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Opposite effects of CCK_B agonists in grooming behaviour in rats: further evidence for two CCK_B subsites

[1]Nathalie Ladurelle, [1]Angélique Sebret, [1]Christiane Garbay, [1]Bernard P. Roques & [1,2]Valérie Daugé

[1]Département de Pharmacochimie Moléculaire et Structurale, U 266 INSERM, URA D 1500 CNRS, Faculté des Sciences Pharmaceutiques et Biologiques, 4, avenue de l'Observatoire–75270 Paris Cedex 06, France

1 The hypothesis of the existence of two CCK_B receptor subsites, CCK_{B1} and CCK_{B2} corresponding probably to different coupling states of CCK_B receptors, was studied by measuring grooming behaviour in rats.

2 The B1 receptor agonist, BC197 (300 μg kg^{-1}, i.p.) produced a 45–50% decrease in grooming activity, which was prevented by both the CCK_B receptor antagonists CI-988 (20 μg kg^{-1} i.p.) and L-365,260 (200 μg kg^{-1}, i.p.).

3 In contrast, 3, 10 and 30 μg kg^{-1}, i.p., of the potent B2 receptor agonist, BC264, enhanced grooming (150–190%). This effect was prevented by previous injection of 75 μg kg^{-1} of L-365,260 while higher doses (200 μg kg^{-1}, i.p.) produced only a partial antagonism. Moreover, CI-988 (20 μg kg^{-1}, i.p.), showed an opposite effect in potentiating the responses induced by BC264. However, 200 μg kg^{-1} of CI-988 tended to suppress the increase of grooming induced by BC264.

4 The effects of BC264 were prevented by the D_1 receptor (SCH 23390) and D_2 receptor (sulpiride) antagonists, while those of BC197 were only antagonized by sulpiride, emphasizing the existence of a link between peptidergic (CCK) and dopaminergic systems.

5 This study brings additional evidence for the existence of the two CCK_B receptor subsites and suggests that particular attention should be focused on the selectivity of CCK_B receptor agonists, notably to explain the fact that some compounds such as Boc-CCK4 induce anxiogenic-like effects while others, including BC264, are devoid of these effects.

Keywords: CCK_B receptor subsites, BC197; BC264; grooming activity; dopamine

Introduction

There is now substantial evidence that the C-terminal sulphated octapeptide of cholecystokinin (CCK8), which constitutes the major CCK fragment in the mammalian brain, is involved in the control of several behaviours such as feeding, pain, memory or sexual and reproductive behaviour (reviews in Crawley & Corwin, 1994; Daugé & Roques, 1995).

CCK exerts its actions by interacting with two specific seven transmembrane G-coupled protein families of binding sites, the CCK_A and CCK_B receptors (Wank *et al*., 1992; Lee *et al*., 1993). CCK_A receptors are mainly localized at the level of peripheral organs, while CCK_B receptors predominate in the central nervous system. However, CCK_A receptors have been shown to be present in a few brain regions, including the nucleus of the tractus solitarius, the area postrema, the hypothalamus and the interpeduncular nucleus (Moran *et al*., 1986; Hill *et al*., 1987). In contrast, CCK_B receptors are widely distributed throughout the brain, particularly in limbic and cortical regions (Gaudreau *et al*., 1983; Pélaprat *et al*., 1987). Over the last few years, the use of selective agonists and antagonists has shown that CCK_B receptors seem to be involved in anxiogenic responses (review in Harro *et al*., 1993). Thus, the panic attacks induced in man by i.v. administration of low doses of CCK-4 are suppressed by the CCK_B receptor antagonist L-365,260 (Bradwejn *et al*., 1994). In line with this result, a variety of CCK_B receptor antagonists, such as CI-988 and L-365,260 display an anxiolytic-like profile in rodents (Hughes *et al*., 1990; Costall *et al*., 1991; Rataud *et al*., 1991; Singh *et al*., 1991; Rex *et al*., 1994). However, several studies have shown that the anxiogenic-like effects of CCK_B receptor agonists are dependent on the experimental conditions used and the compounds studied (Derrien *et al*., 1994; Charrier *et al*., 1995; reviews in Harro *et al*., 1993; Daugé & Roques, 1995). Thus, BC264 (Charpentier *et al*., 1988a), a highly selective CCK_B receptor agonist which crosses the blood brain barrier (Daugé *et al*., 1992; Ruiz-Gayo *et al*., 1992) did not produce anxiogenic-like effects but displayed psychostimulant properties and increases in attention and/or memory processes in rats after i.p. administration of very low doses (μg kg^{-1}) (Ladurelle *et al*., 1997). On the other hand, other CCK_B receptor agonists, such as BC197 or Boc-CCK4, were shown to produce anxiogenic-like responses in rodents (Derrien *et al*., 1994; Daugé & Roques, 1995; Ladurelle *et al*., 1997). Furthermore, BC264 and BC197 have a low affinity for CCK_A receptors. One hypothesis to explain this dissociation could be the existence of two CCK_B receptor subsites (B1 and B2), which could correspond to two different activation states of a single molecular entity, as suggested by various studies (Durieux *et al*., 1986; 1988; Knapp *et al*., 1990; Huang *et al*., 1994; Talkad *et al*., 1994; Harper *et al*., 1996; Perez *et al*., 1996; Léna *et al*., 1997; Leff *et al*., 1997). Another important feature is that CCK is a member of a family of peptides referred as 'brain-gut peptides', and gives ingestion-produced 'satiety signals' and functions both in the short-term control of feeding behaviour by interacting with peripheral receptors and in the regulation of several adaptative behaviours, such as grooming, by stimulation of central receptors. Accordingly, peripheral administration of ceruletide or CCK8 did not change grooming behaviour but inhibited food intake, thus inducing a 'satiety signal', by interaction with CCK_A receptors (Kulkosky, 1988). On the other hand, intracerebroventricular

[2]Author for correspondence.

injection of these peptides did not change feeding behaviour but elicited excessive grooming. However, in the latter situation the types of CCK receptors involved have not been investigated (Kulkosky, 1988). In order to understand better the role of CCK_B receptors and that of B1 and B2 subsites in the central effects of CCK8, the effects of BC197 and BC264 on grooming behaviour have been compared in rats. Moreover, the action of the CCK_B receptor antagonists L-365,260 (Lotti & Chang, 1989) and CI-988 (Hughes *et al*., 1990) were studied on the responses induced by these two compounds. Finally, given the strong relationships between peptidergic (CCK) and dopaminergic systems (review in Crawley, 1991) and the well known involvement of dopamine in the modulation of grooming in rodents, the effects of the dopamine D_1 and D_2 receptor antagonists, SCH23390 and sulpiride, respectively, were studied on the responses induced by CCK agonists.

Methods

Animals

The experiments were carried out on male Wistar rats (Elevage Depré, Saint-Doulchard, France) weighing 200–220 g. They were housed four per cage under standard laboratory conditions (controlled temperature (22±1°C) and humidity (50±5%), 12 h light-dark cycle, lights on at 08 h 00 min, food and water available *ad libitum*. Animals were used only once. The animals were treated as approved by the local committee and in accordance with the NIH guidelines for the Care and Use of Laboratory Animals, 1985.

Behavioural measurements

Measurements of grooming Three hours before behavioural measurements, animals were placed in individual cages, identical to their housing cages. This period of isolation was carried out to accustom the animal to the absence of its congener, which could perturb further grooming measurements. Under these conditions, the number of grooming periods and their duration were measured by an experimenter during a session of 15 min. A grooming bout was defined according to Richmond & Sachs (1980), as a succession of events which commonly start with nose wipes by the forepaws, followed by licking or biting actions on the body from the posterior to the head (flank, ventrum) and most often end with anogenital and tail grooming.

Measurements of motor activity Concurrently with the quantification of grooming, the global motor activity of rats was estimated by placing the cage on an Animex meter type S. This apparatus counts the number of perturbations of an electromagnetic field which are generated by the movement of the animals. In our conditions, the detection threshold of movements was set up to detect only the locomotor activity.

Injection procedures

Experiments with BC264 The CCK_B agonist, BC264, was administered at the doses of 0.3, 3, 10, 30 or 300 $\mu g\ kg^{-1}$, i.p., 30 min before the beginning of behavioural measurements, $n=6-7$.

The two CCK-B antagonists were administered 30 min before BC264. L-365,260 (L) was given at doses of 75, 200 or 500 $\mu g\ kg^{-1}$, i.p., in three separate experiments: L 75 $\mu g\ kg^{-1}$: vehicle (CMC, saline) group, BC264 3 $\mu g\ kg^{-1}$, L, L + BC 264: $n=8-12$; L 200 $\mu g\ kg^{-1}$: vehicle (CMC, saline) group, BC264 3 $\mu g\ kg^{-1}$, L, L + BC 264 : $n=8-9$; L 500 $\mu g\ kg^{-1}$: vehicle (CMC, saline) group, BC264 3 $\mu g\ kg^{-1}$, L, L + BC 264: $n=8$. CI-988 (CI) was given at doses of 20 $\mu g\ kg^{-1}$ or 200 $\mu g\ kg^{-1}$, i.p., in two separate experiments: CI 20 $\mu g\ kg^{-1}$: vehicle (CMC, saline) group, BC264 3 $\mu g\ kg^{-1}$, CI, CI + BC 264: $n=8-11$; CI 200 $\mu g\ kg^{-1}$: vehicle (CMC, saline) group, BC264 3 $\mu g\ kg^{-1}$, CI, CI + BC 264: $n=10-11$.

The two dopamine antagonists were injected 15 min before BC264. SCH23390 was administered at the dose of 25 $\mu g\ kg^{-1}$, i.p., and sulpiride at the dose of 50 mg kg^{-1}, i.p. These doses of dopamine receptor antagonists were chosen according to previous experiments (Mazurki & Beninger, 1991; Sampson *et al*., 1991; Ladurelle *et al*., 1997). BC264 was injected at the active dose of 3 $\mu g\ kg^{-1}$, i.p. Six groups of rats were studied in this experiment: vehicle (saline, saline) group, BC264 3 $\mu g\ kg^{-1}$, SCH23390 (SCH), SCH + BC 264, sulpiride (Sulp), sulp + BC 264: $n=8$.

Experiments with BC197 The CCK_B agonist, BC197 was administered at doses of 0.3, 3, 30 or 300 $\mu g\ kg^{-1}$, i.p., 30 min before the beginning of behavioural measurements, $n=8$.

The effects of the CCK-B receptor antagonists on the response induced by BC197 were studied in one experiment. Vehicle (CMC, saline) group, BC197 300 $\mu g\ kg^{-1}$, CI 20 $\mu g\ kg^{-1}$, CI + BC 197, L 75 $\mu g\ kg^{-1}$, L + BC 197, L 200 $\mu g\ kg^{-1}$, L + BC 197: $n=10-15$.

The two dopamine-receptor antagonists were injected 15 min before BC264. SCH23390 was administered at a dose of 25 $\mu g\ kg^{-1}$, i.p., and sulpiride at a dose of 50 mg kg^{-1}, i.p. BC197 was injected at the active dose of 300 $\mu g\ kg^{-1}$. Six groups were examined for this experiment: vehicle (saline, saline) group, BC197, SCH23390, SCH + BC 197, sulpiride, sulp + BC 197: $n=10-11$. All the experiments were conducted blind and performed from 12 h 00 min to 18 h 00 min.

Drugs

The two CCK_B agonists BC264 (Boc-Tyr(SO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_2) and BC197 (c-(Boc-D-Asp-Tyr(SO_3H)-Nle-D-Lys)-Trp-Nle-Asp-Phe-NH_2) were synthesized in the laboratory following the procedure described by Charpentier *et al*. (1988a, b) except that all coupling steps were performed with BOP (benzotriazol-l-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate) as a reagent. They were dissolved in 0.9% aqueous NaCl.

The two CCK_B receptor antagonists, L-365,2600 (3**R**-(+)-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4 benzodiazepin-3-yl)-N'-(3-methylphenyl)-urea) (Lotti & Chang, 1989) 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) (Hughes *et al*., 1990) were both suspended in carboxymethylcellulose (CMC, 0.5%).

The D_1 receptor antagonist SCH 23390 ((+)-8-chloro-2, 3, 4, 5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol) and the D_2 receptor antagonist sulpiride (5-(aminosulphonyl)-N-((1-ethyl-2-pyrolidinyl)methyl)-2-methoxybenzamide) were dissolved in 0.9% aqueous NaCl.

Data expression and statistical analysis

For time course data, the results are expressed as means±s.e. mean. Significances were determined by a two-way repeated

measure (treatment and time) analysis of variance (ANOVA) and if it was significant, a one-way (treatment) ANOVA followed by Dunnett's *t*-test for comparison to control group was performed. For the other experiments, data were expressed as the percentage of change compared to control animals, then, for each group, means and s.e.mean were calculated. The statistical analysis consisted of a one-way (treatment) analysis of variance (ANOVA) followed by a Dunnett's *t*-test for comparison to control group or a Duncan-test for multiple comparisons. The 5% level for significance was chosen *a priori*.

Results

Effects of the CCK_B agonist, BC264

The i.p. administration of BC264 did not induce any modification of the locomotor activity of rats (data not shown), but it increased the number (data not shown) and the duration of grooming ($F(5,33)=6.323$, $P=0.0003$; Figure 1a). Among the five doses tested, 3, 10 and 30 μg kg^{-1} induced significant effects while 300 μg kg^{-1} had no effect compared to the control group. It seems that BC264 may give an all-or-none response whether results are expressed as percentage (Figure 1a) or as raw data (s) (controls = 137.3 ± 16.4, BC264 0.3 μg kg^{-1} = 118.8 ± 14.4; 3 μg kg^{-1} = 201.5 ± 16.5; 10 μg kg^{-1} = 203.9 ± 23; 30 μg kg^{-1} = 199.4 ± 14.3; 300 μg kg^{-1} = 127.6 ± 12.9). Figure 2 shows the time course of the effect with the dose of 3 μg kg^{-1} i.p. The increase in grooming was not immediate but appeared progressively and was only significant at the end of the session, factor treatment: $F(1,11)=7.851$, $P=0.0172$, factor time: $F(2,22)=1.972$, $P=0.163$, interaction: $F(2,22)=2.686$, $P=0.0904$.

To confirm the occurrence of a CCK_B receptor mechanism in this response, two CCK_B receptor antagonists, L-365,260 and CI-988, were administered to the animals before BC264 was injected at a dose of 3 μg kg^{-1}. These two compounds had no intrinsic effect on grooming (Figures 3 and 4). L-365,260 totally prevented the effects induced by 3 μg kg^{-1} BC264 when it was injected at a dose of 75 μg kg^{-1} ($F(3,40)=2.885$, $P=0.00474$; Figure 3a). Interestingly, this antagonism was only partial with both of the higher doses tested, 200 μg kg^{-1} ($F(3,30)=3.079$, $P=0.0424$; Figure 3b) and 500 μg kg^{-1} ($F(3,28)=6.322$, $P=0.0021$; Figure 3c). On the other hand, a low dose of CI-988 (20 μg kg^{-1}), did not prevent the increase in grooming, as conversely, it potentiated the effects of BC264 ($F(3,36)=13.794$, $P=0.0001$; Figure 4a), while a higher dose (200 μg kg^{-1}) tended to suppress the response of BC264 ($F(3,39)=3.342$ $P=0.0289$), (Figure 4b). Administration of

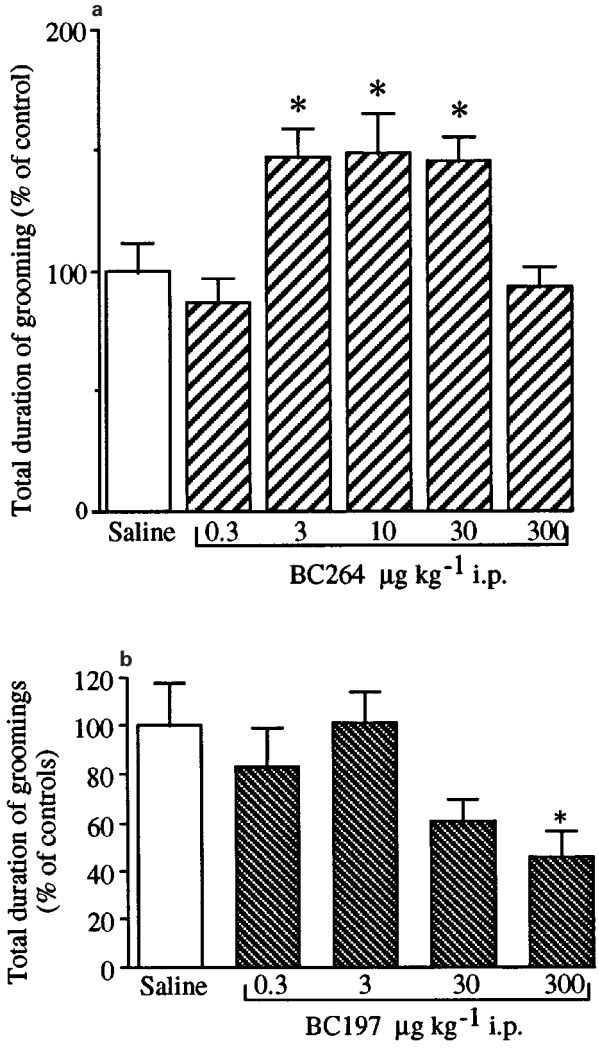

Figure 1 (a) Cumulative duration of grooming bouts during a session of 15 min, measured 30 min after the i.p. administration of 0.3 to 300 μg kg^{-1} of BC264 in rats ($n=6-7$). (b) Cumulative duration of grooming bouts during a session of 15 min, measured 30 min after the i.p. administration of 0.3 to 300 μg kg^{-1} of BC197 in rats ($n=8$). *$P\leqslant0.05$ *vs* saline group, Dunnett's *t*-test.

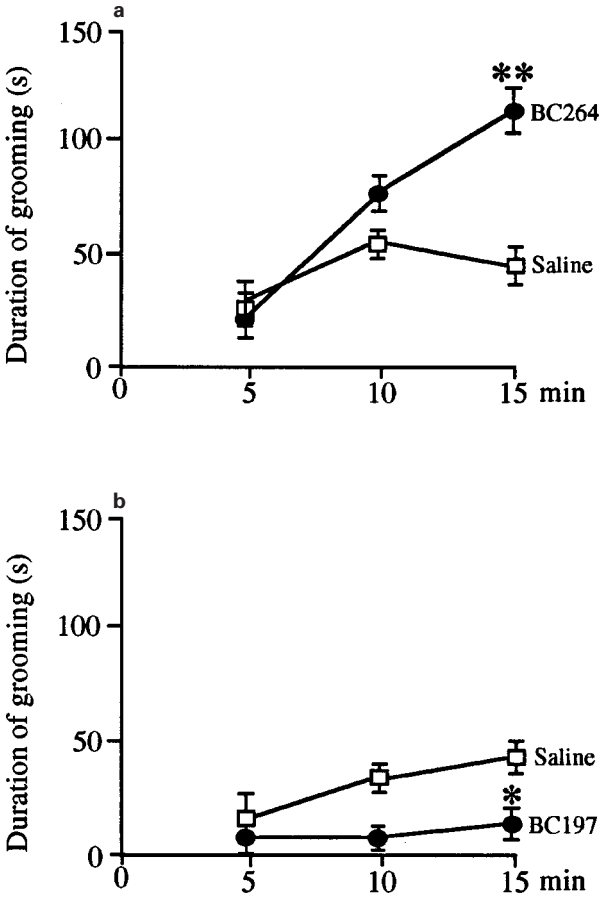

Figure 2 Time course of changes in the grooming duration measured by periods of 5 min in a session of 15 min. Measurements were performed 30 min after i.p. administration of 3 μg kg^{-1} of BC264 (a) and 300 μg kg^{-1} of BC197 (b) ($n=6-8$). *$P<0.05$, **$P<0.01$ *vs* saline group, Dunnett's *t*-test.

both the D_1, SCH 23390, and the D_2, sulpiride, receptor antagonists counteracted the effect of BC264 ($F(5,42)=3.943$, $P=0.0051$; Figure 5a).

Effects of the CCK_B agonist, BC197

The i.p. administration of BC197 did not induce any modification of the locomotor activity of rats which were accustomed to their cages (data not shown), but in contrast to BC264, the injection of 30 or 300 μg kg^{-1}, i.p., of BC197 reduced the number (data not shown) and the duration of grooming periods ($F(4,34)=3.124$, $P=0.0268$). This effect was statistically significant at the dose of 300 μg kg^{-1} (Figure 1b). Figure 2b shows the time course of the effect with the dose of 300 μg kg^{-1}, i.p. The decrease in grooming induced by BC197 appears to have the same time course as the increase observed with BC264: this effect took place progressively and was significant at the end of the session, factor treatment: $F(1,44)=6.646$, $P=0.0219$, factor time: $F(2,28)=2.594$, $P=0.0926$, interaction: $F(2,28)=1.548$, $P=0.230$.

Both CCK_B receptor antagonists were able to prevent the effect of BC197 ($F(7,81)=2.304$, $P=0.0341$; Figure 6): 20 μg kg^{-1}, i.p., of CI-988 administered 30 min before BC197 entirely prevented the reduction of grooming induced by this compound. Furthermore a complete antagonism of this effect by L-365,260 was obtained after administration of 200 μg kg^{-1}, i.p., but not of 75 μg kg^{-1}, i.p.

The decrease in the duration of grooming induced by 300 μg kg^{-1} BC197 was completely suppressed by the D_2 receptor antagonist, sulpiride (50 mg kg^{-1}), but not by the D_1 receptor antagonist, SCH23390 (25 μg kg^{-1}), $F(5,58)=3.226$, $P=0.0125$; Figure 5b.

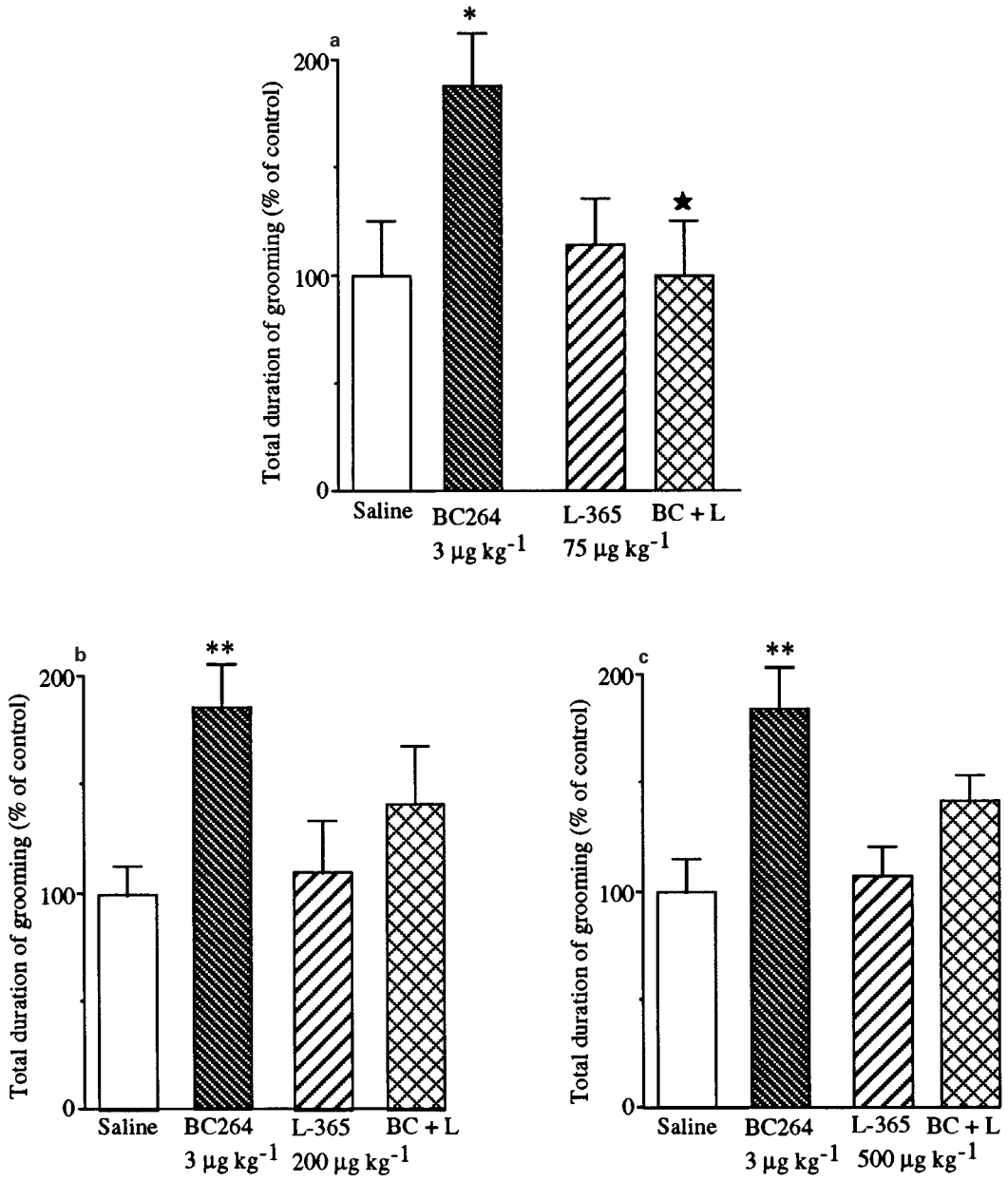

Figure 3 Effects of various doses (a, 75 μg kg^{-1}; b, 200 μg kg^{-1}; c, 500 μg kg^{-1}, i.p.) of the CCK_B receptor antagonist L-365,260 administered 30 min before BC264 (3 μg kg^{-1}, i.p.) on the increase in grooming induced by this agonist ($n=8-12$). *$P\leqslant0.05$; **$P\leqslant0.01$ *vs* saline group; ★$P\leqslant0.05$ vs BC264 group, Duncan test.

Discussion

In this work, the pharmacological effects of two potent and selective CCK_B receptor agonists, BC197 and BC264, were investigated on grooming activity in rats.

The results show that injection of low doses of BC264 (3, 10 and 30 $\mu g\ kg^{-1}$, i.p.) increased grooming bouts. Excessive grooming has been previously found after injection of non-selective CCK compounds, such as CCK8 or ceruletide into ventricles, caudate, substantia nigra or nucleus accumbens, but not after peripheral injection (Diamond *et al*., 1984; Kulkosky, 1988). Conversely, the i.p. administration of BC197 at a dose of 300 $\mu g\ kg^{-1}$ decreased grooming behaviour. It is interesting to note that both effects took place progressively, starting at 10 min during the 15 min period and were significant at the end of this period. The two CCK_B receptor agonists have previously been shown to have a low affinity for CCK_A receptors (rat pancreas: $K_i = 355 \pm 98$ nM for BC264, $K_i = 2120 \pm 303$ nM for BC197) (Derrien *et al*., 1994). Furthermore, the active doses of BC264 (3 $\mu g\ kg^{-1}$) and BC197 (300 $\mu g\ kg^{-1}$) were shown to produce CCK_B but not CCK_A receptor responses (Derrien *et al*., 1994; Ladurelle *et al*., 1997). Finally, the effect induced by BC264 was suppressed by a low dose of the selective CCK_B receptor antagonist, L-365,260 and a relatively high dose of the selective CCK_B receptor antagonist, CI-988. In addition, the effect of BC197 was antagonized by a low dose of CI988 and relatively high dose of L365,260. This indicates that the responses induced by both agonists very probably result from their interaction with CCK_B receptors.

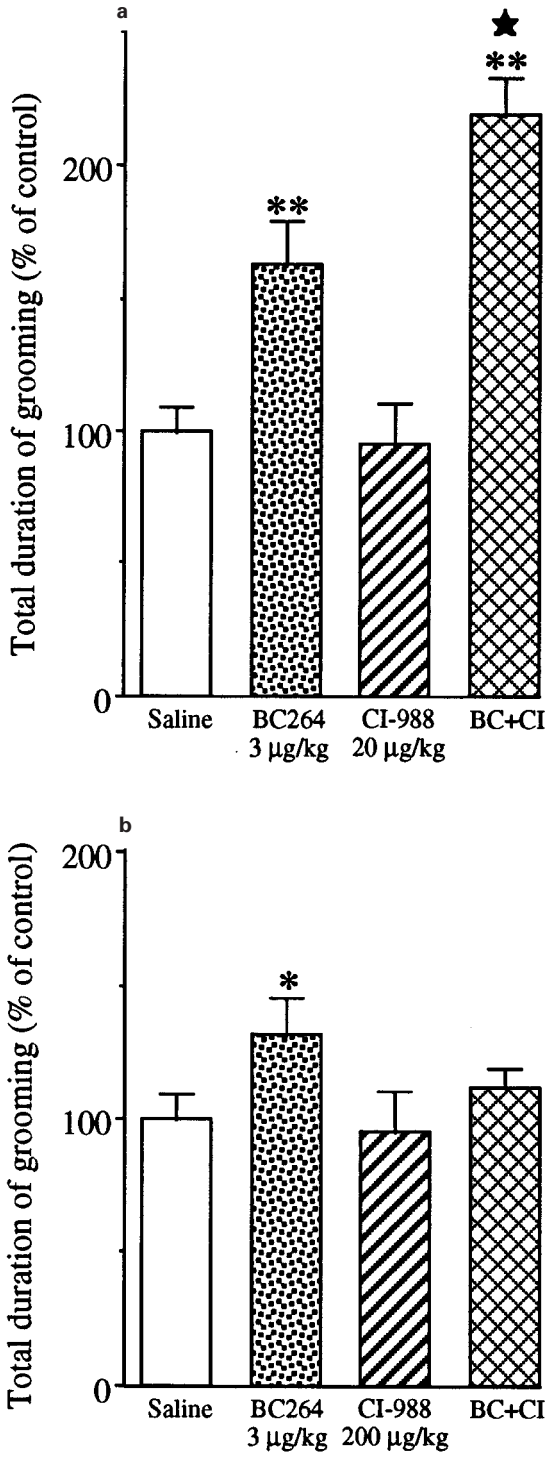

Figure 4 (a) Potentiation of the increase in grooming induced by BC264 (3 $\mu g\ kg^{-1}$, i.p.) using the CCK_B receptor antagonist CI-988 (20 $\mu g\ kg^{-1}$, i.p.) injected 30 min before the CCK_B receptor agonist (b) Partial antagonism of the increase in grooming induced by BC264 (3 $\mu g\ kg^{-1}$) by 200 $\mu g\ kg^{-1}$ of CI988, i.p., injected 30 min before the CCK_B agonist ($n = 8-11$). $^*P \leqslant 0.05$; $^{**}P \leqslant 0.01$ *vs* saline group; ★$P \leqslant 0.05$ *vs* BC264 group, Duncan test.

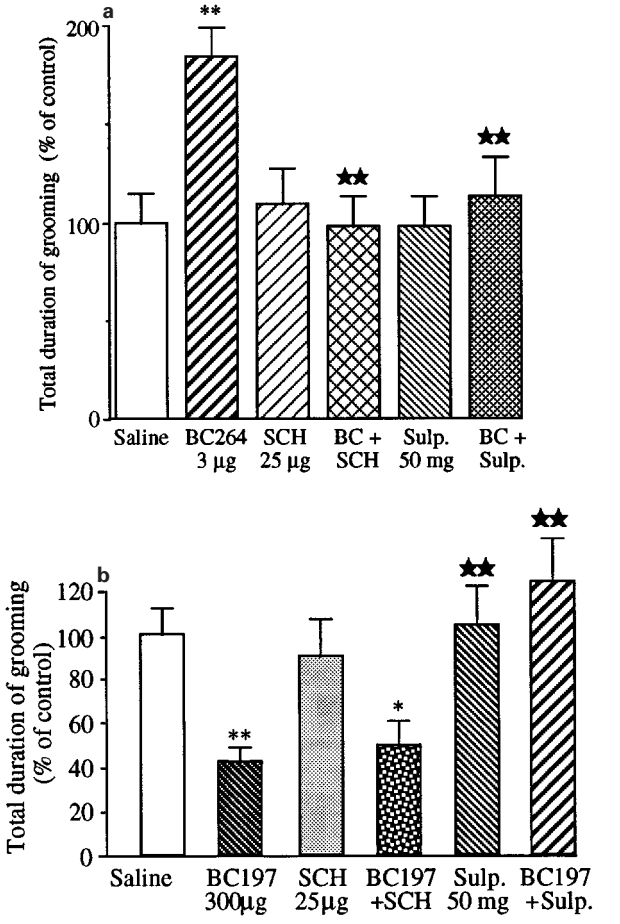

Figure 5 (a) Antagonism of the BC264-induced increase of grooming by the D_1, SCH23390 (25 $\mu g\ kg^{-1}$, i.p.) and the D_2, sulpiride (50 mg kg^{-1}, i.p.) receptor antagonists injected 15 min before BC264 ($n = 8$). (b) Antagonism of the BC197-induced decrease of grooming by the D_2, sulpiride (50 mg kg^{-1}, i.p.) but not by the D_1, SCH23390 (25 $\mu g\ kg^{-1}$, i.p.) receptor antagonists. The antagonists were injected 15 min before BC197 (300 $\mu g\ kg^{-1}$, i.p.) ($n = 10-11$). $^*P < 0.05$; $^{**}P \leqslant 0.01$ *vs* saline group; ★★$P \leqslant 0.01$ *vs* BC 264 or BC197 group, Duncan test.

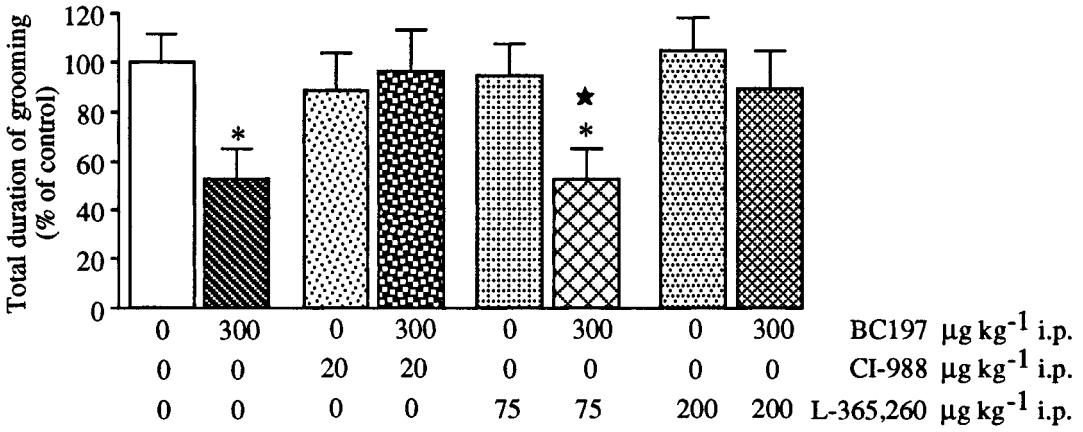

Figure 6 Effects of the CCK_B receptor antagonists L-365,260 (75 and 200 μg kg^{-1}, i.p.) and CI-988 (20 μg kg^{-1}, i.p.) administered 30 min before BC197 (300 μg kg^{-1}, i.p.) on the decrease in grooming induced by this agonist ($n=10-15$). *$P\leqslant0.05$ *vs* saline group; ★$P\leqslant0.05$ *vs* L-365,260 (75 μg kg^{-1}) group, Duncan test.

The opposing behavioural responses produced by these two selective CCK_B receptor agonists could be explained by the existence of two CCK_B subsites. Indeed, binding experiments have shown that BC197 interacts with a high affinity to one class of binding sites (CCK_{B1}, $K_i=2.3$ nM) and to another site with a lower affinity (CCK_{B2}, $K_i=540$ nM). In contrast, BC264 appears to have the same affinity ($K_i=0.39$ nM) for both subsites (Derrien *et al*., 1994). Moreover, BC197 has been shown to induce anxiogenic-like effects in rats and to increase the K^+-evoked release of dopamine from slices of rostral nucleus accumbens, while BC264 does not modify the level of anxiety and successively decreases (low doses) and enhances (high doses) the release of dopamine (Derrien *et al*., 1994; Léna *et al*., 1997).

It can be hypothesized that the stimulation of the B1 subsite by BC197 was responsible for the inhibition of grooming observed in this study. In contrast, the simultaneous activation of both B1 and B2 subsites by BC264 resulted in an enhancement of grooming. This last response might mean that B1 and B2 sites act in an opposing manner and that the B2 sites, the stimulation of which would lead to an increase in grooming, might be in higher concentrations and/or be more efficient, in the rat. Such a hypothesis has recently been suggested in *in vitro* experiments on dopamine release by Léna *et al*. (1997). To explore better the characteristics of the opposing effects induced by BC197 and BC264, the two CCK_B receptor antagonists L-365,260 and CI-988 were used. Our results showed that a small dose (75 μg kg^{-1}) of L-365,260 was able to block completely the effect induced by 3 μg kg^{-1} of BC264, while higher doses only produced a partial antagonism. Thus, supposing that L-365,260 preferentially binds to B2 subsites, the loss of activity with increasing doses of this antagonist could be due to its progressive action, firstly only on B2 sites (complete antagonism) and secondly on both B1 and B2 sites (partial antagonism). The previous administration of CI-988 at a dose of 200 μg kg^{-1} partially suppressed the effect induced by this agonist, while the dose of 20 μg kg^{-1} significantly binding to the B1 sites, blocking the decrease resulting from their stimulation by BC264, and thus potentiating the increase occurring from the activation of the B2 sites. The antagonism experiments carried out with BC197 seem to confirm this hypothesis, since the reduction of grooming induced by this compound was completely reversed by CI-988, while a dose of L-365,260 (200 μg kg^{-1}, i.p.), stronger than that required to antagonize BC264 responses, was needed to prevent the effects of BC197. As previously suggested by several authors, these two CCK_B receptor subsites could correspond to the existence of two binding states of the receptor (Durieux *et al*., 1986; 1988; Knapp *et al*., 1990; Derrien *et al*., 1994; Huang *et al*., 1994; Talkad *et al*., 1994; Harper *et al*., 1996; Perez *et al*., 1996; Léna *et al*., 1997)

It is well known that the peptidergic (CCK) and the dopaminergic systems are closely linked. For instance, CCK and dopamine were shown to coexist in a large proportion of neurones from the mesolimbic pathways (Hökfelt *et al*., 1980) and numerous dopaminergic responses are modulated by CCK (review in Crawley, 1991). Furthermore the agonists of dopamine receptors increased grooming behaviour, probably by interacting with the D_1 type receptor (Molloy & Waddington, 1984; Starr & Starr, 1986). The prior administration of both D_1 (SCH23390) and D_2 (sulpiride) receptor antagonists suppressed the increase of grooming induced by BC264, while the decrease of grooming induced by BC197 was only suppressed by sulpiride. Therefore, these two opposite responses are dependent on the dopaminergic system, but they differ with regard to the dopamine receptor types involved.

Grooming is elicited by a large number of peptides such as pro-opiomelanocortin, bombesin and melanopeptides. Furthermore certain peptides such as CCK, found in the gut and brain, function as ingestion-produced 'satiety signals' and induce excessive grooming which forms part of the behavioural sequence of satiety in the rat (Kulkosky, 1988). The results obtained with BC264 are in favour of the involvement of CCK_{B2} receptors in this behaviour. Nevertheless, it has been suggested that grooming serves functions other than cleaning and body maintenance, such as social signalling and increasing or decreasing arousal and self-stimulation. Grooming has also been considered as an index of behavioural adaptation to mild stressful or conflicting situations. Even mild stress such as placing a rat in a novel environment causes increased grooming (Mood *et al*., 1988 and reference herein). On the other hand, intense prolonged stress decreases grooming (Colbern and Gispen, 1988). Therefore, the decrease of grooming induced by BC197 could be a consequence of its previously described anxiogenic-like effects (Derrien *et al*., 1994). This is in contrast to BC264, which did not induce anxiogenic-like responses (Derrien *et al*., 1994; Charrier *et al*., 1995; Ladurelle *et al*., 1997), but was shown to increase attention and/or memory processes when rats were placed in a novel environment (Ladurelle *et al*., 1997).

Finally, the results described in this study provide additional evidence for the existence of two CCK_B receptor subsites and suggest that CCK_B receptor agonists and antagonists could be selected as a function of their affinity for the B1 and the B2 subsites. This dichotomy might be of critical interest, notably in regard to the anxiety processes commonly related to CCK_B receptor stimulation, since only one subsite appears to be responsible for these effects (Derrien *et al*., 1994).

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