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Cholecystokinin-8 regulation of NGF concentrations in adult mouse brain through a mechanism involving CCK_A and CCK_B receptors

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1 Nerve growth factor (NGF), a powerful agent for the growth, differentiation and regeneration of lesioned cells of the central and peripheral nervous systems, has in recent years been indicated as a potential therapeutic agent capable of reversing the processes of cell damage in neurodegenerative events in man. Since NGF does not cross the blood-brain barrier and central NGF administration requires invasive surgical procedures, the discovery of substances modulating *in vivo* NGF synthesis in the brain will be extremely useful for a possible clinical use of NGF.

2 The aim of the present study to analyse if the content of NGF in the brain of adult mice can be affected by peripheral administration of cholecystokinin-8 (CCK-8), a well known neuropeptide which has stimulant actions on neurons in the brain and promotes a variety of neurobehavioural effects both in man and rodents.

3 The dose-response and time course effects of an i.p. injection of CCK-8 on the NGF concentrations in the hippocampus, cortex, hypothalamus and pituitary of adult male mice were analysed by use of a sensitive immunoenzymatic assay for NGF. The effects of pretreatment with selective CCK_A and CCK_B receptor antagonists and atropine on the NGF response to CCK injection were also studied.

4 The effects of CCK-8 were dose- and time-dependent and the injection of 8 nmol kg^{-1} resulted in a 3 fold increase of NGF levels in the hypothalamus and pituitary, and about a 60% increase in the hippocampus. No effects were observed in the cortex. Pretreatment with a selective CCK_A receptor antagonist blocked the CCK-induced NGF increase in the hypothalamus and pituitary. In the hippocampus the same effect was obtained with a CCK_B receptor antagonist. Pretreatment with atropine suppressed the CCK-induced effects on NGF levels in all the brain regions examined.

5 Our results showing that i.p. injection with CCK-8 can modulate NGF levels in the brain through a mechanism which seems, in part, to be mediated via the vagal afferents, indicate that this neuropeptide may represent a useful pharmacological approach to enhance endogenous NGF levels in neuropathologies associated with a neurotrophin deficit.

Keywords: Nerve growth factor (NGF); cholecystokinin-8 (CCK-8); CCK-8 receptors; CCK_A; CCK_B; brain; hypothalamus, hippocampus; pituitary; atropine

Introduction

Nerve growth factor (NGF), the first and best characterized member of the neurotrophin family which includes BDNF and NT3-5 (Ebendal, 1992), is known to play a major role in growth, differentiation and maintenance of peripheral sensory and sympathetic neurones and neurones of the basal forebrain (Levi-Montalcini, 1987; Thoenen *et al*., 1987). NGF prevents acute cholinergic cell damage in the medial septum and diagonal Broca's band following surgical lesion of the fimbria-fornix in rodents (Hefti, 1986; Yunshao *et al*., 1991; Lapchak & Hefti, 1992) and monkeys (Koliatos *et al*., 1990). Moreover, NGF is known to increase acetylcholine turnover (Kewitz *et al*., 1990), neurite outgrowth (Levi-Montalcini, 1987), neuronal survival and resistance to insults (Aloe, 1987; Shinego *et al*., 1991; Holtzman *et al*., 1996) and to protect neurones against hypoglycaemia and exocytotoxicity by stabilizing intracellular calcium (Cheng *et al*., 1993). A decrease or lack of NGF or other neurotrophins has been proposed to induce several neuropathologies, including Alzheimer's disease (Eide *et al*., 1993; Scott & Crutcher, 1994). Thus, NGF seems to have a number of potentially important clinical applications, some of which may benefit patients with neurological brain diseases (Olson *et al*., 1992). Based on these findings, NGF has, in recent years, attracted considerable interest as a potential therapeutic agent capable of reversing the processes of cell damage in neurodegenerative events (Hefti & Shneider, 1989; Scott & Crutcher, 1994; Fricker, 1997). A key limitation for the clinical use of NGF is that this protein does not cross the blood-brain barrier and invasive neurosurgical procedures, such as infusion into the ventricular space, or directly into brain parenchyma, are necessary for its delivery into the central nervous system (CNS; Harbaugh, 1989; Hefti & Shneider, 1989; Saffran, 1992). It is therefore conceivable that the identification of molecules which can promote the synthesis of endogenous NGF in the brain would be extremely useful.

In recent years findings from our and other laboratories have indicated that hormones, such as glucocorticoids (Aloe, 1989; Barbany & Persson, 1992; Lindholm *et al*., 1992) and testosterone (Katoh-Semba *et al*., 1994; Tirassa *et al*., 1997) can influence basal levels of brain NGF. Although hormone therapy might be feasible in certain pathological conditions, it is known that hormones may present a number of undesirable side-effects in the brain (Sapolsky, 1992; Tirassa *et al*., 1997).

The aim of the present *in vivo* study was to investigate the role played by cholecystokinin-8 (CCK-8) in the regulation of

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constitutive NGF content in the brain of adult mice. CCK-8 (Dockray, 1976; Beinfeld *et al*., 1981) and its receptors CCK_A and CCK_B (Hill *et al*., 1987; Wank *et al*., 1992) are largely distributed in the hippocampus, hypothalamus and cortex. In these brain regions CCK-8 acts as a neurotransmitter producing excitatory effects on neurones, which seem to be mediated by CCK receptors and blocked by selective CCK_A and CCK_B antagonists (Dodd & Kelly, 1981; Boden & Hill, 1988; Hicks *et al*., 1993). Peripheral CCK-8 administration results in the stimulation of neuropeptide and hormone synthesis and/or release (Verbalis *et al*., 1986; Page *et al*., 1990; Kamilaris *et al*, 1992), and promotes a variety of physiological and behavioural responses (Weatherford *et al*., 1992; Salorio *et al*., 1994). Some of these CCK-8 effects are mediated by the activation of vagal afferent fibres, since the response is abolished by selective CCK_A antagonists and by vagotomy (McCann *et al*., 1988; Kamilaris *et al*., 1992; Wettstein *et al*., 1994).

In this study we have analysed the dose- and time-dependent effects of intraperitoneal (i.p.) CCK-8 administration on the NGF levels in the hippocampus, hypothalamus, cortex and pituitary, which represent the principal sites for NGF (Korshing *et al*., 1985; Spillantini *et al*., 1989) synthesis in brain. The effects of pretreatment with atropine, CCK_A and CCK_B receptor antagonists on the NGF response to i.p. injection with CCK-8 were also evaluated.

Methods

Subjects

Adult male CD1 mice (35–40 g) were obtained from Charles River (Italy). They were housed 5 to a cage at constant room temperature (21 ± 1°C) with free access to water and standard food, and with a 12 h light-dark cycle.

Experiment 1: establishment of a dose-response curve for i.p. CCK-8-induced effects on NGF levels in brain

Vehicle or different doses of CCK-8 ranging from 2.0–32 nmol kg^{-1} were i.p. injected (500 μl volume) in mice to evaluate the effects of this peptide on NGF content in various brain regions (pituitary, hypothalamus, hippocampus and cerebral cortex). Animals (eight per dose/group) were killed 15 min post-injection. Brains were removed and dissected following the Glowinsky and Iversen method (1965).

Experiment 2: time course for CCK-8 induced effects on brain NGF levels

CCK-8 (8 nmol kg^{-1}) or vehicle were injected i.p. (500 μl volume). The mice were killed at 15, 30, 60 or 120 min after the injection ($n = 8$/group). Hypothalamus, hippocampus, cerebral cortex and pituitary were dissected (37), and tissues were stored at −70°C until required.

Experiment 3: evaluation of the effects of CCK receptor antagonists on CCK-8-induced brain NGF levels

To determine if the effects of the maximal stimulating dose of CCK-8 administration on brain NGF levels were mediated by peripheral and/or central CCK receptors, mice were pretreated with an i.p. injection of a selective CCK_A receptor antagonist, CR1409 (10 mg kg^{-1}), or a selective CCK_B receptor antagonist, PD135-158 (0.5 mg kg^{-1}), 20–30 min before the i.p. treatment with 8 nmol kg^{-1} CCK-8 or vehicle. Animals (eight per group of treatment) were killed 15 min after the CCK injection. Brains were removed, dissected and tissues were stored at −70°C until used.

Experiment 4: effects of pretreatment with atropine on NGF response to i.p. injection with CCK-8

Twelve mice received an i.p. injection of 5 mg kg^{-1} atropine, 20–30 min before being injected with 8 nmol kg^{-1} CCK-8 or saline. The mice ($n = 6$/group) were killed 15 min after the CCK injection. The brain tissues removed were stored at −70°C until used.

In these experiments a group of five untreated mice (UT) was used as control of baseline NGF brain levels. Saline + saline treated mice (S + S) were considered as the internal control group in the experiments 3 and 4.

NGF measurement by enzyme immunoassay (ELISA)

The tissues were sonicated in extraction buffer (0.1 M Tris-HCl pH 7.00; 400 mM NaCl, 0.1% Triton X100; 0.05% NaN_3; 2% BSA; 0.5% gelatine; 4 mM EDTA; 40 u ml^{-1} aprotinin; 0.2 mM PMSF; 0.2 mM benzetonium chloride; 2 mM benzamidine) followed by centrifugation at 15 000 r.p.m. for 30 min. The supernatants were used for the assay. The bioactive form of 2.5S NGF, purified from mouse submaxillary glands and prepared in our laboratory according to the method of Bocchini and Angeletti, was used as standard. NGF was dissolved in extraction buffer and the standard curve was in a range of 0.015 pg ml^{-1} and 1 ng ml^{-1}. ELISA was performed as described by Weskamp and Otten (1987). Specific NGF binding was assessed by use of a moncolonal mouse anti β-2.5S NGF (Boehringer Mannheim) which reacts with both the 2.5S and 7S NGF biologically active forms. Absorbance of samples and standards was corrected for non-specific binding (i.e. the absorbance in a well coated with purified mouse IgG). The NGF content in the samples was determined in relation to NGF standard curve. Data were not corrected for recovery of NGF from samples, which was routinely 70–90%, and was accepted only when the values were >2 s.d. above the blank. With these criteria, the limit of sensitivity of NGF ELISA averaged at 0.5 pg per assay. Moreover, the specificity of NGF measurement by ELISA was assessed by use of recombinant and biologically active NGF as described previously (Weskamp & Otten, 1987; Bracci-Laudiero *et al*., 1992). The NGF data are presented as mean ± s.d. The Kruskal-Wallis non-parametric analysis of variation with multiple comparisons were used for significance testing. P values less than 0.05 were considered significant.

NGF characterization by high-performance liquid chromatography (h.p.l.c.)

The entire brain (excluding the cerebellum) of untreated mice and saline- or CCK-8 (8 nmol kg^{-1})-treated mice ($n = 3$/group) was homogenized by sonication in 20 mM Tris, 40 mM NaCl (pH 7.2) and centrifuged. The supernatants were dialyzed against 5 mM Tris, 10 mM NaCl pH 7.2 for 16 h at 4°C followed by a second over-night dialysis against 50 mM sodium acetate, 10 mM NaCl pH 5.0. Before the samples were lyophilized, the supernatants were dialyzed against 5 mM sodium acetate, pH 5.0, and re-centrifuged to obtain clean samples. The lyophilized brain samples were re-dissolved in 500 μl of 10 mM sodium acetate, 100 mM NaCl, pH 5.0, before being applied to the h.p.l.c. analysis.

A progel TSK (3000PW-dp 10 μm, 7.5 mm i.d. $\times$ 30 cm) column equipped with a TSK guard column was used. The column was eluted (0.5 ml min^{-1}) with 10 mM sodium acetate, 100 mM NaCl (pH 5.0) for 60 min at room temperature. Fractions of 1 ml were collected and utilized to assess the presence of NGF by ELISA. The h.p.l.c. column was calibrated with 40 μg of purified and bioactive murine 2.5 S NGF extracted from submaxillary glands. The above standard was also added to the brain samples from the untreated mice. To avoid possible contamination of the h.p.l.c. column, the samples from the saline and CCK-8-treated mice were analysed first.

Drugs

The following drugs were used: cholecsytokinin-8 (CCK 26-33) was obtained from Peninsula Lab (U.S.A.); CCK_B receptor antagonist: PD 135-158 (N-methyl-D-glucamine salt) and CCK_A receptor antagonist: CR1409 (lorglumide sodium salt ($C_{22}H_{31}Cl_2N_2O_4Na$) were obtained from Research Biochemical International (U.S.A.); atropine sulphate salt (Sigma Chemicals Italy). All drugs were administered intraperitoneally (i.p.).

Results

Intraperitoneal injection with CCK-8 increased NGF levels in the hippocampus, hypothalamus and pituitary, while in the cortex no significant changes were observed. Table 1 shows the dose-response effects of i.p. CCK injection on brain NGF levels (Experiment 1). The highest NGF increase was observed at the dose of 8 nmol kg^{-1} which caused an NGF increase of about 3 fold in hypothalamus and pituitary and about 60% in the hippocampus ($P<0.01$). Compared to saline-treated mice, the CCK doses of 4 and 16 nmol kg^{-1} were also able to upregulate the basal NGF levels in hippocampus, hypothalamus and pituitary, but no changes were seen in the cortex. The dose of 8 nmol kg^{-1} was used to characterize the CCK-induced NGF release in the brain by h.p.l.c. and to explore the time-course of the NGF response to i.p. injection of CCK-8 (Experiment 2).

Brain samples from untreated mice, with (Figure 1c) or without (Figure 1b) addition of NGF standard, run on the h.p.l.c. column showed only a single immunoreactive component eluting in the position of purified and bioactive 2.5S NGF standard (Figure 1a). The elution profile detected with ELISA corresponded with the one detected by u.v. No other immunoreactive components than the one found in the untreated animals or the standard samples were found in the saline (Figure 2a)- or CCK-8 (Figure 2b)-treated animal brains.

The time-course study (Experiment 2) showed that CCK-8 induced a significant increase in NGF levels in the hippocampus 15 and 30 min after the injection and NGF levels returned to basal levels at 60 min (see Figure 3a), whereas in the hypothalamus, although the NGF levels of CCK-treated mice remained higher than those of untreated ones at all time points examined ($P<0.01$ vs untreated mice), they were statistically different from the saline treated ones only at 15 min (see Figure 3b). In the pituitary, where the NGF levels of saline-treated mice increased during the time of

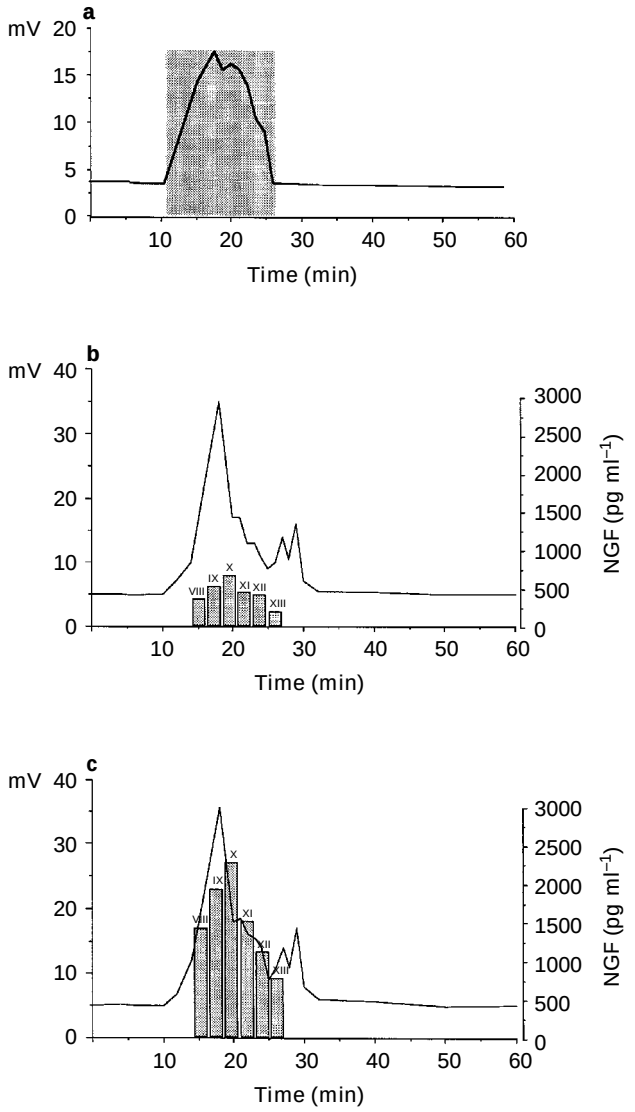

Figure 1 H.p.l.c. elution profiles for purified murine 2.5S NGF standard (a), mouse brain sample (b) and brain sample + NGF (c). In both brain samples NGF was eluted in the position of NGF standard. The bars represent the NGF levels (pg ml^{-1}) in the h.p.l.c. fractions detected by NGF ELISA (from VIII to XIII). See Methods section for details.

Table 1 Brain NGF levels (pg g^{-1} tissue) of mice injected, i.p., with different doses of CCK-8 (nmol kg^{-1}) and killed 15 min after injection

	UT	*Saline*	*CCK 2 nmol*	*CCK 4 nmol*	*CCK 8 nmol*	*CCK 16 nmol*	*CCK 32 nmol*
Hippocampus	3973 $\pm$ 104	4143 $\pm$ 305	4893 $\pm$ 895	5549 $\pm$ 324**	6754 $\pm$ 966**	5084 $\pm$ 530*	4685 $\pm$ 765
Hypothalamus	385 $\pm$ 41	544 $\pm$ 61	1212 $\pm$ 628	1604 $\pm$ 319**	1716 $\pm$ 436**	849 $\pm$ 85*	539 $\pm$ 226
Cortex	839 $\pm$ 77	852 $\pm$ 158	900 $\pm$ 83	957 $\pm$ 117	1106 $\pm$ 345	1009 $\pm$ 185	897 $\pm$ 112
Pituitary	1286 $\pm$ 46	1322 $\pm$ 160	1522 $\pm$ 480	3041 $\pm$ 660**	4626 $\pm$ 430**	2764 $\pm$ 89**	2047 $\pm$ 400*

Values are expressed as mean $\pm$ s.d. *$P<0.05$; **$P<0.01$ vs saline. UT = untreated mice.

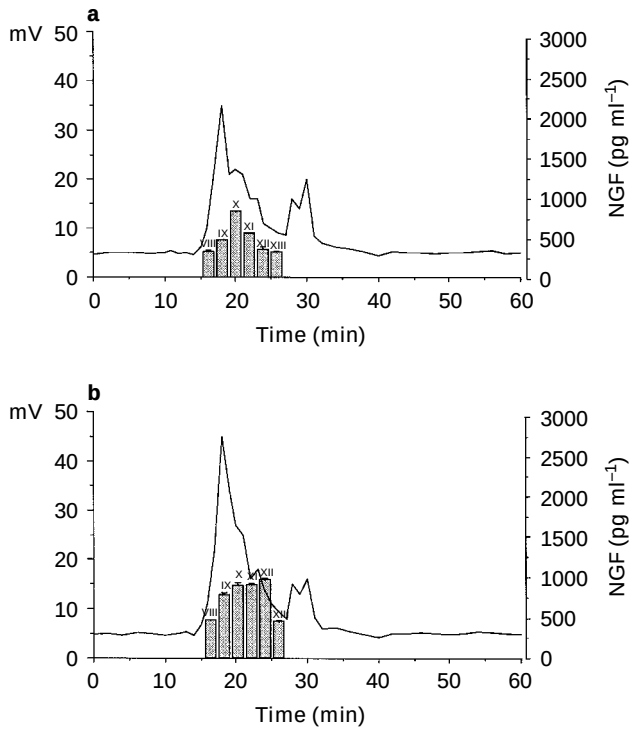

Figure 2 H.p.l.c. analysis of NGF in brain samples of saline- (a) and CCK-8 i.p. treated mice. (b) In both sample groups NGF eluted as a single immunoreactive peak in the position of murine 2.5S NGF (see Figure 1a). The bars represent the NGF levels ± s.d. (pg ml^{-1}, $n = 3$/group) in the h.p.l.c. fractions detected by NGF ELISA (see Methods section).

observation (15 min = 1322 ± 160; 30 min = 4451 ± 1000*; 60 min = 7694 ± 1751* NGF pg g^{-1} tissue; *$P < 0.01$ vs UT), the NGF content of the CCK-treated mice was significantly increased compared with both control and saline-treated mice. The time course study showed that in CCK-treated mice the pituitary NGF levels were about 3 fold higher than in the saline-treated ones at all the time points examined (15 min = 4626 ± 430; 30 min = 14 000 ± 2855; 60 min = 26.000 ± 1815 NGF pg g^{-1} tissue; $P < 0.0001$ vs saline NGF values at corresponding time). In the cortex, although a small increase in NGF levels was observed 30 and 60 min after the CCK-8 injection, it was significant when compared with saline-treated mice (data not shown).

The results of the effects of pretreatment with CCK receptor antagonists (Experiment 3) on the NGF response to CCK injection, are shown in Figures 4a and b. Pretreatment with the CCK_B receptor antagonist (PD 135-158) blocked the CCK-induced NGF level increase in the hippocampus, while pretreatment with the CCK_A receptor antagonist (CR1409) did not (see Figure 4a). Opposite effects were found in the hypothalamus (Figure 4b), where the NGF response to CCK injection was suppressed by pretreatment with CCK_A receptor antagonist but not by the CCK_B receptor antagonist.

Compared to the internal control group (saline + saline) no significant effects of treatment with either of the CCK receptor antagonists alone on NGF levels in brain were noted (see Figure 4a and b).

In mice pretreated with atropine, the ability of CCK-8 to increase the brain NGF levels after i.p. injection was abolished in both the regions examined (Figure 5). No changes in NGF levels were noted in the brain of mice treated with atropine + saline when compared to the saline + saline treated mice (Figure 5).

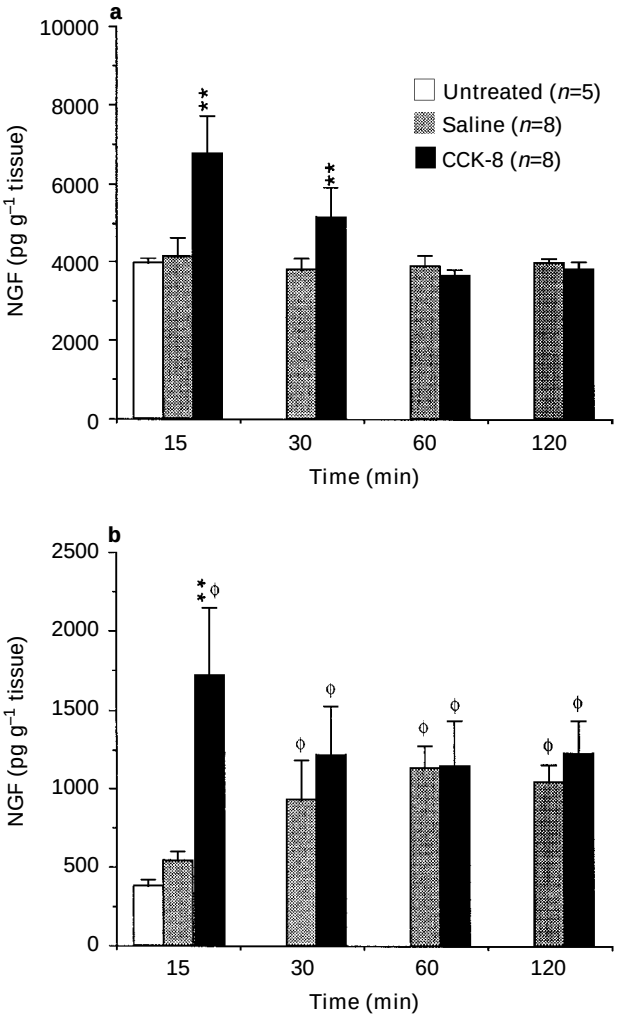

Figure 3 Time course of CCK-8 effects on NGF levels (pg g^{-1} tissue) in the hippocampus (a) and hypothalamus (b) of adult male mice (Experiment 2, see Methods section). Values are expressed as means ± s.d. $\phi P < 0.01$ statistically different from untreated; **$P < 0.01$ statistically different from saline. Untreated = white bars ($n = 5$); Saline = grey bars ($n = 8$); CCK-8 = black bars ($n = 8$).

Conclusion

In the present study we demonstrated that i.p. injection with CCK-8 induced a significant increase of NGF in adult mouse hippocampus, hypothalamus and pituitary. No changes of NGF levels were observed in the cortex. However, it is possible that cortical NGF levels may be affected at different times and/or doses of CCK, which have not been considered in the present study. CCK-8 does not cross the blood-brain barrier and is rapidly processed to smaller biologically active and inactive fragments (Davis & Konings, 1993). Thus it may be possible that the form of CCK-8 used in this study, was not active long enough to produce prolonged and/or extensive effects in brain. Although these latter aspects require further investigation, the present findings indicate that CCK affects NGF production in two of the major NGF-synthesizing brain regions: the hippocampus (Korshing *et al.*, 1985) and hypothalamus (Spillantini *et al.*, 1989). In these regions, as well as in the pituitary, the effects of CCK were observed in a dose range of 4–16 nmol kg^{-1}. The strongest effects on NGF were obtained with 8 nmol kg^{-1}. Similar CCK doses have previously been demonstrated to stimulate the synthesis of

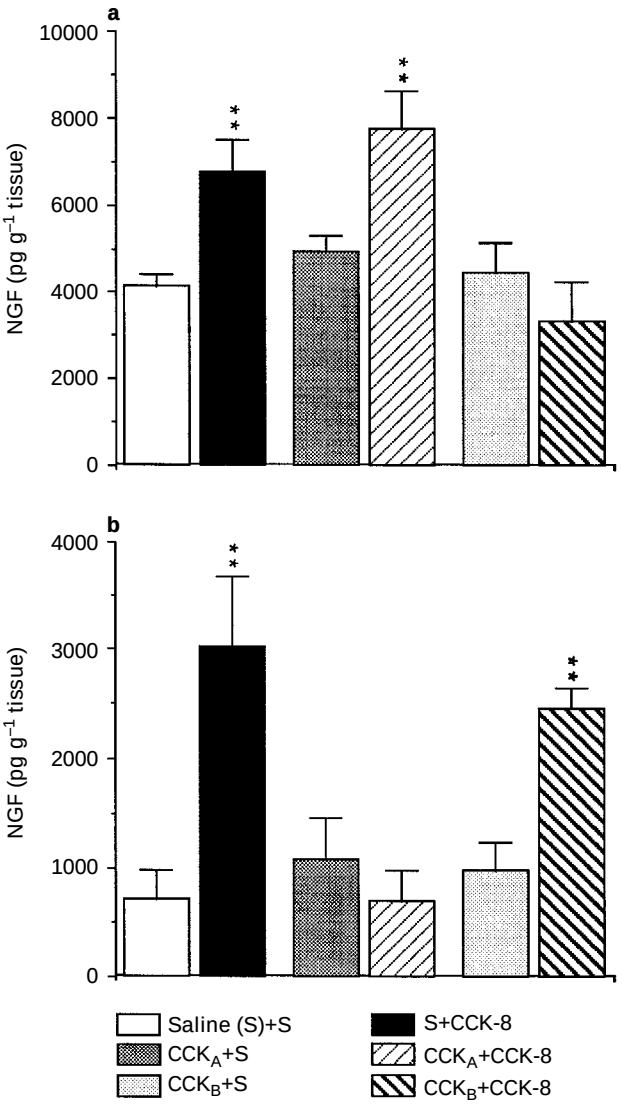

Figure 4 NGF concentrations in hippocampus (a) and hypothalamus (b) of adult mice pretreated with CCK receptor antagonists (indicated as CCK_A and CCK_B in the graph) 20–30 min before receiving an injection of CCK-8 (S+CCK-8; CCK_A+CCK-8 and CCK_B+CCK-8 groups) or saline (S+S; CCK_A+S and CCK_B+S groups). Mice were killed 15 min after treatment with CCK-8. Values are expressed as means±s.d. ($n=8$). $^{**}P<0.01$ statistically different from the saline+saline group.

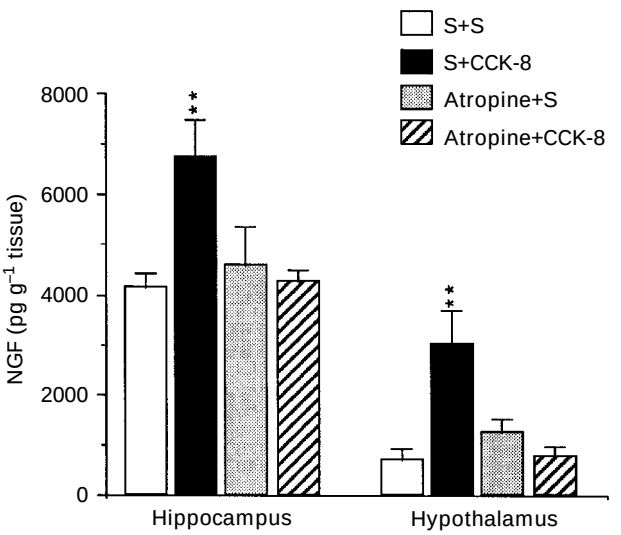

Figure 5 Effects of pretreatment with atropine on NGF concentrations in hippocampus and hypothalamus. In these two brain regions the NGF levels of atropine pretreated mice (both atropine+S and atropine+CCK-8) were similar to saline+saline treated ones. Values are expressed as means±s.d. ($n=6$/group). $^{**}P<0.01$ versus saline+saline.

other neuropeptides and hormones in the brain (Verbalis *et al.*, 1986; Page *et al.*, 1990; Kamilaris *et al.*, 1992), to induce immediate early gene expression (Day *et al.*, 1994) and to influence centrally-mediated behavioural effects (Bloch *et al.*, 1987; Weatherford *et al.*, 1992; Salorio *et al.*, 1994). Thus, our findings showing that CCK alters brain NGF levels, extended these observations and confirm that peripheral CCK-8 administration can have effects on the brain.

The h.p.l.c. analysis of the brain samples from saline- and CCK-8-treated mice confirms that the CCK-8-induced NGF increase in the brain is predominantly due to an increase of the biologically active form of NGF. The results of the h.p.l.c. analysis are consistent with results obtained by NGF-ELISA and, in agreement with previous data (Weskamp & Otten, 1987; Bracci-Laudiero *et al.*, 1992) showing that the ELISA is specific for the bioactive 2.5S NGF form and does not seem to detect the proteins which may contaminate the NGF preparation, and/or NGF forms different from 2.5S or 7S NGF. However, in our experimental conditions, we cannot exclude the possibility that CCK-8 acts through a mechanism which increases the processing of NGF precursor and/or the NGF utilization by brain cells.

We also observed that the NGF response to CCK administration was abolished following pretreatment with the selective CCK_A and CCK_B receptor antagonists and atropine. While CCK_A and CCK_B receptor antagonists have opposite effects in the hypothalamus and hippocampus, pretreatment with atropine blocks the NGF response to CCK in both brain regions. Further studies are needed to understand the role of the two CCK receptors in mediating the effects on NGF levels in the brain. Nevertheless, it is interesting to note that CCK_A and CCK_B receptor antagonists given alone have no effect on the NGF brain levels. CCK does not readily cross the blood-brain barrier (Oldendorf, 1981), which indicates that the NGF increase in brain following CCK-8 i.p. injection is mediated by the activation of both the CCK receptors localized outside the blood-brain barrier. Moreover, in agreement with previous observations (McCann *et al.*, 1988; Kamilaris *et al.*, 1992; Wettstein *et al.*, 1994), the finding that atropine can block the CCK effect supports the hypothesis that the central effects of peripheral CCK administration are at least in part mediated by vagal afferent activity.

This hypothesis may also be supported by the fact that CCK, when peripherally injected, can have access to specific receptors located at some specific brain sites where the blood-brain barrier is incomplete, i.e. the nucleus tractus solitarius (NTS; Harro *et al.*, 1993). In this nucleus, which receives a large number of vagal afferents and sends a very dense network of fibre to different superior brain regions, CCK_A and CCK_B receptors have been localized (Corp *et al.*, 1993). Transection of vagal afferents entering in the hindbrain results in substantial loss of CCK binding density in NTS, indicating that CCK receptors in this nucleus are associated with terminals of vagal origin (Moran *et al.*, 1990). Furthermore, it has been observed that neither CCK_A nor CCK_B antagonists alone specifically inhibited CCK binding in the vagus, while

combined treatment with the two antagonists saturated the vagal CCK binding sites (Mercer & Lawrence, 1992; Corp *et al*., 1993).

Although a functionally significant role for vagal CCK_A receptors has been suggested by behavioural and electrophysiological studies (for a review see Moran & Schwartz, 1994), a role for the vagal CCK_B receptors remains to be determined. Nevertheless, it may be possible that the NGF increase in brain following CCK-8 i.p. injections is associated with different physiological effects of this peptide which are mediated by the two classes of vagal receptors.

Alternatively, the converse effects of the CCK_A and CCK_B receptor antagonists may be related to the interaction of the different receptor subtypes with different CCK fragments derived from the processing of the unstabilized form of CCK-8 administered. CCK-8 is rapidly metabolized and converted into bioactive and inactive fragments, including CCK-4 which is known to bind with high affinity to CCK_B but not CCK_A. Similarly to CCK-8, CCK-4 produces effects in brain (Moran & Schwartz, 1994), thus it is possible that regional effects of the antagonist pre-treatment reflect the action of these two peptides and the shift from CCK_A to CCK_B sensitivity by a mechanism similar to that suggested for the longitudinal muscle (Davis & Konings, 1993).

Although further studies will be necessary to elucidate the mechanism by which CCK-8 and its receptors modulate the central release of bioactive proteins and peptides, our findings, that amounts of CCK very close to the physiological circulating levels (Linden *et al*., 1989) enhance brain NGF levels through a peripheral injection can be helpful to understand better the regulation of NGF production in brain, as well as that of the other neurotrophins.

Moreover, since a therapeutic role has been proposed for exogenous NGF administration (Hefti & Shneider, 1989; Scott & Crutcher, 1994; Fricker, 1997) but its clinical utilization is currently limited by the fact that significant amounts of NGF do not cross the blood-brain barrier and that cerebral NGF treatment is too invasive (Harbaugh, 1989; Hefti & Shneider, 1989; Saffran, 1992), the possibility that peripheral treatment with CCK enhances NGF in brain may provide a pharmacological tool in some neuropathologies characterized by a deficit of neutrophins.

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