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Heterogeneity of CCK-B Receptors Involved in Animal Models of Anxiety

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DERRIEN, M., I. McCORT-TRANCHEPAIN, B. DUCOS, B. P. ROQUES AND C. DURIEUX. *Heterogeneity of CCK-B receptors involved in animal models of anxiety.* PHARMACOL BIOCHEM BEHAV **49**(1) 133–141, 1994.—The effects of the selective CCK-B agonists, BC 264 and BC 197, and the nonselective CCK agonist BDNL were investigated in the elevated plus-maze in rats. BDNL and BC 197 induced anxiogeniclike effects, in contrast to BC 264, which had no effect. The behavioral responses induced by BDNL were not significantly blocked by L-365,260, but were suppressed by CI-988, another selective CCK-B antagonist, and by high doses of L-364,718, a selective CCK-A antagonist. BC 197-induced effects were also blocked by CI-988. Competition experiments performed with [^{3}H]pBC 264 using brain membranes of guinea pig, mouse, and rat were significantly better fitted when analyzed by a two site model than by a one site model with BC 197 but not with BC 264. Moreover, BC 264 produced anxiogeniclike effects when administered with increasing doses of L-365,260 and opposing effects with increasing doses of CI-988. Together these results give pharmacological and behavioral evidence for the existence of CCK-B receptor subtypes.

Cholecystokinin CCK-B agonists CCK-B receptors Subtypes Elevated plus-maze Amphetamine
Scopolamine Chlordiazepoxide FG 7142 Rat Mouse Guinea pig

THERE is now substantial evidence in the literature suggesting that cholecystokinin, a neuropeptide extensively distributed in the central nervous system as well as in the gastrointestinal tract (46), could be involved in mechanisms related to anxiety. Clinical studies have shown that the *C*-terminal tetrapeptide of cholecystokinin (CCK-4), intravenously injected in healthy volunteers or in patients with panic disorders, induces panic attacks (1,12). In rodents, systemic injection of CCK-4 or ceruletide (20,21) was found to decrease the exploratory behavior measured in the elevated plus-maze, an animal model of anxiety (36). Central injection of CCK-8, ceruletide, or pentagastrin also produced anxiogeniclike effects in rats and mice (9,44,45).

CCK-8 interacts with a similar nanomolar affinity with the recently cloned CCK receptors designated CCK-A and CCK-B receptors (29,48). In contrast, CCK-4 and pentagastrin have a better selectivity for CCK-B receptors. CCK-A receptors have been detected in high concentrations throughout the gastrointestinal tract and in a few discrete brain regions, including the nucleus of the tractus solitarius, area postrema, hypothalamus, and interpeduncular nucleus in rats (23,32). However, behavioral and electrophysiological data suggest the existence of CCK-A receptors in other brain regions, particularly in the nucleus accumbens or in the ventral tegmental area (9,10,24). CCK-B receptors are widely distributed throughout the brain, particularly in regions such as limbic structures and cortical areas (16,19).

The roles of CCK-A and CCK-B receptors have been investigated in animal models of "anxiety" by using selective antagonists. Thus, selective antagonists for CCK-B receptors such as L-365,260 and CI-988 (2,25), peripherally administered to rats or mice were reported to produce anxiolyticlike effects (6,25,38-40,44,45). Furthermore, CI-988 antagonized the anxiogeniclike effect of intracerebroventricularly administered pentagastrin. These results support the hypothesis that central CCK-B receptors could be critically involved in the monitoring of anxiety processes (44). However, central CCK-A receptors could also be involved in these processes. This is because injection of CCK-8, but not the selective CCK-B agonist BC 264 (4), into the posterior nucleus accumbens induced anxiogeniclike effects that were blocked by the CCK-A antagonist L-364,718 (9,10). This selective CCK-A antagonist was also

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shown to induce anxiolyticlike effect in rats (6,39) and a recent study has reported that the two CCK-B agonists (nonsulfated CCK-8 and pentagastrin) and the CCK-A antagonist L-364,718 had some anxiolyticlike effects in mice (22).

In order to clarify the contribution of CCK receptors in the elevated plus-maze model of anxiety in rats, the effects induced by intraperitoneal administration of BC 264 (Boc-Tyr(SO_3H)-gNle-mGly Trp-(NMe)Nle-Asp-Phe-NH_2) and BC 197 [c-(Boc-D-Asp-Tyr(SO_3H)-Nle-D-Lys)-Trp-Nle-Asp-Phe-NH_2], two selective CCK-B agonists able to cross the blood-brain barrier (18), and of BDNL (42), a CCK-8 analogue were investigated in the presence or in the absence of L-364,718, a selective CCK-A antagonist and two selective CCK-B antagonist, L-365,260 and CI-988 were investigated. Moreover, the binding properties of BC 264 and BC 197 were analysed in rat and guinea pig cortex as well as in mouse brain to investigate the possible occurrence of CCK binding subtypes.

MATERIALS AND METHODS

Animals

Male Wistar rats (Depré, Saint-Doulchard, France) weighing 200–220 g were housed in groups of five with food and water available ad lib. Animals were used only once. For binding experiments the animals used were male Sprague–Dawley rats (140–180 g) (Depré), Swiss mice (25–30 g) (Depré), and guinea pigs (300 g) (Ruvel, Thiron, France).

Chemicals and Injection Procedures

BDNL (Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) (42), BC 264 (Boc-Tyr(SO_3H)-gNle-mGly Trp-(NMe)Nle-Asp-Phe-NH_2) (4), BC 197 [c-(Boc-D-Asp-Tyr(SO_3H)-Nle-D-Lys,-Trp-Nle-Asp-Phe-NH_2] (5), L-364,718 (3S(-)-N-(2,3 dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide) (3), L-365,260 (3R-(+)-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4 benzodiazepin-3-yl)-N′-(3-methylphenyl)-urea) (2), and CI-988 ([R-(R*,R*)]-4-[[2-[[3-(1H-Indol-3-yl)2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]-amino]propyl]-amino]-1-phenylethyl]amino]-4-oxobutanoic acid) (25) were synthetized as previously described. [^{3}H]pCCK-8 (60 Ci/mmol) was purchased from Amersham, and [^{3}H]pBC 264 (98–100 Ci/mmol) was synthesized in the laboratory using (N-succinimidyl [2,3^{3}H]propionate (Amersham) as labelling agent as previously reported (7). The validity and the sensitivity of the parameters used in these experiments were first investigated by administrating to the rats, chlordiazepoxide and a β carboline, FG 7142 (generous gifts of Rhône Poulenc Rorer Laboratories, Vitry/Seine, France), two compounds that have been used to reveal anxiolytic and anxiogeniclike effects, respectively (36). Chlordiazepoxide and FG 7142 were suspended in carboxymethylcellulose (0.5%) and intraperitoneally (IP) administered 30 min before the experiments. Two drugs that may modify attention and/or memory, scopolamine hydrobromide (Merck) and (D,L) amphetamine (Calaire Chimie S.A., Calais, France), were also investigated. Scopolamine and amphetamine were dissolved in saline (0.9% NaCl) and IP administered 30 min before the experiments. CCK antagonists were suspended in carboxymethylcellulose and injected IP 45 min before the experiments. BDNL, BC 264, and BC 197 were dissolved in saline and administered IP 30 min before the behavioral test. IP injections (1 ml/kg) were carried out at the doses given in results.

Binding Experiments

Animals were killed by decapitation, and their brains quickly removed. Brain cortices from rats and guinea pigs, and whole brain (minus cerebellum) of mice were homogenized (12 ml/g of tissue wet weight) at 4°C in 50 mM Tris-HCl buffer, pH 7.4 containing 5 mM $MgCl_2$. The homogenate was centrifuged at 4°C for 35 min at 100 000 g, and the resulting pellet was rehomogenized in a large excess of ice-cold buffer and centrifuged under the same conditions. The final pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 0.2 mg/ml bacitracin and 5 mM $MgCl_2$. The homogenate was used immediately. Protein concentration was determined by the Pierce BCA protein assay using bovine serum albumin standards.

Rat pancreata were quickly dissected and placed in ice-cold 10 mM Pipes-HCl buffer, pH 6.5 containing 30 mM $MgCl_2$. After careful removal of the fat, the tissue was homogenized in 25 volumes of the same buffer at 4°C, the homogenate was filtered on gauze, and the filtrate was centrifuged twice at 50 000 g for 10 min with an intermediate rehomogenization of the pellet in fresh buffer. The final pellet was resuspended in 2 volumes of fresh buffer and stored at −80°C until use.

The binding assays were performed as described (4,17) in Tris-HCl buffer, pH 7.4, 0.2 mg/ml bacitracin, and 5 mM $MgCl_2$ in the presence of brain membranes (0.6 mg protein/tube), or in 10 mM Pipes-HCl buffer, pH 7.4, 30 mM $MgCl_2$, 0.2 mg/ml bacitracin, 0.2 mg/ml soybean trypsin inhibitor in the presence of pancreatic membranes (0.2 mg protein/tube). Incubations were carried out at 25°C for 60 min using 0.2 nM [^{3}H]pBC 264 with brain membranes and for 120 min using 0.1 nM [^{3}H]pCCK-8 for pancreatic membranes in the presence of eight to ten increasing concentrations of the competitor. Nonspecific binding was determined in the presence of 1 μM CCK-8. Incubation was terminated by filtration through Whatman GF/B filters precoated by incubation in buffer containing 0.1% (w/v) bovine serum albumin. The filters were rinsed twice with 5 ml of ice-cold buffer, dried, and the radioactivity counted.

The K_I values were calculated using the Cheng-Prusoff equation. Competition curves were analysed with a two-site model using the nonlinear curve fitting program LIGAND. A two-site fit was accepted only when it resulted in a significant improvement of the fit in a F-test with $p < 0.05$ and if the "runs test" was not significant. The runs test is an indication of the goodness of the fit. When the runs test is not significant, indicating a random distribution of the points around the fitted line, the model is appropriate (33). All values are the mean ± standard error (SEM) of at least three determinations.

Behavioral Tests

The elevated plus-maze test was used in these experiments as previously described by Pellow et al. (36). The wooden apparatus consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) with open tops arranged such that the two open arms were opposite to each other. The maze was elevated (50 cm) and illuminated from the top (300 lx) and stand in a soundproof room. At the beginning of experiments rats were placed in the middle of the maze facing one of the open arms. The total number of visits to the open arms, the total number of visits to the closed arms, the cumulative time spent in the open arms, and the cumulative time spent in the closed arms were then measured for 5 min. An arm visit was

TABLE 1

APPARENT AFFINITIES OF CCK ANALOGS ON RAT PANCREAS (CCK-A) AND BRAIN CORTEX (CCK-B) BINDING SITES

	K_I (nM)		Relative CCK-B Selectivity
	Brain CCK-B	Pancreas CCK-A	
B agonist BC 264	0.39 ± 0.08	355 ± 98	910
B agonist BC 197	160 ± 52	2120 ± 303	13
B antagonist L-365,260	10.9 ± 2.0	1170 ± 330	107
B antagonist CI 988	6.1 ± 0.5	2382 ± 514	390
A antagonist L-364,718	549 ± 69	1.55 ± 0.15	0.0028

Results are mean ± SEM of three independent determinations performed in triplicate. [^{3}H]pBC 264 = 0.2 nM in rat brain (K_D = 0.24 nM) and [^{3}H]pCCK$_8$ = 0.1 nM in rat pancreas (K_D = 1.02 nM).

recorded when a rat moved all four paws into the arm. Results are expressed as the number of entries into open arms divided by the total number of entries into open or closed arms (O/[O + F]), and the time spent in open arms over the time spent in open and closed arms (O/[O + F] duration). Behavioral experiments were performed in the afternoon. Animals were used only once.

Statistical Analysis

Data were analysed by one-way analysis of variance (ANOVA), followed by a pairwise comparison Dunnett's test or Newman-Keuls test.

RESULTS

Binding Studies

The affinities and selectivities of the agonists and antagonists used in this study for rat CCK-A (pancreas) and CCK-B (brain cortex) binding sites are shown in Table 1. BC 197 exhibited a tenfold lower selectivity in the rat than that previously described in the guinea pig (5), the selectivity factor K_I CCK-A/K_I CCK-B being 13 and 180 in rat and guinea pig, respectively. As shown in Table 2, the affinity of this compound for CCK-B binding sites, obtained from nonlinear analysis, assuming a one-site model, was 3-fold and 17-fold less in mouse and rat, respectively, than in guinea pig. Nevertheless, in all species studied, the Hill slope value (nHill) for BC 197 was significantly less than unity, 0.61, 0.71, and 0.66 in guinea pig, rat, and mouse, respectively (Table 2).

These low values could suggest the presence of multiple binding sites. Reanalysis of the data with a two-site model (Table 2) showed that the inhibition curves were significantly best fitted by a two-site model than a one-site model ($p < 0.05$ in guinea pig and rat and $p < 0.01$ in mouse brain) as illustrated in Fig. 1.

The relative proportion of CCK-A and CCK-B sites differed among species. The high affinity site represented around 15% of the total in rat brain cortex, 45% in guinea pig brain cortex, and 70% in mouse whole brain. Under these condi-

TABLE 2

APPARENT AFFINITIES OF THE AGONISTS BC 197 AND BC 264 FOR CCK-B BINDING SITES OF DIFFERENTS SPECIES

		One-Site Model		Two-Site Model K_I (nM)	
		K_I (nM)	nHill	Site 1	Site 2
Guinea pig	BC 197	9.2 ± 1.3	0.61 ± 0.05	0.20 ± 0.04 (45)	38.5 ± 5.7* (55)
	BC 264	0.15 ± 0.01	0.97 ± 0.03	–	–
Rat	BC 197	160 ± 52	0.71 ± 0.07	2.3 ± 0.8 (15)	540 ± 60* (85)
	BC 264	0.39 ± 0.08	1.02 ± 0.05	–	–
Mouse	BC 197	26.8 ± 2.7	0.66 ± 0.03	7.8 ± 0.8 (70)	219 ± 9† (30)
	BC 264	0.32 ± 0.02	0.91 ± 0.03	–	–

The K_I values are the mean ± SEM of three independent determinations, each in triplicate. A two-site model was analyzed using Ligand program. Apparent improvement in fit of two-site model over that for one-site model was tested for significance using the F-ratio test, *$p < 0.05$, †$p < 0.01$. The percentage of each site is noted in parentheses.

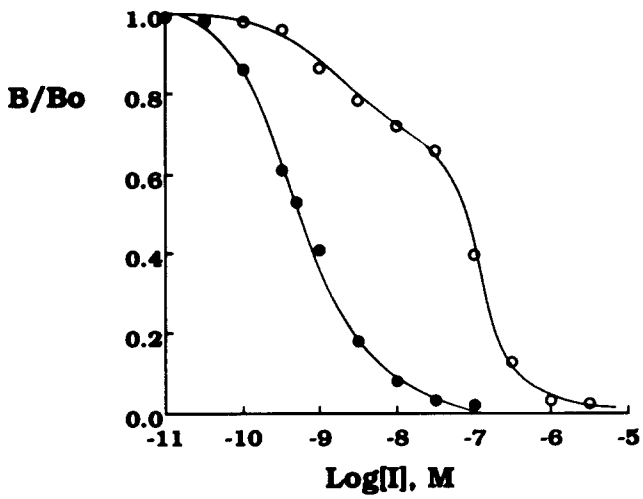

FIG. 1. Inhibition of [^{3}H]pBC 264 binding to guinea-pig brain cortex by selective CCK-B agonists BC 264 (●) and BC 197 (○). Each point represents the mean value of triplicate determination, and the experiment was done three times.

tions, the potency of BC 197 for the high affinity site was larger in rat brain (K_I = 2.3 nM) than in mouse brain (K_I = 7.8 nM). In contrast, BC 264 exhibited a high affinity for all the species, and in all experiment the Hill coefficient was close to unity, 0.97, 1.02, and 0.91 in guinea pig, rat, and mouse brain respectively (Table 2). The inhibition curves could not be fitted with a two-site model (Fig. 1).

Elevated Plus-Maze Tests

Validation. As shown in Table 3, chlordiazepoxide (5 mg/kg) significantly increased the percentage of time spent in open arms (O/[O + F] duration), $F(3, 37) = 3.72$, $p < 0.01$, and significantly enhanced the percentage of open arm visits (O/[O + F] number), $F(3, 37) = 4.22$; $p < 0.01$. The total number of entries into the arms of the maze was also significantly increased in rats treated with 3 and 5 mg/kg chlordiazepoxide, $F(3, 37) = 8.95$ $p < 0.01$.

FG 7142 induced a significant decrease [$F(3, 25) = 4.95$, $p < 0.01$] in the percentage of time spent in the open arms at doses of 5 and 10 mg/kg but did not significantly change [$F(3, 25) = 1.85$, $p = 0.16$] the percentage of open arm visits. The total number of visits was not changed significantly by FG 7142 although no individual group difference could be identified.

Scopolamine (2.5 mg/kg) induced a significant increase [$F(2, 28) = 3.73$, $p < 0.05$] in the percentage of time spent in open arms, but not open arms visits, [$F(2, 28) = 3.45$, $p = 0.04$] or the total number of entries into the arms, [$F(2, 28) = 0.26$, $p = 0.76$]. Amphetamine induced a significant [$F(3, 30) = 3.00$, $p < 0.05$] decrease in the percentage of time spent in open arms, at a dose of 2 mg/kg, but not at 4 mg/kg. Amphetamine also significantly decreased [$F(3, 30) = 3.57$, $p < 0.05$] the percentage of open arms visits only at the dose of 2 mg/kg. A significant [$F(3, 30) = 7.26$ $p < 0.01$] increase in the total number of entries into the arms of the maze occurred after doses of 2 and 4 mg/kg. These studies indicate that under our experimental conditions, the most sensitive parameter to the action of these drugs is the fraction of time spent in the open arms.

CCK agonists. As shown in Fig. 2A, 0.3-3.0 mg/kg BDNL induced a significant [$F(4, 37) = 5.29$ $p < 0.01$] decrease in the percentage of time spent in the open arms.

TABLE 3

EFFECTS OF IP ADMINISTRATION OF CHLORDIAZEPOXIDE, FG 7142, AMPHETAMINE, AND SCOPOLAMINE MEASURED IN THE ELEVATED PLUS-MAZE TEST IN RATS

Treatments		*N*	O/O + F Duration	O/O + F Number	O + F Number
Control (CMC)		10	10.13 ± 2.13	21.66 ± 2.51	9.16 ± 0.92
Chlordiazepoxide	1 mg/kg	11	11.31 ± 3.14	19.48 ± 3.33	13.45 ± 2.03
Chlordiazepoxide	3 mg/kg	9	15.95 ± 3.05	26.79 ± 3.09	22.71 ± 2.71*
Chlordiazepoxide	5 mg/kg	11	21.27 ± 2.64*	33.04 ± 3.03*	15.63 ± 1.57*
Control (CMC)		8	12.32 ± 1.55	19.86 ± 4.73	8.87 ± 0.63
FG 7142	0.5 mg/kg	6	11.72 ± 3.73	19.61 ± 2.97	10.66 ± 0.66
FG 7142	5 mg/kg	7	3.06 ± 1.39*	8.57 ± 4.32	6.60 ± 0.86
FG 7142	10 mg/kg	8	5.17 ± 1.64*	11.62 ± 3.84	8.12 ± 0.77
Control (saline)		12	10.13 ± 2.13	21.66 ± 2.51	9.16 ± 0.92
Scopolamine	0.5 mg/kg	10	9.47 ± 3.27	15.10 ± 4.43	10.30 ± 1.24
Scopolamine	2.5 mg/kg	9	18.60 ± 1.95*	29.23 ± 4.08	10.00 ± 1.43
Control (saline)		10	12.73 ± 1.84	23.40 ± 2.14	8.90 ± 1.09
Amphetamine	0.2 mg/kg	8	8.46 ± 2.55	13.91 ± 3.33	11.25 ± 1.72
Amphetamine	2 mg/kg	8	3.34 ± 0.98*	8.45 ± 3.77*	17.50 ± 1.98*
Amphetamine	4 mg/kg	8	10.00 ± 3.32	14.30 ± 4.46	18.00 ± 2.12†

Effects of chlordiazepoxide, FG 7142, scopolamine, and amphetamine (injected 30 min before the experiments) in rats observed after IP administration of drugs. The behavioral responses of rats were measured in the elevated plus-maze for 5 min and are expressed as the percentage of time spent in open arms (O/[O + F] duration), as the percentage of entries in open arms (O/[O + F] number), and as the total number of arm entries (O + F number). *$p < 0.05$; †$p < 0.01$ Dunnett's test.

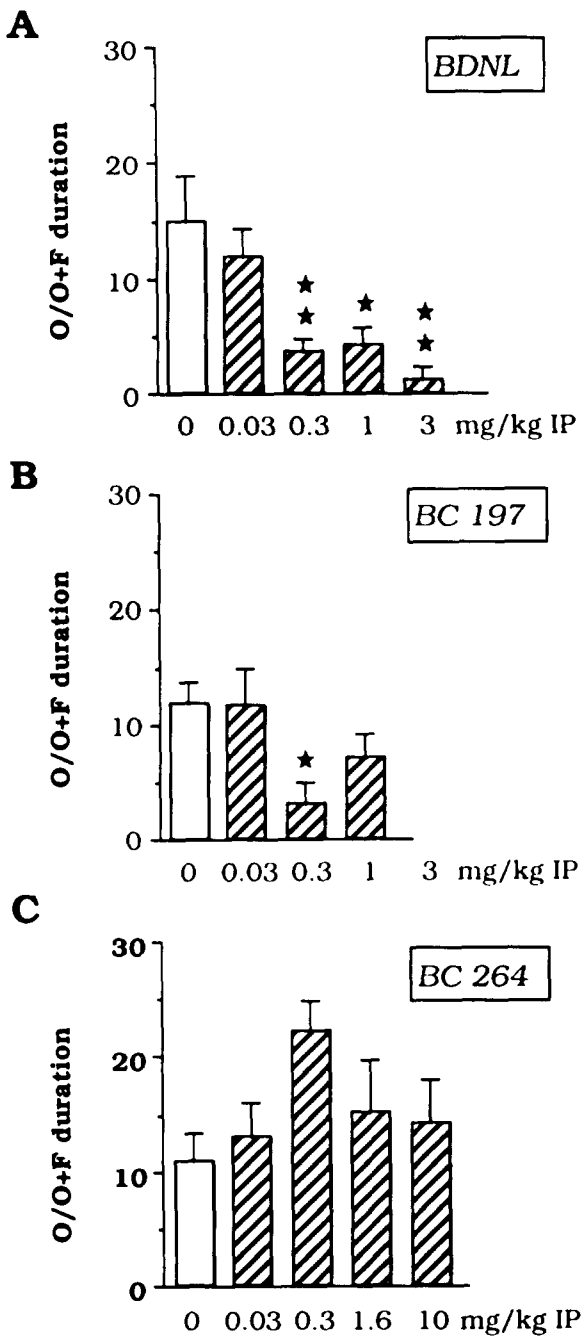

FIG. 2. Effects of intraperitoneal (IP) injection of A) BDNL, B) BC 197, and C) BC 264 administered 30 min before the experiment. The behavioral responses of rats were measured in the elevated plus-maze for 5 min and are expressed as the percentage of time spent in open arms (O/[O + F] duration). ★$p < 0.05$; ★★$p < 0.01$ Dunnett's test.

BC 197 (0.3 mg/kg) decreased the percentage of time spent in open arms (Fig. 2B) [$F(3, 34) = 3.08, p < 0.05$].

BC 264 induced no significant variation in the percentage of time spent in the open arms (Fig. 2C) [$F(4, 45) = 1.72, p = 0.16$].

CCK antagonists and BDNL. CI-988 alone did not modify the percentage of time spent in the open arms (Fig. 3A). The selective CCK-B antagonist, intraperitoneally injected at a dose of 0.02 mg/kg, significantly suppressed the decrease in the percentage of time spent in the open arms induced by BDNL (0.3 mg/kg IP) (Fig. 3A) [$F(7, 72) = 4.15, p < 0.01$].

As shown in Fig. 3B, IP injection of L-365,260 did not significantly modify the significant decrease [$F(9, 88) = 6,93$ $p < 0.01$] in the percentage of time spent in the open arms induced by BDNL (0.3 mg/kg IP), although a partial suppres-

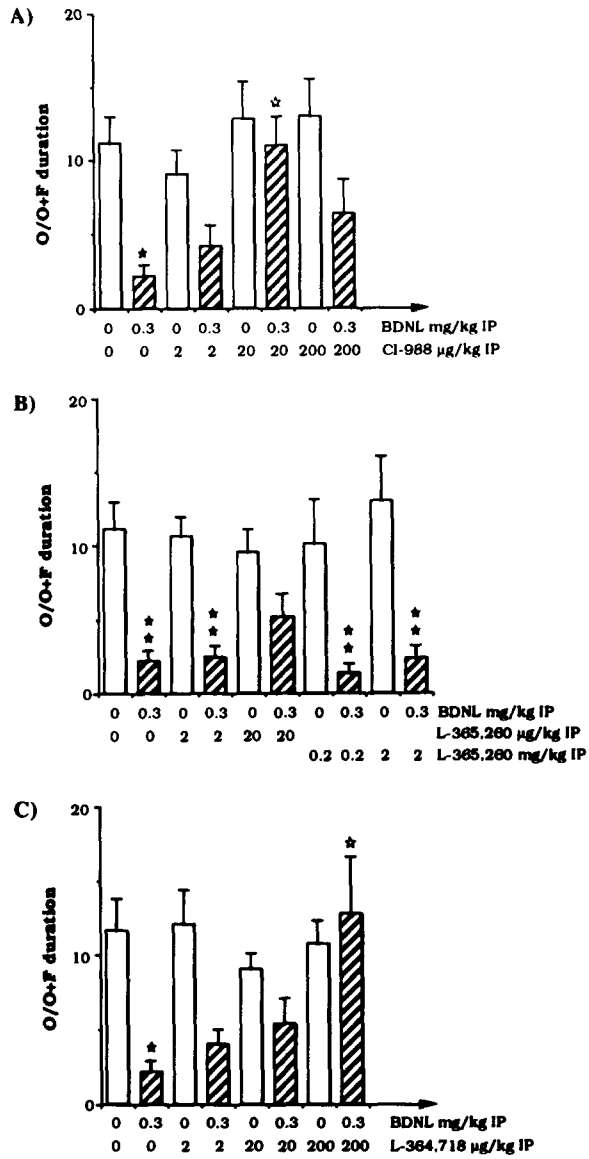

FIG. 3. Effects of increasing doses of A) CI-988, B) L-365,260, and C) L-364,718, administered 45 min before the experiment, on the decrease in the percentage of time spent in open arms (O/[O + F] duration) induced by BDNL (0.3 mg/kg IP, 30 min before the experiment). The behavioral responses of rats were measured in the elevated plus-maze for 5 min and are expressed as the percentage of time spent in open arms (O/[O + F] duration). IP = intraperitoneal; ★$p <$ 0.05, ★★$p < 0.01$ as compared to the control group; ☆$p < 0.05$ as compared to BDNL group Newmann-Keuls test.

sion of the effects of the CCK agonist was observed by L-365,260 at a dose of 20 μg/kg.

The CCK-A antagonist L-364,718 did not modify the percentage of time spent in the open arms (Fig. 3C). L-364,718 (0.2 mg/kg) did significantly [$F(7, 65) = 3.66, p < 0.01$] suppress the decrease in the percentage of time spent in the open arms induced by BDNL (0.3 mg/kg IP) (Fig. 3C).

CCK antagonists and BC 197. The selective CCK-B antagonist CI-988, significantly suppressed [$F(4, 35) = 4.80, p < 0.01$] the decrease in the percentage of time spent in the open arms induced by BC 197 (0.3 mg/kg IP) (Fig. 4).

CCK-B antagonists with BC 264. A significant decrease [$F(7, 67) = 3.88, p < 0.01$] in the percentage of time spent in the open arms occurred in animals treated with the selective CCK-B antagonist L-365,260, intraperitoneally injected at a dose of 0.2 mg/kg 15 min before BC 264 (0.3 mg/kg IP), as compared to the control group, the BC 264 group or the L-365,260 group (Fig. 5A). Neither L-365,260 (0.02, 0.2 and 2 mg/kg) nor BC 264 (0.3 mg/kg) alone produced any significant change in the percentage of time spent in the open arms (Fig. 5A).

A significant increase [$F(3, 34) = 5.24, p < 0.01$] in the percentage of time spent in the open arms occurred in animals treated with the selective CCK-B antagonist CI-988 (0.02 mg/kg) 15 min before BC 264 (0.3 mg/kg IP), as compared to the control group, the BC 264 group and the CI-988 group (Fig. 5B). The increased dose of 0.2 mg/kg CI-988, however, did not change the percentage of time spent in the open arms after BC 264 (0.3 mg/kg IP) [$F(3, 33) = 0.84 p = 0.48$] (Fig. 5B).

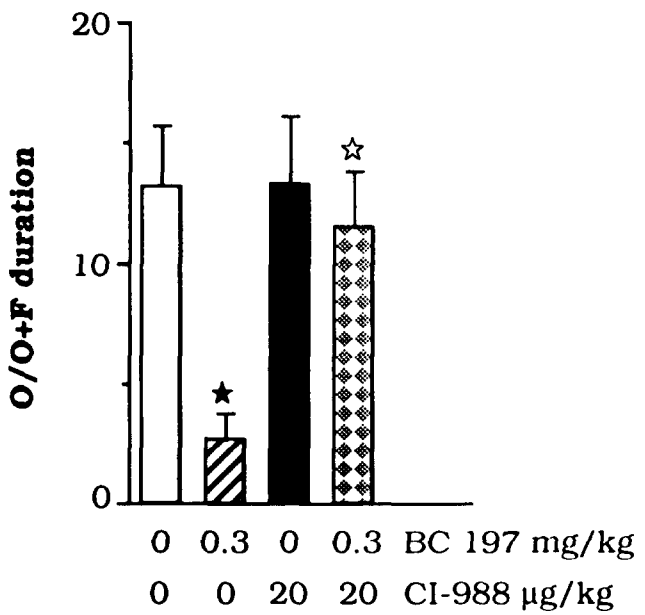

FIG. 4. Effects of CI-988 (20 μg/kg IP), administered 45 min before the experiment, on the decrease in the percentage of time spent in open arms (O/[O + F] duration) induced by BC 197 (0.3 mg/kg IP, 30 min before the experiment). The behavioral responses of rats were measured in the elevated plus-maze for 5 min and are expressed as the percentage of time spent in open arms (O/[O + F] duration). ★$p < 0.05$ as compared to the control group; ☆$p < 0.05$ as compared to BC 197 group Newmann-Keuls test.

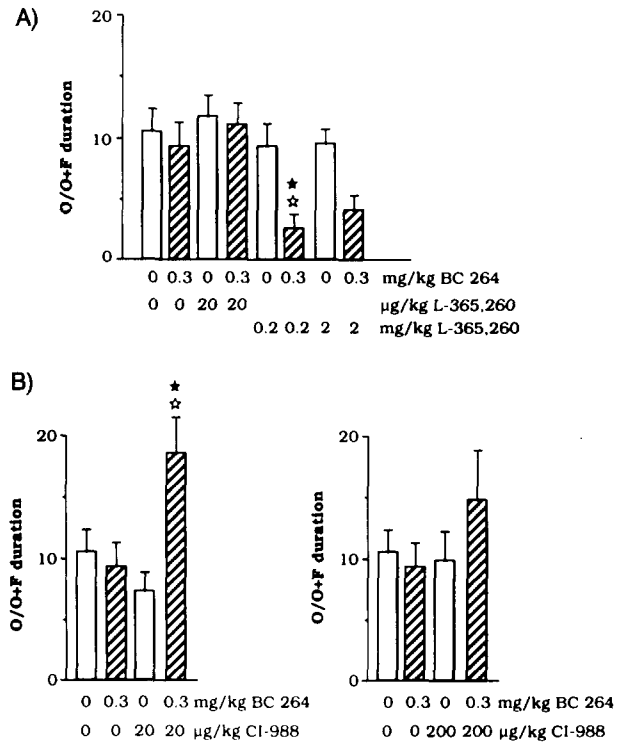

FIG. 5. Effects of increasing doses of A) L-365,260 and B) CI-988 administered 15 min before BC 264 (0.3 mg/kg intraperitoneally njected 30 min before the experiment). The behavioral responses of rats were measured in the elevated plus-maze for 5 min and are expressed as the percentage of time spent in open arms (O/[O + F] duration). ★$p < 0.05$ as compared to the control group; ☆$p < 0.05$ Newmann-Keuls test as compared to BC 264 and to L-365,260 or CI-988 group.

DISCUSSION

The pharmacological profiles of CCK-related compounds were investigated in the elevated plus-maze test. Injection of chlordiazepoxide and FG 4172 confirmed a previous report that administration of anxiolytic and anxiogenic drugs modifies the percentage of entries and time spent in open arms of the maze (36). Moreover, these studies indicated that under our experimental conditions, the most sensitive parameter to the action of these drugs is the fraction of time spent in the open arms. In addition, scopolamine, thought to disrupt attention, induced an anxiolyticlike effect, whereas amphetamine (thought to increase attention) induced opposite effects, as previously reported (36).

IP injection of the nonselective CCK-A/CCK-B agonist, BDNL, significantly decreased the percentage of time spent in the open arms in the elevated plus-maze. This result is in agreement with previous experiments showing that peripheral or intracerebroventricular injection of ceruletide, another CCK-8 analogue, produced anxiogeniclike effects (20,45).

CI-988 at a dose of 20 μg/kg, but not 200 μg/kg, reversed the decrease in the percentage of time spent in the open arms induced by BDNL, whereas at a dose of 20 μg/kg, L-365,260 partially antagonized the effects of BDNL. In both experi-

ments, the dose-response curves were bell-shaped. The CCK-A antagonist, L-364,718 was also found to antagonize the effects of BDNL, but at a dose (0.2 mg/kg) tenfold higher than CI-988, and at which L-364,718 very likely interacts with both CCK-A and CCK-B receptors (34). Peripheral injection of the selective CCK-B agonist, BC 197, at 0.3 mg/kg decreased the percentage of time spent in the open arms, and this was suppressed by a dose of CI 988 which antagonizes the effects of BDNL. Together these results are in agreement with the preferential involvement of CCK-B receptors in the induction of anxiogeniclike effect, also evidenced in the black and white box in mice and the elevated plus maze in rats (44,45).

One of the most interesting results of this study is the difference observed in the behavioral responses induced by the two CCK-B agonists, BC 264 and BC 197. Thus, peripheral injection of BC 197 decreased the percentage of time spent in the open arms, whereas BC 264 did not significantly change the behavior of rats tested in the elevated plus-maze. Both compounds interacted with a low affinity with CCK-A receptors, but BC 264 had a better affinity for CCK-B receptors than BC 197, especially in rats. Previously reported differences in affinities of CCK analogues for CCK-B receptors of rat, mouse, guinea pig, and human (8,16,17,51) suggest the existence of slight differences in the structure of brain CCK receptors. This has recently been confirmed by the cloning of CCK-B/gastrin receptors from rat, human, and dog showing that the sequences of these receptors are close but not identical (28,29,37,48). However, in all species studied, competition experiments performed with BC 197 were significantly better when the results were analyzed by a two-site than by a one-site model. In rat, the affinities of BC 197 were 2.3 nM and 540 nM for the high and low affinity binding site, respectively. Thus, BC 197 could interact with two different states of affinity of the CCK-B receptor, whereas BC 264 could have the same affinity for the two states. This finding could explain why BC 197 interacted with a high in vivo affinity with CCK-B receptors despite its weak in vitro affinity in contrast to BC 264, which displayed high affinity both in vivo and in vitro (18). A heterogeneity of CCK-B binding sites has previously been reported in guinea pig brain from studies using linear and cyclic analogues (15,27,41). The existence of two binding states has been clearly demonstrated for CCK-A receptors by Wank et al. (49) using COS-7 cells transfected with CCK-A receptor cDNA. These two affinity states could correspond to different states of coupling to G proteins, as previously suggested for CCK-B receptors (17,27), or to linkage with different second messenger systems, as proposed for delta opioid receptor subtypes (26,50).

The hypothesis that BC 197 interacts differently with two CCK-B receptor subtypes, could explain the bell-shaped dose response curves produced by BC 197 in the elevated plus-maze. It is possible that BC 197 at a dose of 0.3 mg/kg stimulates only one of the CCK-B binding sites, producing a decrease in the percentage of time spent in the open arms, and that the stimulation of the other CCK-B site by BC 197 at a higher dose compensates for the effects produced by activation of the first site. In contrast, BC 264 may simultaneously activate both binding sites, resulting in no behavioral change in this particular test. The higher proportion of the CCK-B subsite 2 in rats might allow to reveal the anxiolyticlike effects more easily than the anxiogeniclike effects using BC 264. Nevertheless, higher doses of BC 264 (up to 30 mg/kg) did not modify the behavior of rats in the elevated plus-maze (unpublished data). In mice, in which the proportions of CCK-B subsites are inversed, both, BC 197 and BC 264 have been found to increase stress-induced immobility (13). The affinity of BC 197 for CCK-B subsite 1 was sixfold lower than the affinity of BC 264 (2.3 nM vs. 0.39 nM), however previous study has shown that BC 197 was able to cross the blood-brain barrier tenfold better than BC 264 (18). These data suggested that these two agonists might be active as the same dose.

The pharmacological relevance of these two binding states was investigated by administering increasing doses of L-365,260 and CI-988, 15 min before BC 264 injection. Under these conditions BC264 with L-365,260 at a dose of 0.2 mg/kg, decreased time spent in the open arms. In contrast, with CI-988, administered at a dose found to suppress the anxiogeniclike effects of BDNL (20 μg/kg), it increased time spent in the open arms. Together these results show that BC 264 induces opposite effects in rats treated with two different CCK-B antagonists. L-365,260 and CI988 might selectively block one of the CCK-B receptor subsites, thus permitting BC 264 to interact with the other subsite. In agreement with this result, CCK-B antagonists both produced anxiolyticlike effects and also potentiated the anxiogeniclike effect of CCK-4 (21) and of *N*-methyl-D-aspartate (47). CCK-B antagonists also stimulated β-endorphin and ACTH secretion from the rat pituitary during stressful events (31).

Consistent with our findings, both anxiolyticlike and anxiogeniclike effects of two other CCK-B agonists, the nonsulfated CCK-8 and pentagastrin have been reported to depend on the perception of threat intensity occurring in different behavioral tests (22). Individual sensitivity of animals could also account for these results. In agreement with this hypothesis, BC 264 produced anxiolyticlike effects in rats previously selected for their high locomotor activity in the open field test, and anxiogeniclike effect in rats with low activity; L-365,260, at a dose of 0.2 mg/kg, which allowed the anxiogeniclike effects of BC 264 to be revealed in this study, also produced opposite effects in rats selected for their high or low locomotor activity (14). An individual sensitivity of patients with panic disorders to the panicogenic effects of CCK-4 has been also reported (1), which could result from differences in CCK receptor sensitivity and/or in endogenous CCK levels (30). Moreover, certain benzodiazepines were found to decrease anxiety in anxious patients and to increase emotional tension in patients with low levels of emotivity (11).

The effects observed in this study in BC 264-treated animals could be also related to a modification in the vigilance state. In agreement with this hypothesis, scopolamine was found to increase the percentage of time spent in the open arms of the elevated plus-maze. Moreover, a recent study indicated that BC 264 induced a slight increase in awakeness in rats (43) and produced paranoidlike effects in monkeys (35). According to the present results, it remains possible that the emotional state of rats, or more precisely the way by which they react to a stressful situation might be conducted by at least two CCK-B receptor subsites. One could accentuate immobility or freezing, the other could facilitate escape or avoidance.

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