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Impairment of stress adaptive behaviours in rats by the CCK_A receptor antagonist, devazepide

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1 Cholecystokinin (CCK) is released during stress both in limbic and hypothalamic areas suggesting that CCK could participate in modulating neuroendocrine as well as behavioural responses to stress.

2 In this study we have examined the effect of CCK receptor antagonists on the retention of the immobility response to a forced-swim stress in rats. In this test, rats are forced to swim during 15 min (conditioning period) and 24 h later, the duration of immobility is measured during a period of 5 min (re-test period). During the conditioning period rats display a period of vigorous activity, followed by progressive inactivity. During the re-test period rats remain 70-80% of the time in an immobile posture.

3 The CCK_A receptor antagonist, devazepide (MK-329) but not the CCK_B receptor antagonist, L-365,260, administered s.c. immediately before the conditioning period, decreased the duration of acquired immobility during the re-test period. The effect of devazepide was prevented by cholecystokinin octapeptide (CCK-8; 40 $\mu g\ kg^{-1}$, s.c.) as well as by the selective glucocorticosteroid G_{II} receptor agonist, dexamethasone (30 $\mu g\ kg^{-1}$, s.c.).

4 Neither corticosterone nor ACTH plasma levels measured both after the re-test period and after the conditioning period were modified by devazepide treatment.

5 The results suggest a role for CCK in the behavioural adaptation to stress and indicate a relationship between CCK systems and glucocorticoids in the neuronal mechanisms involved in the acquisition of adaptive behaviours to stress.

Keywords: CCK; devazepide; L-365,260; stress adaptive behaviours; dexamethasone; hypothalamo-pituitary-adrenal axis; hippocampus; depression; forced-swimming test

Introduction

Cholecystokinin (CCK) is a gastrointestinal peptide also found in the mammalian central nervous system (CNS). The sulphated C-terminal octapeptide of CCK (CCK-8) binds to at least two different receptors: the CCK_A receptor, also called the peripheral receptor although it has also been identified in the CNS, and the CCK_B receptor, which is the most abundant form in the brain. The main central physiological actions of CCK include modulation of pain perception (Baber *et al.*, 1989) and dopaminergic activity (Crawley, 1991). A role for CCK in the regulation of higher brain functions, such as emotivity (reviewed by Ravard & Dourish, 1990; Harro *et al.*, 1993) and memory processes (reviewed by Itoh & Lai, 1990), has also been proposed.

CCK-8 has been shown to be released during stress both in limbic areas (Siegel *et al.*, 1985) and in the hypothalamus (Siegel *et al.*, 1987) suggesting that CCK may participate, to some extent, in both the emotive and the neuroendocrine responses to stress. This has been related to the potential antidepressant (Hernando *et al.*, 1994; Derrien *et al.*, 1994) as well as the anxiolytic-like properties (reviewed by Ravard & Dourish, 1990 and by Harro *et al.*, 1993) of CCK_B receptor antagonists. On the other hand, it has been demonstrated that CCK modulates the activity of the hypothalamic-pituitary-adrenal axis both *in vivo* and *in vitro*. This regulation seems to occur both at the hypothalamic (Matsumura *et al.*, 1983; Kamilaris *et al.*, 1992; Calogero *et al.*, 1993) and hypophyseal level (Reisine & Jensen, 1986; Millington *et al.*, 1992). Accordingly, it has been proposed that CCK could be physiologically involved, as a second-rank secretagogue, in regulating ACTH release during stress (Reisine & Jensen, 1986).

CCK systems are involved in memory formation (Itoh *et al.*, 1992 and references cited therein) as demonstrated in avoidance paradigms as well as in spatial learning tasks. It has been suggested that stress may be an important factor leading to the development of depression (Willner, 1990 and references cited therein) or anxiety (Hamon, 1994 and references cited therein). It has been proposed that occupation of hippocampal glucocorticoid receptors during stress is necessary for the behavioural adaptation to stress (de Kloet *et al.*, 1988; Papolos *et al.*, 1993). By using the forced-swimming test, de Kloet *et al.* (1988) have demonstrated that the manifestation of immobility in rats during a second trial is conditioned by the occupation of hippocampal glucocorticoid receptors during the first one. Papolos *et al.* (1993) have suggested that glucocorticoids could exert a long-term influence on stress-induced behaviour by affecting glucocorticoid-responsive neurotransmitter and receptor genes.

The goal of this work was to study the involvement of CCK systems in the retention of behaviours associated with stress. For this purpose we investigated the effect of selective CCK_A and CCK_B receptor antagonists on the retention of immobility in the forced-swimming test in rats. The modulation by glucocorticoids of the CCK receptor antagonists effect was also examined by using dexamethasone, as a selective glucocorticoid receptor agonist.

Methods

Animals

Male Wistar rats (Charles River, Spain) weighing 150-180 g at the moment of the experiment were housed under controlled light/dark cycle (12h:12h) for a week before the experiment. Water and food were available *ad libitum*. All experiments were performed between 11 h 00 min and 16 h 00 min. Each animal was used only once.

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Forced-swimming test procedure

The forced-swimming test (FST) was performed in an apparatus identical to that described by Porsolt *et al.* (1978). Briefly, each rat was placed for 15 min in a vertical plexiglass cylinder (height 25 cm diameter 18 cm) containing water to a depth of 15 cm at 25°C ('conditioning period', CP). After 24 h, animals were put into the swimming pool again and the duration of the immobility measured during a period of 5 min ('re-test period', RP). Only active swimming, not floating movements, were taken into account for immobility measurement. Non-conditioned animals were forced to swim only during 5 min, 24 h after pharmacological treatment. Results are expressed as the percentage of immobility over a period of 5 min.

$$\% \text{ immobility} = [\text{Duration of immobility (s)}/300 \text{ s}] \times 100$$

Injection procedure

All drugs were administered subcutaneously. L-365,260 and devazepide were given in ethanol:polyethyleneglycol 200 (5:95) in a volume of 0.2 ml, 5 min prior to the 15 min CP. Dexamethasone free base was given in the same vehicle, 15 min after the CP. CCK-8 was administered in saline, 5 min after the CP. Animals were not re-treated before the 5 min re-test period. Non-conditioned animals were forced to swim only 24 h after the pharmacological treatment.

Measurement of ACTH and corticosterone plasma levels

Rats were killed by decapitation, (i) immediately after the RP, or (ii) at different time intervals (0, 15, 30, 60 or 150 min) after the CP. Trunk blood was collected in chilled EDTA-coated tubes and plasma obtained by centrifugation and kept at −80°C until radioimmunoassay (RIA).

ACTH was measured by double antibody precipitation RIA, as described by Woods *et al.* (1992). Rabbit IgG-ACTH-1 was used as the primary antiserum. Separation of the bound fraction was carried out by precipitation with a second antiserum (IgG-GARGG) and PEG 6000, 6%. Synthetic human ACTH was used as a reference standard and [^{125}I]-ACTH as a tracer. Under the conditions employed, the assay detects 0.5 fmol/tube of human ACTH, the intra-assay coefficient of variation being 10% and inter-assay coefficient of variation 13%.

Corticosterone was determined according to the method described by Armario & Castellanos (1984). Briefly, plasma samples were incubated for 1 h with trypsin, when the digestion was stopped with a trypsin inhibitor (30 min). Specific corticosterone antiserum and tracer were then added, and the mixture incubated at 4°C for 24 h. Free tracer was precipitated with charcoal 1%. Corticosterone was used as reference standard and [^{3}H]-corticosterone as tracer. The sensitivity was 12 pg/tube.

Chemicals

L-365,260 ((3**R**)-(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-3-methyl phenylurea; Lotti & Chang, 1989) and devazepide ((3**S**)-(−)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide; Chang & Lotti, 1986) were kindly provided by Merck Sharp and Dohme Research Laboratories (U.S.A.). CCK-8 and human ACTH were supplied by Bachem (Switzerland). Dexamethasone and corticosterone were obtained from Sigma (U.S.A.). [^{3}H]-corticosterone and [^{125}I]-ACTH were obtained from Amersham (U.K.). ACTH antiserum (IgG-ACTH-1) and second goat antirabbit serum (IgG-GARGG) were purchased from IgG-Corporation (U.S.A.). Corticosterone antiserum was supplied by Bioclin (U.K.). Trypsin and trypsin inhibitor were supplied by Boehringer Mannheim (Germany).

Analysis of data

In behavioural experiments, individual group comparisons were made using a two-way ANOVA. The factors of variation were treatment and conditioning. Individual dose-effects within a given group (conditioned or non-conditioned) were analyzed by one-way ANOVA. *Post hoc* comparisons were made with the Newman-Keuls' test. Corticosterone and ACTH plasma levels were compared, at each time, by two-way ANOVA (Snedecor & Cochran, 1980).

Results

Effect of the CCK-$_A$ receptor antagonist, devazepide and of the CCK$_B$ receptor antagonist, L-365,260, on retention of acquired immobility in the Porsolt swimming test

The effect of both devazepide (0.03, 0.1, 0.3 and 1 mg kg^{-1}) and L-365,260 (0.01, 0.1, 1 and 10 mg kg^{-1}) on the retention of immobility in the FST was examined.

In the case of devazepide, two-way ANOVA revealed significant treatment ($F_{(4,74)}=3.316$; $P<0.05$) and conditioning effects ($F_{(1,74)}=16.284$; $P<0.001$) without significant interaction between treatment and conditioning ($F_{(4,74)}=1.247$; NS). In conditioned animals, devazepide significantly decreased the duration of immobility ($F_{(4,46)}=6.98$; $P<0.01$; Figure 1a) whereas in non-conditioned rats, devazepide was without effect ($F_{(4,28)}=0.97$; NS; Figure 1b).

For the CCK$_B$ receptor antagonist, L-365,260, two-way ANOVA revealed a significant conditioning effect ($F_{(1,68)}=93.18$; $P<0.001$). Treatment effect and the interaction between both treatment and conditioning had no statistical significance ($F_{(1,68)}=1.308$, NS; $F_{(3,68)}=1.804$, NS respectively; Figures 2a and 2b).

In a single experiment, devazepide was administered either 5 min before or 3 h (the time at which corticosterone and ACTH plasma levels may be assumed to have reached basal levels) after the conditioning period (CP). As illustrated in Figure 3, devazepide was only effective in preventing the retention of immobility when administered before the CP. One-way ANOVA revealed a significant effect in the experiment carried out in animals treated with devazepide before the CP ($F_{(3,28)}=7.17$; $P<0.01$; $^{*}P<0.05$ for the dose of 0.1 mg kg^{-1}, $^{**}P<0.01$ for the dose of 0.3 mg kg^{-1}, Newman-Keuls test) but not in animals that received the drug after the CP ($F_{(3,25)}=0.44$; NS).

In non-conditioned animals the immobility was measured during a period of 15 min. Neither devazepide nor L-365,260 had an effect during the periods 0–5 min, 5-10 min or 10–15 min (data not shown). In addition, neither devazepide nor L-365,260 elicited a significant effect during the 15 min of CP (data not shown).

Effect of CCK-8 on retention of acquired immobility in the forced-swimming test

As illustrated in Figure 4, the effects induced by CCK-8 (1, 40 and 100 μg kg^{-1}) on the retention of immobility in the FST were studied. Two-way ANOVA revealed a significant conditioning effect ($F_{(1,50)}=61.662$; $P<0.001$). Treatment effect, as well as the interaction between both conditioning and treatment, was without statistical significance ($F_{(3,50)}=2.358$, NS; $F_{(3,50)}=0.863$, NS respectively).

As illustrated in Figure 5, the ability of CCK-8 (40 μg kg^{-1}) to inhibit the response elicited by devazepide (0.3 mg kg^{-1}) was evaluated. Two-way ANOVA revealed a significant treatment effect ($F_{(3,93)}=7.232$; $P<0.001$) and conditioning effect ($F_{(1,93)}=27.982$, $P<0.001$), without interaction between treatment and conditioning ($F_{(3,93)}=1.852$, NS). In conditioned animals, CCK-8 suppressed the decrease of the duration of

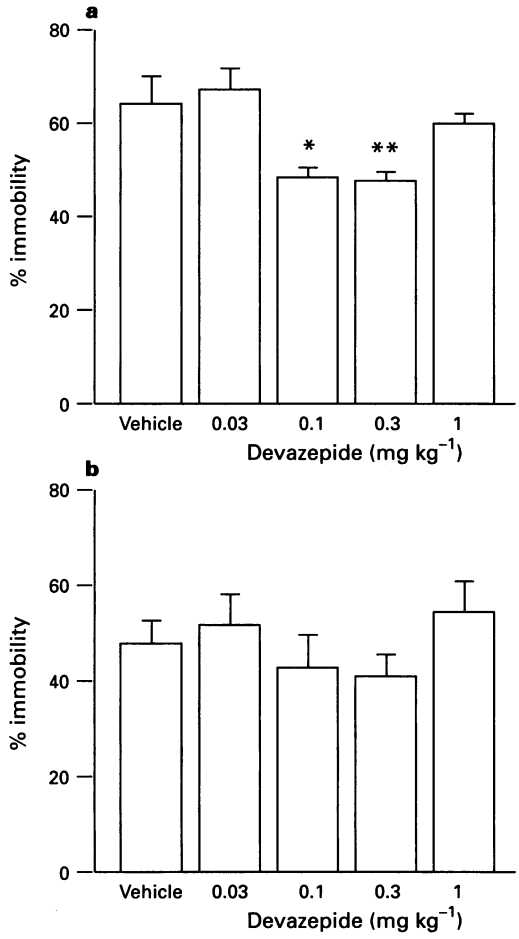

Figure 1 Dose-effect relationship of the CCK-$_A$ receptor antagonist, devazepide, on the duration of immobility in the forced-swimming test. The doses administered were 0.03 to 1 mg kg^{-1} (s.c.) (a) Conditioned animals were tested 24 h after a previous conditioning period (15 min forced-swim), performed 5 min after devazepide administration. (b) Non-conditioned animals were tested 24 h after devazepide administration. Newman-Keuls test indicates a significant effect for doses of 0.1 and 0.3 mg kg^{-1} in conditioned animals (*$P<0.05$; **$P<0.01$). No effect of devazepide was observed in non-conditioned animals. The results are expressed as % immobility ± s.e.mean over a period of 5 min. $n=7-9$ animals per group.

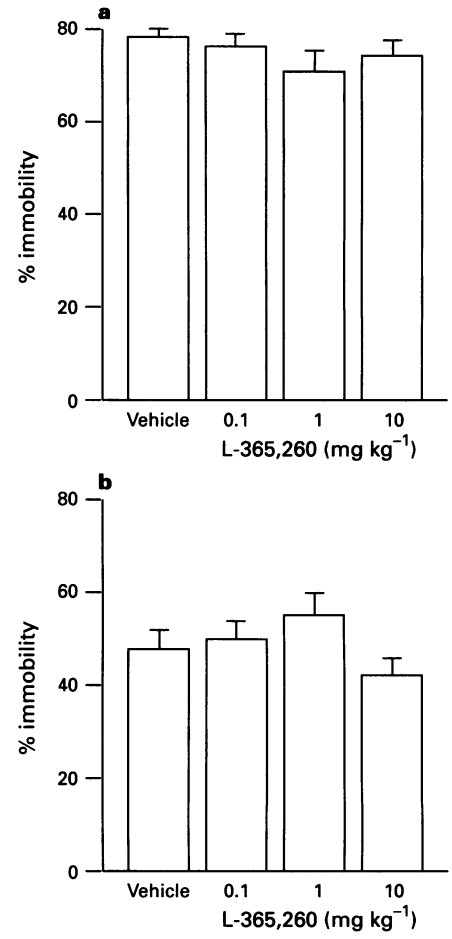

Figure 2 Dose-effect relationship of the CCK$_B$ receptor antagonist, L-365,260, on the duration of immobility in the forced-swimming test. The doses administered were 0.1 to 10 mg kg^{-1} (s.c.). (a) Conditioned animals were tested 24 h after a previous conditioning period (15 min forced-swim), performed 5 min after L-365,260 administration. (b) Non-conditioned animals were tested 24 h after L-365,260 administration. L-365,260 had effect neither in conditioned nor in non-conditioned animals (Newman-Keuls test). The results are expressed as % immobility ± s.e.mean over a period of 5 min. $n=8-9$ animals per group.

immobility induced by devazepide ($F_{(3,68)}=6.76$; $P<0.01$). In non-conditioned animals CCK-8 was without effect ($F_{(3,25)}=2.34$; NS).

Effect of dexamethasone on the retention of acquired immobility in the forced-swimming test

As illustrated in Figure 6, the effect of the glucocorticoid G$_{II}$ agonist, dexamethasone (10, 30 and 100 μg kg^{-1}) was evaluated. Two-way ANOVA was significant for both the conditioning effect ($F_{(1,44)}=49.160$, $P<0.001$) and interaction between treatment and conditioning ($F_{(3,44)}=7.333$; $P<0.01$). Treatment effect was not significant ($F_{(3,44)}=0.738$, NS). In conditioned animals, dexamethasone decreased the duration of immobility at the dose of 100 μg kg^{-1} ($F_{(3,23)}=3.37$; $P<0.05$). In non-conditioned animals, dexamethasone (100 μg kg^{-1}) increased the duration of immobility ($F_{(3,21)}=7.64$; $P<0.01$).

The ability of dexamethasone (30 μg kg^{-1}) to block the response elicited by devazepide (0.3 mg kg^{-1}) was evaluated (Figure 7). Two-way ANOVA revealed significant treatment ($F_{(3,70)}=11.755$; $P<0.01$) and conditioning ($F_{(1,70)}=58.938$; $P<0.01$) effects, as well as a significant interaction between treatment and conditioning ($F_{(3,70)}=3.232$; $P<0.05$). In con-

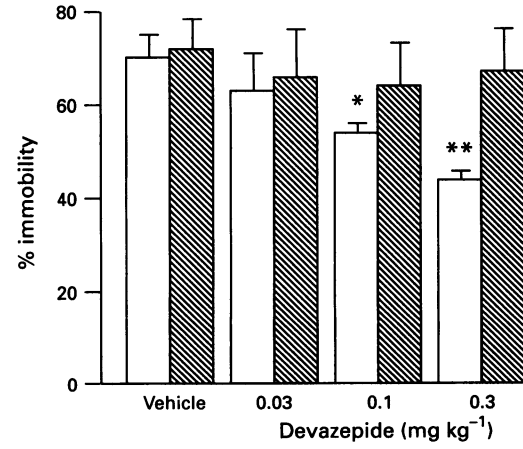

Figure 3 Dose-effect relationship of the CCK$_A$ receptor antagonist, devazepide, on the duration of immobility during the re-test period in conditioned rats. Devazepide (0.03, 0.1 and 0.3 mg kg^{-1}) was administered 5 min before (open columns) or 3 h after (hatched columns) the conditioning period (CP). Newman Keuls test indicates a significant treatment effect in animals that received devazepide 5 min before the CP, at a dose of 0.1 (*$P<0.05$) and 0.3 (**$P<0.01$) mg kg^{-1}. The results are expressed as % of immobility ± s.e.mean over a period of 5 min. $n=6-9$ animals per group.

ditioned animals dexamethasone blocked the effect of devazepide ($F_{(3,47)}=13.07$; $P<0.01$). Treatment was without effect in non-conditioned animals ($F_{(3,23)}=0.65$; NS).

Dexamethasone was without effect during the 15 min of conditioning period (data not shown).

Effect of devazepide on ACTH and corticosterone plasma levels after 15 min forced-swim stress (FSS)

The effect of devazepide (0.3 mg kg^{-1}) on both ACTH and corticosterone plasma levels was determined 0, 15, 30, 60 and 150 min after 15 min FSS. As indicated in Table 1, two-way ANOVA revealed a significant effect of FSS on plasma levels of ACTH only at time 0 ($F_{(1,20)}=16.53$; $P<0.01$). At this time, neither treatment nor the interaction between stress and treatment was significant. In the case of corticosterone (Table 2), two-way ANOVA indicated a stress effect, 0 ($F_{(1,20)}=25.15$; $P<0.01$), 15 ($F_{(1,24)}=26.56$; $P<0.01$), 30 ($F_{(1,40)}=50.66$; $P<0.01$) and 60 min ($F_{(1,21)}=0.037$; $P<0.05$) after FSS. Neither treatment nor the interaction between stress and treatment was different at any time.

The effect of devazepide (0.3 mg kg^{-1}) on ACTH and corticosterone plasma levels was also determined just after the re-test period. At this time no significant differences were found either in corticosterone (320±28 ng ml^{-1} vs 380±42 ng ml^{-1} for control and devazepide treated animals; Values are mean ±s.e.mean of 19 animals; Student's t test) or in ACTH plasma levels (128±11 fmol ml^{-1} vs 161±17 fmol ml^{-1} for control and devazepide-treated animals; Values are mean±s.e.mean of 19 animals; Student's t test).

Discussion

In this study we examined the effect of CCK receptor antagonists on the retention of immobility in Porsolt's forced-swimming test in rats. Animals were treated s.c. with CCK_A or CCK_B receptor antagonists just prior to 15 min of forced-swim stress ('conditioning period'; CP). 24 h later, animals were put in the swimming pool again, without previous pharmacological treatment, and the duration of immobility measured during 5 min ('re-test period'; RP). Under these conditions rats react, during the CP, by a vigorous initial attempt to escape followed by immobility (rats remain immobile about 40% of the time over the first 5 min). During the RP, untreated animals are immobile 70–80% of the total time. This suggests that animals 'learn' to be immobile (retention of immobility) as a consequence of an identical previous stressful experience. This behavioural pattern is responsive to antidepressant treatment

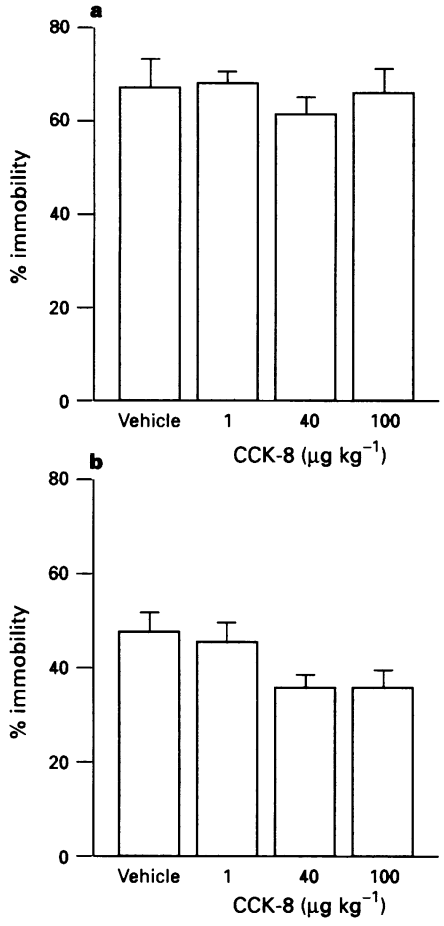

Figure 4 Dose-effect relationship of CCK-8 on the duration of immobility in the forced-swimming test. The doses administered were 1 to 100 μg kg^{-1} (s.c.). (a) Conditioned animals were tested 24 h after a previous conditioning period (15 min forced-swim). CCK-8 was administered 5 min after the conditioning period. (b) Non-conditioned animals were tested 24 h after CCK-8 administration. CCK-8 was without effect both in conditioned and in non-conditioned animals (Newman-Keuls test). The results are expressed as % immobility±s.e.mean over a period of 5 min. $n=5-7$ animals per group.

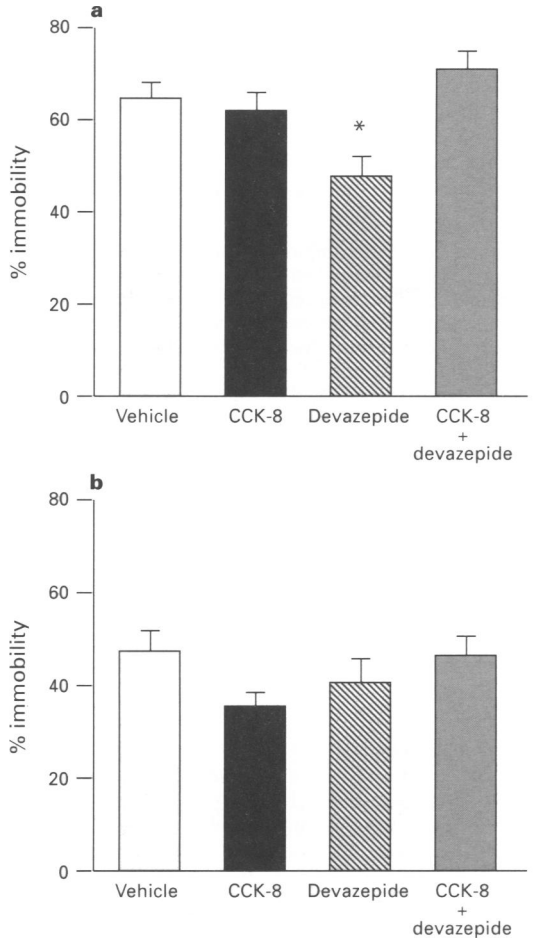

Figure 5 Effect of CCK-8 (40 μg kg^{-1}, s.c.) on the response induced by devazepide (0.3 mg kg^{-1}, s.c.). (a) Conditioned animals were tested 24 h after a previous conditioning period (CP; 15 min forced-swim). CCK-8 was administered 5 min after the CP. Devazepide was administered 5 min before the CP. (b) Non-conditioned animals were tested 24 h after administration of drugs. CCK-8 prevented the effect of devazepide in conditioned animals (*$P<0.05$, compared to the other three groups; Newman-Keuls test). No effect of treatment was observed in non-conditioned animals. The results are expressed as % immobility±s.e.mean over a period of 5 min. $n=9-11$ animals per group.

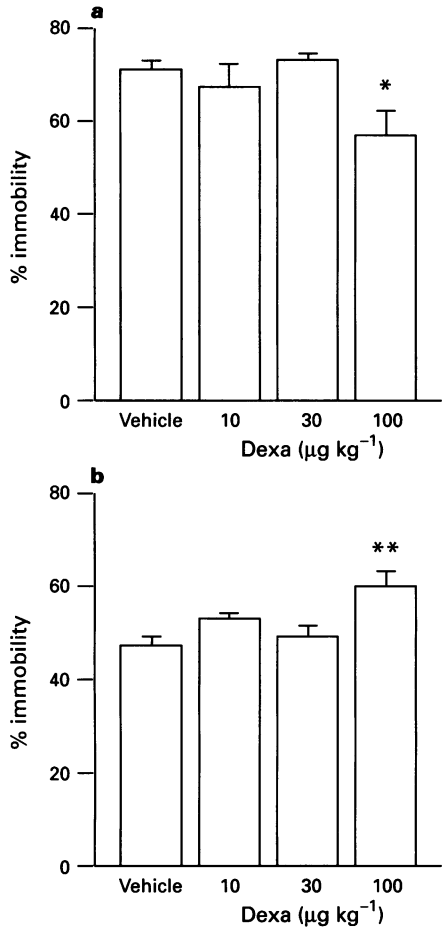

Figure 6 Dose-effect relationship of the glucocorticoid (G_{II}) receptor agonist, dexamethasone (Dexa), on the duration of immobility in the forced-swimming test. The doses administered were 10 to 100 $\mu g\,kg^{-1}$ (s.c.). (a) Conditioned animals were tested 24 h after a previous conditioning period (15 min forced-swim). Dexamethasone was administered 15 min after forced-swim stress. (b) Non-conditioned animals were tested 24 h after dexamethasone administration. In conditioned animals dexamethasone decreased the duration of immobility (*$P<0.05$, compared to the vehicle group; Newman-Keuls test). In non-conditioned animals dexamethasone increased the duration of immobility (**$P<0.01$, compared to the vehicle group; Newman-Keuls test). The results are expressed as % immobility ± s.e.mean over a period of 5 min. $n=5-7$ animals per group.

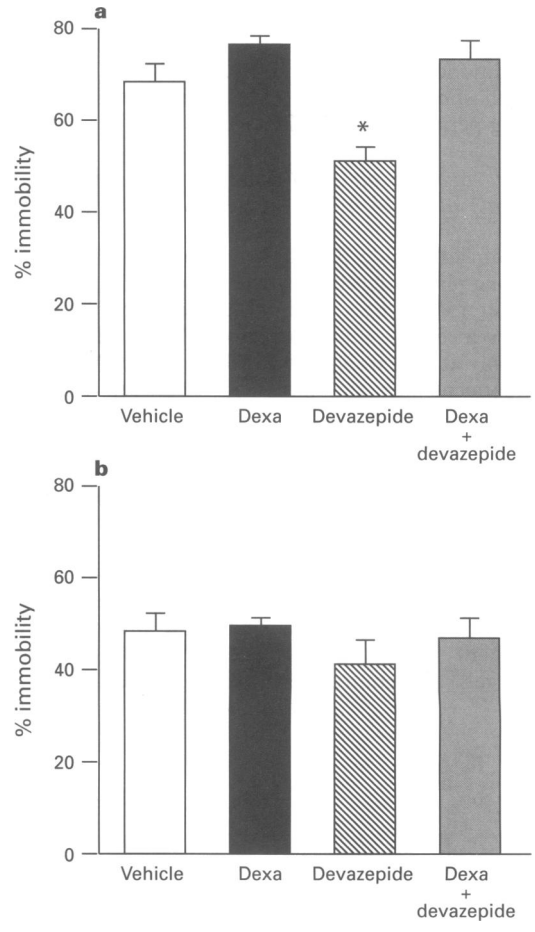

Figure 7 Effect of dexamethasone (30 $\mu g\,kg^{-1}$, s.c.) on the response induced by devazepide (0.3 $mg\,kg^{-1}$, s.c.). (a) Conditioned animals were tested 24 h after a previous conditioning period (CP; 15 min forced-swim). Dexamethasone was administered 15 min after the CP. Devazepide was administered 5 min before the CP. *$P<0.05$ Newman-Keuls test. (b) Non-conditioned animals were tested 24 h after administration of drugs. In conditioned animals, dexamethasone blocked the effect of devazepide (*$P<0.05$ compared to the other three groups; Newman-Keuls test). The results are expressed as % immobility ± s.e.mean over a period of 5 min. $n=8-10$ animals per group.

Table 1 Time-course of devazepide (0.3 $mg\,kg^{-1}$) effect on plasma ACTH levels (fmol ml^{-1}) in basal conditions (a) and after 15 min forced-swim stress (b)

	Time after forced swimming (min)				
Group	*0*	*15*	*30*	*60*	*150*
Control + vehicle[a]	167 ± 17	155 ± 16	52 ± 11	73 ± 3	29 ± 3
Control + devazepide[a]	220 ± 54	129 ± 13	42 ± 7	94 ± 19	29 ± 2
Stress + vehicle[b]	435 ± 81**	164 ± 16	67 ± 10	92 ± 17	26 ± 3
Stress + devazapide[b]	421 ± 57†	166 ± 17	85 ± 11	120 ± 9	30 ± 4

The vehicle or devazepide were given 5 min prior to 15 min forced-swim. **$P<0.01$ when compared to control + vehicle group. †$P<0.05$ when compared to control ± devazepide group (Student's t test). Values, from 6–10 animals, are mean ± s.e.mean.

in rodents (Porsolt *et al.*, 1978). The duration of acquired immobility in male rats during the RP was reduced in animals that received the CCK_A receptor antagonist, devazepide (MK-329) prior to the CP. The CCK_B receptor antagonist, L-365,260 had no effect over a wide range of doses (0.1 to 10 mg kg^{-1}). Neither CCK_A nor CCK_B receptor antagonists had any effect on the duration of immobility during the CP (data not shown). The effect elicited by devazepide was prevented by CCK-8. The selective G_{II} glucocorticoid agonist, dexamethasone, administered after the CP, also reversed the effect of devazepide. In contrast, neither devazepide nor L-365,260 had an effect in non-conditioned animals that received the drug 24 h prior to 5 min of forced-swim stress.

Devazepide decreased the duration of immobility during the RP following a U-shaped dose-response relationship. U-shaped dose-responses often characterize the effect of CCK

Table 2 Time-course of devazepide (0.3 mg kg^{-1}) effect on plasma corticosterone levels (ng ml^{-1}) in basal conditions (a) and after 15 min forced-swim stress (b)

	Time after forced swimming (min)				
Group	*0*	*15*	*30*	*60*	*150*
Control + vehicle[a]	470±88	439±68	218±32	111±44	127±20
Control + devazepide[a]	505±88	436±42	229±43	228±49	152±30
Stress + vehicle[b]	764±57*	1327±248**	618±78**	196±49	119±20
Stress + devazepide[b]	932±76†	1108±154††	791±108††	278±40	95±13

The vehicle or devazepide were given 5 min prior to the 15 min swimming period. *$P<0.05$, **$P<0.01$ when compared to their respective control + vehicle groups. †$P<0.05$, ††$P<0.01$ when compared to the control + devazepide group (Student's t test). Values are mean±s.e.mean of 6–10 animals.

agonists and antagonists on behaviour and electrophysiological activity (Crawley *et al.*, 1985; Daugé *et al.*, 1992). This response pattern has been interpreted as the result of an opposite action in the modulation of neuronal activity and behaviour by CCK_A and CCK_B receptors (Vasar *et al.*, 1991; O'Neill *et al.*, 1991; Millington *et al.*, 1992; Hernando *et al.*, 1994) and thus high doses of devazepide may result in the behavioural pattern observed by us. The effect observed seems to be due only to the blocking of CCK_A receptors by devazepide, since the CCK_B antagonist, L-365,260, even at the dose of 10 mg kg^{-1}, was not effective in decreasing the duration of immobility. Although our results have no bearing on whether this effect is mediated by a central mechanism, it could be expected that, in addition to the blocking of CCK_A peripheral receptors, devazepide could also block CCK_A receptors in the brain. In fact, it has been reported that devazepide (1 mg kg^{-1}) induces memory impairment in rats, after i.p. administration, by blocking central CCK_A receptors (Itoh *et al.*, 1992).

The ability of CCK-8, given s.c. at low doses, to block the effect of devazepide, suggests a peripheral site of action for this peptide. However, it has been previously proposed that stimulation of CCK receptors in the periphery might activate central CCK systems (Hernando *et al.*, 1994). In accordance with this, it has been demonstrated that vagotomy suppresses some behavioural changes elicited by peripheral CCK-8 (Itoh & Katsuura, 1986 and references cited therein; Crawley *et al.*, 1981). It has been demonstrated that low peripheral doses of both CCK-4 and CCK-8 elicit marked behavioural effects (De Montigny, 1989; Bradwejn *et al.*, 1991; Hernando *et al.*, 1994) while i.v. administered [^{125}I]-BH-CCK_4 can reach central structures (Merani *et al.*, 1994). The effect of CCK agonists could also be due to their interaction with CCK receptors located in central structures lacking a blood-brain barrier, such as the *nucleus tractus solitarius* (Branchereau *et al.*, 1992).

The lack of effect of devazepide in non-conditioned rats indicates that the behaviour observed is not due to a long-lasting effect of devazepide. On the basis of these results, the activation of neuronal pathways involving CCK-ergic systems that probably occurs during forced-swimming seems to be required for the devazepide effect to be observed and suggests that CCK may influence post-stress information storage. It has been reported that CCK-related peptides prevent experimental amnesia in rats (Katsuura & Itoh, 1986; Maurice *et al.*, 1994). Accordingly, CCK_A receptor antagonists induce memory impairment in rats (Itoh *et al.*, 1992 and references cited therein).

The occupation of G_{II} receptors in the hippocampus has been shown to be necessary for the behavioural adaptation to stress (De Kloet *et al.*, 1988; Papolos *et al.*, 1993) such as the enhancement of immobility in the forced-swimming test (FST). Direct application of the glucocorticoid G_{II} antagonist RU-24,486 in the hippocampal formation, as well as adrenalectomy, impairs retention of immobility in the FST without modifying the activity of the HPA axis (De Kloet *et al.*, 1988). In addition, corticosterone may modulate hippocampal functioning during memory formation (Micheau *et al.*, 1985). In our study, the effect of devazepide was prevented by treatment with dexamethasone, a selective G_{II} agonist. This result suggests that CCK_A receptor blocking has an opposite behavioural effect to that elicited by occupation of central glucocorticoid receptors. An alternative explanation for the interaction observed could be a decrease in the activity of the HPA during stress elicited by devazepide. CCK is released during stress from hypothalamic neurones and it has been demonstrated that CCK can stimulate the activity of the HPA axis both *in vivo* and *in vitro* (Kamilaris *et al.*, 1992; Millington *et al.*, 1992). We have examined the effect of devazepide on the activity of the HPA axis by measuring both corticosterone and ACTH plasma levels in rats exposed to forced-swim stress. Devazepide did not modify the forced-swim stress-induced increase in plasma levels of either hormone measured 0, 15, 30, 60 and 150 min after the CP. These results suggest that the effect of the CCK_A receptor antagonist, although blocked by dexamethasone, does not appear to be related to the HPA axis inhibition. Moreover, ACTH and corticosterone plasma levels, determined after the RP, were identical in devazepide-treated and untreated animals. Our results lead us to speculate that the blocking of CCK receptors in the CNS modulates glucocorticoid-mediated behaviours without altering HPA axis activity.

The blocking of the devazepide effect by dexamethasone suggests that both G_{II} and CCK receptor activation could have similar effects on neuronal activity in brain areas involved in the retention of immobility, and thus dexamethasone could compensate for the effect of CCK receptor blocking. Interestingly, when devazepide was administered 3 h after the CP (a period at which corticosterone plasma level can be assumed to be at basal level) no effect on immobility was detected during the RP.

It should be pointed out that in a previous study in our laboratory (Hernando *et al.*, 1994) we observed that the CCK_B receptor antagonist, L-365,260, but not devazepide, elicits an antidepressant-like effect in the FST in mice. This apparent contradiction could reflect the different animal species and substantially different protocol that was used. L-365,260 was administered, just prior to the test (30 min before), in non-conditioned animals, and its effect can be considered as a typical antidepressant-like response in the FST. In the present work, the devazepide effect is linked to post-stress information storage rather than with a classical antidepressant-like effect.

In summary our data have shown that the blocking of CCK_A receptors by the selective CCK_A receptor antagonist, devazepide impairs the acquisition of adaptive behaviours to stress, such as the retention of immobility in the FST. The effect of devazepide is abolished by the glucocorticoid agonist, dexamethasone, indicating that both glucocorticoid and CCK receptors are involved in similar behavioural responses induced by stress. Our results would suggest that CCK_A receptor antagonists could be useful drugs for protecting limbic areas from the noxious effects of stress, which have been related to the development of psychological disorders, such as depression and anxiety.

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References

ARMARIO, A. & CASTELLANOS, J.M. (1984). A simple procedure for direct corticosterone radioimmunoassay in the rat. *Rev. Esp. Fisiol.*, **40**, 437–442.

BABER, N.S., DOURISH, C.T. & HILL, D.R. (1989). The role of CCK, caerulein, and CCK antagonists in nociception. *Pain*, **39**, 307–328.

BRADWEJN, J., KOSZYCKI, D. & SHRIQUI. (1991). Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder. Clinical and behavioural findings. *Arch. Gen. Psychiatry*, **48**, 603–610.

BRANCHERAU, P., BÖHME, C.A., CHAMPAGNAT, J., MORIN-SURUN, M.P., DURIEUX, C., BLANCHARD, J.C., ROQUES, B.P. & DENAVIT-SAUBIE, M. (1992). Cholecystokinin A and cholecystokinin B receptors in neurons of the brainstream solitary complex of the rat: pharmacological identification. *J. Pharmacol. Exp. Ther.*, **260**, 1433–1440.

CALOGERO, A.E., NICOLISI, A.M.G., MONCADA, M.L., CONIGLIONE, F., VICARI, E., POLOSA, P. & D'AGATA, R. (1993). Effects of cholecystokinin octapeptide on the hypothalamic-pituitary-adrenal axis function and on vasopressin, prolactin and growth hormone release in humans. *Neuroendocrinology*, **58**, 71–76.

CHANG, R.S. & LOTTI, V.J. (1986). Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4923–4926.

CRAWLEY, J.N. (1991). Cholelcystokinin-dopamine interactions. *Trends Pharmacol. Sci.*, **12**, 232–235.

CRAWLEY, J.N., HAYS, S.E. & PAUL, S.M. (1981). Vagotomy abolishes the inhibitory effects of cholecystokinin on rat exploratory behaviours. *Eur. J. Pharmacol.*, **73**, 379–380.

CRAWLEY, J.N., HOMMER, L.R. & SKIRBOLL, L.R. (1985). Topographical analysis of nucleus accumbens sites at which cholecystokinin potentiates dopamine-induced hypolocomotion in rats. *Brain Res.*, **335**, 337–341.

DAUGE, V., DERRIEN, M., BLANCHARD, J.C. & ROQUES, B.P. (1992). The selective CCK-B agonist, BC-264, injected in the antero-lateral part of the nucleus accumbens, reduces the spontaneous alternation behaviour of rats. *Neuropharmacology*, **31**, 67–75.

DE KLOET, E.R., DE KOCK, S. & VELDHUIS, H.D. (1988). Antiglucocorticoid RU 38486 attenuates and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites. *Neuroendocrinology.*, **47**, 109–115.

DE MONTIGNY, C. (1989). Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers. *Arch. Gen. Psychiatry*, **46**, 511–517.

DERRIEN, M., DURIEUX, C. & ROQUES, B.P. (1994). Antidepressant-like effects of CCK_B antagonists in mice: antagonism by naltrindole. *Br. J. Pharmacol.*, **111**, 956–960.

HAMON, M. (1994). Neuropharmacology of anxiety: perspectives and prospects. *Trends Pharmacol. Sci.*, **15**, 36–39.

HARRO, J., VASAR, E. & BRADWEJN, J. (1993). CCK in animal and human research on anxiety. *Trends Pharmacol. Sci.*, **14**, 244–249.

HERNANDO, F., FUENTES, J.A., ROQUES, B. & RUIZ-GAYO, M. (1994). The CCK-B receptor antagonist, L-365,260, elicits antidepressant-type effects in the forced-swimming test in mice. *Eur. J. Pharmacol.*, **261**, 257–263.

ITOH, S. & KATSUURA, G. (1986). Behavioural effect of cholecystokinin octapeptide in vagotomized rats. *Can. J. Physiol. Pharmacol.*, **64**, 745–747.

ITOH, S. & LAI, H. (1990). Influences of cholecystokinin and analogues on memory processes. *Drug Develop. Res.*, **21**, 257–266.

ITOH, S., TAKASHIMA, A. & MAEDA, Y. (1992). Memory impairments induced by peripherally administered cholecystokinin A-type receptor antagonists in rats. *Drug Develop. Res.*, **26**, 89–99.

KAMILARIS, T.C., JOHNSON, E.O., CALOGERO, A.E., KALOGERAS, K.T., BERNARDINI, R., CHOROUS, G.P. & GOLD, P.W. (1992). Cholecystokinin-octapeptide stimulates hypothalamic-pituitary-adrenal function in rats: Role of corticotropin-releasing hormone. *Endocrinology*, **130**, 1764–1774.

KATSUURA, G. & ITOH, S. (1986). Preventive effect of cholecystokinin octapeptide on experimental amnesia in rats. *Peptides*, **7**, 105–110.

LOTTI, V.J. & CHANG, R.S.L. (1989). A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand L-365,260. *Eur. J. Pharmacol.*, **162**, 273–280.

MATSUMURA, M., YAMANOI, A., YAMAMOTO, S. & SAITO, S. (1983). In vivo and in vitro effects of cholecystokinin octapeptide on the release of β-endorphin-like immunoreactivity. *Neuroendocrinology*, **36**, 443–448.

MAURICE, T., HIRAMATSU, M., KAMEYAMA, T., HASEGAWA, T. & NABESHIMA, T. (1994). Cholecystokinin-related peptides, after systemic or central administration, prevent carbon monoxide-induced amnesia in mice. *J. Pharmacol. Exp. Ther.*, **269**, 665–673.

MERANI, S., GUTKOWSKA, J., BRADWEJN, F.R. & ERVIN, F.R. Behavioural effects of CCK-4: Central or peripheral? *Society for Neuroscience. 24th Annual Meeting*, November 13–18, 1994. Abst. No 163.3.

MICHEAU, J., DESTRADE & SOUMIREU-MOURAT, B. (1985). Time-dependent effects of posttraining intrahippocampal injections of corticosterone on retention of repetitive learning tasks in mice. *Eur. J. Pharmacol.*, **106**, 39–46.

MILLINGTON, W.R., MUELLER, G.P. & LAVIGNE, G. (1992). Cholecystokinin type A and type B receptor antagonists produce opposing effects on cholecystokinin-stimulated β-endorphin secretion from the rat pituitary. *J. Pharmacol. Exp. Ther.*, **612**, 454–461.

O'NEILL, M.F., DOURISH, C.T. & IVERSEN, S.D. (1991). Hypolocomotion induced by peripheral or central injection of CCK in the mouse is blocked by the CCK-A receptor antagonist devazepide, but not by the CCK-B receptor antagonist L-365,260. *Eur. J. Pharmacol.*, **193**, 203–208.

PAPOLOS, D.F., EDWARDS, E., MARMUR, R., LACHMAN, H.M. & HENN, F.A. (1993). Effects of the antiglucocorticoid RU 38486 on the induction of learned helpless behaviour in Sprague-Dawley rats. *Brain Res.*, **615**, 304–309.

PORSOLT, R., ANTON, G., BLAVET, N. & JALFRE, M. (1978). Behavioural despair in rats: A new model sensitive to Anti-depressant treatments. *Eur. J. Pharmacol.*, **47**, 379–383.

RAVARD, S. & DOURISH, C.T. (1990). Cholecystokinin and anxiety. *Trends Pharmacol. Sci.*, **11**, 271–273.

REISINE, T. & JENSEN, R. (1986). Cholecystokinin-8 stimulates adrenocorticotropin release from anterior pituitary cells. *J. Pharmacol. Exp. Ther.*, **236**, 621–626.

SIEGEL, R.A., DÜKER, E.-M., FUCHS, E., PAHNKE, U. & WUTTKE, W. (1985). Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal system to stress, in the male rat. *Neurochem. Int.*, **6**, 783–789.

SIEGEL, R.A., DÜKER, E-M., PAHNKE, U. & WUTTKE, W. (1987). Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. *Neuroendocrinology*, **46**, 75–81.

SNEDECOR, G.W. & COCHRAN, W.G. (1980). *Statistical Methods.* 7th Edition. Iowa: The Iowa State University Press.

VASAR, E., HARRO, J., LANG, A., POLD, A. & SOOSAAR, A. (1991). Differential involvement of CCK-A and CCK-B receptors in the regulation of locomotor activity in the mouse. *Psychopharmacology*, **105**, 393–399.

WILLNER, P. (1990). Animal models of depression: an overview. *Pharmacol. Ther.*, **45**, 425–455.

WOODS, M.D., SHIPSTON, J., MULLENS, E.L. & ANTONI, F.A. (1992). Pituitary corticotrope tumor (AtT20) cells as a model system for the study of early inhibition by glucocorticoids. *Endocrinology*, **131**, 2873–2880.

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