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ORIGINAL INVESTIGATION

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Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents

Comparison with diazepam and buspirone

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Abstract The present series of experiments compared the behavioral effects of the novel non-peptide CRF antagonist CP-154,526 with those of diazepam and the 5-HT_{1A} receptor partial agonist buspirone in classical animal models of anxiety including conflict tests (punished lever pressing and punished drinking tests in rats) and exploratory models (elevated plus-maze test in rats, light/dark choice and free-exploration tests in mice), and a recently developed mouse defense test battery (MDTB) which has been validated for the screening of anxiolytic drugs. Results from both conflict procedures showed that diazepam (2.5–10 mg/kg, IP) produced clear anxiolytic-like effects, whereas buspirone (2.5 mg/kg, IP) displayed anticonflict activity in the punished drinking test only. CP-154,526 (0.6–20 mg/kg) was devoid of significant activity in both procedures. In the elevated plus-maze, diazepam (2 mg/kg, IP) produced significant effects on traditional (i.e. spatio-temporal) and ethologically derived (i.e. risk assessment and directed exploration) indices of anxiety. Buspirone (1–4 mg/kg, IP) reduced risk assessment activities only, and CP-154,526 (0.6–20 mg/kg, IP) did not modify the indices of anxiety in the elevated plus-maze. In the light/dark test, diazepam (2.5–5 mg/kg, IP) and CP-154,526 (10–40 mg/kg, IP) affected all behavioral indices of anxiety, while buspirone reduced risk assessment activities at the highest doses only (10 and 15 mg/kg, IP). In the free-exploration test, diazepam (1 mg/kg, IP) reduced avoidance responses towards novelty, as indicated by the increase in exploratory activity in a novel compartment and the decrease in risk assessment. CP-154,526 failed to affect the former behavior and weakly reduced the latter (5 and 20 mg/kg, IP). Buspirone (1.25–5 mg/kg, IP) was inactive in this test. Finally, in the MDTB, diazepam (0.5–3 mg/kg, IP) attenuated all defensive reactions of mice confronted with a rat stimulus (i.e. flight, risk assessment and defensive attack) or with a situation associated with this threat (i.e. contextual defense). Buspirone (1.25–5 mg/kg, IP) reduced defensive attack and contextual defense, while CP-154,526 (5–20 mg/kg, IP) affected all defensive behaviors, with the exception of one risk assessment measure. The finding that CP-154,526 displayed positive effects in mice but not in rats may be due to increased sensitivity to environmental stress of the strains used (i.e. BALB/c, Swiss) and/or to the fact that animals were exposed to unavoidable stress stimuli which may lead to a significant activation of the CRF system. Although in mice the anxiety-reducing potential of CP-154,526 is superior to that of the atypical anxiolytic buspirone, it is smaller in terms of the magnitude of the effects and the number of indices of anxiety affected than that of diazepam.

Key words CRF antagonist · CP-154,526 · Anxiety model · Conflict test · Exploration test · Defensive behavior · Diazepam · Buspirone

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Introduction

Corticotropin-releasing factor (CRF) is a 41-residue peptide originally isolated by Vale et al. (1981). CRF is widely distributed in the brain, with highest concentrations found in the hypothalamus where it is produced and secreted by the parvocellular neurons of the hypothalamic paraventricular nucleus. It is the major hypophysiotropic factor regulating basal and stress-induced release of adrenocorticotropic hormone (ACTH) and β-endorphin (Vale et al. 1981, 1983). Moderate and low levels of CRF are also present in cortical and limbic structures, respectively (Orth 1992). In addition, high densities of CRF receptors have been

detected in the cerebral cortex, olfactory bulb, amygdala and hippocampus (Chalmers et al. 1995).

In recent years, substantial evidence has accumulated from both laboratory and clinical studies that CRF plays a primary role in coordinating the overall response of the body to stressors. Much of the evidence comes from studies showing that intracerebroventricular (ICV) application of CRF in rodents produces behavioral effects similar to those observed when animals are exposed to stress, including decreased food intake (Britton and Britton 1981; Britton et al. 1982; Levine et al. 1983; Morley and Levine 1983), altered locomotor activity (Sutton et al. 1982; Berridge and Dunn 1986; Sherman and Kalin 1987; Takahashi et al. 1989; Spadaro et al. 1990), sleep disruption (Ehlers et al. 1983) and anxiety (Britton 1985; Swerdlow et al. 1986; Dunn and File 1987; File et al. 1988; Adamec et al. 1991; Baldwin et al. 1991; Britton et al. 1992; Liang et al. 1992). Importantly, these effects appear to be independent of direct activation of the hypothalamo-pituitary axis, since they were observed in hypophysectomized and dexamethasone-treated rats (Eaves et al. 1985; Britton et al. 1986a, 1986b; Adamec and McKay 1993). These observations led to the suggestion that extrahypothalamic CRF receptors probably participate in the behavioral effects of CRF (Koob 1991). Consistent with this idea is the finding that chronic infusion of a CRF_1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats (Liebsch et al. 1995). In addition, a recent study by Stenzel-Poore et al. (1996), who developed a CRF transgenic mouse line overexpressing CRF, further emphasized the anxiogenic properties of CRF overproduction, since these mice exhibited a behavioral state resembling that produced by anxiety. In clinical studies, patients with panic disorder and generalized anxiety disorder (GAD) have been found to exhibit signs of CRF hypersecretion as revealed by a blunted ACTH response to intravenously administered CRF (Roy Byrne et al. 1986). In this context, it was postulated that CRF receptor antagonists may represent novel agents for the treatment of anxiety disorders (Chalmers et al. 1996).

Several peptide CRF receptor antagonists, including α-helical $CRF_{9–41}$, a fragment of CRF (Rivier et al. 1984), have been studied extensively. α-Helical $CRF_{9–41}$ has been shown to block nearly all of the behavioral effects of exogenously applied CRF, including the anxiogenic-like effects (Britton et al. 1986c; Berridge and Dunn 1987; Swerdlow et al. 1989; Adamec et al. 1991; Liang et al. 1992). In addition, α-helical $CRF_{9–41}$ was found to reduce anxiety-related responses (e.g. freezing, defensive withdrawal) following stress exposure (e.g. inescapable electric shocks or conspecific aggression) (Tazi et al. 1987; Kalin et al. 1988; Takahashi et al. 1989; Smagin et al. 1996). However, the usefulness of peptide antagonists is limited because of poor access to the brain following systemic administration and poor oral bioavailability. Recently, several classes of non-peptide antagonists of CRF receptors have been identified. For example, CP-154,526 is a pyrrolo[2,3-*d*]pyrimidine derivative with high affinity for CRF receptors ($Ki < 10$ nM) and low affinity (> 1 μM) for other receptors (Chen et al. 1997). Previous investigations have shown that CP-154,526 antagonized the CRF-induced increase in plasma ACTH levels in rats and inhibited the excitation of locus coeruleus neurons induced by ICV CRF (Schulz et al. 1996). In addition, CP-154,526 was able completely to reverse the enhancement in startle amplitude induced by ICV CRF in an acoustic startle procedure (Schulz et al. 1996), and anxiolytic-like activity was demonstrated in the fear-potentiated startle (Schulz et al. 1996) and the elevated plus-maze tests in rats (Lundkvist et al. 1996).

The aim of the present study was to examine further the anxiolytic-like properties of CP-154,526 in classical animal models of anxiety including conflict procedures (punished lever pressing and punished drinking tests in rats) and exploratory models (elevated plus-maze test in rats, light/dark and free-exploration tests in mice), and in a recently developed mouse defense test battery (MDTB) which was found to be useful for the screening of anxiolytic drugs (Griebel et al. 1995a, 1996a). In addition, a more ethologically orientated scoring method was used with the exploration tests, as there is increasing evidence that sensitivity to drug effects may be increased when such techniques are employed (Rodgers and Cole 1994; Griebel et al. 1997a). Effects were compared with those of the benzodiazepine (BZ) diazepam and the $5\text{-}HT_{1A}$ partial agonist buspirone. While diazepam has well-established efficacy in GAD and may also be used (at higher doses) in the treatment of panic disorder, buspirone has demonstrated efficacy in GAD only.

Materials and methods

All procedures described here are in compliance with French legislation on animal experimentation.

Animals

Male Wistar rats (Charles River France, Saint-Aubin-les-Elbeuf) were used in the punished lever pressing procedure. They weighed 180–200 g at the beginning of training and 400–500 g at the time of drug testing. Male Sprague-Dawley rats (Iffa Credo, L'Arbresle and Charles River France) weighing 180–230 g at time of testing were used in the Vogel drinking and the elevated plus-maze tests. Male Long Evans rats (400–500 g) (Iffa Credo) were used as a threat stimulus in the MDTB. BALB/c mice (7 weeks old) (Iffa Credo) were used in the light/dark and free-exploration tests, and Swiss mice (10 weeks old) (Iffa Credo) were used in the MDTB. Rats used in the elevated plus-maze and in the Vogel drinking tests were housed in groups of eight, whereas those used in the punished lever

pressing procedure were housed singly. BALB/c mice were housed in groups of six and Swiss mice were isolated 1 week prior to testing. All animals were maintained under standard laboratory conditions (22–23°C) and kept on a 12:12-h light-dark cycle with light onset at 7 a.m. Rats used in the punished lever pressing procedure were restricted to the food obtained during sessions and a daily ration of 15–20 g standard laboratory chow given at the end of each weekday and over the weekend.

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing 1 or 2 drops of Tween 80. They were injected in a volume of 2 ml/kg (rats) or 20 ml/kg (mice). The drugs used were diazepam, buspirone and CP-154,526 (*N*-butyl-*N*-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-*N*-ethylamine) (all synthesized by the Department of Chemistry, Synthélabo Recherche). Drugs were given IP 30 min before experiments. Testing was performed between 9 a.m. and 3 p.m. Doses are expressed as the bases. The doses of CP-154,526 were chosen on the basis of findings with this compound in published studies (Lundkvist et al. 1996; Schulz et al. 1996; Chen et al. 1997; Mansbach et al. 1997).

Punished lever pressing

The procedure was a modification of that described previously (Sanger et al. 1985). Animals were tested in standard rat operant test chambers (MED Associates, Inc., Georgia, USA) placed in sound-attenuated boxes with ventilation fans. Each chamber was fitted with a stainless steel grid floor. Electric shocks could be delivered to each grid by a shock generator and scrambler (MED Associates, Inc.). A total of 11 rats was trained initially to press a lever for food reward (45 mg precision food pellets, PJ Noyes, Inc., Lancaster, USA). As training progressed, schedule parameters were gradually changed to a variable interval (VI 30 s) schedule of food reinforcement during daily 15-min sessions. After several sessions of VI 30 s responding, five 60-s periods of a visual stimulus were presented during a 25-min session. Each visual stimulus consisted of three stimulus lights situated above the food pellet dispenser and to the right of the response lever, which flashed at a rate of 1 s on, 1 s off. In this component, a footshock punishment schedule consisting of two independent VI schedules (VI 30 s for food, VI 10 s for shock) was in operation. Footshock was initially set at 0.1 mA. The first stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 s after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3 to 0.65 mA) to obtain stable baselines of responding (i.e. an average lever pressing rate of 8 ± 2 presses in each 60-s punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were initiated.

Drug injections were given once or twice each week with at least two non-drug days intervening between two drug administrations. Vehicle was injected on all non-drug days. Drugs and doses were given in a mixed order. Diazepam was first tested in all 11 rats. Subsequently, eight rats were tested with all doses of buspirone and six rats with CP-154,526. The effects of drugs were assessed on punished and unpunished responses rates. The former corresponds to those recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60-s periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the non-drug session preceding the drug injection sessions were used as the control values. Thus, drug effects were analyzed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's analysis of variance (ANOVA).

Punished drinking

The procedure was a modification of the technique described by Vogel et al. (1971). At the beginning of the experiment, rats, deprived of water for 48 h prior to testing, were placed in cages ($27 \times 22 \times 21$ cm) with a stainless steel grid floor. Each cage contained a drinking tube connected to an external 50 ml buret filled with tap water. Trials were started only after the animal's tongue entered in contact with the drinking tube for the first time. An electric shock (0.3 mA) was delivered to the tongue after every 20 licks. The number of shocks was recorded automatically during a 3-min period. Results were analyzed by the non-parametric Kruskal-Wallis test.

Elevated plus-maze

The test apparatus is based on that described by Pellow et al. (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open (50×10 cm) and two enclosed arms ($50 \times 10 \times 50$ cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10×10 cm). To prevent rats falling off, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. 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. The light intensity on the central platform was 10 lux. 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 video-link by an observer located in an adjacent room. This permitted the recording of the more ethologically orientated measures: (a) attempt: attempt at entry into open arms followed by avoidance responses. This includes stretched attend posture (the rat stretches forward and retracts to original position); (b) head-dipping: protruding the head over the edge of an open arm and down towards the floor (this response can occur while the animal's body is in a closed arm, central square or on an open arm). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of both closed and open arm entries, mean total number of closed arm entries, mean total number of attempts and mean total number of head-dips. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Light/dark choice test

The test apparatus is based on that described by Misslin et al. (1989). It consisted of two polyvinylchloride boxes ($20 \times 20 \times 14$ cm) covered with Plexiglas. One of these boxes was darkened. A neon tube fixed on the ceiling provided the room illumination so that the light intensity in the centre of the illuminated box was 150 lux. An opaque plastic tunnel ($5 \times 7 \times 10$ cm) separated the dark box from the illuminated one. At the beginning of the experiment, a mouse was placed in the illuminated box, facing the tunnel. Recording started when the animal entered the tunnel for the first time. The apparatus was equipped with infrared beams and sensors capable

of recording the following three parameters during a 4-min period: (a) time spent by mice in the lit box; (b) attempt at entry into the lit box followed by avoidance responses. This includes stretched attend posture (the mouse stretches forward and retracts to original position); (c) total number of tunnel crossings. Data were analyzed with the Kruskal-Wallis test.

Free-exploration test

The procedure was a modification of the technique described in a previous paper (Griebel et al. 1993). 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 72 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 test 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) novel unit entries; (c) familiar unit entries; (d) attempts at entry into the novel compartment followed by avoidance responses. This included stretch attend posture. The results were expressed as mean percentage of time spent in the novel compartment, mean total number of novel unit changes, mean total number of familiar unit changes and mean total number of attempts. The experimenter was unaware of the drug treatment. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Mouse defense test battery (MDTB)

The procedure has been extensively described in a previous paper (Griebel et al. 1997b). 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 × 0.30 × 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. In addition, the apparatus was equipped with infrared beams and sensors capable of measuring the velocity of the animal during the chase/flight test. 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

Pre-test: 3-min familiarization period. A subject was placed into the runway for a 3-min. familiarization period, in which line crossings were recorded (min 1–3).

The rat avoidance test [min 4–6]. Immediately after the 3-min familiarization period, the experimenter introduced a hand-held dead rat (killed by CO_2 inhalation just before the beginning of the experiment) five times at one end of 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.

The chase test (min 7–8). The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. The number of stops (pause in movement) during the chase was recorded.

The straight alley test (min 9–11). After the chase was completed, the runway was converted to a straight alley by closing two doors (60 cm distant from each other). The dead rat was placed in one end of the straight alley and the number of approach/withdrawal responses (subject must move more than 0.2 m forward from the closed door, then return to it) was measured during a 30-s period. Stops and approach/withdrawal responses are described as risk assessment activities (Griebel et al. 1995a).

The forced contact test (min 12–13). Finally, the experimenter brought the rat up to contact the subject in the straight alley. For each such contact, defensive attack responses (i.e. bites) were noted. This was repeated three times.

Post-test: contextual defense. Immediately after the forced contact test, the rat was removed and the doors were opened. Escape attempts (wall rears, wall climbs, and jump escapes) were recorded during a 3-min session (min 14–16).

Data were analyzed with a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Results

Punished lever pressing

Figure 1 shows that the rates of responding decreased by the punishment contingency were significantly increased by diazepam ($\chi^2 = 10.9$, $P < 0.05$) at the doses of 2.5 and 5 mg/kg. In contrast, neither buspirone nor CP-154,526 produced any statistically significant increases in rates of punished responding. Unpunished responding was increased by diazepam at 1.25 and 2.5 mg/kg ($\chi^2 = 11.97$, $P < 0.05$) and decreased by buspirone at 2.5 mg/kg ($\chi^2 = 20.24$, $P < 0.001$).

Punished drinking test

Table 1 shows that diazepam (2.5–5 mg/kg) and buspirone (2.5 mg/kg) significantly increased the number of punished licks ($K = 16.3$, $P < 0.001$ and $K = 9.2$, $P < 0.05$, respectively). By contrast, CP-154,526 failed to modify significantly punished responding in this test.

Elevated plus-maze test

Figure 2 shows that diazepam (2 mg/kg) significantly increased both the percentage of time spent [$F(4,30) = 3.4$, $P < 0.05$] and the percentage of entries made

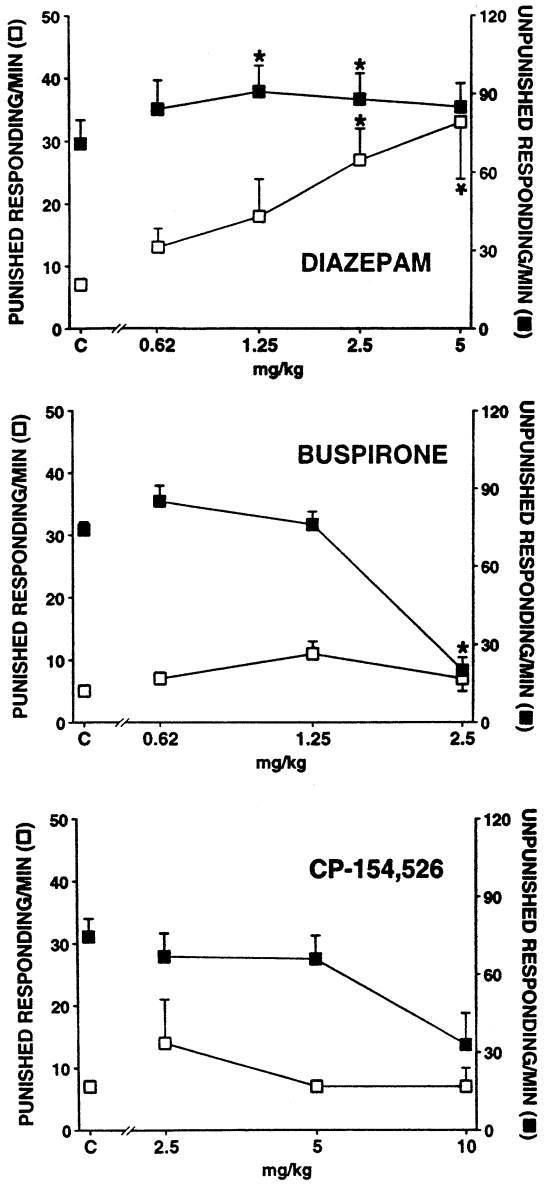

Fig. 1 Effects of diazepam ($n = 11$), buspirone ($n = 8$) and CP-154,526 ($n = 6$) on rates of punished and unpunished responding in rats. Drugs were administered IP 30 min before testing. Data represent mean ± SEM lever presses/min. $n = 6$–11 $^*P < 0.05$ (Friedman)

[$F(4,30) = 4.9$, $P < 0.01$] into open arms. By contrast, buspirone and CP-154,526 affected neither measure in a significant manner. With respect to the ethologically derived measures, diazepam reduced the number of attempts at entry into open arms followed by avoidance responses [$F(4,30) = 2.2$, $P < 0.05$], and increased directed exploration (head-dippings) [$F(4,30) = 6.3$, $P < 0.001$]. The latter measure remained unaffected by buspirone and CP-154,526, but the 5-HT_{1A} receptor agonist significantly reduced attempts from 1 to 4 mg/kg [$F(4,30) = 5.6$, $P < 0.01$). Closed arm entries remained unchanged in all groups, while the total number of arm entries was increased by 5 mg/kg CP-154,526 [$F(6,48) = 2.6$, $P < 0.05$) (Table 2).

Table 1 Effects of diazepam, buspirone and CP-154,526 in the punished drinking conflict test in rats. Data represent mean ± SEM. Drugs were administered IP 30 min before the beginning of the experiment. $n = 7$–10. $^*P < 0.05$ (Kruskal-Wallis test)

	Dose (mg/kg)	Number of shocks
Diazepam	0	6.4 ± 1.0
	1.25	15.7 ± 3.9
	2.5	21.9 ± 3.2*
	5	28.0 ± 5.2*
	10	24.8 ± 2.0*
Buspirone	0	7.4 ± 1.4
	1.25	15.5 ± 3.7
	2.5	17.0 ± 3.3*
	5	12.6 ± 2.3
	10	7.4 ± 2.2
CP-154,526	0	9.2 ± 2.1
	0.62	12.0 ± 3.6
	1.25	12.7 ± 3.3
	2.5	11.8 ± 2.5
	5	11.2 ± 3.9
	10	13.4 ± 3.1
	20	12.3 ± 3.5

Light/dark test

Figure 3 shows that diazepam (2.5 and 5 mg/kg) and CP-154,526 (10–40 mg/kg), but not buspirone, significantly increased time spent by mice in the lit box ($K = 38.2$, $P < 0.001$ and $K = 14.5$, $P < 0.01$, respectively). The number of attempts at entry into the lit box was significantly reduced by all compounds (diazepam: $K = 34.6$, $P < 0.001$; buspirone: $K = 12.7$, $P < 0.05$; CP-154,526: $K = 25.8$, $P < 0.001$). Diazepam (1.25–5 mg/kg) and CP-154,526 (10 and 20 mg/kg), but not buspirone significantly increased total number of tunnel crossings ($K = 26.1$, $P < 0.001$ and $K = 15.1$, $P < 0.01$, respectively).

Free-exploration test

Table 3 shows that diazepam (1 mg/kg), but not buspirone or CP-154,526 significantly increased the proportion of time spent in the novel units [$F(3,36) = 3.1$, $P < 0.05$] and the number of novel unit changes [$F(3,36) = 5$, $P < 0.01$]. Diazepam (1 and 2 mg/kg) [$F(3,36) = 4.2$, $P < 0.01$] and CP-154,526 (5 and 20 mg/kg) [$F(3,36) = 3.46$, $P < 0.05$], but not buspirone significantly decreased the number of attempts at entry into the novel units. The measure of general activity (familiar unit changes) was decreased by CP-154,526 [$F(3,36) = 9.18$, $P < 0.001$] at 20 mg/kg, but remained unchanged in all other groups.

The mouse defense test battery

Table 4 shows that diazepam significantly reduced the stimulus-subject distance at which avoidance occurred

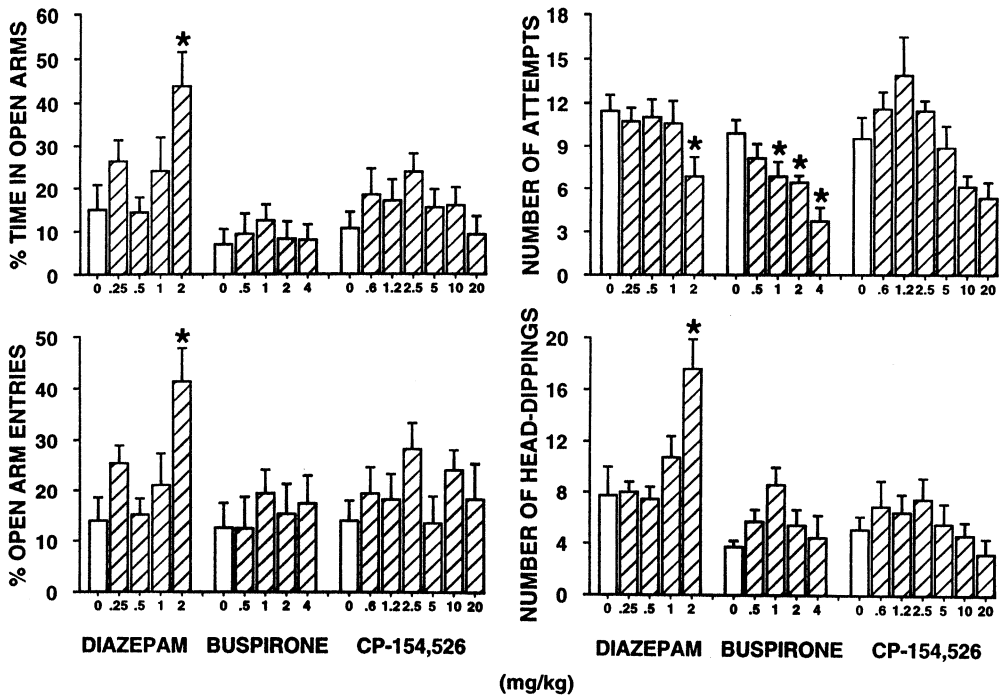

Fig. 2 Effects of diazepam, buspirone and CP-154,526 on the behavior of rats on the elevated plus-maze. Drugs were administered IP 30 min before testing. Data represent mean ± SEM. $n = 7–14$ *$P<0.05$ (Dunnett's t-test)

Table 2 Effects of diazepam, buspirone and CP-154,526 on measures of general activity in the elevated plus-maze test. Drugs were administered IP 30 min before testing. Data represent mean ± SEM. *$P < 0.05$

	Dose (mg/kg)	Total arm entries	Closed arm entries
Diazepam	0	13.0 ± 1.4	10.9 ± 0.7
	0.25	13.4 ± 1.0	9.9 ± 0.6
	0.5	14.7 ± 1.2	12.4 ± 0.9
	1	16.7 ± 2.6	12.7 ± 1.6
	2	16.3 ± 1.2	9.4 ± 1.1
Buspirone	0	8.4 ± 1.4	7.3 ± 1.3
	0.5	11.3 ± 1.3	9.6 ± 0.9
	1	11.9 ± 1.0	9.4 ± 0.6
	2	8.7 ± 2.0	7.0 ± 1.2
	4	5.4 ± 1.5	4.3 ± 1.2
CP-154,526	0	9.9 ± 0.8	8.4 ± 0.7
	0.6	10.9 ± 1.0	8.6 ± 0.6
	1.2	12.0 ± 0.7	9.7 ± 0.5
	2.5	12.9 ± 0.7	9.3 ± 0.9
	5	13.7 ± 0.3*	11.8 ± 0.8
	10	10.5 ± 0.9	8.0 ± 0.8
	20	9.8 ± 1.4	8.2 ± 1.5

[$F(3,28) = 4.81$, $P < 0.01$], the number of stops during the chase test [$F(3,36) = 15$, $P < 0.001$], and the frequency of defensive biting upon forced contact [$F(3,36) = 2.93$, $P < 0.05$]. The drug also increased the number of approaches followed by withdrawal responses in the straight alley [$F(3,36) = 3.94$, $P < 0.05$] and counteracted the potentiation of escape attempts from the runway cage after the removal of the rat [$F(3,36) = 28.24$, $P < 0.001$]. All these effects appear to be specific as indicated by the lack of significant effect of diazepam on the number of line crossings recorded before the exposure to the rat. CP-154,526 decreased avoidance distance [$F(3,31) = 8.32$, $P < 0.001$], stops [$F(3,36) = 3.35$, $P < 0.05$], bitings [$F(3,36) = 25.61$, $P < 0.001$] and post-rat escape attempts [$F(3,36) = 4.13$, $P < 0.05$], but did not affect approach/withdrawal responses and line crossings. Buspirone reduced bitings [$F(3,36) = 16.72$, $P < 0.001$] and escape attempts [$F(3,36) = 39.1$, $P < 0.001$], but failed to modify significantly the other defensive responses. At 5 mg/kg, buspirone also reduced the number of line crossings before the exposure to the rat stimulus [$F(3,36) = 12.78$, $P < 0.001$].

Discussion

The results of the present series of experiments showed that the non-peptide CRF receptor antagonist CP-154,526 failed to elicit anxiolytic-like effects in both conflict procedures and in the elevated plus-maze test in rats. By contrast, the drug produced some evidence of reduced anxiety-related responses in the light/dark test, the MDTB and, to a lesser extent, in the free-exploration test in mice. Overall, these behavioral effects of CP-154,526 in anxiety models differed from those observed with the BZ diazepam and the 5-HT_{1A} receptor partial agonist buspirone.

In the punished lever pressing and the punished drinking tests in rats, diazepam produced an increase in rates of responding suppressed by punishment. By contrast, neither buspirone nor CP-154,526 produced

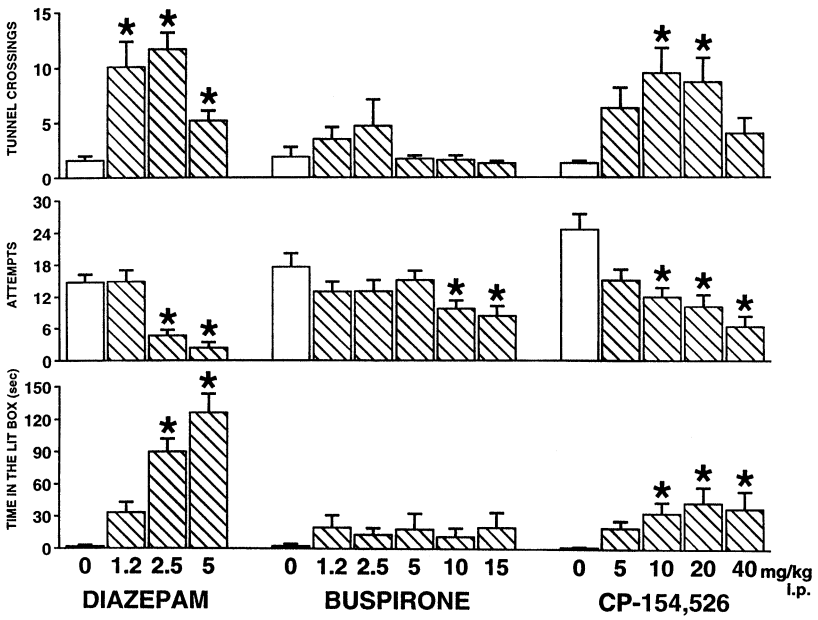

Fig. 3 Effects of diazepam, buspirone and CP-154,526 on the behavior of BALB/c mice exposed to the light/dark choice test. Drugs were administered IP 30 min before testing. Data represent mean ± SEM. $n = 15–16$. $^*P < 0.05$ (Kruskal-Wallis test)

Table 3 Effects of diazepam, buspirone and CP-154,526 on several behavioral responses displayed by BALB/c mice in the free-exploration test. Drugs were administered IP 30 min before the beginning of the experiment. Data represent mean ± SEM. $n = 10$. $^*P < 0.05$ (Dunnett's *t*-test)

	Dose (mg/kg)	% time in novel units	Novel unit changes	Familiar unit changes	Attempts
Diazepam	0	18.8 ± 8	18.9 ± 8.7	33.1 ± 5.6	23.4 ± 4.8
	0.5	25.8 ± 9	17.4 ± 5.9	22.0 ± 3.5	15.3 ± 3.4
	1	46.7 ± 6*	48.8 ± 6.5*	33.1 ± 3.8	6.8 ± 2.1*
	2	19.4 ± 7	16.7 ± 6.5	18.8 ± 5.6	9.7 ± 3.4*
Buspirone	0	20.6 ± 8	16.3 ± 6.5	21.8 ± 3.3	29.0 ± 7.6
	1.25	6.8 ± 7	5.3 ± 5.3	12.0 ± 3.1	23.8 ± 4.1
	2.5	10.8 ± 8	8.6 ± 5.8	17.2 ± 4.1	15.9 ± 2.4
	5	15.8 ± 8	8.3 ± 3.9	18.0 ± 1.9	19.0 ± 2.9
CP-154,526	0	19.0 ± 8	12.1 ± 5.3	24.6 ± 2.1	25.6 ± 4.9
	5	28.1 ± 9	26.6 ± 9.7	30.1 ± 5.4	14.7 ± 4.6*
	10	8.7 ± 6	4.5 ± 3.1	16.5 ± 2.7	24.8 ± 3.1
	20	11.5 ± 8	5.6 ± 3.9	7.2 ± 1.7*	10.9 ± 2.8*

Table 4 Effects of diazepam, buspirone and CP-154,526 on several behavioral responses displayed by Swiss mice before (*locomotor activity*), during (*flight, risk assessment* and *defensive attack*) and after (*contextual defense*) exposure to a Long Evans rat in the mouse defense test battery. Drugs were administered IP 30 min before the beginning of the experiment. Data represent mean ± SEM. $n = 10$. $^*P < 0.05$ (Dunnett's *t*-test)

	Dose (mg/kg)	Locomotor activity (line crossings)	Flight [avoidance distance (cm)]	Risk assessment		Defensive attack (bitings)	Contextual defense (escape attempts)
				Stops	Approaches-withdrawals		
Diazepam	0	125.5 ± 11.6	100.3 ± 8.5	10.0 ± 1.3	0.6 ± 0.2	1.5 ± 0.3	34.4 ± 10.9
	0.5	132.7 ± 14.3	70.7 ± 11.1	8.5 ± 1.3	2.4 ± 0.6*	0.7 ± 0.3	28.7 ± 9.1
	1	118.6 ± 12.7	71.1 ± 8.1	2.7 ± 0.9*	2.6 ± 0.6*	0.8 ± 0.3	15.7 ± 5.0*
	3	106.8 ± 12.7	53.4 ± 12.5*	1.7 ± 0.5*	1.6 ± 0.4	0.4 ± 0.2*	1.4 ± 0.4*
Buspirone	0	131.1 ± 12.5	108.3 ± 11.4	11.1 ± 0.9	0.3 ± 0.2	2.2 ± 0.2	35.9 ± 11.4
	1.25	102.8 ± 6.8	70.8 ± 10.1	10.1 ± 1.4	0.5 ± 0.2	0.9 ± 0.2*	28.9 ± 9.1*
	2.5	117.2 ± 9.3	81.8 ± 6.7	11.1 ± 0.9	0.1 ± 0.1	0.9 ± 0.3*	23.8 ± 7.5*
	5	54.9 ± 7.4*	71.9 ± 13.4	11.8 ± 2.2	0.1 ± 0.1	0.2 ± 0.1*	13.1 ± 4.4*
CP-154,526	0	137.2 ± 15.9	112.4 ± 10.0	11.7 ± 1.0	0.4 ± 0.2	2.8 ± 0.1	39.1 ± 12.4
	5	134.8 ± 7.2	66.9 ± 6.1*	8.3 ± 1.3*	0.7 ± 0.3	2.4 ± 0.3	27.4 ± 8.7*
	10	120.3 ± 10.5	73.1 ± 4.8*	11.1 ± 0.8	0.4 ± 0.3	0.0 ± 0.0*	28.7 ± 9.1*
	20	108.9 ± 11.7	68.6 ± 7.2*	8.4 ± 0.7*	1.2 ± 0.4	1.1 ± 0.4*	31.4 ± 9.9

a significant increase in punished responding in the lever pressing test, although a tendency to an increase was observed with buspirone at 1.25 mg/kg and with CP-154,526 at 2.5 mg/kg. Similar weak effects of buspirone have been found in an earlier study with this procedure (Sanger 1992). In the punished drinking procedure, buspirone but not CP-154,526 displayed anticonflict activity. The reason for the difference in behavioral profiles of buspirone in the two conflict tests is unclear. However, it is noteworthy that buspirone significantly reduced unpunished responding in the lever pressing test at the dose (2.5 mg/kg) which produced an increase in responding in the punished drinking test, suggesting that the anticonflict action may have been contaminated by motor impairment. Thus, it can be speculated that the lack of anticonflict effects of buspirone in the lever pressing test may be due to behavioral impairment. In the punished drinking test, one can assume that motor deficits interfered less with responding so that anticonflict effects were still detectable. The amount of data that has been accumulated on the effects of buspirone in conflict models is vast (Griebel 1995). Although several studies demonstrated that the drug produced anxiolytic-like effects, numerous reports failed to show a positive action of the compound in these tests. The reasons for this inconsistency in drug profiles have been extensively discussed in several review articles and are still a matter of debate (Barrett and Vanover 1993; De Vry 1995; Handley 1995). The lack of significant action of CP-154,526 on both punished and unpunished responses suggests that conflict models are of limited utility in the study of the behavioral action of CRF antagonists. These results corroborate findings from operant studies showing that ICV infusion of the peptide CRF antagonist α-helical CRF_{9-41} failed to produce a significant release of punished responding in a Geller-Seifter conflict test and did not reverse the suppression of responding produced by the conditioned stimulus presentation in a conditioned suppression task (Britton et al. 1986c; Koob 1991).

In the elevated plus-maze test in rats, diazepam produced significant effects on all anxiety-related measures. Thus, on traditional behavioral indices, it increased percentage of time spent in open arms and number of open arm entries. Regarding the ethologically derived measures, the BZ markedly decreased attempts and increased head-dippings. The $5\text{-}HT_{1A}$ partial agonist buspirone failed to modify both spatio-temporal measures and head-dippings, but significantly decreased attempts from 1 to 4 mg/kg. The behavioral profile of buspirone in this study somewhat contrasts with a recent finding from this laboratory showing that the drug increased the percentage of time spent in open arms and head-dipping (Griebel et al. 1997a). However, the use of a different administration route in the previous study (subcutaneous) may account for this discrepancy. The present results with buspirone provided further evidence that the incorporation of risk assessment (RA) measures (i.e. attempts) into the scoring of the plus-maze may be useful when screening drugs acting at $5\text{-}HT_{1A}$ receptors (Rodgers and Cole 1994; Griebel et al. 1997a). However, it is not clear yet whether an effect on RA only is indicative of a weak anxiolytic-like action or a behavioral disruption unrelated to anxiety. A recent factor analysis of spatio-temporal and ethological measures in the rat plus-maze showed that although RA (i.e. stretch attend behaviour) and conventional anxiety measures loaded on the same factor, the former also loaded on a separate factor thought to be related to more cognitively orientated aspects (i.e. decision-making) of anxiety (Cruz et al. 1994). This suggests that the marked effects of buspirone on RA may reflect modulation of a specific, perhaps more cognitively related, aspect of anxiety responses. Although no predictor symptoms have been clearly identified, there is suggestive clinical evidence that buspirone may more effectively treat the cognitive aspects of anxiety (Rickels et al. 1982). The CRF receptor antagonist CP-154,526, though tested over a wide dose-range (from 0.6 to 20 mg/kg), was devoid of significant effects on all indices of anxiety. This profile contrasts with that observed by Lundkvist et al. (1996) in the elevated plus-maze. These authors showed that CP-154,526 produced a specific increase in open arm exploration at 1 mg/kg but not at 3 or 10 mg/kg. This discrepancy cannot be attributed to differences in rat strains (Sprague-Dawley were used in both studies) or to administration route and pretreatment (similar in both studies). However, it is worth mentioning that in the present situation control rats spent about 11% of the total time in the open arms, whereas in the Lundkvist study baseline levels barely reached 4%. This may indicate that basal levels of stress or anxiety in control subjects were somewhat higher in the latter study, suggesting a greater production of CRF in these animals. As a consequence, we would predict greater efficacy of a CRF antagonist in reducing stress-related responses. Interestingly, findings from Adamec and colleagues (1991) have revealed that repeated handling altered the anxiolytic-like effects of the CRF antagonist α-helical CRF_{9-41} in the elevated plus-maze. More recently, two studies showed that α-helical CRF_{9-41} produced anxiolytic-like effects in the elevated plus-maze only after animals had been stressed by exposure to conspecific aggression (Heinrichs et al. 1992; Menzaghi et al. 1994). Taken together, these findings indicate that baseline levels of stress are of crucial importance when investigating the behavioral action of CRF antagonists in the elevated plus-maze test and call for more careful attention of experimental stress when such studies are carried out.

Previous studies with the light/dark choice task and the free-exploration test demonstrated that the administration of BZs (e.g. alprazolam, chlordiazepoxide) increased time spent by BALB/c mice in the aversive parts of the apparatus (i.e. illuminated box and novel

units, respectively) and locomotor activity (i.e. number of tunnel crossings and novel unit changes), while decreasing aborted attempts at entry in the aversive areas, a profile which is consistent with an anxiolytic-like action (Griebel et al. 1993, 1996b). The results obtained in this study with diazepam agree with these data, as the drug displayed clear anxiolytic-like effects in both procedures. Buspirone did not modify the spatio-temporal measures in either test but decreased attempts at 10 and 15 mg/kg in the light/dark test. However, it is not clear whether this action is indicative of a specific decrease in anxiety or is secondary to behavioral impairment since baseline levels of the presumed measure of motor activity (i.e. tunnel crossings) were too low (less than two crossings) to be decreased further. CP-154,526 showed clear evidence for reduced anxiety-related responses in the light/dark test. The drug affected both indices of anxiety and increased tunnel crossings over a wide dose-range (from 10 to 40 mg/kg). However, the magnitude of these effects was less than that of diazepam. In the free-exploration test, CP-154,526 modified RA responses only. The drug reduced attempts at 5 and 20 mg/kg. However, at this latter dose, the drug also reduced familiar unit changes suggesting that the effects may have been confounded by behavioral suppression.

BALB/c mice are described as "emotional" animals (Robertson 1979; Peeler and Nowakowsky 1987; Makino et al. 1991; Beuzen and Belzung 1995). For example, Makino et al. demonstrated that BALB/c mice showed strong and long-lasting stretching immediately after their introduction into an open-field, while C57BL/6 and DBA/2 mice never displayed such behavior. Instead, they immediately started to move around. These authors interpretated their findings in terms of "emotional arousal", with the BALB/c strain being more "anxious" than the two other lines. More recently, several studies, using the light/dark choice test and/or the free-exploration procedure confirmed that these tests represent for BALB/c mice more stressful situations when compared with other strains (e.g. C57BL/6, DBA/2) (Griebel et al. 1993; Beuzen and Belzung 1995). The present data are in line with these findings as they showed that, in the light/dark test, control animals spent less than 1% of the total time in the illuminated box. Thus, if BALB/c mice are genetically predisposed to increased behavioral responsivity to stress, and if endogenous CRF has a role in this predisposition, the present results with CP-154,526 in the light/dark test would be expected. Similar findings have been reported by Conti et al. (1994), who showed that α-helical CRF_{9-41} was more efficacious and more potent in BALB/c mice than in three other strains (i.e. NIH Swiss, CF-1, CD) in the elevated plus-maze. The weak activity of CP-154,526 in the free-exploration procedure may be explained by the fact that this test is devoid of intrinsic stressful elements (Misslin and Cigrang 1986). Baseline levels of time spent in the novel units reached about 20% of the total time in all groups, indicating that this procedure represents a less stressful situation than the light/dark test. Assuming that basal release of CRF is less in animals exposed to the free-exploration test, we would predict weaker effects of a CRF antagonist.

In the MDTB, diazepam clearly affected all defensive responses, thereby confirming previous findings from this test battery on the sensitivity of specific defense responses to BZ receptor ligands (Griebel et al. 1995a,1996c). Prominent effects of diazepam were observed on RA activities. Thus, the drug reduced RA (i.e. stops) when subjects were chased by the rat and increased RA (i.e. approaches followed by withdrawal responses) in the straight alley situation. Furthermore, diazepam reduced flight responses (i.e. avoidance distance when the threat stimulus was first placed into the runway apparatus) and defensive attack reactions upon forced contact with the rat and, finally, prevented the increase in escape attempts following the removal of the rat from the runway apparatus. Buspirone reduced defensive attack reactions and contextual defense at all doses (1.25–5 mg/kg), but did not significantly affect any of the other defensive behaviors, although it is noteworthy that avoidance distance was somewhat reduced over the entire dose-range. Importantly, the effects observed at the highest dose of buspirone may have been contaminated by behavioral impairment as spontaneous locomotor activity was reduced during the pretest. Overall, results obtained with buspirone in the MDTB are in agreement with those observed with two other 5-HT_{1A} receptor agonists (8-OH-DPAT and gepirone) in this test (Griebel et al. 1995b). These compounds reduced defensive attack and contextual defense, while they did not specifically modify the other defensive reactions. CP-154,526 reduced flight, RA during the chase test, defensive biting and contextual defense, but failed to affect RA activities in the straight alley test. In addition, it is important to note that for some undetermined reasons the effects of CP-154,526 were not dose-dependent.

Swiss mice are among the most aggressive laboratory strains with reference to both offensive and defensive forms of intraspecific attack (Parmigiani et al. 1989). When tested in the MDTB, Swiss mice were found to display higher levels of avoidance responses and RA activities than other strains (i.e. C57BL/6, CBA) but they showed similar pattern of defensiveness than BALB/c mice (Griebel et al. 1997b). In addition, the MDTB appears to be particularly stressful for animals, since they have no possibility to escape from the runway cage and confrontation with the threat stimulus is unavoidable. Thus, assuming that the CRF system contributes significantly to the emotional responses displayed by Swiss mice in this test battery, we would expect a CRF antagonist to attenuate these reactions.

Importantly, the fact that CP-154,526 modifies the behavioral responses in certain but not all paradigms

that are also sensitive to the BZ diazepam does not necessarily imply that the CRF receptor antagonist possesses anxiolytic-like properties. It is possible that CP-154,526 alters behavioral and/or cognitive processes associated with stress or arousal, but not directly related to anxiety, and that positive effects in the mouse tests reflect alterations in these non-anxiety processes. However, in the case of the MDTB, the extensive behavioral and pharmacological evaluation of this test has demonstrated that the defensive behaviors elicited in mice by the exposure to a natural threat stimulus may relate to certain aspects of human anxiety (Griebel et al. 1995a,b, 1996a,c,d, 1997c). For example, it has been shown that clinically effective anti-panic drugs (e.g. clonazepam, diazepam, chronic alprazolam, imipramine, fluoxetine, moclobemide) specifically reduce animals' flight responses. Notably, avoidance responses when the rat is placed into the runway appear to be particularly sensitive to such drug treatment. Anti-GAD agents such as other BZ receptor ligands (e.g. chlordiazepoxide) and 5-HT_{1A} receptor agonists (e.g. gepirone) either failed to affect flight responses or had inconsistent effects. However, these compounds affected RA, defensive threat/attack reactions and escape attempts, thereby suggesting that these defense responses more likely relate to certain aspects of GAD. Taken together with the present results, these latter findings suggest that CP-154,526 may have some efficacy in the clinical management of both GAD and panic attacks.

In summary, the results of the present experiments indicate that the non-peptide CRF antagonist CP-154,526 is devoid of activity in traditional conflict procedures and in the elevated plus-maze test in rats. By contrast, the compound reduced anxiety-related responses in the light/dark test, the MDTB and, to a lesser extent, in the free-exploration test. It is suggested that positive effects in the mouse models may be due to increased sensitivity to environmental stress of the strains used and/or to the fact that animals are exposed to unavoidable stress stimuli which may lead to a significant activation of the CRF system.

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