Psychopharmacology (1996) 124 : 245–254

ORIGINAL INVESTIGATION

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The use of the rat elevated plus-maze to discriminate between non-selective and BZ-1 (ω_1) selective, benzodiazepine receptor ligands

Received: 19 October 1995 / Final version: 22 November 1995

Abstract The behavioral effects of a wide range of BZ (ω) receptor ligands, including non-selective full (alprazolam, clorazepate, chlordiazepoxide and diazepam) and partial (bretazenil, imidazenil and Ro 19-8022) agonists, and selective BZ-1 (ω_1) (abecarnil, CL 218,872, CL 284,846 and zolpidem) receptor ligands, were compared in the rat elevated plus-maze test. Behaviors recorded comprised the traditional indices of anxiety as well as a number of ethologically derived measures. In addition, the specificity of drug effects was evaluated by measuring spontaneous locomotor activity in activity cages in separate groups of animals. Results showed that all compounds tested not only increased the proportion of time spent and proportion of entries into the open arms of the maze (considered as traditional indices of anxiety) but also affected head-dippings and attempts at entry into open arms, which can be considered as indices of risk assessment responses. However, the magnitude of these effects was generally smaller with the BZ-1 (ω_1) selective agents. Moreover, additional differences were apparent on the total number of arm entries measure, which was significantly increased by most full and all partial agonists, but was unaffected by the selective BZ-1 (ω_1) compounds. If it is assumed that total arm entries are contaminated by anxiety, the latter finding indicates a weaker anxiety-reducing potential of selective BZ-1 (ω_1) ligands. Importantly, the increase in total arm entries induced by the non-selective agents was not associated with a similar effect on locomotion as revealed in the actimeter. Finally, anxiolysis produced by the BZ-1 (ω_1) ligands was invariably observed at doses which reduced locomotor activity, suggesting that the anxiolytic-like effects of these compounds are confounded by decreases in locomotor activity.

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Key words Benzodiazepines · BZ-1 (ω_1) receptor · Anxiety · Elevated plus-maze · Risk assessment · Locomotor activity · Rat

Introduction

Benzodiazepines (BZs) are the most frequently used psychotropic agents and the mainstay of drug treatment for anxiety disorders. These agents are very effective at reducing anxiety with a rapid onset of action, but can be associated with sedation, amnesia, muscle relaxation, tolerance and dependence (Lader 1994). BZs produce their effects by acting at BZ sites associated with $GABA_A$ receptors and it is now widely acknowledged that there exist two subtypes of BZ receptors called BZ-1 and BZ-2 (Squires et al. 1979; Sieghart and Schuster 1984) designated as ω_1 and ω_2, respectively (Langer and Arbilla 1988). Recent work in molecular biology has demonstrated that the former corresponds to the $GABA_A$ receptors containing the α_1 subunit while the latter forms a part of the $GABA_A$ receptor complex having α_2, α_3 or α_5 subunits (Pritchett et al. 1989; Sieghart 1995).

Classical BZs, such as diazepam, interact with nearly all receptor subtypes with high affinity and high efficacy. In contrast, partial agonists, typified by bretazenil or imidazenil, display high affinity at nearly all receptors but act with reduced efficacy compared to diazepam at all receptors (Haefely et al. 1990; Puia et al. 1992; Wafford et al. 1993). Hence, it was hypothesized that these compounds may produce fewer unwanted effects (e.g. sedation and amnesia) than full agonists, but retain anxiolytic and anticonvulsant properties (Haefely et al. 1990). In addition, there are BZ receptor ligands which display affinities and/or efficacies that vary depending on the $GABA_A$/BZ subtype. This is exemplified by the imidazopyridine zolpidem and the triazolopyridazine CL 218,872, which

show selectivity for receptors containing the α_1 subunit (Pritchett and Seeburg 1990; Faure-Halley et al. 1993). On the basis of these findings, it has been speculated that there might be a correlation between the behavioral profile of these BZ (ω) receptor ligands and subtype specificity (Sanger et al. 1994). Several preliminary investigations support this view. As an illustration, Zivkovic et al. (1992) demonstrated that zolpidem and CL 218,872 induced myorelaxant effects at doses which were much greater than those decreasing exploration, while the non-selective BZ (ω) receptor full agonist triazolam primarily affected muscle strength, suggesting that myorelaxant activities are not related to an interaction with BZ-1 (ω_1) receptors. More recently, Sanger (1995) showed that BZ-1 (ω_1) selective agents only weakly affected punished operant responding and antagonism of the pentylenetetrazole cue, while non-selective BZ (ω) receptor agonists produced clear-cut effects in both procedures.

In an attempt to explore further the possibility that some behavioral actions of BZ (ω) ligands may be associated with actions at a defined receptor subtype, the present study compared the effects of several classical and novel BZ (ω) receptor ligands in an experimental procedure designed for evaluating anxiety-modulating agents in rats, namely the elevated plus-maze test. The drugs used included non-selective BZ (ω) receptor full (i.e. alprazolam, clorazepate, chlordiazepoxide and diazepam) and partial (i.e. bretazenil, imidazenil and Ro 19-8022) (Martin et al. 1988; Jenck et al. 1992; Giusti et al. 1993) agonists, and selective BZ-1 (ω_1) (i.e. abecarnil, CL 218,872, CL 284,846 and zolpidem) receptor ligands (Lippa et al. 1979; Depoortere et al. 1986; Stephens et al. 1990; Day et al. 1992).

The elevated plus-maze is based upon the finding that rodents are highly thigmotactic when exposed to a novel environment (Treit et al. 1993). As traditionally employed, the key indices of anxiety in this test are the open arm entries and the time spent on the open arms (Pellow et al. 1985). However, several authors recently reported on the potential utility of recording additional behavioral measures (Rodgers et al. 1992a; Rodgers and Cole 1993; Cruz et al. 1994). These comprise entry latency, non-exploratory behavior, and a cluster of behaviors collectively referred to as "risk assessment". This latter concept has emerged from work on antipredator defense in rodents. It typically refers to a pattern of responses (scanning, stretch attend, flat back approach) invariably observed in potentially dangerous situations and which is particularly sensitive to anxiety-modulating drug treatment (Blanchard et al. 1990a, b; Griebel et al. 1995). In the plus-maze, risk assessment is reflected in high levels of stretch attend postures and head-dippings. Recent studies with classical (BZs) and novel (5-HT_{1A} receptor agonists, 5-HT reuptake inhibitors) anxiolytics have revealed the utility of these measures (Cole and Rodgers 1993, 1994; Griebel et al. 1994). In the present study, we used several risk assessment measures in addition to the traditional parameters. Also, special attention was given to the total arm entries measure, as several reports suggested that this response is contaminated by anxiety (Lister 1987; Handley and McBlane 1993; Cruz et al. 1994), thereby suggesting that it may be used as an index of anxiety. To this end, the specificity of effect on total arm entries was determined by measuring spontaneous locomotor activity.

Materials and methods

All procedures described here are in compliance with ethical principles and guidelines for scientific experiments on animals.

Animals

Male Sprague-Dawley rats weighing 180–220 g at time of testing were used. All animals were housed in groups of five and maintained under standard laboratory conditions with free access to food and water. They were kept on a 12:12-h light-dark cycle with light onset at 6 a.m. Animals were supplied by Charles River France (Saint-Aubin-les-Elbeuf, France) and Iffa Credo (L'Arbresle, France).

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing 1 or 2 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 and CL 284,846 (both courtesy of Dr. B. Beer, American Cyanamid). All doses are expressed as the bases. Drugs were administered intraperitoneally (IP) in a constant volume of 2 ml/kg 30 min before experiments were carried out.

Behavior on the elevated plus-maze

All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. It consisted of a maze elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). Although the floor was rubberized, a rim of Plexiglas 0.5 cm high surrounding open arms was necessary to avoid rats falling off. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. At the beginning of the experiment, rats were placed in the centre of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries and number of closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via a video camera by an observer located in an adjacent room. This permitted the recording of the additional risk assessment measures: (a) *attempt*: attempt at entry into open arms followed by avoidance responses. This includes stretch attend posture (the rat stretches forward and retracts to original position);

(b) *head-dipping*: protruding the head over the ledge of an open arm and down towards the floor (this response can occur while the animal's body is in the closed arms, central square or on open arms). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean total number of open arm entries, mean total number of entries in both open and closed arms, mean total number of attempts and mean total number of head-dips. Testing was performed between 8.30 a.m. and 1 p.m. The following drugs and doses were tested: clorazepate: 0, 1, 3 and 10 mg/kg (n = 7–8); diazepam: 0, 1, 1.5 and 2 mg/kg (n = 11–12); alprazolam: 0, 0.1, 0.3 and 1 mg/kg (n = 12); chlordiazepoxide: 0, 1.25, 2.5 and 5 mg/kg (n = 11–12); Ro 19-8022: 0, 1, 3 and 10 mg/kg (n = 11–12); bretazenil: 0, 0.1, 0.3 and 3 mg/kg (n = 11–12); imidazenil: 0, 0.3, 1 and 3 mg/kg (n = 12); zolpidem: 0, 0.1, 0.3 and 1 mg/kg (n = 10–12); abecarnil: 0, 0.1, 0.3 and 0.6 mg/kg (n = 12); CL 284,846: 0, 0.1, 0.3 and 1 mg/kg (n = 12); CL 218,872: 0, 1, 3 and 10 mg/kg (n = 11–12).

Effects on spontaneous locomotor activity: the actimeter

Testing was conducted in square, clear Plexiglas boxes (40 × 40 × 15 cm) equipped with infrared beams and sensors and placed in sound attenuated cupboards. Horizontal locomotor activity was recorded for 5 min immediately after placing rats in the centre of the apparatus 30 min after an IP injection. Testing was performed between 8.30 a.m. and 1 p.m. The following drugs and doses were tested: clorazepate: 0, 0.3, 1, 3, 10 and 30 mg/kg (n = 8); diazepam: 0, 0.5, 1, 1.5, 2 and 4 mg/kg (n = 8); alprazolam: 0, 0.1, 0.3, 1, 3 and 10 mg/kg (n = 8); chlordiazepoxide: 0, 0.62, 1.3, 2.5, 5 and 10 mg/kg (n = 8); Ro 19-8022: 0, 0.3, 1, 3, 10 and 30 mg/kg (n = 8); bretazenil: 0, 0.03, 0.1, 0.3, 1 and 3 mg/kg (n = 8); imidazenil: 0, 0.1, 0.3, 1, 3 and 10 mg/kg (n = 8); zolpidem: 0, 0.3, 1 and 3 mg/kg (n = 8); abecarnil: 0, 0.06, 0.1, 0.3, 0.6 and 1 mg/kg (n = 8); CL 284,846: 0, 0.03, 0.1, 0.3, 1 and 3 mg/kg (n = 8) and CL 218,872: 0, 0.3, 1, 3, 10 and 30 mg/kg (n = 7–8).

Statistical analysis

Each dose-response curve was assessed by a one-way analysis of variance (ANOVA) followed by a Dunnett's a posteriori t-test.

Results

Elevated plus-maze test

For purposes of comparing drug effects, the total number of arm entries are displayed with the locomotor activity data in Figs 4, 5 and 6. Other measures of behavior on the plus-maze are shown in Figs 1, 2 and 3. Closed arm entries are not presented as statistical analysis did not reveal any significant drug effects on this measure.

Non-selective BZ (ω) receptor full agonists

Figure 1 shows that all four compounds significantly increased percentage of time spent by rats on open arms

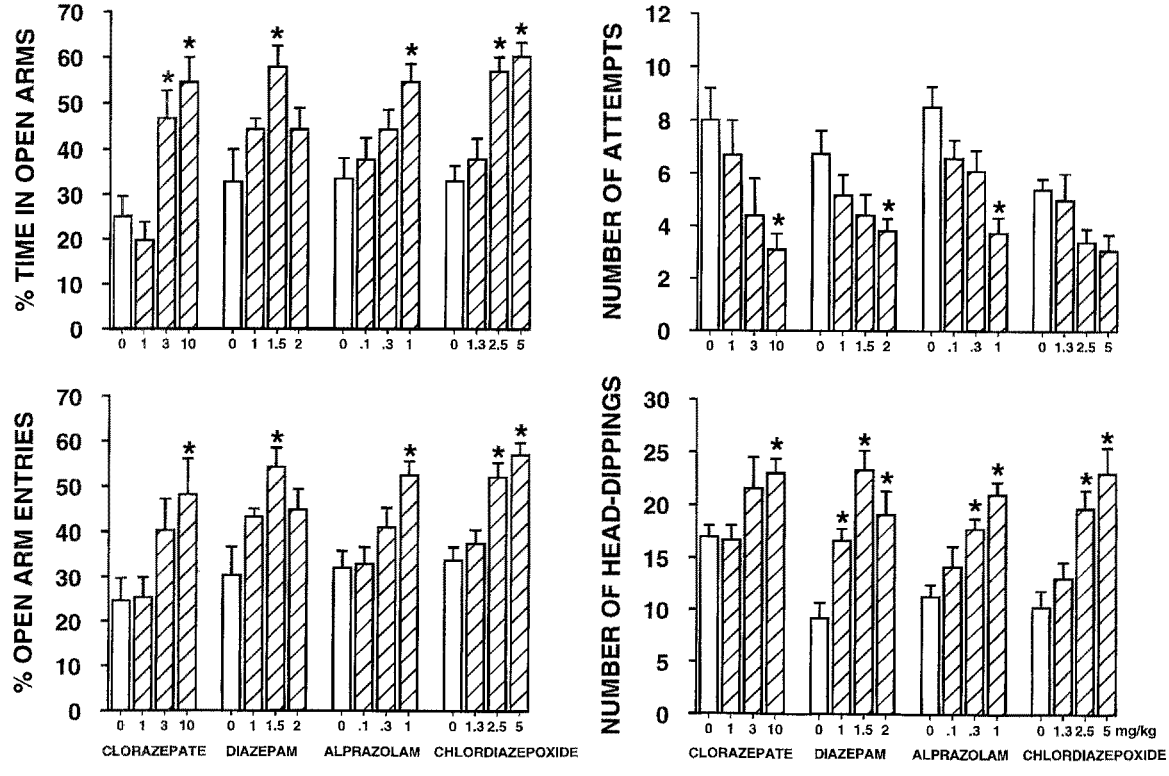

Fig. 1 Effects of four non-selective BZ (ω) receptor full agonists on the behavior of rats exposed to the elevated plus-maze test on traditional indices of anxiety (% time in open arms, number of open arm entries) and ethologically derived measures (number of attempts, number of head-dippings). Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

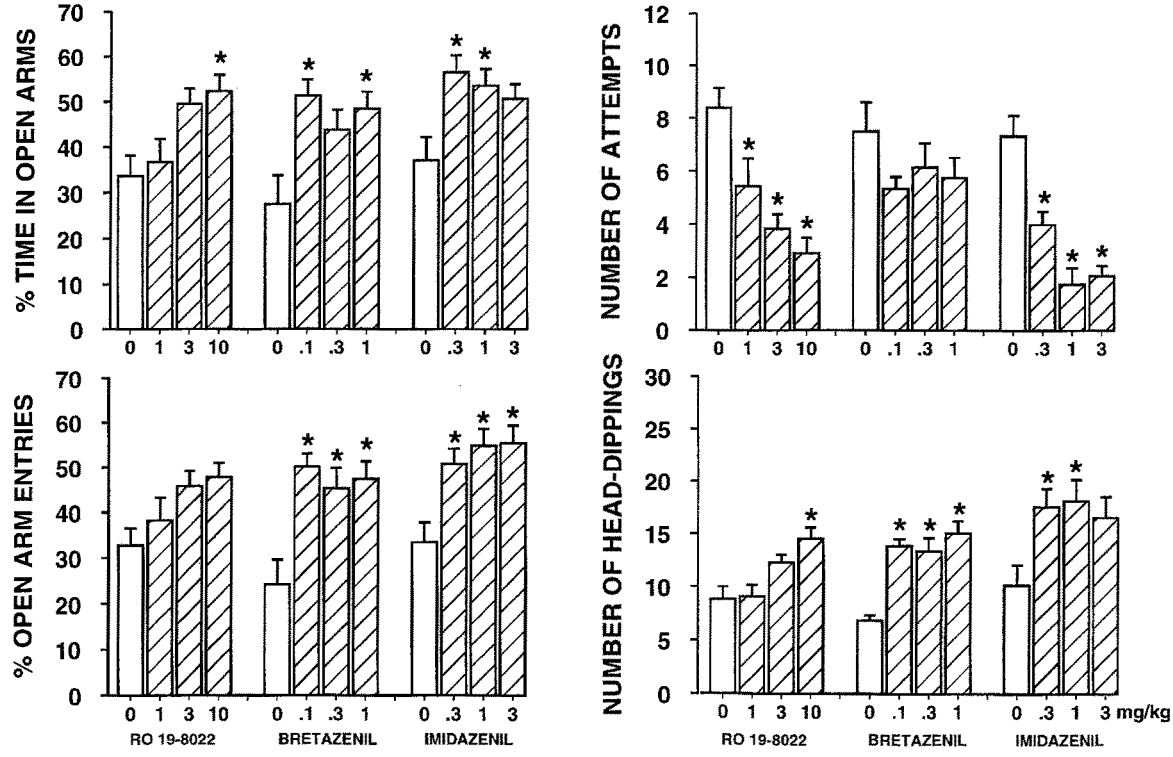

Fig. 2 Effects of three non-selective BZ (ω) receptor partial agonists on the behavior of rats exposed to the elevated plus-maze test. Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

[clorazepate: $F(3, 24) = 10.75$, $P < 0.001$; diazepam: $F(3, 43) = 2.96$, $P < 0.05$; alprazolam: $F(3, 44) = 3.27$, $P < 0.03$ and chlordiazepoxide: $F(3, 41) = 10.21$, $P < 0.001$], the percentage of open arm entries [clorazepate: $F(3, 24) = 10.75$, $P < 0.001$; diazepam: $F(3, 43) = 2.96$, $P < 0.05$; alprazolam: $F(3, 44) = 3.27$, $P < 0.05$ and chlordiazepoxide: $F(3, 41) = 10.21$, $P < 0.001$] and the number of head-dippings [clorazepate: $F(3, 24) = 2.96$, $P < 0.05$; diazepam: $F(3, 43) = 12.24$, $P < 0.001$; alprazolam: $F(3, 44) = 9.51$, $P < 0.001$ and chlordiazepoxide: $F(3, 41) = 9.41$, $P < 0.001$]. Clorazepate [$F(3, 24) = 3.54$, $P < 0.05$], diazepam [$F(3, 43) = 2.86$, $P < 0.05$] and alprazolam [$F(3, 44) = 7.66$, $P < 0.001$] significantly reduced the number of attempts at entry in open arms. The effect of the doses of chlordiazepoxide studied was not statistically significant on this measure. Finally, with the exception of alprazolam, the drugs increased the total number of arm entries [clorazepate: $F(3, 24) = 3.85$, $P < 0.05$; diazepam: $F(3, 43) = 9.04$, $P < 0.001$ and chlordiazepoxide: $F(3, 41) = 6.97$, $P < 0.001$] (Fig. 4).

Non-selective BZ (ω) receptor partial agonists

Figure 2 shows that the drugs significantly increased the percentage of time spent on open arms [Ro 19-8022: $F(3, 43) = 3.69$, $P < 0.05$; bretazenil: $F(3, 41) = 3.49$, $P < 0.05$ and imidazenil: $F(3, 44) = 3.46$, $P < 0.05$] and the two latter also increased the percentage of open arm entries [bretazenil: $F(3, 41) = 5.49$, $P < 0.01$ and imidazenil: $F(3, 44) = 5.21$, $P < 0.01$]. With the exception of bretazenil, the drugs also significantly decreased the number of attempts at entry into open arms [Ro 19-8022: $F(3, 43) = 10.5$, $P < 0.001$ and imidazenil: $F(3, 44) = 18.84$, $P < 0.001$]. Moreover, all three compounds increased the frequency of head-dippings [Ro 19-8022: $F(3, 43) = 6.66$, $P < 0.001$; bretazenil: $F(3, 41) = 14.17$, $P < 0.001$ and imidazenil: $F(3, 44) = 3.51$, $P < 0.05$]. Finally, Fig. 5 shows that like the full agonists, the three partial agonists increased total number of arm entries [Ro 19-8022: $F(3, 43) = 4.58$, $P < 0.01$; bretazenil: $F(3, 41) = 8.11$, $P < 0.001$ and imidazenil: $F(3, 44) = 3.54$, $P < 0.05$].

Selective BZ-1 (ω_1)receptor agonists

Figure 3 shows that the effects of the BZ-1 (ω_1) selective agents on percentage of time spent on open arms were in general similar to the effects of non-selective full and partial agonists [zolpidem: $F(3, 41) = 6.33$, $P < 0.001$; CL 284,846: $F(3, 44) = 3.47$, $P < 0.05$ and CL 218,872: $F(3, 43) = 6.19$, $P < 0.001$]. Although the effect of abecarnil did not reach statistical significance for this measure, a tendency to an increase was observed. In addition, while zolpidem, abecarnil and CL 218,872 significantly increased the percentage of entries into open arms [zolpidem: $F(3, 41) = 5.9$,

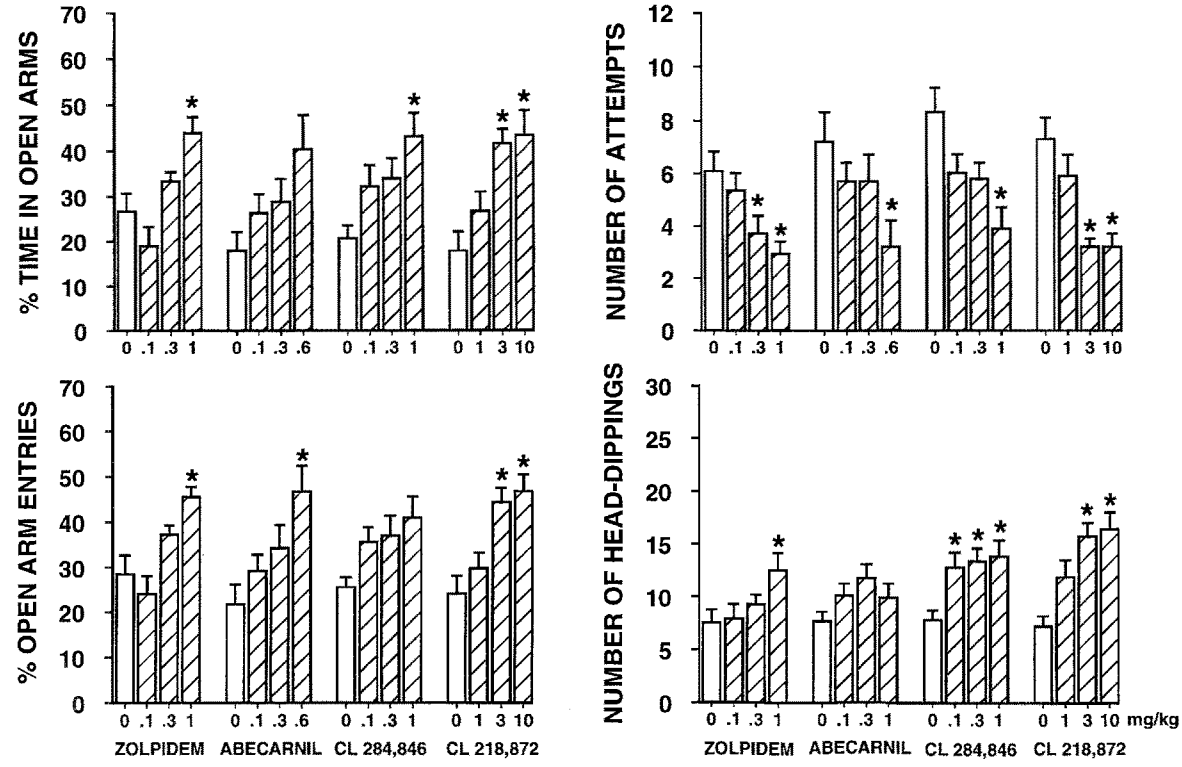

Fig. 3 Effects of four selective BZ-1 (ω_1) receptor ligands on the behavior of rats exposed to the elevated plus-maze test. Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

$P < 0.01$; abecarnil: $F(3, 44) = 3.83$, $P < 0.05$ and CL 218,872: $F(3, 43) = 8.18$, $P < 0.001$], CL 284,846 failed to affect this response. Regarding the ethologically based measures, all four drugs significantly decreased the number of attempts at entry into open arms [zolpidem: $F(3, 41) = 5.04$, $P < 0.01$; abecarnil: $F(3, 44) = 2.92$, $P < 0.05$; CL 284,846: $F(3, 44) = 5.44$, $P < 0.001$; CL 218,872: $F(3, 43) = 10.43$, $P < 0.001$] and zolpidem [$F(3, 41) = 2.8$, $P < 0.05$], CL 284, 846 [$F(3, 44) = 4.59$, $P < 0.01$], CL 218,872 [$F(3, 43) = 9.21$, $P < 0.001$] but not abecarnil also increased the number of head-dippings. Finally, Fig. 6 shows that, in contrast to the results obtained with the non-selective full and partial agonists, the BZ-1 (ω_1) selective compounds did not increase the total number of entries.

Spontaneous locomotor activity

Non-selective BZ (ω) receptor full agonists (Fig. 4)

Spontaneous horizontal locomotor activity was significantly affected by all compounds tested: clorazepate: $F(5, 42) = 10.32$, $P < 0.005$; diazepam: $F(5, 42) = 6.68$, $P < 0.001$; alprazolam: $F(5, 42) = 12.63$, $P < 0.001$ and chlordiazepoxide: $F(5, 42) = 5.68$, $P < 0.001$. Dunnett comparisons indicated a significant decrease in the number of beams crossed at the highest dose of clorazepate, diazepam and chlordiazepoxide (30, 4 and 10 mg/kg, respectively). Alprazolam significantly decreased this measure at 1, 3 and 10 mg/kg. None of the tested doses of these four BZs produced increases in locomotor activity during the 5-min test.

Non-selective BZ (ω) receptor partial agonists (Fig. 5)

Only imidazenil significantly affected locomotor activity [$F(5, 42) = 7.17$, $P < 0.001$]. Subsequent post-hoc comparisons revealed that the drug significantly decreased this response at doses of 1, 3 and 10 mg/kg.

Selective BZ-1 (ω_1) receptor agonists (Fig. 6)

All four drugs belonging to this group significantly decreased horizontal locomotor responses: zolpidem: $F(3, 28) = 25.59$, $P < 0.001$; abecarnil: $F(5, 42) = 10.58$, $P < 0.001$; CL 284,846: $F(5, 42) = 13.01$, $P < 0.001$ and CL 218,872: $F(5, 39) = 13.42$, $P < 0.001$. Post-test comparisons indicated that zolpidem and CL 284,846 significantly depressed this behavior from the dose of 1 mg/kg, while abecarnil and CL 218,872 produced a similar effect from 0.3 and 3 mg/kg, respectively.

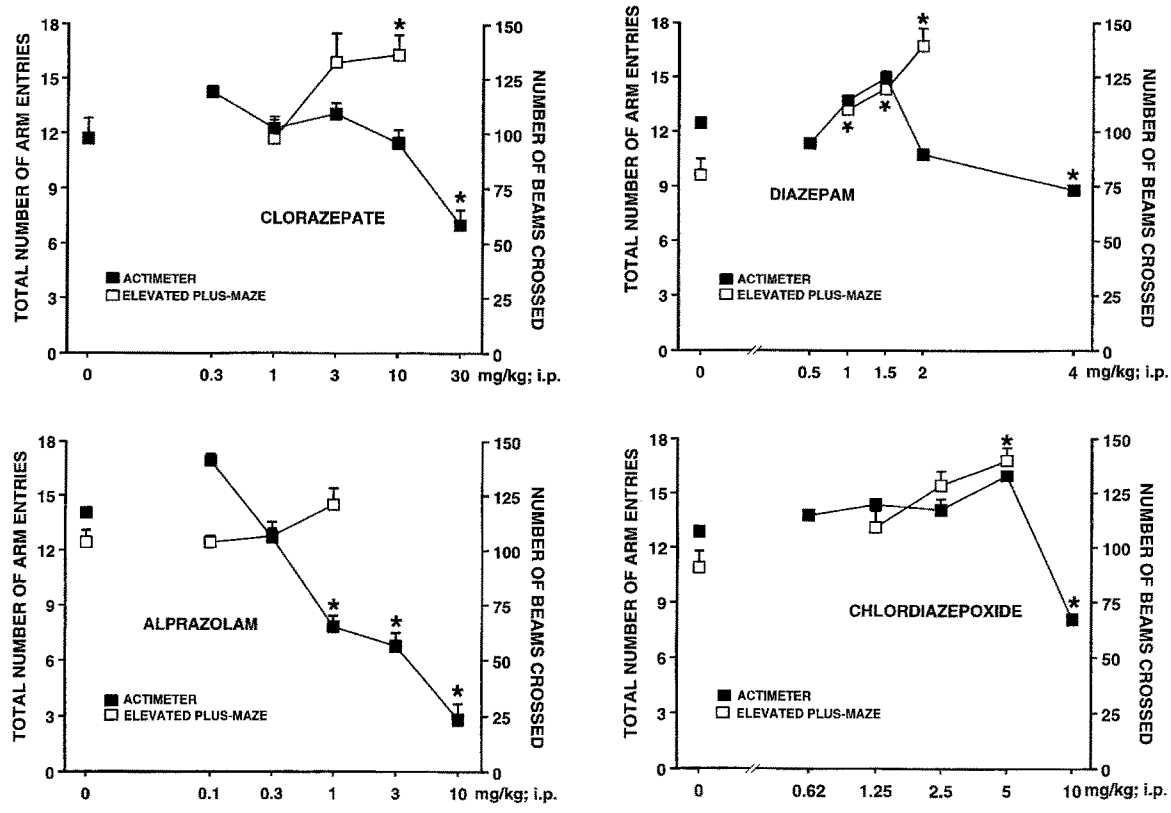

Fig. 4 Effects of four non-selective BZ (ω) receptor full agonists on horizontal locomotor activity in an actimeter (*solid symbols*) and on total number of arm entries in the elevated plus-maze test (*open symbols*). Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

Discussion

The present experiments demonstrated that both non-selective BZ (ω) receptor agonists and selective BZ-1 (ω_1) receptor ligands elicited anxiolytic-like effects in the rat elevated plus-maze test. However, the behavioral profiles of selective and non-selective agents differ somewhat, notably in terms of the magnitudes of the effects observed.

The present data generally agree with previous reports on the sensitivity of the elevated plus-maze test to the anxiolytic effects of non-selective BZ (ω) receptor full and partial agonists (e.g. Pellow et al. 1985; Pellow and File 1986; Rodgers et al. 1992b; Cole and Rodgers 1993). Thus, on traditional behavioral indices, they increased percentage of time spent in open arms and percentage of open arm entries. Traditionally, among the basic parameters scored in plus-maze studies, total arm entries are often considered as an index of general activity, while the number of open arm entries and the time spent in the open arms are the primary indices of anxiety. However, in a factor-analytic study, Lister (1987) reported that total arm entries also loaded, albeit less strongly, on the "anxiety factor". Consequently, a change in the total number of arm entries may not merely reflect changes in activity but is also probably sensitive to changes in anxiety. Although the initial report of Pellow et al. (1985) failed to reveal any changes in total arm entries after the administration of non-sedative doses of chlordiazepoxide and diazepam, several recent studies using such agents, including chlordiazepoxide, found a clear-cut increase in this measure at similar doses (Cruz et al. 1994; Dawson et al. 1995). In agreement with these latter findings, the present observations showed that, with the exception of alprazolam and imidazenil, non-selective BZ (ω) receptor full and partial agonists simultaneously increased total number of arm entries and open arm activities, while having no direct effect on motor activity as revealed by the actimeter test. Overall, these plus-maze findings are consistent with the observation that BZs produce some evidence of low dose behavioral stimulation in exploratory models of anxiety (Treit 1985).

The failure of alprazolam to produce a similar increase in total arm entries might be attributable to the fact that the dose at which anxiolytic effects are observed is close to those at which sedative/myorelaxant effects appear. The present data strongly support this view, as alprazolam was the only compound in this category which produced anxiolytic activity in the plus-maze at doses which decreased motor activity (actimeter data). Based on this latter finding, it can tentatively be suggested that, in the case of alprazolam, the total arm entries variable was

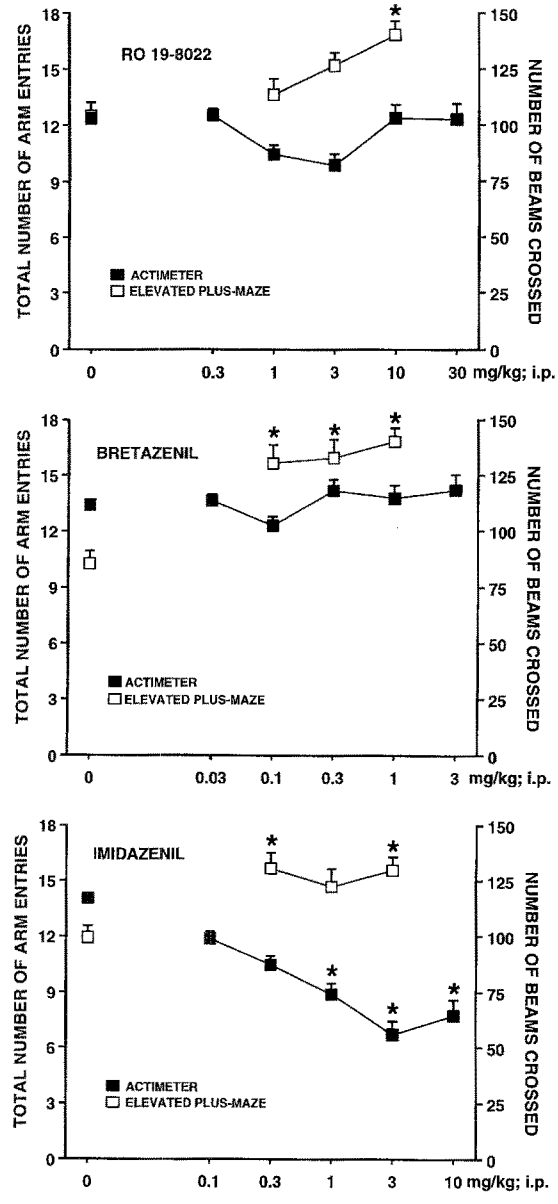

Fig. 5 Effects of three non-selective BZ (ω) receptor partial agonists on horizontal locomotor activity in an actimeter (*solid symbols*) and on total number of arm entries in the elevated plus-maze test (*open symbols*). Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

contaminated by sedation and/or myorelaxation. Regarding imidazenil, it is striking that the increase in total arm entries was associated with behavioral suppression (actimeter data). This finding thus provides further evidence that the total arm entries measure is not a reliable index of general motor activity, but is heavily contaminated by anxiety. Clearly, in the plus-maze, responses to aversive stimuli (i.e. novelty combined with elevated open spaces) involve central mechanisms that can override the strong hypolocomotor effect seen in the actimeter, a less aversive situation in which the level of arousal is probably lower.

Regarding the novel, ethologically derived, measures, the present results with the non-selective BZ (ω) receptor full and partial agonists confirmed that head-dippings and attempts at entry into open arms may be viewed as valid indexes of anxiety (Cole and Rodgers 1993; Cruz et al. 1994; Griebel et al. 1994). All compounds markedly increased head-dippings and decreased attempts, but this latter effect was not statistically significant for chlordiazepoxide and bretazenil. The reason for this is unclear, but it can be speculated that, at least in the case of chlordiazepoxide, control values of attempts were too low to be further decreased. Together, these "risk assessment" data indicate that rats treated with such agents show a reduced reluctance to leave relatively safe areas of the maze (decreased attempts) and an enhanced tendency to actively explore the potentially dangerous open arms (increased head-dipping), a behavioral pattern that strengthens the conclusion of an anxiolytic-like action based upon the traditional indices of anxiety.

Overall, the present findings with non-selective BZ (ω) partial agonists are in agreement with previous preclinical reports indicating that these partial agonists are potential anxiolytic drugs (Belzung et al. 1989; Jenck et al. 1992; Giusti et al. 1993; Griebel et al. 1993; Martin et al. 1993; Dazzi et al. 1995) and, especially, that bretazenil reduced risk assessment behavior in a murine plus-maze (Cole and Rodgers 1993). In addition, the observation that bretazenil and Ro 19-8022 did not decrease locomotor activity in the actimeter over the entire dose range (0.03–3 mg/kg and 0.3–30 mg/kg, respectively) also agrees with the suggestion that these drugs may have low propensity in producing sedation and/or myorelaxation while retaining the therapeutic effectiveness of classical BZs (Haefely et al. 1990). However, on a clinical level, only few trials with such agents have been carried out so far and evidence of clear-cut effects in the treatment of anxiety disorders is still lacking (Potokar and Nutt 1994).

The BZ-1 (ω_1) selective agents zolpidem, CL 218,872 and CL 284,846 each increased the percentage of time spent on the open arms and the percentage of open arm entries was increased by the two former and abecarnil. This anxiolytic-like profile was confirmed by their action on the ethological-based measures as all compounds markedly decreased the number of attempts, while three of them (i.e. zolpidem, CL 218,872 and CL 284,846) increased head-dippings. Together, these results are in line with several reports showing that BZ-1 (ω_1) selective agents produce anxiolytic-like activity in conditioned conflict procedures as well as in exploration tests (Stephens et al. 1990; Zivkovic et al. 1990; Jones et al. 1994; Ozawa et al. 1994; Sanger 1995). In particular, they agree with findings from plus-maze studies demonstrating that CL 218,872 and zolpidem increased exploration time in the open arms (Pellow and File 1986; Auta et al. 1993; Davies et al. 1994).

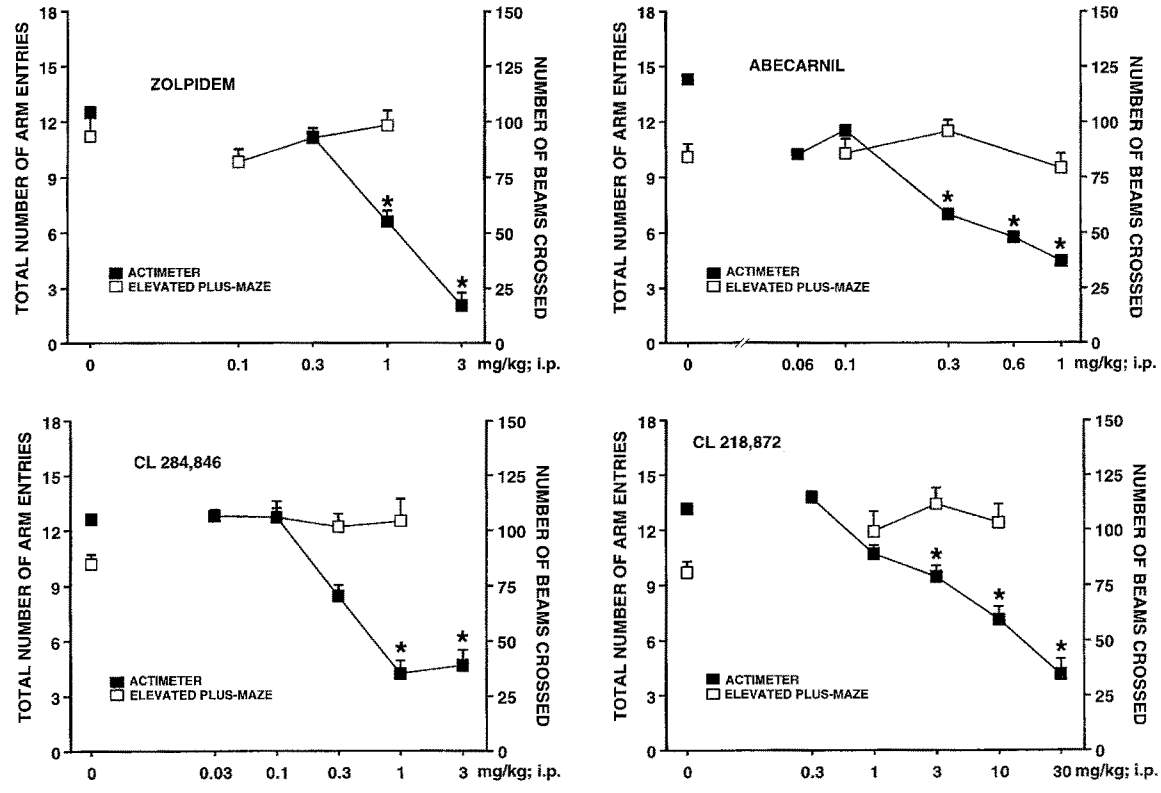

Fig. 6 Effects of four selective BZ-1 (ω_1) receptor ligands on horizontal locomotor activity in an actimeter (*solid symbols*) and on total number of arm entries in the elevated plus-maze test (*open symbols*). Drugs were administered IP 30 min before testing. Data represent means ± SEM. * $P < 0.05$ (Dunnett's test)

When the effects produced by the BZ-1 (ω_1) selective compounds are compared directly with those of the non-selective compounds, the data indicated that the selective BZ-1 (ω_1) ligands generally displayed weaker anxiolytic-like effects. This is exemplified by the effects of the drugs on the number of head-dippings. The magnitude of the effects on this response for the non-selective full and partial agonists is greater than with the BZ-1 (ω_1) selective agents as active doses for the former compounds (with the exception of clorazepate) increased head-dippings by 91–130%, whereas selective BZ-1 (ω_1) agents generally failed to produce quantitatively similar effects. Drug profiles on the percentage of time spent in open arms also seem to indicate such a difference in efficacy between non-selective and selective BZ-1 (ω_1) ligands. However, control values were somewhat lower in the case of the latter agents so that a direct comparison is compromised.

In addition, BZ-1 (ω_1) selective agents also differed from most non-selective compounds in that they failed to increase the total number of arm entries. This finding contrasts with the proposal of Dawson et al. (1995), who suggested that anxiolysis in the elevated plus-maze is associated with changes in locomotor activity (including an increase in total arm entries). However, these authors based their conclusion on results obtained with one anxiolytic drug, namely chlordiazepoxide, which is well known for its strong stimulatory action on locomotor parameters in animal models of anxiety.

Depressant properties of the selective BZ-1 (ω_1) ligands might account for the lack of effect on the total number of arm entries. This did not show up in the data obtained for closed arm entries [a presumed reliable measure of general activity (File 1992; Cruz et al. 1994)] as no drug effects were apparent. However, results from the actimeter support this idea as doses producing anxiolysis also significantly decreased horizontal motor activity. Such an explanation would be in agreement with the conclusion drawn by Sanger (1995), who suggested that the reduced efficacy of BZ-1 (ω_1) selective agents (including the compounds used in the present study) in increasing punished operant responding and in blocking the pentylenetetrazole cue may be related to their marked response rate-decreasing effects. One can assume that the weaker efficacy of BZ-1 (ω_1) selective agents might be related to their lack of activity at certain BZ (ω) receptor subtypes on which non-selective BZ (ω) act.

It has been reported previously that differences in the pharmacological profiles of the BZ-1 (ω_1) selective compounds themselves can also be observed. For instance, Zivkovic and collaborators (1992) demonstrated that CL 218,872 produced anticonflict activity in the punished drinking procedure at doses devoid of

central depressant effect, whereas in the case of zolpidem, the former effect was accompanied by a reduction in general motor activity. Results from the present study also revealed differences in the behavioral profile of BZ-1 (ω_1) agents. While CL 284,846 and CL 218,872 significantly increased both the number of open arm entries and the number of head-dippings at several doses, zolpidem and abecarnil failed to affect the former response and abecarnil did not significantly modify head-dippings. As was recently suggested by Zivkovic and collaborators (1992), differences in intrinsic activity between zolpidem and CL 218,872 may account for the weaker effect of the former. This was confirmed by electrophysiological data showing that zolpidem and abecarnil act as full agonists at BZ-1 (ω_1) receptors, whereas CL 218,872 is a partial agonist at these binding sites (Wafford et al. 1993). This suggests that compounds specifically behaving as full agonists at BZ-1 (ω_1) receptors display weaker anxiolytic effects than partial agonists at these sites.

In the case of abecarnil, our findings are somewhat at variance with the results of other studies in which the drug produced potent and clear-cut anxiolytic-like activities in several models in mice and rats, including the four-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). The current difference in drug effect between abecarnil and the other selective compounds cannot readily be explained by differences in dose range. Neither can it be attributed to rate-dependency factors (i.e. response baselines). It is, however, conceivable that, in comparison with the test situations used in the aforementioned studies, the rat plus-maze is more sensitive in revealing certain pharmacological features of abecarnil. Indeed, it has been shown recently that, in addition to its marked activity at the α_1 subunit, the drug also acts as a full agonist on receptors containing the α_3 subunit (Knoflach et al. 1993). Thus, the different behavioral profile of this β-carboline in the rat plus-maze might be due to an interaction with specific receptor subtypes.

In conclusion, the results of the present study confirmed that classical and novel BZ (ω) receptor agonists produce anxiolytic-like effects in the elevated plus-maze test. The addition of ethological measures strengthens this conclusion. However, the magnitude of behavioral changes induced was weaker in the case of the selective BZ-1 (ω_1) ligands for both traditional and novel measures. Moreover, based on the assumption that total arm entries are contaminated by anxiety, together with the current failure of BZ-1 (ω_1) selective agents to affect this response, anxiolysis induced by these latter drugs also differs from the behavioral profile observed with non-selective compounds. These differences may be related to the preferential sedative properties of BZ-1 (ω_1) selective compounds which could mask some indices of anxiolysis such as low dose behavioral stimulation.

Acknowledgements The skilled technical assistance of Anne-Marie Poisson and Carmen Aliaga is gratefully acknowledged.

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