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"Anxiolytic" Action of Diazepam and Abecarnil in a Modified Open Field Test

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REX, A., D. N. STEPHENS AND H. FINK. *"Anxiolytic" action of diazepam and abecarnil in a modified open field test.* PHARMACOL BIOCHEM BEHAV **53**(4) 1005–1011, 1996.—The effects of acutely administered diazepam and the β-carboline abecarnil were examined in two animal models of anxiety in rats, and for their effects on food intake and locomotor activity. In the elevated x-maze diazepam (0.6–2.5 mg/kg) and abecarnil (0.03–0.3 mg/kg) induced anxiolytic-like effects. The second paradigm is based upon the suppression of feeding by exposure to a novel environment, adapted from Bodnoff et al. (1989). Food-deprived rats were placed in a corner of the open field containing food in the centre. The number of rats beginning to eat in the first 5 min was recorded. Diazepam (2.5–5.0 mg/kg) and abecarnil (0.01–0.3 mg/kg) increased the number of rats eating. Flumazenil, the benzodiazepine antagonist, was without effect, but antagonised the effects of diazepam and abecarnil. In a seperate series of experiments the effects of diazepam and abecarnil on feeding and locomotor activity were excluded as having influenced the the anxiolytic effects of these compounds in the animal models of anxiety.

Abecarnil Diazepam Anxiety Feeding Hyperphagia Benzodiazepine Plus-maze

ANXIETY is an emotional state experienced by people, and is not readily modelled in animals. Nevertheless, a need exists for animal models that can be used to predict the anxiolytic properties of drugs for potential clinical use, and a number of tests have been developed for this purpose [see (17,23,38) for a discussion and critique on animal models in anxiolytic testing, from academic, industrial, and clinical standpoints]. Most such tests are based on the ability of anxiolytic agents to increase the frequencies of behaviours that have been suppressed by punishment, by social threat, or by exposure to natural threats such as brightly lit environments or open spaces.

Although the traditional tests of anxiolytic activity depended on the release of punished behaviour [e.g., (7,16,41)], more recently developed anxiety tests make use of the native behavioural neophobia of rodents (24). The aversive effects of an unfamiliar and aversive environment suppress spontaneous naturalistic responses, such as eating and exploring behaviour; anxiolytic drugs might be expected to reduce the fear associated with the new environment, and hence to release the suppressed behaviour. The elevated plus-maze, based on the Y-maze (29), is now a well-established example of this kind of model for anxiety-like behaviours that has been used in rats (19,31), mice (24,37), and in guinea pigs (34). The elevated plus-maze is a well-validated and reliable method for detecting both "anxiolytic" and "anxiogenic" effects of agents acting at the benzodiazepine receptor (31,32).

In this model, an anxiolytic or anxiogenic effect is evaluated by the relation of entries into the open arms to the total entries and the time spent in the open arms of the elevated plus-maze in comparison to the same parameters of the control group (31). Anxiolytic agents increase and anxiogenic agents decrease the entries into and the time spent in the open arms of the elevated plus-maze. The benzodiazepine receptor antagonist flumazenil prevents the anxiolytic effects of benzodiazepines in the elevated plus-maze (27).

Recently, a similar approach, using the fear of rodents of open spaces, to modulate the frequency of another natural occurring behaviour (feeding) as the dependent variable has been suggested (3,4). In these tests potential anxiolytic drugs are increasing the feeding in a novel and aversive open field. The parameters determined as a measure of anxiolytic drug effect were the reducing of latency to begin eating (3) or the increased amount of food eaten (4). We used an open field and counted the number of food-deprived rats starting to eat in this aversive environment within 5 min after placement in the open field. The potential stress on the rats in the pretesting

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procedure was reduced in our experimental design by shortening the duration of food deprivation compared to the reference test (3). Nevertheless, a possible confounding factor in tests of anxiolytic activity based on feeding is the ability of agonists at the benzodiazepine receptor to induce a hyperphagia in rodents (8,9,21). For this reason, an assessment of possible drug-induced changes in feeding behaviour was obtained by performing a food consumption test after a similar time of food deprivation in the animals home cage, as well as in another measure of drug effects on feeding, the influence on intake in rats trained to consume their daily ration in a 4-h period (food consumption test). Locomotor activity was measured simultaneously to control for sedative or stimulatory drug effects, which might compete with feeding.

The novel β-carboline anxiolytic, abecarnil, has been characterised for its anxiolytic potential in a number of animal models employing punishment (22,39), but data on the anxiolytic-like activity of abecarnil in tests of anxiolytic activity not based on punishment are limited to a report on its effect in the mouse in the plus-maze (22). The anxiolytic profile of a compound cannot be characterised by using only one type of animal model of anxiety-related behaviour, because proposed anxiolytic agents are active in some tests and inactive in others, dependent on the design and the nature of the aversive

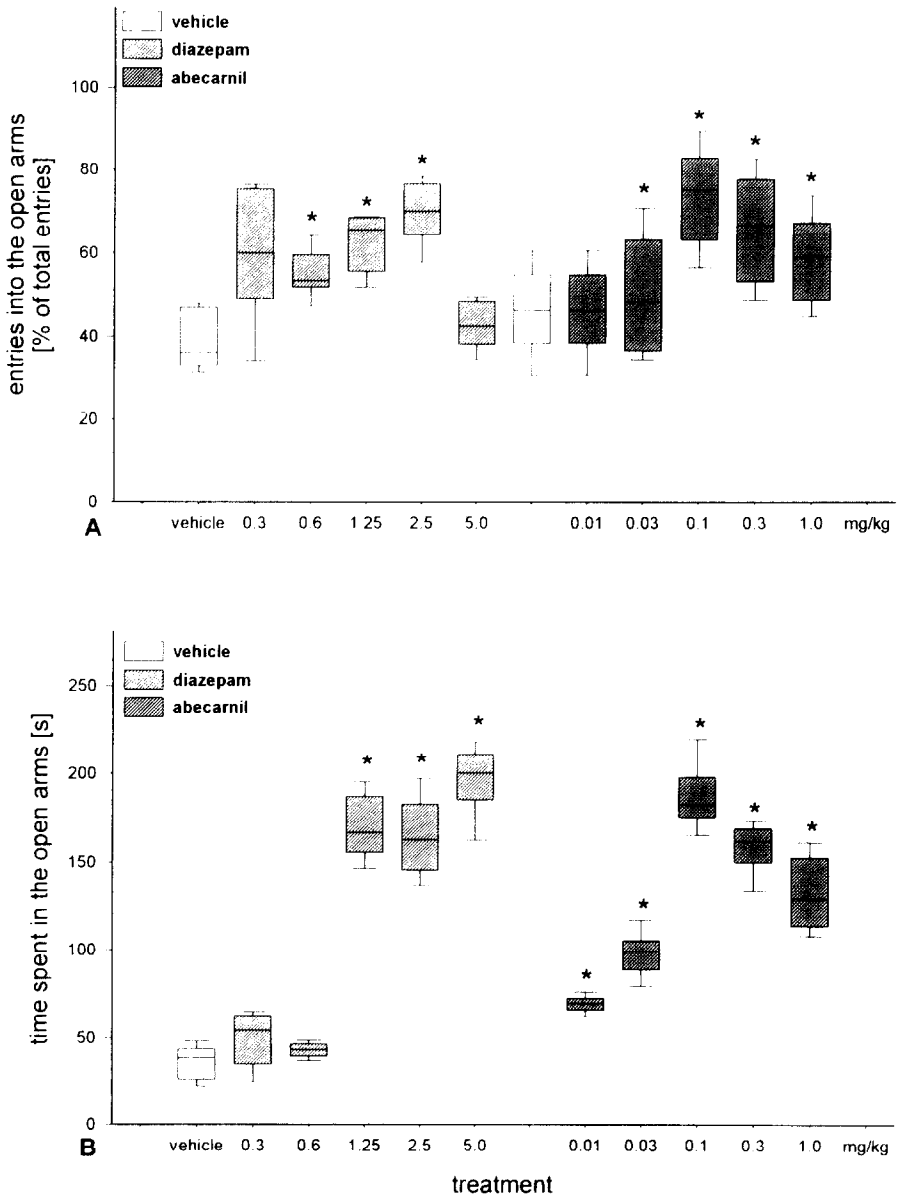

FIG. 1. On exposure of rats to the elevated plus-maze diazepam (0.3–5.0 mg/kg, IP, n = 8–9) and abecarnil (0.001–1.0 mg/kg, IP, n = 8–9) produced an anxiolytic profile with increased entries into the open arms (A) and more time spent on the open arms (B) compared to vehicle-treated animals (n = 10). *$p < 0.05$, Kruskal–Wallis test. Data presented as median with 25th/75th percentiles.

component of the test used (6). For this reason, it was thought useful to compare abecarnil with a standard anxiolytic, diazepam, in rat models of anxiolytic activity not requiring punishment.

METHODS

Animals

Male Wistar rats (from Meichsner, Berlin, and for the antagonist study with flumazenil and abecarnil from Schönwalde GmbH) of 170–230 g body weight were used. They were group housed, 10 per cage (45 × 60 × 25 cm), at room temperature (22°C) with a 12 L : 12 D cycle (light on at 0600 h). Standard pellet food (Altromin 1326) and water were freely available at all times. Rats were housed in the departmental holding room for 2 weeks before testing. The tests were performed in a soundproofed, brightly illuminated room between 0900 and 1100 h.

Drugs and Solutions

The following drugs were used: abecarnil (Schering AG, Berlin, Germany), diazepam (AWD, Dresden, Germany), and flumazenil (Hoffman LaRoche, Basel, Switzerland) or vehicle (0.9% NaCl solution containing 1% Cremophor EL®; BASF, Friedrichshafen, Germany). All drugs were suspended ultrasonically in vehicle immediately prior to use. The drugs were injected IP in a volume of 5 ml/kg.

Apparatus and Procedure

Elevated plus-maze. The behavioural experiments were performed using a brightly illuminated (≈1500 lx) elevated plus-maze (height: 70 cm, arm length: 45 cm, arm width: 15 cm, wall height on two opposite arms: 10 cm). The behavioural parameters measured were: total entries into all arms (measurement of locomotor activity); entries into the open arms; and time spent in the open arms (measures of anxiolytic activity). Rats that jumped off the elevated plus-maze ($n = 5$) were excluded from the study.

Modified open field. Twenty hours before testing the pellets were removed from the animal's home cage; water was still available. One hour before the test the animals were transferred in their home cages from the animal unit to the observation chambers. After this habituation time the rats received the injections. Twenty-five minutes later the animals were individually placed into a corner of the brightly illuminated (1500 lx) white open field (100 × 100 × 40 cm). The familiar food pellets were placed in the centre of the open field. Each rat was observed for 5 min and the time to onset of feeding was recorded. The incidence of food intake (% of rats in a group with same treatment) was registered and dose-response curves determined. If the feeding rate decreased with higher doses after an increase in lower doses, the series was terminated.

The animals were observed using a real-time video system. Measurement of locomotor activity was carried out simultaneously by counting interruptions of 10 infrared light beams in the open field; 9–11 animals were examined for each dose of a drug.

Food consumption test. Rats were isolated and kept in single cages in the familar surrounding. All animals were undergoing a feeding schedule with a 4-h feeding period per day, with water freely available. After 6 days of habituation the rats received a vehicle injection at the beginning of the feeding period. After 2 h the amount of food eaten was determined and used as control. On the next day the same animals received an injection of either diazepam or abecarnil, followed by the same procedure. The consumption of food during the 2 h after treatment was calculated as the percentage of food consumption by the same animals without treatment a day before and was considered as a drug effect.

Statistics

Elevated plus-maze. The data were analysed using Kruskal–Wallis tests ($p < 0.05$) and are expressed as median with 25th and 75th percentile and the extremes.

Incidence of food intake in the modified open field. Data are expressed as percentage of rats eating in each single group and were analysed using the χ^2 test.

Food consumption test. The data from each group were analysed using the paired t-test. Data are expressed as mean ± SEM.

A difference of the means or the medians of two groups of $p < 0.05$ was considered as statistically singnificant.

RESULTS

Elevated Plus-Maze

Vehicle-treated animals spent 24.6 ± 5.2 s in the open arms and made 36.8 ± 9.6% of all entries into the open arms of the elevated plus-maze during the 5-min period (Fig. 1). Diazepam dose-dependently increased the percentage of entries into the open arms up to 61.25 ± 13.75% and also the time spent in the open arms to 148.5 ± 42.1 s (Fig. 1). The ratio of entries into the open arms was decreased at the highest dose of 5.0 mg/kg, whereas the time spent in the open arms continued to increase.

Abecarnil also increased the time spent in the open arms, peaking at 172.2 ± 10.9 s after administration of 0.1 mg/kg. The percentage of entries into the open arms increased to 79.3 ± 10.3% at the same dose of 0.1 mg/kg. Both parameters

TABLE 1

LOCOMOTOR ACTIVITY IN THE PLUS-MAZE

Drug	Dose (mg/kg)	Locomotor Activity Entries/5 min
Vehicle	0.9% + 1% CEL	6.0 (5.0/7.7)
Diazepam	0.3	7.7 (6.4/9.9)
	0.6	6.2 (5.2/7.8)
	1.2	6.4 (5.6/7.8)
	2.5	8.2 (6.8/11.9)
	5.0	5.9 (4.9/7.6)
Abecarnil	0.01	5.5 (4.9/6.5)
	0.03	6.1 (5.3/7.3)
	0.1	8.2 (7.1/10.1)
	0.3	8.4 (7.6/9.6)
	1.0	6.9 (6.2/8.0)

The total number of entries into all arms during 5 min representing the locomotor activity on exposure to the elevated plus-maze following the treatment with diazepam (0.3–5.0 mg/kg, IP, $n = 8$–9) or abecarnil (0.001–1.0 mg/kg, IP, $n = 8$–9) compared to vehicle-treated controls ($n = 10$). Data are expressed as median ± 25th/75th percentile.

were decreased after the administration of higher doses of abecarnil (0.3–1.0 mg/kg). Abecarnil produced an inverted U-shape dose-response curve regarding the parameters for anxiolytic action.

The number of all entries as a measurement of locomotor activity in the control rats (n = 30) was 5.7 ± 0.8 total entries during the 5-min observation period. Diazepam (0.3–5.0 mg/kg, IP, n = 8–9) and abecarnil (0.01–1.0 mg/kg) did not alter locomotor activity significantly (Table 1).

Modified Open Field

The total number of control rats showing feeding during the 5-min test session was 4 out of 55 (i.e., 7.8%). In the antagonist study using flumazenil and abecarnil another rat strain had been used and 40% of the control rats were feeding in the open field.

The benzodiazepine diazepam showed a dose-dependent increase in the ratio of rats feeding to rats not feeding, peaking at 2.5 mg/kg with an effect of 70% rats feeding, followed by a decrease (Fig. 2a). A similar dose-response curve could be shown for the β-carboline abecarnil peaking at 0.03 mg/kg with 50% rats feeding (Fig. 2b). The benzodiazepine antagonist flumazenil on its own did not affect the incidence of food intake. But pretreatment with flumazenil resulted in a dose-dependent reduction of the maximal anxiolytic effects of the benzodiazepine receptor agonists diazepam (Fig. 3a) and abecarnil (Fig. 3b).

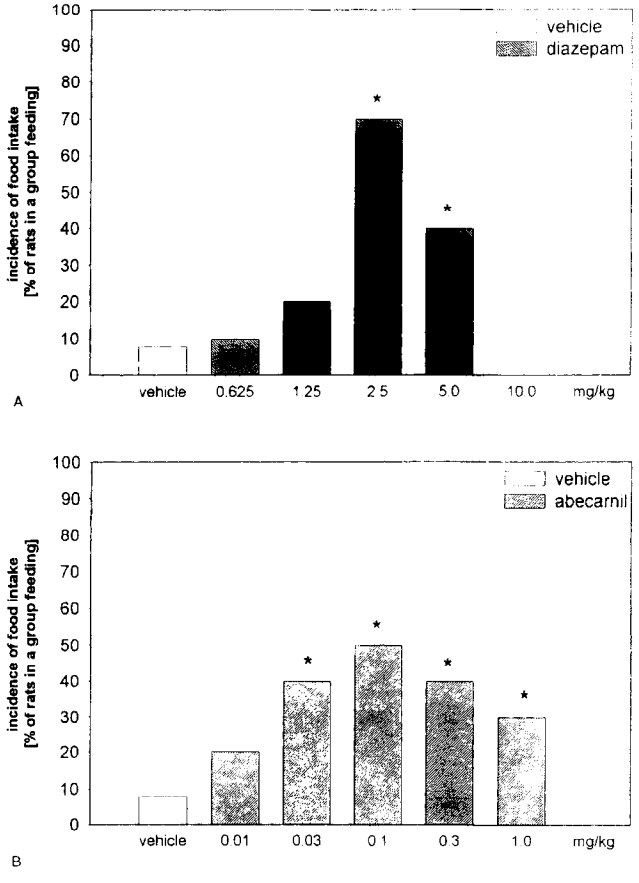

FIG. 2. The incidence of food intake in the open field after pretreatment with diazepam (A) or abecarnil (B). χ^2 test, $^*p < 0.05$, compared to vehicle-treated controls. Data are presented as percent.

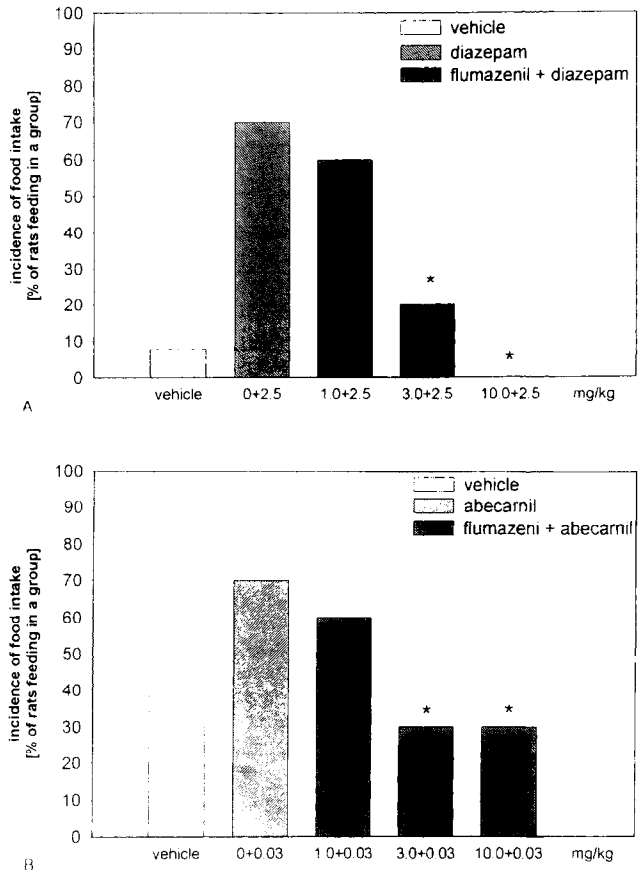

FIG. 3. Dose-dependent attenuation of the diazepam (2.5 mg/kg, A) and abecarnil (0.03 mg/kg, B) induced incidence of food intake in the open field after pretreatment with flumazenil compared to diazepam- and abecarnil-treated animals, respectively. χ^2 test, $^*p < 0.05$. Data are presented as percent.

Locomotor activity of the animals was measured simultaneously. The rats in the control groups crossed 64 ± 11.5 (mean ± SEM) squares in the 5-min observation period. The benzodiazepine diazepam produced a slight hyperactivity in the test after a dose of 10.0 mg/kg (89 ± 9.7 crossings). Abecarnil, and other doses of diazepam, did not alter locomotor activity significantly (Table 2).

Food Consumption Test

Diazepam did not increase the amount of food eaten during the 2-h period following the drug administration, when the animals were trained to the food schedule as described above. After treatment with the highest dose of 12 mg/kg diazepam a decrease occurred (Table 3). Abecarnil had no effect on food consumption at the dose of 0.075 mg/kg, giving rise to the maximal anxiolytic activity, but induced a slight hyperphagia following the administration of 0.15 mg/kg and rising doses (Table 3).

TABLE 2

LOCOMOTOR ACTIVITY IN THE OPEN FIELD

Drug	Dose (mg/kg)	Locomotor Activity (Crossed Light Beams/5 min)
Vehicle	0.9% + 1% CEL	64 ± 11.5
Diazepam	0.625	46 ± 21.2
	1.25	65 ± 14.7
	2.5	72 ± 14
	5.0	58 ± 15.2
	10.0	89 ± 9.7
Abecarnil	0.01	71 ± 13.8
	0.03	47 ± 24.1
	0.1	80 ± 17.5
	0.3	60 ± 25
	1.0	69 ± 12

The locomotor activity in the open field following the treatment with diazepam (0.3-5.0 mg/kg, IP, n = 9-11) or abecarnil (0.001-1.0 mg/kg, IP, n = 9-11) compared to vehicle-treated controls (n = 10). Data are expressed as mean ± SEM.

DISCUSSION

Benzodiazepines have been the most widely used anxiolytics in general practice for many years (20), and are relatively safe drugs for a short-term treatment of anxiety despite their drug-dependence potential and side effects (2). In animal pharmacology, the ability of benzodiazepines to exert an effect is an essential aspect of characterisation of putative models of anxiety. Diazepam is a standard benzodiazepine anxiolytic, and has been frequently employed in animal pharmacology as a reference compound.

In the present experiments, diazepam showed clear dose-dependent increases in both number of entries into the open arms of the plus-maze and in the time spent in the open arms, in agreement with a number of previous reports [e.g., (1,18, 22,30,31,40-42)]. In this model, diazepam first showed significant effects at doses from 0.6 mg/kg. Diazepam is active in most animal tests of anxiolytic activity, including a test measuring the consumption of novel unfamiliar food [(33), effective dose range from 2.5 to 40 mg/kg], in a light/dark choice novelty situation [(28), dose range 1-10 mg/kg), in the two-compartment black and white box [(10), 1.25 mg/kg], the social interaction test [(e.g., (5), 1 mg/kg], and the defensive burying paradigm (36) with doses from 1.0 to 10.0 mg/kg. In our modified open field test doses from 2.5 to 5.0 mg/kg diazepam were significantly effective and increased the incidence of food intake without alteration of the locomotor activity, whereas 10.0 mg/kg induced a hyperactivity.

Diazepam is known to induce a hyperphagia in rodents (8,21,33). But in our food consumption test in the home cages and in the food consumption in the open field (4) diazepam did not increase the feeding of rats. However, it may be that fasting produced an increase in feeding that could not be further stimulated by diazepam (4).

Abecarnil is a β-carboline acting at the benzodiazepine receptor complex (26) whose anxiolytic properties were first described in the four-plate test in mice and the water-lick conflict test in rats, with effective doses of 0.39 and 0.3 mg/kg, respectively (39). Preliminary clinical studies show also anxiolytic effects in patients (2,11). Abecarnil produced an anxiolytic behaviour in a dose range of 0.03-1.0 mg/kg on the elevated plus-maze. The dose-response curves of the parameters reflecting the anxiolytic activities showed a similar shape to diazepam treatment. Abecarnil also showed a clear effect in the modified open field test, displaying a bell-shaped dose-response curve. The effective doses were in a range of 0.003-0.3 mg/kg. The maximally effective dose was much lower than that of diazepam.

TABLE 3

FOOD INTAKE IN THE FOOD CONSUMPTION TEST

Drug	Dose (mg/kg)	Food Intake of Controls in 2 h (g)	Food Intake of Treated Rats in 2 h (g)	Food Intake Compared to Controls (%)
Diazepam	0.35	7.3 ± 1.1	9.0 ± 0.5	123 ± 5.5
	0.7	6.8 ± 0.7	8.3 ± 0.8	122 ± 9.6
	1.5	8.7 ± 1.1	9.8 ± 1.3	112 ± 13.2
	3.0	10.9 ± 0.6	11.2 ± 0.4	102 ± 3.7
	6.0	8.2 ± 0.7	8.9 ± 1.0	108 ± 11.2
	12.0	6.0 ± 0.6	4.5 ± 1.5*	75 ± 33.3*
Abecarnil	0.07	9.4 ± 0.6	8.6 ± 0.9	91 ± 10.5
	0.15	9.1 ± 1.0	11.7 ± 0.9*	123 ± 7.6*
	0.35	9.5 ± 0.5	6.5 ± 1.0*	68 ± 15.3*
	0.7	8.7 ± 0.6	11.0 ± 1.3	126 ± 11.8
	1.5	10.7 ± 0.7	10.7 ± 1.2	100 ± 11.2
	3.0	8.3 ± 0.6	4.2 ± 1.4*	51 ± 33.3*

Food intake in the 2 h following an acute administration of benzodiazepine receptor agonists using single-housed rats in the familar environment after 20 h of food deprivation, compared to direct vehicle-treated controls. The food intake is measured in grams and as percent of the food consumption of the vehicle-treated controls. Data are presented as means ± SEM.

*$p < 0.05$, Kruskal-Wallis test.

A hyperphagia induced by abecarnil (0.3–10 mg/kg, IP) in a familiar environment has been described (9). The same effect was visible in our food consumption test using rats trained to get their food in a 4-h period every day at a dose of 0.15 mg/kg and higher. However, the anxiolytic behaviour induced by abecarnil reached the maximum at a dose of 0.03 mg/kg in the modified open field, well below the hyperphagia-inducing doses. Thus, the increase in the incidence of food intake induced by lower doses of abecarnil in the modified open field seems not to be attributable to hyperphagia, which occurred at a higher dose of abecarnil. Furthermore, the effective anxiolytic effect of abecarnil in the modified open field occurred over a dose range (0.01–0.3 mg/kg) corresponding to that at which an anxiolytic effect was seen in the elevated plus-maze (0.03–1.0 mg/kg). Locomotor activity was not significantly altered by the treatment with abecarnil.

The benzodiazepine antagonist, flumazenil, given alone, was ineffective in the modified open field, but a pretreatment with flumazenil (1.0–10.0 mg/kg) dose-dependently attenuated the anxiolytic action of diazepam. Similar results using the social interaction test have been found with rats (14). Flumazenil (10 mg/kg) also blocked the diazepam-induced anxiolytic behaviour in guinea pigs on exposure to the elevated plus-maze (34). Although acute flumazenil (8–10 mg/kg) alone has been shown to have partial agonistic effects (35) and to enhance exploratory behaviour in a similar manner to chlordiazepoxide (13), it has also been reported to induce anxiogenic effects in the social interaction test (12) and in a punished drinking test (15). However, in our modified open field flumazenil failed to induce any agonist- or inverse agonist-like action. There are only a few studies in the literature to the antagonising effects of flumazenil on anxiolytic effects of abecarnil. In our test flumazenil did antagonise the anxiolytic actions of abecarnil and decreased the number of rats feeding in the aversive open field to baseline level, similar to the antagonism against diazepam.

The dose–response curves induced by the two benzodiazepine receptor agonists in both behavioural paradigms show the form of a inverted U-shaped curve. The descending limb of the inverted U may be attributable to other effects of the anxiolytics interfering with the expression of the behaviour used to measure anxiolysis [e.g., (22)].

This simple paradigm of conflict behaviour in an aversive, unfamiliar environment has been demonstrated to detect anxiolytic agents with a minimum of time and technical equipment. Although many tests for anxiolytic drugs have been described, most of them demand well-trained animals or relatively complex equipment, and may require skilled personnel. Lastly, the use of electric shock, however minimal, as a punishment may make such tests unacceptable, especially in a teaching environment. The present test represents a simple model with minimal requirements for highly trained personnel and equipment and employing a more ethologically relevant induction of anxiety, which would allow the rapid assessment of potential anxiolytics, on the one hand, while demonstrating the principles of action of anxiolytic drugs on the other.

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