Behavioural Pharmacology (1997), **8**, 549–560

CCK receptor antagonists in animal models of anxiety: comparison between exploration tests, conflict procedures and a model based on defensive behaviours

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The present experiments compared the behavioural effects of one cholecystokinin$_A$ (CCK_A; lorglumide) and two CCK_B (PD 135,158 and LY 288513) receptor antagonists in classical animal models of anxiety, including conflict tests (punished lever pressing and Vogel drinking tests in rats) and exploratory models (elevated plus-maze test in rats and light/dark choice test in mice), and a recently developed mouse defence test battery (MDTB) which has been validated for the screening of both anti-panic and classical anxiolytic (i.e. benzodiazepines) drugs. Diazepam was used as a positive control. Results showed that all three CCK receptor antagonists were inactive in both conflict tests. Furthermore, despite the incorporation of more ethologically-derived measures (i.e. risk assessment activities or directed exploration, or both) no effects were observed in the elevated plus-maze and in the light/dark tests. These profiles contrast with that of diazepam which displayed clear anxiolytic-like effects in these models. In the MDTB, the CCK receptor antagonists failed to modify parameters (i.e. risk assessment, defensive threat/attack and escape attempts), which have been shown to be particularly sensitive to drugs effective in the treatment of generalized anxiety. By contrast, the CCK_B receptor antagonists PD 135,158 (0.001–0.01, 1 mg/kg, i.p.) and LY 288513 (1 and 3 mg/kg, i.p.) significantly decreased avoidance distance when the rat was first placed in the test apparatus, an effect which is consistent with an anti-panic-like action. Overall, these findings support the idea that classical animal models of anxiety may not be suitable for evaluation of the behavioural effects of CCK receptor antagonists, whereas tests which may model certain aspects of human panic such as the MDTB appear to be more reliable tools when screening such compounds.

Keywords: Animal models – Anxiety – CCK antagonists – Conflict tests – Defensive behaviours – Exploration models – Lorglumide – LY 288513 – Mouse – Panic – PD 135,158 – Rat

INTRODUCTION

The first report of a possible involvement of the neuropeptide cholecystokinin (CCK) in the aetiology of anxiety was published nearly 20 years ago by Della-Fera and Baile (1979), who observed that intracerebroventricular infusion of the CCK_B receptor agonist pentagastrin in sheep produced behavioural modifications indicative of increased fear. Subsequent experiments with pentagastrin and other CCK_B receptor agonists, including CCK fractions such as CCK-4 and CCK-8s, confirmed the anxiogenic-like effects of these compounds (for a recent review, see Van Megen *et al.*, 1996). These preclinical findings have prompted several research groups to study the effects of CCK_B receptor agonists in humans, and there is now clinical evidence that systemic administration of these agents elicits panic-like symptoms in healthy volunteers, and potentiates the occurrence of panic attacks in panic disorder (PD) patients (for review, see Van Megen *et al.*, 1996).

In recent years, specific and highly potent antagonists for CCK receptors have been discovered and developed (Woodruff and Hughes, 1991). These compounds were found to abolish the anxiogenic-like effects of CCK receptor stimulation in rodents. For example, several authors demonstrated that the CCK_B receptor antagonists L-365,260 and CI-988 attenuated the effects of CCK-4 in the elevated plus-maze test (Harro and Vasar, 1991; Singh *et al.*, 1991; Derrien *et al.*, 1994; Rex *et al.*, 1994). Similarly, human studies revealed that L-365,260 was able to block the CCK-4-elicited panic attacks in PD patients (Bradwejn *et al.*, 1994) and reversed the pentagastrin-induced symptoms of anxiety in healthy volunteers (Lines *et al.*, 1995).

Anxiolytic-like effects of CCK receptor antagonists *per se* (without previous defined anxiogenic challenge) are, however, not always observed in animals. Although several authors showed that CI-988 displayed

anxiolytic-like effects comparable to those of the benzodiazepines (BZ) in various animal models, including the elevated plus-maze, the social interaction test, the human threat model and the light/dark test (Hughes *et al.*, 1990; Costall *et al.*, 1991; Singh *et al.*, 1991; Hinks *et al.*, 1996), others, using mostly conflict tests, either reported less robust effects than with BZs (Powell and Barrett, 1991; Dooley and Klamt, 1993) or failed to show an anxiolytic-like response (Bickerdike *et al.*, 1994; Charrier *et al.*, 1995; Dawson *et al.*, 1995; Molewijk *et al.*, 1996). Similarly, variable results were observed with L-365,260 (Rataud *et al.*, 1991; Chopin and Briley, 1993; Hendrie *et al.*, 1993; Rex *et al.*, 1994; Vasar *et al.*, 1994; Josselyn *et al.*, 1995; Johnson and Rodgers, 1996; Matto *et al.*, 1996). The reasons for this inconsistency in drug effects are not yet clear. It has been suggested that models based on spontaneous or exploratory behaviours are more suitable for the investigation of CCK receptor antagonists than tests based on punished responses (Bourin *et al.*, 1996; Van Megen *et al.*, 1996). These compounds have, however, been reported to have anxiolytic-like effects or no effect in both types of paradigms. Alternatively, it was argued that classical animal models of anxiety are less sensitive to the action of CCK compounds, which may be involved in a type of anxiety that is not assessed in these tests (Charrier *et al.*, 1995; Jenck *et al.*, 1996; Johnson and Rodgers, 1996). Most of these tests have been validated pharmacologically by BZs, which represent the first-choice treatment in generalized anxiety disorders (GAD), and this raises the question of whether routine models are suitable to screen for putative anti-panic agents such as CCK receptor antagonists.

In this context, the present experiments aimed at comparing the effects of several CCK receptor antagonists under identical test conditions in classical animal models of anxiety, including conflict procedures (punished lever pressing and Vogel drinking tests in rats) and exploratory models (elevated plus-maze test in rats and light/dark choice test in mice), and in a recently developed mouse defence test battery (MDTB) which was found to be useful for the screening of both anti-panic and anti-GAD drugs (Griebel *et al.*, 1995, 1996a,c). In addition, a more ethological-orientated scoring method was used with the elevated plus-maze and the light/dark choice 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.*, 1997b). The drugs used were the CCK_A receptor antagonist, lorglumide (Woodruff and Hughes, 1991) and two highly selective CCK_B receptor antagonists, PD 135,158 (Hughes *et al.*, 1990) and LY 288513 (Helton *et al.*, 1996). They were chosen on the basis of results in animal models of anxiety (e.g. Belzung *et al.*, 1994; Charrier *et al.*, 1995; Helton *et al.*, 1996; Izumi *et al.*, 1996; Johnson and Rodgers, 1996). Diazepam was used as a positive control.

METHODS

Subjects

Male Wistar rats (Charles River, Saint-Aubin-les-Elbeuf, France) 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, France and Charles River) weighing 180–230 g at the 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 threat stimulus in the MDTB. BALB/c mice (7 weeks old) and male Swiss mice (10 weeks old) (both supplied by Iffa Credo) were used in the light/dark test and in the MDTB, respectively. 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 before 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 07.00 h. Rats used in the punished lever pressing procedure were restricted to the food obtained during sessions and a daily ration of 15–20 g of 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 one or two drops of Tween 80. They were injected in a volume of 2 ml/kg (rats) or 20 ml/kg (mice). The drugs used were PD 135,158 (4-{[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[[1.7.7-trimethyl-bicyclo[2.2.1]hept-2-yl)oxy] carbonyl]amino]pro-pyl]amino]-1-phenylethyl] amino-1-oxo-[1S-1α.2β[S*(S*)]}4α]butanoate N-methyl-D-glucamine (bicyclo system 1S-endo)) (RBI, Natick, USA), LY 288513 ((4S,5R)-N-(4-bromophenyl)-3-oxo-4,5-diphenyl-1-pyrazolidinecarboxamide) (Eli Lilly and Co., Indianapolis, USA), lorglumide sodium salt (D,L-4-(3,4-dichlorobenzoyl-amino)-5-(diphentyl-amino)-5-oxo-pentanoic acid sodium salt (RBI, Natick) and diazepam (synthesized by the department of chemistry, Synthélabo Recherche).

Drugs were given i.p. 30 min before experiments. Testing was performed between 09.00 and 15.00 h. Doses are expressed as the bases. Doses of diazepam were chosen on the basis of previous results with the drug in the tests employed in this study (Griebel *et al.*, 1996d,e) and doses of the CCK antagonists were chosen on the basis of findings with these compounds in published studies (Costall *et al.*, 1991; Charrier *et al.*, 1995; Helton *et al.*, 1996; Izumi *et al.*, 1996; Johnson and Rodgers, 1996).

Punished lever pressing

This procedure has been described previously (Sanger, 1995). Animals were tested in a standard rat operant test chamber (MED Associates Inc., Georgia, USA) placed in sound-attenuated boxes that were well ventilated. 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). A total of 11 rats were trained initially to press a lever for food reward (45 mg precision food pellets; PJ Noyes Inc., Lancaster, NH, 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 1 min 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.

Injections were given once or twice each week with at least two non-drug days intervening between two administrations. Vehicle was injected on all non-drug days. Drugs and doses were given in a mixed order. 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. Drug effects were thus analysed 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 before 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 came into 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. Because of a great inter-individual variability, results were analysed by the non-parametric Kruskal–Wallis (CCK compounds) or the Wilcoxon two-sample test (diazepam).

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 the 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 ledge of an open arm and down towards the floor (this response can occur while the animal's body is in the closed arms, central square or on the open arms). The results were expressed as mean ratio of time spent in the open arms to total time spent in both the open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of closed arm entries, mean total number of attempts and mean total number of head-dips. Data with the CCK compounds were analysed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test. Data from the diazepam experiment were analysed by a Student'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 box. 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 parameters during a 4 min period: (a) Entry into the lit box. Results were expressed as the total number of mice which entered the lit box and analysed by a Chi-square-independence (χ^2) test; (b) Attempts 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) Tunnel crossings: this parameter was recorded in order to evaluate general motor activity in the same context as the anxiety measures. It must be emphasized, however, that the total number of tunnel crossings may also be an element of anxiety. Data with the CCK compounds were subjected to a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using a Dunnett's *t*-test. Data from the diazepam experiment were analysed by a Student's *t*-test.

Mouse Defence Test Battery (MDTB)

The procedure has been extensively described in a previous paper (Griebel *et al.*, 1997c). 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 ($2.0 \times 0.30 \times 0.06$ 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. The test consisted of the following elements:

(a) Pre-test: 3 min familiarization period. A subject was placed into the runway for a 3 min familiarization period, in which line crossings, wall rears, wall climbs, and jump escapes were recorded (min 1 to 3);
(b) Effects on flight responses: the rat avoidance test (min 4 to 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;
(c) Effects on risk assessment: the chase (min 7 to 8) and the straight alley (min 9 to 11) tests. 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. After the chase was completed, the runway was converted to a straight alley by closing two doors (60 cm distant from each other). The 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.*, 1995);
(d) 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 in the straight alley. For each such contact, defensive threat and attack responses (i.e. bites and

upright postures) were noted. This was repeated three times;

(e) Post-test: contextual defence: 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 to 16).

Data with the CCK compounds were analysed with a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test. Data from the diazepam experiment were analysed by a Student's *t*-test. Pre- versus post-test differences were evaluated by a paired Student'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 [$\chi^2 = 19.78$, $p < 0.001$] at the doses of 2.5 and 5 mg/kg. By contrast, the CCK receptor antagonists did not produce any statistically significant increases in rates of punished responding. Unpunished responding was increased by diazepam at 1.25 mg/kg [$\chi^2 = 11.16$, $p < 0.05$] (Table I).

Vogel drinking test

Table II shows that diazepam (5 mg/kg) significantly increased the number of punished licks [$\chi^2 = 2.13$, $p < 0.05$]. By contrast, Kruskal–Wallis analysis revealed that all three CCK receptor antagonists failed to modify significantly punished responding in this test.

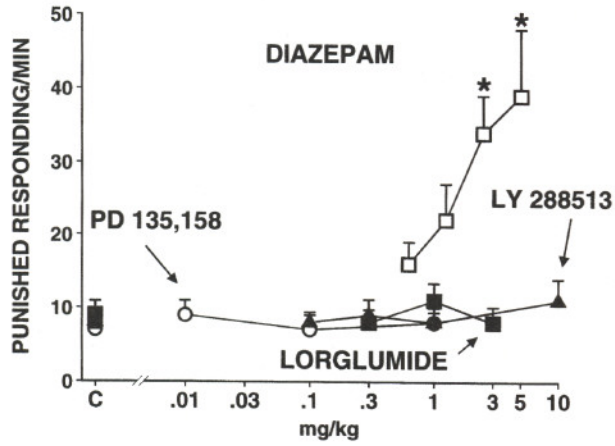

FIG. 1. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on rates of punished responding in rats. Drugs were administered i.p. 30 min before testing. Diazepam was used as a positive control. Data represent mean ± SEM. $n = 7$–14. *$p < 0.05$ (Friedman).

TABLE I. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on rates of unpunished responding in rats

	Dose (mg/kg)	Unpunished responding/min
Diazepam	0	62 ± 5
	0.6	73 ± 7
	1.25	82 ± 8*
	2.5	77 ± 9
	5	53 ± 11
Lorglumide	0	77 ± 6
	0.3	76 ± 15
	1	75 ± 9
	3	75 ± 5
PD 135,158	0	72 ± 7
	0.01	61 ± 11
	0.1	77 ± 9
	1	75 ± 9
LY 288513	0	73 ± 5
	0.1	64 ± 3
	0.3	76 ± 5
	1	74 ± 5
	10	72 ± 11

Drugs were administered i.p. 30 min before testing. Diazepam was used as a positive control. Data represent mean ± SEM. $n = 7$–14. *$p < 0.05$ (Friedman).

TABLE II. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) in the Vogel drinking conflict test in rats

	Dose (mg/kg)	Number of shocks
Diazepam	0	6.3 ± 1.0
	5	20.0 ± 4.6*
Lorglumide	0	4.4 ± 0.4
	0.3	6.4 ± 1.4
	1	7.4 ± 1.2
	3	11.0 ± 4.5
	10	5.4 ± 0.7
PD 135,158	0	7.7 ± 0.8
	0.001	10.7 ± 2.5
	0.01	12.4 ± 2.3
	0.1	12.2 ± 3.1
	1	12.8 ± 3.4
LY 288513	0	6.9 ± 0.9
	0.3	3.9 ± 0.5
	1	13.5 ± 2.9
	3	8.3 ± 2.1
	10	14.1 ± 3.8

Diazepam was used as a positive control. Drugs were administered i.p. 30 min before the beginning of the experiment. Data represent mean ± SEM. $n = 8$–10. *$p < 0.05$ (Wilcoxon two-sample test).

Elevated plus-maze test

Figure 2 shows that diazepam significantly increased both the percentage of time spent [$T = -3.78$, $p < 0.01$] and the percentage of entries made [$T = -2.92$, $p < 0.05$] into the open arms. By contrast, CCK receptor antagonists 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 [$T = 5.75$, $p < 0.001$], and increased directed exploration (head-dippings) [$T = -4.60$, $p < 0.001$]. Both measures remained unaffected by the CCK receptor antagonists. The measure of general activity (closed arm entries) remained unchanged in all groups (Table III).

Light/dark test

In the diazepam experiment, statistical analysis indicated a significant increase in the number of mice that entered the lit box [$\chi^2 = 10.67$, $p < 0.05$] and in the number of tunnel crossings [$T = -2.54$,

TABLE III. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on a measure of general activity in the elevated plus-maze test in rats

	Dose (mg/kg)	Number of closed arm entries
Diazepam	1	7.7 ± 1.6
	1.5	9.6 ± 1.3
Lorglumide	0	10.6 ± 1.4
	0.3	8.4 + 1.0
	1	8.7 ± 0.6
	3	10.3 ± 1.3
PD 135,158	0	9.0 ± 0.7
	0.01	9.1 ± 0.9
	0.1	8.3 ± 0.8
	1	10.1 ± 0.9
LY 288513	0	8.6 ± 1.1
	0.3	8.6 ± 0.9
	1	10.3 ± 0.7
	3	9.1 ± 0.4
	10	10.9 ± 0.8

Drugs were administered i.p. 30 min before testing. Diazepam was used as a positive control. Data represent mean ± SEM. $n = 7$–14.

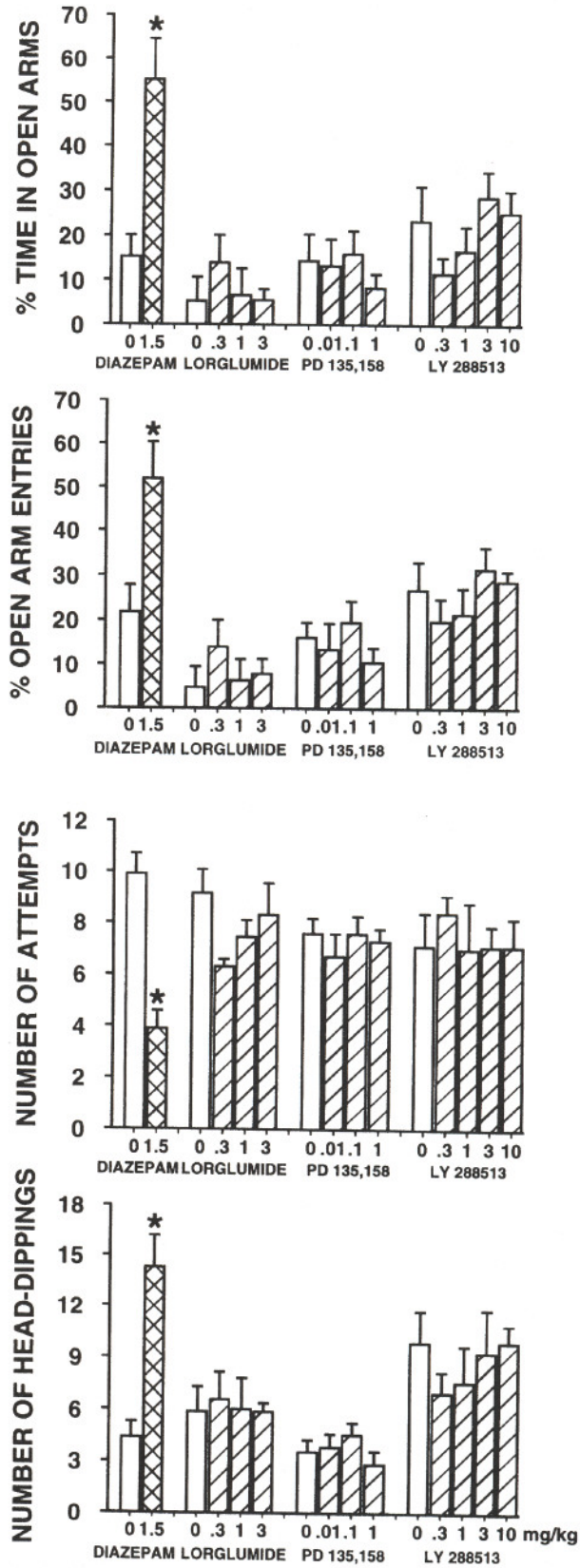

FIG. 2. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on the behaviour of rats on the elevated plus-maze. Drugs were administered i.p. 30 min before testing. Diazepam was used as a positive control. Data represent mean ± SEM. $n = 7$–14. *$p < 0.05$ (Student's *t*-test).

$p < 0.05$], and a decrease in the risk assessment measure (number of aborted attempts) [T = 4.27, $p < 0.001$] (Table IV). None of the parameters was significantly affected by the CCK receptor antagonists.

The mouse defence test battery

Diazepam significantly reduced the stimulus-subject distance at which avoidance occurred [T = 5.52, $p < 0.001$] (Fig. 3), the number of stops during the chase test [T = 4.10, $p < 0.001$], and the frequency of defensive threat and attack responses upon forced contact [upright postures: T = 2.74, $p < 0.05$; bitings: T = 3.79, $p < 0.01$]. This drug also increased the number of approaches followed by withdrawal responses in the straight alley [T = −3, $p < 0.01$] (Table V) and counteracted the potentiation of escape attempts from the runway cage after the removal of the rat [T = −2.2] (Fig. 4). All these effects appear to be

TABLE IV. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) in the light/dark test with BALB/c mice

	Dose (mg/kg)	n =	No. of mice that entered lit box	Attempts	Tunnel crossings
Diazepam	0	12	2	15 ± 2.1	3.6 ± 2.0
	2.5	12	10*	4.0 ± 1.0†	10.6 ± 1.6†
Lorglumide	0	12	1	20.1 ± 2.7	2.4 ± 1.4
	1	10	1	15.7 ± 2.6	2.0 ± 1.0
	3	10	1	21.1 ± 2.4	1.8 ± 0.8
	10	10	1	19.5 ± 1.7	2.0 ± 1.0
PD 135,158	0	12	4	20.0 ± 2.5	3.9 ± 1.5
	0 01	12	1	16.8 ± 2.0	1.2 ± 0.1
	0.1	12	3	14.3 ± 2.0	3.1 ± 1.2
	1	12	3	11.5 ± 2.1	2.2 ± 0.7
	3	12	2	14.5 ± 2.4	2.2 ± 0.8
LY 288513	0	12	1	25.3 ± 2.5	2.0 ± 1.0
	0.1	10	1	17.1 ± 2.4	1.1 ± 0.2
	0.3	10	1	18.3 ± 2.7	1.5 ± 0.6
	1	12	1	21.7 ± 3.1	1.0 ± 0.0
	3	12	1	30.8 ± 2.9	1.3 ± 0.3
	10	12	1	31.1 ± 4.4	1.2 ± 0.2

Diazepam was used as a positive control. Drugs were administered i.p. 30 min before the beginning of the experiment. Data represent mean ± SEM. *$p < 0.05$ (χ^2); †$p < 0.05$ (Student's t-test).

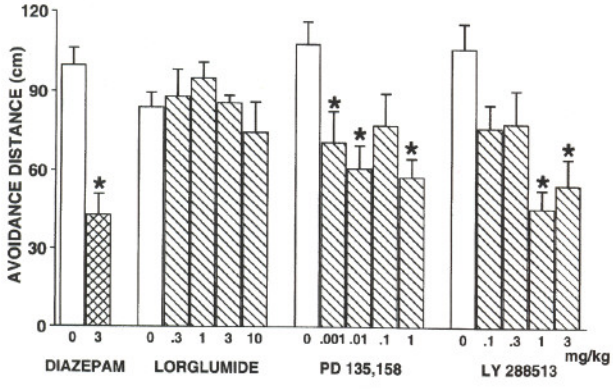

FIG. 3. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on flight reactions of Swiss mice approached by a Long–Evans rat in the mouse defence test battery. Data represent mean ± SEM. $n = 8$–11. *$p < 0.05$ (Dunnett's t-test (CCK compounds) or Student's t-test (diazepam).

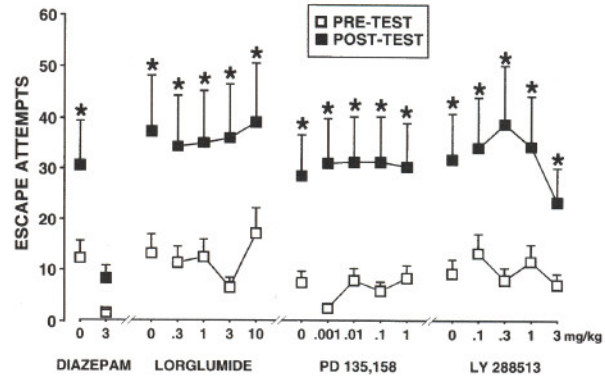

FIG. 4. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on escape attempts of Swiss mice from the runway cage before (open symbols) and after (solid symbols) the exposure to a Long–Evans rat. Diazepam was used as a positive control. Data represent mean ± SEM. $n = 8$–11. *$p < 0.05$ (versus open symbol).

TABLE V. Effects of one CCK_A receptor antagonist (lorglumide) and two CCK_B receptor antagonists (PD 135,158 and LY 288513) on several behavioural responses displayed by Swiss mice before (locomotor activity) and during (risk assessment and defensive threat/attack) exposure to a Long–Evans rat in the Mouse Defence Test battery

		Locomotor activity	Risk assessment		Defensive threat/attack	
	Dose (mg/kg)	Line crossings	Stops	Approaches -withdrawals	Upright postures	Bites
Diazepam	0	113 ± 15.3	11.0 ± 2.0	0.6 ± 0.4	2.6 ± 0.4	1.6 ± 0.3
	3	94.6 ± 12.9	2.4 ± 0.6*	3.1 ± 0.7*	1.1 ± 0.4*	0.3 ± 0.2*
Lorglumide	0	114.8 ± 20.0	11.1 ± 0.9	0.8 ± 0.3	2.3 ± 0.4	1.8 ± 0.4
	0.3	126.6 ± 14.2	11.0 ± 1.0	0.6 ± 0.3	2.6 ± 0.4	1.9 ± 0.4
	1	116.4 ± 8.8	10.5 ± 1.0	1.5 ± 0.3	2.9 ± 0.1	2.0 ± 0.3
	3	110.0 ± 6.7	8.5 ± 0.9	1.6 ± 0.4	3.0 ± 0.1	2.0 ± 0.3
	10	127.8 ± 6.1	10.1 ± 0.8	1.3 ± 0.8	2.9 ± 0.1	1.8 ± 0.4
PD 135,158	0	123.9 ± 5.1	14.5 ± 1.0	0 5 ± 0.2	2.6 ± 0.2	2.5 ± 0.3
	0.001	107.1 ± 11.0	9.7 ± 1.1	1.3 ± 0.4	2.3 ± 0.4	1.9 ± 0.4
	0.01	122.7 ± 14.4	9.3 ± 1.3	0.9 ± 0.5	1.9 ± 0.4	1.5 ± 0.4
	0.1	105.3 ± 12.1	11.5 ± 2.3	0.5 ± 0.2	2.4 ± 0.4	2.1 ± 0.3
	1	129.5 ± 15.4	11.7 ± 1.7	0.5 ± 0.3	2.3 ± 0.3	1.9 ± 0.4
LY 288513	0	141.1 ± 13.1	10.7 ± 1.1	1.1 ± 0.3	2.9 ± 0.1	2.4 ± 0.2
	0.1	128.1 ± 12.3	10.4 ± 1.6	0.8 ± 0.3	2.6 ± 0.4	1.8 ± 0.4
	0.3	130.1 ± 14.9	11.3 ± 1.8	1.3 ± 0.9	3.0 ± 0.0	2.3 ± 0.3
	1	128.0 ± 14.1	12.1 ± 1.8	0.6 ± 0.3	3.0 ± 0.0	2.3 ± 0.3
	3	116.8 ± 15.8	9.3 ± 1.4	0.5 ± 0.4	2.6 ± 0.4	1.5 ± 0.5

Diazepam was used as a positive control. Drugs were administered i.p. 30 min before the beginning of the experiment. Data represent mean ± SEM. $n = 8$–11. $^*p < 0.05$ (Student's t-test).

specific, as indicated by the lack of significant effect of diazepam on the number of line crossings recorded before exposure to the rat (Table V). As shown in Fig. 3, both CCK_B antagonists, but not the CCK_A antagonist lorglumide, decreased avoidance distance [PD 135,158: $F(4,36) = 3.22$, $p < 0.05$; LY 288513: $F(4,38) = 6.54$, $p < 0.001$]. PD 135,158 reduced this measure at all doses, although at 0.1 mg/kg this effect did not reach statistical significance, whereas the effect of LY 288513 was significant at 1 and 3 mg/kg. None of the other behavioural responses was significantly modified by any of the CCK compounds.

DISCUSSION

This study used for the first time classical exploratory- and conflict-based tests, together with a recently designed test battery validated for the screening of both anxiolytic and anti-panic compounds, to evaluate the behavioural effects of CCK receptor antagonists. The results showed that lorglumide, PD 135,158 and LY 288513 failed to produce any significant anxiolytic-like effects in more traditional models, whereas, in the MDTB, the CCK_B receptor antagonists PD 135,158 and LY 288513 displayed a profile which may be consistent with an anti-panic-like action.

In the punished lever pressing and the Vogel drinking conflict tests, in rats, none of the CCK compounds increased punished responding. This was in contrast to the BZ anxiolytic diazepam, used as a positive control in this study, which produced an increase in rates of responding suppressed by punishment. The lack of effect of the CCK compounds in the present study differs from previous reports which showed that two other CCK_B receptor antagonists, CI-988 and L-365,260 were active in procedures using electric shock punishment (Powell and Barrett, 1991; Singh *et al.*, 1991; Dooley and Klamt, 1993). Lorglumide, PD 135,158 and LY 288513 have all been found to have central effects (e.g. anxiolytic-like, potentiation of anti-convulsant effects of diazepam, neuronal excitability) after peripheral administration (Panerai *et al.*, 1987; Hughes *et al.*, 1990; Liu *et al.*, 1994; Harro *et al.*, 1995; Popoli *et al.*, 1995; Izumi *et al.*, 1996). Therefore, it is unlikely that the present negative findings can be attributed to poor bioavailability or brain penetration, or both. Similarly, the lack of effects cannot be attributed to dose range as doses used in this study

overlapped with those from previous studies with these compounds. Importantly, the magnitude of the anxiolytic-like effects reported with CI-988 and L-365,260 in the above studies were small in comparison to BZs and were not dose-dependent. Moreover, the present data are in agreement with more recent findings which indicate that, in punishment procedures (in rats and squirrel monkeys), CCK_A and CCK_B receptor antagonists are devoid of significant anxiolytic-like effects (Charrier *et al.*, 1995; Dawson *et al.*, 1995). It therefore seems that punished procedures are of limited utility for the screening of CCK receptor antagonists.

Most previous studies of CCK receptor antagonists in models of anxiety used exploratory-based procedures, such as the elevated plus-maze test or the light/dark choice task. The present experiments with these models provide no support for an anxiolytic-like action of the CCK compounds tested, whereas the positive control diazepam elicited clear effects on all behavioural parameters in both situations. This latter result, taken together with recent findings obtained with other BZ receptor agonists under identical test conditions (Griebel *et al.*, 1996b,e), indicates that the current methodology is suitable for detecting anxiolytic-like effects. Nevertheless, the absence of significant effects of the CCK compounds in the present experiments stands in marked contrast to those obtained by Hughes *et al.* (1990), Costall *et al.* (1991) and Singh *et al.* (1991), which demonstrated anxiolytic-like effects of CI-988 and/or PD 135,158 in the rat elevated plus-maze and the mouse light/dark tests over a wide dose-range (0.001–10 mg/kg) using different routes of administration (i.e. i.p., s.c., oral). The effects of PD 135,158 in the light/dark test were later confirmed by Belzung *et al.* (1994), who showed that the drug (0.1–1 mg/kg, s.c.) increased the time spent by animals in the aversive part of the apparatus, an effect which is consistent with an anxiolytic-like action. Similar positive effects have been reported with LY 288513 (10 mg/kg, i.p.) and L-365,260 (0.001–1 mg/kg, i.p.) in the elevated plus-maze test (Rataud *et al.*, 1991; Chopin and Briley, 1993; Helton *et al.*, 1996). It is important, however, to note that several laboratories failed to detect any behavioural modification after the administration of CCK receptor antagonists in such models (Harro and Vasar, 1991; Hendrie *et al.*, 1993; Bickerdike *et al.*, 1994; Dawson *et al.*, 1995), thereby questioning the generality of the above positive findings. These inconsistencies in drug profiles prompted Johnson and Rodgers (1996) to characterize fully the behavioural effects of several CCK receptor antagonists in the murine elevated plus-maze to detect subtle or minor changes in behaviour, which cannot be observed when only the usual spatiotemporal measures are recorded. Particular attention was paid to behavioural measures such as risk assessment related to the defensive repertoire. This latter concept refers to a pattern of responses (scanning, stretch-attend, flat-back approach) invariably observed in potentially dangerous situations (Blanchard *et al.*, 1991). In the plus-maze, the most prominent risk assessment measure is the stretched-attend posture, a behaviour that has been of particular interest as it has been shown to be more sensitive to the effects of classical (i.e. BZ receptor ligands) and atypical (i.e. $5\text{-}HT_{1A}$ receptor ligands) anxiolytics than are the traditional indices of anxiety (Rodgers and Cole, 1994; Griebel *et al.*, 1997b). The results showed that, despite detailed analysis, no effects were found after the administration of L-365,260, PD 135,158 or the CCK_A receptor antagonist devazepide. These findings are in line with the lack of effect of the CCK compounds on risk assessment (i.e. aborted attempts) observed in both models in the present study.

These major inconsistencies in the effects of CCK receptor antagonists in the elevated plus-maze and the light/dark tests are difficult to explain. This variability is presumably produced by a multitude of, perhaps small, methodological differences that do not necessarily become clear, even with close scrutiny of published reports. Ultimately, the negative findings argue against the view that exploration models may be more suitable for screening CCK receptor antagonists than are punishment procedures (Bourin *et al.*, 1996; Van Megen *et al.*, 1996).

In the MDTB, pretreatment with the CCK_A receptor antagonist lorglumide did not modify any of the behavioural measures during exposure to the rat. After the administration of PD 135,158 and LY 288513, flight responses (i.e. avoidance distance when the threat stimulus was first placed in the runway apparatus) were significantly decreased, whereas risk assessment (i.e. stops and approaches/withdrawals) and defensive threat and attack reactions (upright and biting) remained unchanged. In addition, none of the CCK receptor antagonists was able to counteract the marked increase in escape attempts from the runway after removal of the rat from the test area. These behavioural profiles differ from that of the positive control diazepam which affected all behavioural responses during and after exposure to the rat.

The extensive pharmacological evaluation of the MDTB has demonstrated that this test may serve as a screening model for both anxiolytic and anti-panic agents (Griebel *et al.*, 1995, 1996a,c, 1997a). It has been shown that panic-modulating compounds specifically affect the flight responses of animals, with panicogenic

treatment (e.g. yohimbine) increasing flight and anti-panic drug challenge (e.g. clonazepam, diazepam, chronic alprazolam, imipramine, fluoxetine, moclobemide) decreasing it. Notably, avoidance responses, when the rat is placed in the runway, appear to be particularly sensitive to panic-modulating drug treatment. Anti-GAD agents such as 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. These compounds, however, affected risk assessment, defensive threat/attack reactions and escape attempts, thereby suggesting that these defence responses probably relate to certain aspects of GAD. Taken together with the present results, these latter findings suggest that CCK receptor antagonists may not be effective drugs for treating GAD. In agreement with this view is a recent report by Adams *et al.* (1995) showing that CI-988 failed to improve patients suffering from GAD in a double-blind, placebo-controlled study. By contrast, the positive effects of PD 135,158 and LY 288513 on avoidance distance fit well with the general assumption that antagonists targeting CCK_B receptors may have some efficacy in the clinical management of PD. A similar conclusion was drawn recently by Jenck *et al.* (1996), who demonstrated that L-365,260 was active in an experimental procedure (aversion induced by electrical stimulation of the dorsal periaqueductal gray matter (DPAG) described as a realistic model of panic attacks. However, on a clinical level, the picture is less clear. Although a few studies demonstrated that L-365,260 reversed panic attacks elicited by pharmacological challenge (e.g. sodium lactate, CCK-4, pentagastrin) (Bradwejn *et al.*, 1994; Van Megen *et al.*, 1994; Lines *et al.*, 1995), a recent placebo-controlled trial of L-365,260 on naturally occurring panic attacks failed to detect clinically significant differences between drug and placebo (Kramer *et al.*, 1995). The authors of this study discussed several possible reasons for the lack of effect of L-365,260 in PD patients, such as the poor bioavailability of the drug or the use of inadequate dosage. Therefore, further clinical trials with CCK_B receptor antagonists are required before any definitive conclusion can be drawn on the clinical potential of these compounds against natural occurring panic attacks and on the predictive value of their effects in animal models of panic.

In conclusion, the present findings are in agreement with recent reports indicating that classical animal models of anxiety, including both punishment procedures and exploratory-based tests, may not be suitable for the evaluation of the behavioural effects of CCK receptor antagonists. By contrast, tests which may model certain aspects of human panic such as the MDTB or the DPAG stimulation procedure appear to be more reliable tools when screening these compounds.

Acknowledgements

The expert technical assistance of Carmen Aliaga, Michelle Lepichon, Monique Lhermitte, Anne-Marie Poisson and Elisabeth Pillière is greatly appreciated. We are also grateful to Bernard Kleinberg for the automation of the runway apparatus, the light/dark and the Vogel conflict tests.

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(Received 1 May 1997; accepted as revised 29 May 1997)