

STUDY OF THE MODULATORY ACTIVITY OF BZ (ω) RECEPTOR LIGANDS ON DEFENSIVE BEHAVIORS IN MICE: EVALUATION OF THE IMPORTANCE OF INTRINSIC EFFICACY AND RECEPTOR SUBTYPE SELECTIVITY

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Abstract

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1. This study examined the hypothesis that the anxiolytic effects of benzodiazepine (BZ (ω)) receptor ligands may be associated with actions at a defined receptor subtype and/or their level of intrinsic activity using the mouse defense test battery.
2. This test 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.
3. The drugs used were the non-selective BZ (ω) receptor full agonist diazepam, the non-selective BZ (ω) receptor partial agonist bretazenil and the β -carboline abecarnil which acts as a full agonist on GABA_A receptors containing the α 1- and the α 3-subunits and as a partial agonist at receptors containing the α 2- and the α 5-subunits. The drugs were given alone and diazepam was co-administered with either bretazenil or abecarnil.
4. When administered alone, diazepam attenuated several defensive responses including risk assessment activities, defensive threat/attack reactions upon forced contact with the rat and escape attempts following the removal of the rat from the apparatus. Unlike diazepam, bretazenil was devoid of significant activity on defense and abecarnil displayed depressant activity.
5. Bretazenil blocked all behavioral effects of diazepam on defense behaviors. The co-administration of diazepam and abecarnil produced a behavioral profile similar to that observed when diazepam was administered alone, indicating that abecarnil did not influence the effects of diazepam on defense. By contrast, diazepam completely antagonized the sedative effects of abecarnil.
6. These findings indicate that only BZ (ω) ligands with high intrinsic efficacy at all BZ (ω) receptor subtypes display clear and specific effects on defensive behaviors in mice, and suggest that GABA_A receptors containing the α 3 subunit might represent the primary target involved in the modulatory action of diazepam on defensive behaviors.

Keywords: abecarnil, anxiety, benzodiazepines, bretazenil, BZ (ω) receptors, defensive behaviors, diazepam, intrinsic efficacy, subtype-selectivity, Swiss mice

Abbreviations: benzodiazepine (BZ), mouse defense test battery (MDTB)

Introduction

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 receptors (Squires *et al.*, 1979; Sieghart and Schuster, 1984). These receptors have also been designated as ω 1 and ω 2, respectively (Langer and Arbilla, 1988). Recent work has shown that there is considerable heterogeneity of GABA_A receptors. At least 15 different subunits have been identified in the mammalian CNS (α 1-6, β 1-3, γ 1-3, ρ 1-2 and δ 1) and it is now widely acknowledged that the BZ-1 (ω 1) subtype corresponds to GABA_A receptors containing the α 1 subunit, while the BZ-2 (ω 2) subtype represents a heterogeneous population of receptors possessing α 2, α 3 or α 5 subunits (Luddens *et al.*, 1995; Sieghart, 1995).

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). Diazepam produces anticonvulsant, anxiolytic, muscle relaxant and sedative effects, whereas bretazenil is mainly anticonvulsant and anxiolytic, but has only weak or no myorelaxant and sedative activities (Haefely *et al.*, 1990). Partial agonists require a higher fractional receptor occupancy than full agonists to elicit a given response *in vivo* (Facklam *et al.*, 1992a,b). In the presence of full agonists, partial agonists compete for the receptor and may attenuate the actions of full agonists, indicating that partial agonists can exhibit antagonistic effects in those circumstances where they display limited or no effect. For example, numerous studies showed that partial agonists (e.g. bretazenil, Ro 19-8022, imidazenil) antagonized sedation and muscle relaxation induced by full agonists (Martin *et al.*, 1988; Jenck *et al.*, 1992; Giusti *et al.*, 1993; Martin *et al.*, 1993).

A number of BZ (ω) receptor subtype-selective compounds are now available. For example, the imidazopyridine zolpidem exhibits high binding affinity for GABA_A receptors containing the α 1-subunit, displays lower affinity for receptors containing the α 2- and α 3-subunits and no affinity for receptors containing the α 5-subunit (Pritchett and Seeburg, 1990; Faure-Halley *et al.*, 1993). The β -carboline abecarnil exhibits 30-fold higher affinity for α 1-containing than α 3-containing subunits combinations but, unlike zolpidem, has high affinity for α 5-containing receptors (Pribilla *et al.*, 1993). In addition to their subtype selectivity, zolpidem and abecarnil differ in terms of their intrinsic efficacies. Zolpidem is a full agonist at a limited number of subtypes (i.e. receptors containing α 1- and the α 3-subunits), whereas abecarnil acts as a full agonist on receptors containing the α 1- and the α 3-subunits but as a partial agonist at receptors containing the α 2-

or $\alpha 5$ -subunits (Knoflach et al., 1993; Pribilla et al., 1993; Wafford et al., 1993). Based on the lack of ataxic and myorelaxant effects of zolpidem and abecarnil, it has been suggested that BZ-1 ($\omega 1$) receptors may not be involved in the muscle relaxation produced by BZs (Perrault et al., 1990; Zivkovic et al., 1992; Turski and Stephens, 1993). In addition, several studies in rodents showed that zolpidem displayed weak or non-specific anxiolytic-like activity (Depoortere et al., 1986; Sanger, 1995; Griebel et al., 1996a,b,c), while abecarnil produced clear anxiolytic-like effects (Stephens et al., 1990; Jones et al., 1994; Ozawa et al., 1994). These observations suggest that anxiety does not involve primarily BZ-1 ($\omega 1$) receptors, as originally proposed (Lippa et al., 1979). Taken as a whole, these findings suggest that there might be a relationship between the behavioral profiles of BZ (ω) receptor ligands and subtype-specificity (Sanger et al., 1994).

In order to explore further the idea that the anxiolytic effects 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 investigated the effects of diazepam alone and in the presence of bretazenil or abecarnil in a recently developed 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). When confronted with a rat, Swiss mice show a characteristic pattern of defensive behaviors including flight, risk assessment, escape attempts, vocalization, and defensive threat/attack, with each behavior induced by specifiable characteristics of the threat stimulus and situation (Griebel et al., 1995a). Recent results showed that diazepam attenuated several defensive behaviors (e.g. escape attempts, risk assessment, defensive threat/attack), whereas bretazenil and abecarnil exhibited weak and non-specific effects, respectively (Griebel et al., 1996c). In view of the pharmacology of diazepam, abecarnil and bretazenil (Table 1), a prediction from the theory of partial agonism is that bretazenil should antagonize all the effects of diazepam on defense, whereas abecarnil which acts as a full agonist at GABA_A receptors containing the $\alpha 1$ - and $\alpha 3$ subunits might antagonize those behaviors involving primarily GABA_A receptors containing the $\alpha 2$ - and/or the $\alpha 5$ -subunits, upon which it acts as a partial agonist (Pribilla et al., 1993).

Methods

Animals

Subjects were naive male Swiss mice aged 9 weeks at the time of testing, and male Long Evans rats (400-500g). They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental

testing, they were housed singly in standard cages (mice: 30 x 20 x 14 cm; rats: 44 x 30 x 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 hr light/dark cycle with light onset at 7 a.m.

Table 1

Efficacies of Diazepam, Abecarnil and Bretazenil in Modulating GABA Response in BZ (ω) Receptors Expressing Different α -Subunits (Adapted from Pribilla *et al.*, 1993).

	BZ-1 (ω 1)		BZ-2 (ω 2)	
	α 1	α 2	α 3	α 5
Diazepam	full	full	full	full
Abecarnil	full	partial	full	partial
Bretazenil	partial	partial	partial	partial

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing one or two drops of Tween 80. The drugs used were diazepam (synthesized by the chemistry department, Synthélabo Recherche), bretazenil (courtesy of Drs Q. Branca and P. Weber, F. Hoffman-La Roche Ltd) and abecarnil (courtesy of Schering). Doses are expressed as the bases and were chosen on the basis of previous results in the MDTB (Griebel *et al.*, 1996c). Drugs were administered intraperitoneally (*i.p.*) in a constant volume of 20 ml/kg 30 minutes before experiments were carried out.

Mouse Defense Test Battery (MDTB)

Apparatus

The procedure has been extensively described in a previous paper (Griebel *et al.*, 1997). The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by

a median wall (2.0 x 0.30 x 0.06 m). The apparatus was elevated to a height of 0.80 m from the floor. 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. The room illumination was provided by one red neon tube fixed on the ceiling and two desk lamps with red bulbs placed respectively on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lux. The experimenter was unaware of the drug treatment.

Procedure

Evaluation of the Depressant Effects Before the Exposure to the Rat: The 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.

Effects on Flight Responses: The Rat Avoidance Test [min. 4 to 6]: Immediately after the 3-min. familiarization period, a hand-held dead rat (killed by CO₂ 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.

Effects on Risk Assessment (RA): The Chase Test [min. 7 to 8]: The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. During the chase, the number of stops (pause in movement), orientations (subject stops, then orients the head toward the rat) and reversals (subject stops, then runs in the opposite direction). was recorded. These responses are described as RA activities (Griebel et al., 1995a).

Evaluation of the Depressant Effects During Exposure to the Rat: The Straight Alley Test [min. 9 to 11]: After the chase was completed, 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 and the immobility time was recorded.

Effects on Defensive Threat/Attack Responses: The Forced Contact Test [min. 12 to 13]: Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites and vocalizations by the subjects were noted. This was repeated three times.

Effects on Contextual Defense: The Post-Test [min. 14 to 16]: Immediately after the forced contact test, the rat was removed and the door was opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session.

Actimeter

Because of the unexpected results obtained after the co-administration of diazepam and abecarnil during the pre-test and in the straight alley, an additional experiment was carried out in order to study further the interaction between the two compounds in a test based on spontaneous locomotor activity.

Testing was conducted in square, clear Plexiglas boxes (22 x 27 x 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 8.30 a.m. and 1. p.m.

Statistical analysis

Data on the effects of diazepam alone and in combination with bretazenil or abecarnil were analysed using the nonparametric Kruskal-Wallis test. Results from the actimeter were analyzed using a two-way (dose x pre-treatment) ANOVA followed by a Newman-Keuls test.

Results

Data on avoidance distance are not presented as statistical analysis did not reveal any significant drug effects during this phase.

Pre-Test (Table 2): Neither diazepam nor bretazenil significantly affected spontaneous horizontal locomotor activity before the rat was placed into the runway. By contrast, in the experiment on the interaction between diazepam and abecarnil, the latter significantly decreased this activity ($H=10.42$, $P<0.05$), an effect which was reversed by diazepam.

Chase Test (Fig. 1): Diazepam significantly decreased the number of reversals ($H=14.99$, $P<0.05$), orientations towards the rat ($H=26.8$, $P<0.001$) and stops ($H=26.75$, $P<0.001$) when compared to vehicle-treated animals. Bretazenil failed to affect in a significant manner these behavioral responses, although a slight decrease of the orientations and the stops was observed. An antagonistic effect was demonstrated for bretazenil as it reduced the action of diazepam on all measures. However, this antagonism was only partial as orientations ($P<0.05$) and stops

($P < 0.05$) were still significantly reduced as compared to control mice. In the second experiment, abecarnil did not produce any behavioral modifications, while diazepam significantly decreased all three RA measures (reversals: $H = 16.84$, $P < 0.05$; orientations: $H = 18.12$, $P < 0.05$; stops: $H = 19.33$, $P < 0.01$). In association with diazepam, abecarnil did not modify the profile observed with diazepam alone.

Table 2

Effects of the Co-Administration of Diazepam and Bretazenil or Abecarnil on Spontaneous Locomotor Activity of Swiss Mice Exposed to the Oval Runway Cage Before the Introduction of the Rat

	Dose (mg/kg)	Number of line crossings
Control		139.8 \pm 8.39
Bretazenil	10	177.1 \pm 12.50
Diazepam	3	120.2 \pm 11.76
Diazepam+bretazenil	3+10	169.2 \pm 14.34
Control		140.9 \pm 12.03
Abecarnil	0.3	83.9 \pm 12.12*
Diazepam	3	116.8 \pm 10.89
Diazepam+abecarnil	3+0.3	110.1 \pm 7.01

Animals were observed during a 3-min period. Drugs were administered i.p. 30 min before experiments were carried out. Data represent mean \pm SEM. $n = 11$. * $P < 0.05$ (vs Control, Kruskal-Wallis test).

Straight alley test (Fig. 2): No behavioral modifications were evident after the administration of diazepam and bretazenil alone or in combination. By contrast, abecarnil significantly increased immobility time in this situation ($H = 17$, $P < 0.001$). Diazepam completely antagonized this effect.

Forced contact test (Fig. 3): Diazepam significantly decreased the number of defensive vocalizations ($H = 18.13$, $P < 0.001$) and bitings ($H = 17.84$, $P < 0.001$) upon contact with the rat, when compared to vehicle-treated animals. Bretazenil failed to affect significantly these behavioral responses, although a small decrease of both responses was observed, but exhibited antagonistic activity as it completely blocked the effects of diazepam. In the second experiment, abecarnil did

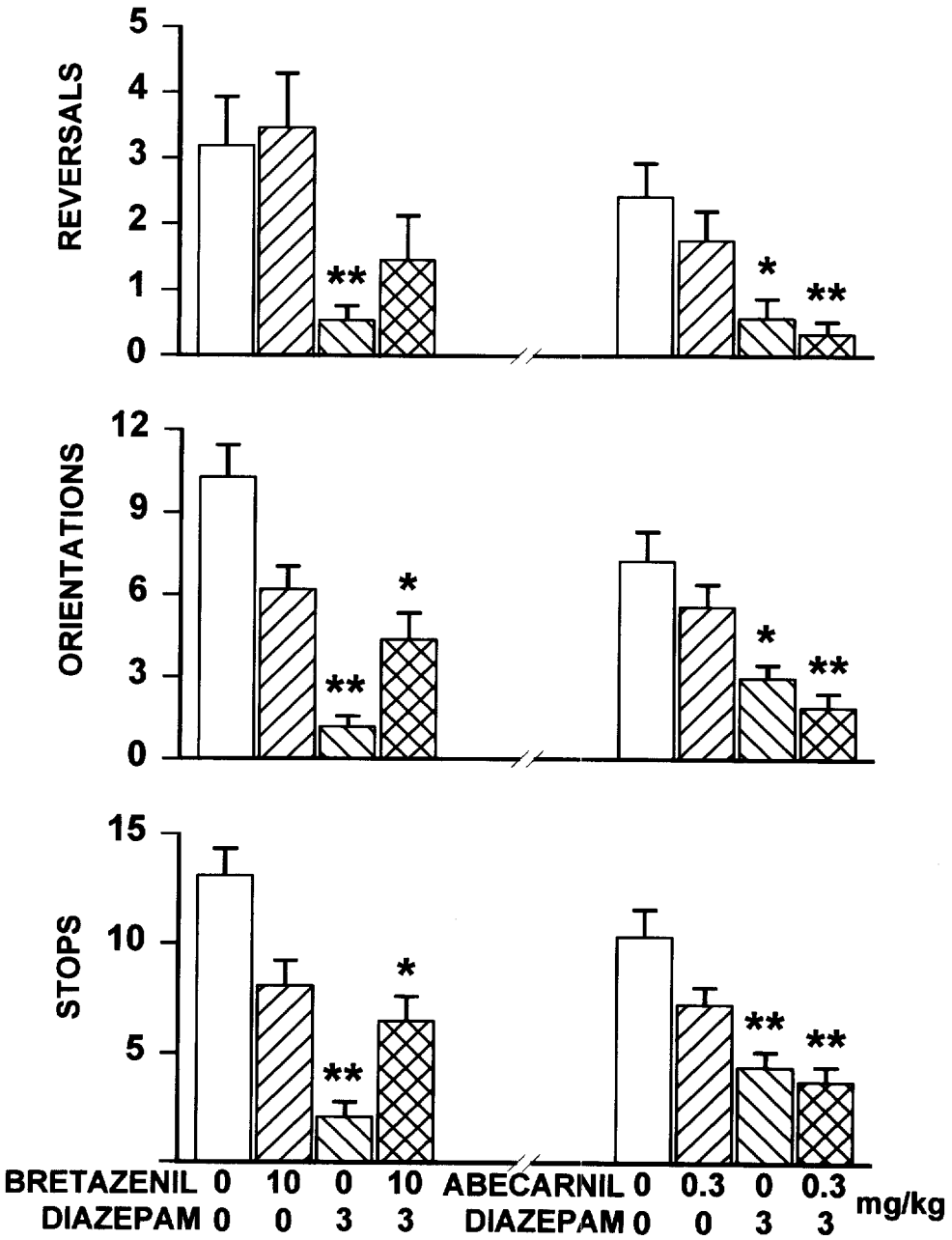


Fig. 1. Effects of diazepam alone and in combination with bretazenil or abecarnil on risk assessment activities exhibited by Swiss mice chased by a hand-held rat in the mouse defense test battery. Data represent mean±SEM. *n*=11-12. **P*<0.05, ***P*<0.01 (vs Control, Kruskal-Wallis test).

not significantly modify defensive threat and attack reactions, whereas diazepam significantly decreased both responses (vocalizations: $H=17.07$, $P<0.05$; bitings: $H=18.19$, $P<0.01$). The co-administration of abecarnil and diazepam produced a profile comparable to that observed with diazepam alone.

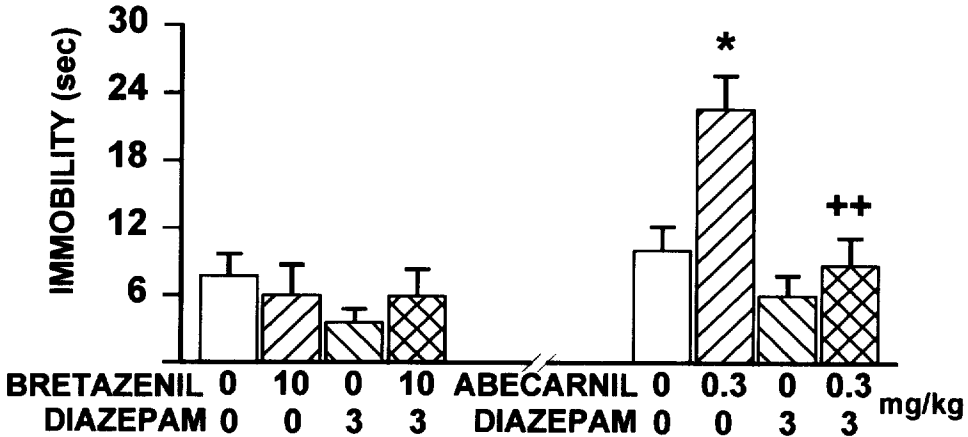


Fig. 2: Effects of diazepam alone and in combination with bretazenil or abecarnil on a measure of sedation in the straight alley situation in the mouse defense test battery. Data represent mean \pm SEM. * $P<0.05$ (vs Control), ++ $P<0.01$ (vs Abecarnil alone, Kruskal-Wallis test).

Post-Test (Fig. 4): In the first experiment, diazepam ($H=22.09$, $P<0.01$) counteracted the increase in escape attempts following the removal of the rat from the runway apparatus, while bretazenil had no significant influence on these responses when compared to saline-treated animals. However, the partial agonist completely antagonized the effect of diazepam. In the second experiment, both abecarnil and diazepam decreased escape attempts ($H=18.55$, $P<0.001$). A similar profile was observed when the drugs were co-administered.

Actimeter: Two-way ANOVA indicated a significant effect of abecarnil dose ($F(3,72)=20.81$, $P<0.001$), a significant effect of diazepam treatment ($F(1,72)=4.93$, $P<0.05$) and a significant interaction between abecarnil and diazepam treatment ($F(3,72)=5.52$, $P<0.01$). Figure 5 shows that abecarnil dose-dependently reduced the number of beams crossed. The dose-effect curve of abecarnil was shifted to the right by 0.5 mg/kg of diazepam, indicating that the benzodiazepine antagonized the hypoactivity induced by abecarnil.

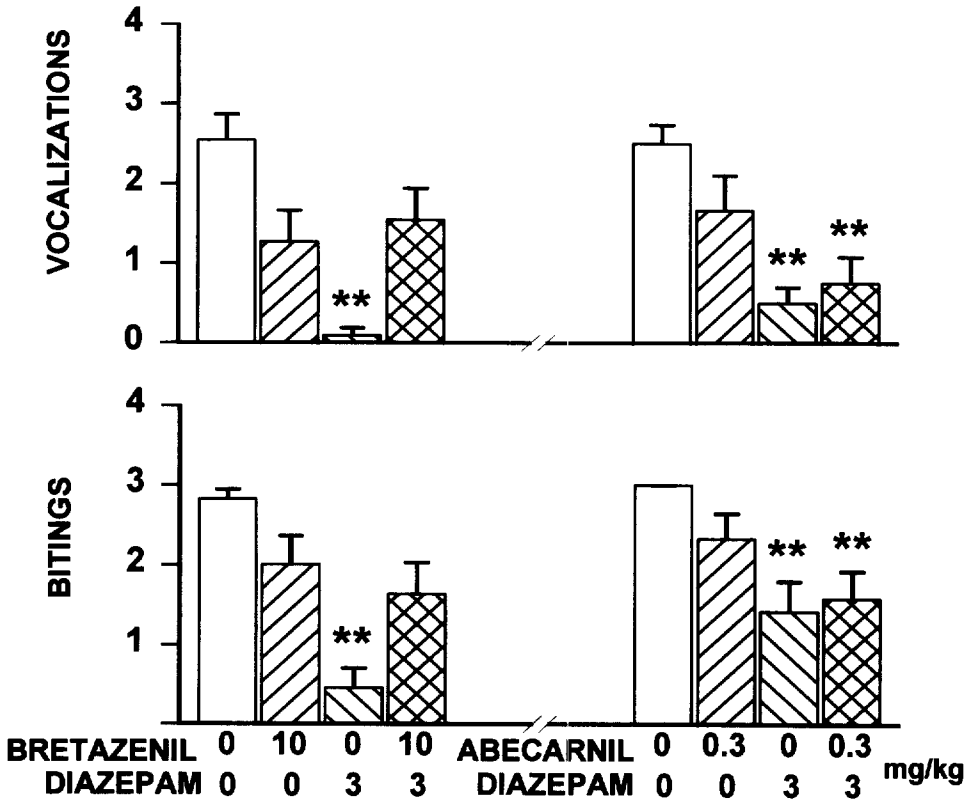


Fig. 3: Effects of diazepam alone and in combination with bretazenil or abecarnil on defensive threat and attack reactions of Swiss mice upon forced contact with a hand-held rat in the mouse defense test battery. Data represent mean±SEM. ** P<0.01 (vs Control, Kruskal-Wallis test).

Discussion

The results of this study showed that diazepam displayed anxiolytic-like effects in the MDTB at the dose tested, whereas bretazenil was inactive. When bretazenil was co-administered with diazepam, it antagonized all effects of diazepam. Administered alone, abecarnil failed to modify defensive behaviors, but it increased immobility. Surprisingly, this effect was fully reversed by diazepam.

Effects of Diazepam, Bretazenil and Abecarnil Alone in the MDTB

When administered alone, diazepam clearly affected several defensive responses, thereby

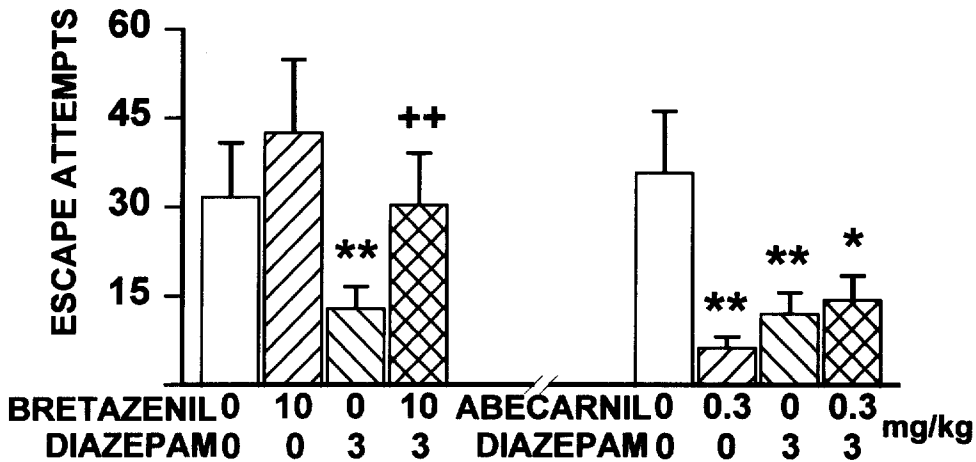


Fig. 4. Effects of diazepam alone and in combination with bretazenil or abecarnil on escape attempts from the runway apparatus after the removal of the rat in the mouse defense test battery. Data represent mean \pm SEM. * $P < 0.05$, ** $P < 0.01$ (vs Control), ++ $P < 0.01$ (vs Diazepam alone, Kruskal-Wallis test).

confirming previous findings from this test battery on the sensitivity of specific defense responses to BZ (ω) receptor full agonists (Griebel et al., 1995a, 1996c). For instance, prominent effects of diazepam were observed during the chase test where the drug reduced risk assessment activities (i.e. reversals, orientations, stops). Furthermore, diazepam decreased defensive threat/attack reactions (i.e. vocalizations, bitings) upon forced contact with the rat and, finally, prevented the increase in escape attempts following the removal of the rat from the runway apparatus. Unlike diazepam, bretazenil was devoid of significant activity on defense, whereas abecarnil decreased locomotor activity during the pre-test and increased immobility time in the straight alley, a profile which is consistent with sedative activity. Although in a previous study with the MDTB, bretazenil was found to decrease one risk assessment measure (i.e. stops) and defensive biting at a single dose (10 mg/kg), the overall profile of bretazenil in this study is in agreement with these earlier findings (Griebel et al., 1996c). Similarly, the profile displayed by abecarnil in this study confirmed the depressant activity of this compound in the MDTB (Griebel et al., 1996c). Taken together, these findings support further the idea that only BZ (ω) ligands with high intrinsic efficacy at all BZ (ω) receptor subtypes display clear and specific effects on defensive behaviors of mice confronted with a natural threat (Griebel et al., 1995a, 1996c).

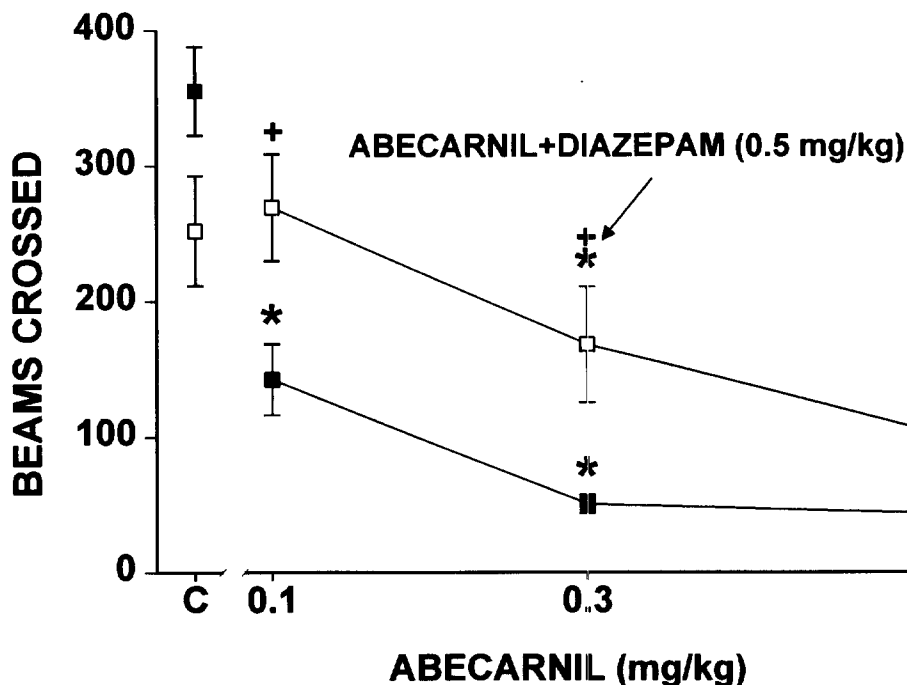


Fig. 5. Effects of abecarnil alone and in combination with diazepam on spontaneous activity of mice in the actimeter test. Data represent mean \pm S.E.M. $n=10$. * $P<0.001$ (vs Control); + $P<0.05$ (vs Abecarnil alone, Newman-Keuls test).

Effects of the co-administration of diazepam and bretazenil in the MDTB

Bretazenil blocked all behavioral effects of diazepam on defense behaviors, including risk assessment activities, defensive threat and attack reactions, and post-test escape attempts. While an antagonistic activity of bretazenil has been demonstrated on the effects of full agonists on muscle relaxation and ataxia (Martin *et al.*, 1988, 1993; Jenck *et al.*, 1992), no study has so far investigated possible interactions between bretazenil and diazepam with respect to their anxiolytic-like actions. This can be readily explained by the fact that bretazenil produced anxiolytic effects *per se* and one can assume that an inactive dose of bretazenil might potentiate, rather than antagonize, the effects of a full agonist. However, bretazenil did not exhibit anxiolytic-like activity in the MDTB, even at doses up to 30 mg/kg as was shown recently (Griebel *et al.*, 1996c). This may indicate that bretazenil has minimal intrinsic efficacy in this test, and hence behaves as

a neutral ligand (i.e. an antagonist). It is therefore not surprising that bretazenil blocked the effects of diazepam in the MDTB. However, since the MDTB is based on response suppression, one can argue that bretazenil actually antagonized sedative rather than anxiolytic-like effects of diazepam. Nonetheless, the lack of effect of diazepam on spontaneous locomotor activity during the pre-test and on immobility time in the straight alley, clearly indicate that diazepam did not produce sedation and thus exhibited specific anxiolytic-like effects. In addition, results obtained with abecarnil also argue against the idea that reduced defensiveness is necessarily associated with behavioral suppression as the drug impaired motor activity without significant effects on defense. Similar observations have been described in the MDTB with other BZ (ω) receptor agonists (e.g. zolpidem) and 5-HT receptor ligands (e.g. 8-OH-DPAT, gepirone, pirenperone) (Griebel et al., 1995b, 1996c). Although numerous studies in rats reported that bretazenil displayed the same or even greater efficacy than full agonists in reducing anxiety-related behaviors (Jenck et al., 1992; Martin et al., 1993; Sanger, 1995; Witkin et al., 1996), studies using mice as subjects demonstrated either weak or no effects of this partial agonist in anxiety models, especially those based on spontaneous responses (Cole and Rodgers, 1993; Jones et al., 1994; Sanger et al., 1995; Griebel et al., 1996a,c). Interestingly, Jones and colleagues (1994) demonstrated that bretazenil had weak effects in the mouse plus-maze test even at BZ (ω) receptor occupancies approaching 90%. In total, these findings suggest that bretazenil displays a very low efficacy in mouse models of anxiety, and thus may behave in these tests as an antagonist of the effects of full agonists.

Effects of the co-administration of diazepam and abecarnil in the MDTB

Abecarnil did not influence the effects of diazepam on defense. It may be speculated that a higher dose of abecarnil (>0.3 mg/kg) would have produced some antagonism. However, the drug displayed depressant effects at the dose employed. Hence, the use of higher doses (Griebel et al., 1996c) would have been inappropriate as depressant effects would have contaminated the action of both drugs on defense. In view of the pharmacology of abecarnil (see Table 1), these findings suggest that defensive behaviors do not primarily involve GABA_A receptors containing the α 2 and α 5 subunits, whereas GABA_A receptors containing α 1 and/or α 3 subunits may play a key role in the modulation of these responses. However, recent findings from the MDTB have demonstrated that selective BZ-1 (ω 1) receptors ligands (e.g. zolpidem, CL 218,872) did not specifically (i.e. at non motor-impairing doses) modify defensive behaviors (Griebel et al., 1996c). These findings, taken together with the current failure of abecarnil to affect defensive behaviors, suggest that GABA_A receptors containing the α 1 subunit also may not be involved in the

modulation of defense. Thus, GABA_A receptors containing the $\alpha 3$ subunit may represent the primary target involved in the modulation of defensive behaviors. However, this hypothesis does not appear to be consistent with the lack of action of abecarnil on defense, as this drug is a full agonist at these receptors. It is possible that the marked depressant effects displayed by abecarnil in the MDTB, may have masked a more specific action on defense. Furthermore, it is important to note that abecarnil exhibits a 30-fold lower affinity for $\alpha 3$ -containing receptors than diazepam (Pribilla *et al.*, 1993).

Effects of the Co-Administration of Diazepam and Abecarnil in the Actimeter

In addition, the results of the co-administration of abecarnil and diazepam showed that the latter completely antagonized the depressant effects of the β -carboline during the pre-test and in the straight alley. These findings were confirmed in the actimeter test where diazepam reversed the hypoactivity induced by abecarnil. Given the full agonist profile of diazepam at all BZ (ω) receptors, the antagonism of the behavioral effects of abecarnil was unexpected and contrasts with previous findings showing that abecarnil blocked some behavioral effects of diazepam. For example, abecarnil antagonized the diazepam-induced potentiation of the hexobarbital-induced loss of the righting reflex and the ataxic effects of diazepam in mice, thereby indicating partial agonist properties (Stephens *et al.*, 1990; Ozawa *et al.*, 1994). However, there is some evidence that abecarnil can decrease locomotor activity in mice at levels of receptor occupancy which are very low (11%) relative to those of diazepam (82%) (Stephens *et al.*, 1990). Hence, diazepam may be able to antagonize the effects of abecarnil in those circumstances where it displays a lower intrinsic efficacy than this β -carboline. In view of the low affinity of abecarnil for GABA_A receptors containing the $\alpha 3$ subunit, the blockade of the depressant effects of abecarnil by diazepam presumably involved BZ-1 ($\omega 1$) receptors where both drugs display comparable high affinities (Pribilla *et al.*, 1993), but where abecarnil shows higher intrinsic activity as revealed by the potentiation of the GABA-induced chloride currents (Vorobjev *et al.*, 1995). Moreover, there is now growing evidence that BZ-1 ($\omega 1$) receptors are of major importance for the sedative activity of BZ (ω) receptors ligands since BZ-1 ($\omega 1$) selective drugs (e.g. zolpidem) exhibit primarily sedative effects (e.g. Depoortere *et al.*, 1986; Perrault *et al.*, 1990; Zivkovic *et al.*, 1992).

Conclusion

These findings confirmed the idea that only BZ (ω) ligands with high intrinsic efficacy at all BZ (ω) receptor subtypes display clear and specific effects on defensive behaviors. In addition, this

study demonstrated that bretazenil exhibited antagonistic activity of all effects of diazepam on defense. Finally, the co-administration of diazepam and abecarnil suggests that BZ (ω) receptors containing the $\alpha 3$ subunit may represent the primary target involved in the modulatory action of diazepam on defensive behaviors.

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