT, only abecarnil affected both anxiety measures. An anxiolytic-like profile of abecarnil in the LDT would be in agreement with the results of other studies in which the drug produced clear anxiolytic-like activities in several models in mice and rats, including the 4-plate test, the murine elevated plus-maze and the water-lick conflict test (Stephens *et al*., 1990; Sanger *et al*., 1991; Jones *et al*., 1994; Stephens and Voet, 1994). It has been shown that, in addition to its selectivity for $GABA_A$ receptors containing the α1 subunit, abecarnil also acts as a full agonist on receptors containing the α3 subunit but as a partial agonist at receptors containing the α2 and α5 subunits (Knoflach *et al*., 1993; Pribilla *et al*., 1993). Thus, it is conceivable

that the different behavioural profile of this β-carboline in the LDT might be due to an interaction with specific receptor subtypes and/or its different intrinsic activities at these sites.

The findings from the FET are in agreement with a previous report showing that non-selective BZ (ω) receptor agonists increased the percentage of time spent in the unfamiliar area (Griebel *et al*., 1993). All four compounds also reduced the number of attempts at entry into this compartment. Interestingly, the drugs decreased the risk assessment activity at all dose-levels, while the time measure was increased at a single dose for most compounds. Only diazepam significantly affected this parameter at 0.5 and 2 mg/kg. This finding underlines the relevance of recording risk assessment responses in the FET, a measure which appears to be particularly sensitive to anxiety-reducing drugs. The concept of risk assessment derives from the work of Blanchard and colleagues on the antipredator defensive repertoire of wild and laboratory rats. These authors demonstrated that risk assessment responses were very sensitive in the analysis of drug effects on antipredator defense (Blanchard *et al*., 1991). More recently, Rodgers and Cole reported that, in the murine elevated plus-maze, risk assessment measures were generally more sensitive to drug action than were the traditional indices of anxiety in this test (Rodgers and Cole, 1994). This principle appears to generalize to the FET and, as was suggested by Rodgers and Cole (1994), may also be applicable to other animal models of anxiety.

In addition to the drug effects on time spent in the novel area and on risk assessment activities, the FET also revealed that total numbers of unit changes were increased by several non-selective BZ (ω) receptor agonists. Although a similar effect on locomotor activity failed to show up in the actimeter, these data are consistent with the observation that BZs can produce behavioural stimulation in some exploratory models of anxiety (Treit, 1985). It must also be emphasized that, unlike the results from the LDT, no consistent differences in the magnitude of drug-effects were found between non-selective full and partial agonists in the FET. This suggests that while both full and partial agonists are equally effective against "trait" anxiety, full agonists may be superior in reducing "state" anxiety.

As was the case in the LDT, the anxiolytic-like effects of selective BZ-1 (ω1) receptor ligands in the FET were weaker and generally non-specific. Thus, none of the drugs were able to increase the time spent in the novel compartment. Furthermore, the reduction of attempts induced by zolpidem, abecarnil and the highest dose of CL 218,872 was associated with a decrease in motor activity as revealed by the actimeter data. These results can be added to those obtained with another selective BZ-1 (ω1) receptor agonist in this test, alpidem (Griebel *et al*., 1993), and thus confirm the weak neophobia-reducing potential of these agents.

In conclusion, the present findings from the LDT are in agreement with a recent study using a rat model of "state" anxiety (i.e the elevated plus-maze) showing that the anxiolytic-like effects of selective BZ-1 (ω1) receptor ligands are somewhat weaker than those observed with non-selective BZ (ω) receptor agonists and are confounded by decreases in locomotor activity (Griebel *et al*., 1996). Furthermore, these results revealed that partial agonists do not show the same efficacy as full agonists in the LDT. Finally, the data from the FET demonstrated a lack of specific effect of selective BZ-1 (ω1) receptor ligands in reducing neophobia, thereby suggesting that "trait" anxiety does not involve primarily BZ (ω) receptors containing the α1 subunit.

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