Involvement of cholecystokinin receptor types in pathways controlling oxytocin secretion

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1 Intravenous administration of cholecystokinin (CCK) results in a transient activation of oxytocin neurones in the rat, and hence to oxytocin secretion: this activation is followed by expression of *c-fos* mRNA and of Fos-like immunoreactivity (Fos-LI) in magnocellular oxytocin neurones. Fos-like immunoreactivity is also induced in the regions of the brainstem that are thought to relay information from the periphery to the hypothalamus.

2 Administration of the selective CCK_A receptor antagonist MK-329, but not the CCK_B receptor antagonist L-365,260, prior to CCK injection, prevented oxytocin release as measured by radioimmunoassay and oxytocin neuronal activation as measured by electrophysiology and by the lack of induction of *c-fos* mRNA.

3 MK-329 abolished the release of adrenocorticotrophic hormone (ACTH) following injection of CCK.

4 MK-329 prevented the expression of Fos-LI in the hypothalamic magnocellular nuclei and in the area postrema and dorsal vagal complex of the brainstem.

5 L-365,260 had no effect on the expression of Fos-LI in the brainstem, but attenuated that seen in the hypothalamic magnocellular nuclei.

6 We conclude that CCK acts on CCK_A receptors, either in the area postrema or on peripheral endings of the vagus nerve, to cause the release of hypothalamic oxytocin and ACTH. Information may be carried to the hypothalamus in part by CCK acting at CCK_B receptors.

Keywords: Adrenocorticotrophic hormone; CCK_A receptors; *c-fos*; cholecystokinin; oxytocin

Introduction

The magnocellular oxytocin neurones of the rat supraoptic and paraventricular nuclei respond to intravenous administration of cholecystokinin octapeptide (CCK) with a highly reproducible excitation, lasting 10–15 min and with a mean peak response of about 2 spikes s⁻¹ above basal firing rates. The neuronal excitation (see Renaud *et al.*, 1987; Leng & Dyball, 1991) results in an elevation of plasma oxytocin concentrations, rising 5 min after CCK injection to about 50 pg ml⁻¹ above basal levels (Verbalis *et al.*, 1986a,b; Blackburn & Leng, 1990). Systemically administered CCK also activates centrally-projecting oxytocin neurones, which form one limb of a gastric reflex arc, and also corticotrophin-releasing factor (CRF) neurones, that results in the release of ACTH (Verbalis *et al.*, 1991b; Kamilaris *et al.*, 1992). The profile of neuronal activation in the hypothalamus following systemic administration of CCK has been studied using products of the immediate-early gene *c-fos* as a marker. Within 10 min of CCK injection, *c-fos* mRNA is expressed in neurones of the supraoptic and paraventricular nuclei (Hamamura *et al.*, 1991). In the supraoptic nucleus, Fos-like immunoreactivity (Fos-LI) is found exclusively in magnocellular oxytocin neurones following CCK injection, while in the paraventricular nucleus at least three neuronal populations express Fos-LI: the CRF neurones, and both magnocellular and parvocellular oxytocin neurones (Verbalis *et al.*, 1991b).

There are two distinct CCK receptor types that are present in both the central nervous system and peripheral tissues (Hill *et al.*, 1987). In the periphery, CCK_A receptors predominate, though they are also present in the medial nucleus tractus solitarius (NTS) and the area postrema of the brainstem. The brain possesses mainly the CCK_B type of receptor. Though the supraoptic and paraventricular nuclei themselves possess CCK receptors (Day *et al.*, 1989; Blackburn *et al.*,

1990), systemically administered CCK is unlikely to access them directly, since these sites are within the blood-brain barrier. Instead CCK probably influences hormone secretion by a primary action at one of the circumventricular sites which lack an effective blood-brain barrier, or at the peripheral endings of the afferent vagus (Verbalis *et al.*, 1986b; Carter & Lightman, 1987). Thus, it has been possible to show the induction of Fos-LI in the area postrema, a circumventricular organ, and in the dorsal vagal complex, that contains the NTS, following administration of CCK (Luckman, 1992). The afferent pathway to the hypothalamus has been proposed to involve a direct vagal projection to noradrenergic neurones within the NTS, and thence either directly to the hypothalamus or indirectly via, for example, the parabrachial nucleus. CCK and CCK receptors are present in neurones throughout this proposed pathway, and it has therefore been suggested that CCK activates a chain of CCK-coding neurones, which may utilize CCK as a central neurotransmitter.

The present study employed two potent CCK receptor antagonists, one selective for CCK_A receptors and one for CCK_B receptors, to establish which receptor type was involved in neuroendocrine activation following systemic administration of CCK. We measured plasma concentrations of oxytocin and of ACTH, the electrical activity of magnocellular oxytocin neurones and the expression of *c-fos* mRNA in the hypothalamus after CCK following receptor antagonism. To determine possible sites of action of the antagonists, the expression of Fos-LI was examined throughout the brain. Preliminary results have been published in abstract form (Leng *et al.*, 1992; Luckman *et al.*, 1993).

Methods

Rats from the AFRC Babraham Wistar colony (body weight 250–300 g) were used in all studies. CCK-8 (CCK26-33,

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sulphated; Sigma, UK) was dissolved in isotonic saline (1 mg ml⁻¹). MK-329 (3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide; also known as L-364,718 or devazepide) and L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-N'-(3-methylphenyl)urea) (both Merck Sharp & Dohme, UK), selective antagonists for the CCK_A and CCK_B receptor types, were dissolved in 20% absolute ethanol/80% propan-1,2-diol.

For the electrophysiological study, female rats were anaesthetized with urethane (ethyl carbamate; 1.25 g kg⁻¹, i.p.). The supraoptic nucleus and the neural stalk were exposed by ventral surgery (Leng & Dyball, 1991); a bipolar stimulating electrode was placed upon the neural stalk and a microelectrode introduced into the nucleus by direct visual control. Single neurones, recorded extracellularly from the supraoptic nucleus region were identified antidromically as projecting to the neural lobe and as putative oxytocin neurones by their continuous pattern of discharge activity, and by their lack of response to i.v. injection of phenylephrine (10 µg; Sigma, UK): phenylephrine injections result in a selective transient inhibition of vasopressin neurones mediated by baroreceptor activation (see Leng & Dyball, 1991). Three injections of CCK-8 were given (20 µg kg⁻¹, i.v.), which were separated by injections of L-365,260 (1 mg kg⁻¹, i.v.) and MK-329 (1 mg kg⁻¹). Only one neurone was tested for these effects in each rat.

To assess the effects of the antagonists on the release of oxytocin induced by CCK, female rats were anaesthetized with urethane to allow cannulation of jugular and femoral veins. Two hours after surgery, rats were given two injections of CCK-8 (20 µg kg⁻¹, i.v.) 40 min apart; 30 min after the first CCK injection MK-329 or L-365,260 were administered at doses of 0.001, 0.01, 0.1 or 1 mg kg⁻¹. Blood samples of 0.3 ml, replaced with an equal volume of heparinized saline, were taken immediately before and 5, 10 and 30 min after each CCK injection. Oxytocin in the plasma was measured by specific radioimmunoassay (Higuchi *et al.*, 1985).

For the *in situ* hybridization study, male rats had a jugular vein cannulated with polyethylene tubing under brief tribromoethanol (10 ml kg⁻¹, i.p.) anaesthesia. The following day the conscious, freely-moving rats were injected with MK-329 (1 mg kg⁻¹, i.v.) or vehicle; 15 min later CCK-8 was injected (50 µg kg⁻¹, i.v.) and the rats were subsequently decapitated at 0, 10, 30 and 240 min. Two control groups were killed 30 min after injection of the antagonist or vehicle without injection of CCK. Oxytocin was measured as described by Higuchi *et al.* (1985) and ACTH was determined by kits derived from Diagnostic Corp (DPC, Los Angeles CA, U.S.A.) in a single assay.

The *in situ* hybridization technique for measurement of *c-fos* mRNA has been described in full elsewhere (Hamamura *et al.*, 1991; 1992). The probe was a synthetic oligonucleotide (48-mer) complementary to rat *c-fos* mRNA (Curran *et al.*, 1987), 3' end-labelled using α[³⁵S]-dATP (New England Nuclear, UK) and terminal deoxynucleotidyl transferase (Pharmacia, UK). Northern analysis has confirmed that probe binding is specifically to mRNA corresponding in size to that reported for rat *c-fos* mRNA (Wisden *et al.*, 1990). Hybridization in the hypothalamus is localized to neuronal cell bodies, and is blocked in the presence of an excess of cold oligonucleotide and by RNase A pretreatment of tissue sections (Hamamura *et al.*, 1991; 1992). Coronal sections of the hypothalamus (20 µm) were fixed, prehybridized and hybridized overnight with the probe, and treated sections were apposed to autoradiographic film (Amersham, UK) for 3 weeks. The apposition time was selected so that the optical density of film images did not exceed 0.7, as above this level the film response deviates from linearity. For quantification of the *c-fos* mRNA signal, the optical density of the autoradiographic film image was measured by a Magiscan Image Analysis System (Joyce-Loebl, UK). The autoradiographic film was viewed under a microscope, and a circular-counting

frame (125 µm diameter) was centred on the region of most intense signal. The mean optical density within each such counting frame, after subtraction of the background optical density, was calculated for each paraventricular and supraoptic nucleus on each section, and the mean values calculated for each animal.

For the functional mapping study using Fos-LI as an activity marker, male rats had a jugular vein cannulated under tribromoethanol anaesthesia 2 days prior to experimentation. On the day of experimentation the conscious, freely-moving rats were injected i.v. with either MK-329, L-365,260 (each at 100 µg kg⁻¹) or vehicle (100 µl kg⁻¹); 20 min later rats were injected with CCK-8 (20 µg kg⁻¹, i.v.) or isotonic saline. After a further 90 min the rats were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.v.) and

Figure 1 (a) Electrical activity of a single oxytocin neurone recorded from the supraoptic nucleus of a urethane-anaesthetized rat, showing responses to three injections of CCK-8 (20 µg kg⁻¹, i.v.), separated by i.v. injection of the CCK_B receptor antagonist L-365,260 and the CCK_A receptor antagonist MK-329 (1 mg kg⁻¹, i.v.). The CCK_B receptor antagonist was ineffective, while the CCK_A receptor antagonist abolished CCK-induced excitation. (b) Mean responses of four oxytocin neurones, each recorded from the supraoptic nucleus of different urethane-anaesthetized rats. Each neurone was tested as in (a). The data for each cell was first expressed as total spikes in each minute of recording. Firing rates were then normalized by subtraction of the mean basal firing rate, calculated separately for each test exposure as the mean firing rate in the 10 min prior to CCK injection. The figure gives the mean ± s.e.mean of the normalized counts, to show the mean neuronal activation following CCK in each of the three tests. The CCK_A receptor antagonist virtually eliminated the neuronal response to CCK, whereas the CCK_B receptor antagonist at the same dose was ineffective. The inset gives the mean neuronal response, calculated as the mean additional number of spikes recorded in the 10 min following each CCK injection compared to the control period, for the initial injection of CCK (C); CCK injection following the CCK_B receptor antagonist (+B); and CCK injection following the CCK_A receptor antagonist (+A).

perfused with fixative for standard immunocytochemistry using a polyclonal antibody raised against the N-terminal amino acids 2–17 of the Fos protein, as previously described (Luckman, 1992). The mean number of Fos-LI nuclear profiles per 30 μm section was counted in a variety of brain regions. These figures were then further meaned to give a value for each experimental group.

The Mann Whitney U-test (two tailed) was used for all statistical comparisons.

Results

Effects of CCK antagonists on the electrical activity of supraoptic neurones

The electrical activity of supraoptic neurones, identified antidromically as projecting to the posterior pituitary, was recorded from four urethane-anaesthetized rats. In each experiment, we selected a single supraoptic neurone which showed a continuous firing pattern, excitation after injection of CCK-8 ($20 \mu\text{g kg}^{-1}$, i.v.), and a lack of response to injection of phenylephrine ($10 \mu\text{g}$, i.v.).

Figure 2 Oxytocin release following i.v. injections of CCK-8 ($20 \mu\text{g kg}^{-1}$) in anaesthetized rats, measured following administration of CCK_A receptor antagonist MK-329 (a) and the CCK_B receptor antagonist L-365,260 (b) at various concentrations. In (a) concentration of receptor antagonist; $\square$, 0; ▨ , 0.001; ▩ , 0.01; $\blacksquare$, 0.1; $\blacksquare$, 1 mg kg⁻¹, in (b): $\square$, 0; ▨ , 0.01; ▩ , 0.1; $\blacksquare$, 1 mg kg⁻¹. Columns are means $\pm$ s.e. mean plasma oxytocin at 0, 5, 10 and 30 min after CCK injection, $n \geq 9$ for each group of rats (numbers in each group is in parentheses). CCK-induced oxytocin release was unaffected by the CCK_B receptor antagonist but blocked in a dose-related manner by the CCK_A receptor antagonist at similar concentrations. The open columns show oxytocin release in response to the first (control) CCK injection given to all rats. Attenuation of CCK-evoked release is significant ($P < 0.01$) at all doses of the CCK_A receptor antagonist above 0.001 mg kg^{-1} .

Injection of CCK-8 ($20 \mu\text{g kg}^{-1}$, i.v.) transiently and significantly increased the firing rate of each of the oxytocin neurones (Figure 1a; $P < 0.01$ comparison of pre- and post-injection firing rates). Injection of L-365,260 had no significant effect either on the basal firing rate or on the excitatory response to CCK. On the other hand, MK-329 clearly suppressed the excitatory response in each neurone without affecting spontaneous discharge rate. Figure 1b shows the averaged response to CCK with or without prior injection of MK-329 or L-365,260. For each cell the CCK-induced excitation after injection of MK-329 was significantly smaller than the responses observed before and after injection of L-365,260 ($P < 0.01$).

Effects of CCK receptor antagonist on plasma concentrations of oxytocin

In 39 urethane-anaesthetized female rats, injection of CCK ($20 \mu\text{g kg}^{-1}$, i.v.) increased significantly ($P < 0.001$) the immunoreactive oxytocin concentration in the plasma (Figure 1a). This initial injection of CCK was followed by the CCK_A antagonist MK-329 i.v. at doses of 0.001, 0.01, 0.1 or 1 mg kg⁻¹ (Figure 2a). Injection of MK-329 itself had no effect on the plasma oxytocin concentration at any dose used. The oxytocin release in response to a subsequent injection of CCK was attenuated in a dose-dependent manner by MK-329; the attenuation was significant at a dose of 0.01 mg kg^{-1} ($P < 0.01$), and higher doses ($P < 0.001$) abolished the response.

Figure 3 Mean concentrations of oxytocin (a) and ACTH (b) in trunk blood of rats killed for analysis of *c-fos* mRNA expression in the hypothalamus. Rats were given an i.v. injection of either the CCK_A receptor antagonist, MK-329 (1 mg kg^{-1} , open columns) or vehicle (solid columns), followed 30 min later by i.p. injection of CCK-8 ($50 \mu\text{g kg}^{-1}$). Rats were killed 10, 30 or 240 min later, or were killed without CCK injection; $n = 5$ or 6 in each group. CCK induced significant increases in oxytocin and ACTH in rats killed 10 min after injection in the vehicle pretreated, but not the antagonist pretreated group. * $P < 0.05$, ** $P < 0.01$ compared to rats killed immediately after CCK.

Figure 4 Photomicrographs showing Fos-LI before (a,c,e) and 90 min after (b,d,f) injection of CCK-8 ($20 \mu\text{g kg}^{-1}$, i.v.). (a and b) Brainstem at level of area postrema (ap); the NTS lies between the area postrema and the central canal. (c and d) Supraoptic nucleus; Fos-LI nuclear profiles are in the dorsal region of the supraoptic nucleus of the stimulated brain, the area in which oxytocin neurones predominate; oc = optic chiasm. (e and f) Paraventricular nuclei on either side of the third ventricle (3v). Bars represent $10 \mu\text{m}$.

A second group of 25 female rats similarly showed a significant increase in plasma oxytocin concentration following an initial injection of CCK ($20 \mu\text{g kg}^{-1}$, i.v.). Thirty minutes after the first injection, the CCK_B receptor antagonist L-365,260 was administered i.v. at doses of 0.01, 0.1 or 1 mg kg^{-1} . L-365,260 had no significant effect either upon basal oxytocin release or upon the oxytocin release in response to CCK at any dose (Figure 2b).

Effects of CCK_A receptor antagonism on c-fos mRNA expression in the hypothalamus

Radioimmunoassay of the trunk blood samples confirmed significant elevation of both oxytocin ($P < 0.01$) and ACTH

($P < 0.05$) plasma concentrations 10 min after CCK in the vehicle pre-treated rats, but not in the MK-329 pre-treated rats (Figure 3).

In the two control groups not injected with CCK, no *c-fos* mRNA signal was detected in any of 6 rats pretreated with MK-329, but measurable signal was present in two of six rats pre-treated with vehicle. In these two rats, the relative optical densities ($\times 100$) of the *c-fos* mRNA signal in the supraoptic nucleus were 21.8 and 17.5 respectively and in the paraventricular nucleus were 19.7 and 24.6 respectively. These two rats had elevated concentrations of oxytocin in the circulation at the time of killing (39.5 and 53.4 pg ml^{-1} respectively, the highest values of this group and higher than any rat in the vehicle pretreated group killed 30 min or 4 h after CCK

injection). One of these rats also had markedly elevated plasma ACTH concentration (249.4 ng ml^{-1}), although one rat in this group showed a still higher plasma ACTH level (638 ng ml^{-1}) not accompanied by any detected signal in the paraventricular nucleus.

In rats killed 10 or 30 min after CCK injection, *c-fos* mRNA was measurable on the film images corresponding to the paraventricular nuclei of every rat in the vehicle pretreated group, but of no rat in the MK-329 pretreated group. The mean relative optical density ($\times 100$) of the *c-fos* mRNA signal in the paraventricular nucleus was 21.0 ± 1.9 ($n = 5$ rats) and 20.2 ± 2.4 ($n = 5$) in rats killed 10 and 30 min after CCK in vehicle pretreated rats (values not significantly different between these two time points). In the supraoptic nucleus, again *c-fos* mRNA signal was detected in every rat killed 10 or 30 min after CCK injection in the vehicle-pretreated group: the mean relative optical density ($\times 100$) of the *c-fos* mRNA signal being 12.4 ± 2.6 ($n = 5$) and 16.1 ± 1.8 ($n = 5$) at 10 and 30 min respectively. In MK-329 pretreated rats, measurable *c-fos* mRNA signal was detected in the supraoptic nucleus of only two of 12 rats (relative O.D. $\times 100$, 14.0 and 16.8), both at 30 min after CCK injection. These two rats had elevated plasma oxytocin levels at the time of killing (67 and 50.9 pg ml^{-1} respectively, higher than in any of the other 18 antagonist-pretreated rats), but ACTH levels in the normal range for this experiment (40 and 63.7 ng ml^{-1} respectively). No *c-fos* mRNA signal was detected in any of the rats killed 4 h after injection of CCK, whether in rats pretreated with vehicle ($n = 5$) or antagonist ($n = 5$).

Effect of receptor antagonists on Fos-LI in the brain following CCK

Systemic injection of CCK-8 caused significant increases in the number of Fos-LI nuclear profiles in the dorsal vagal complex and the area postrema of the brainstem and the supraoptic and paraventricular nuclei of the hypothalamus (Figure 4). In each of these instances, the increase in Fos-LI was abolished by pretreatment with the CCK_A receptor antagonist MK-329 (Figure 5). The CCK_B receptor antagonist, L-365,260 did not affect staining in the dorsal vagal complex or the area postrema, but severely attenuated the response in the supraoptic and paraventricular nuclei. The effect of L-365,260 was only statistically significant in the case of the supraoptic nucleus. CCK did not significantly alter the amount of Fos-LI in the ventrolateral medulla, the region of

Figure 5 Number of Fos-LI nuclear profiles in four brain areas following injections of isotonic saline, CCK-8 alone ($20 \mu\text{g kg}^{-1}$, i.v.), or CCK with prior injection of MK-329 or L-365,260 ($100 \mu\text{g kg}^{-1}$, i.v.). Columns represent means $\pm$ s.e.mean, $n = 5-6$. □ Saline; ■ CCK; ▨ MK-329; ▩ L-365,260. DVC, dorsal vagal complex; AP, area postrema; SON, supraoptic nucleus; PVN, paraventricular nucleus. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.005$ compared to isotonic saline; † $P < 0.05$ compared to CCK only.

the locus coeruleus or the parabrachial nucleus (results not shown).

Discussion

The oxytocin response to i.v. injection of CCK-8 is abolished in the presence of the specific CCK_A receptor antagonist MK-329 ($100 \mu\text{g kg}^{-1}$, i.v.) but unaffected in the presence of the CCK_B receptor antagonist L-365,260 at a higher concentration. The two antagonists are chemically related (Evans *et al.*, 1986), but binding studies indicate that their relative affinities for CCK_A and CCK_B receptors differ by over two orders of magnitude (Lotti & Chang, 1989). The effect of CCK_A receptor antagonism in blocking oxytocin secretion following intraperitoneal CCK has been recently reported by others (Miller *et al.*, 1993). In the present study, neither antagonist produced a significant change in the basal firing rate of oxytocin neurones, or in the basal plasma concentrations of oxytocin, hence it appears that the pathway activated by CCK may not be tonically active. The measurements of ACTH and of the hypothalamic profile of *c-fos* mRNA expression indicate that CCK_A receptors are also involved in activation of parvocellular neurosecretory neurones in the paraventricular nucleus.

Selective gastric vagotomy eliminates the oxytocin response to CCK (Verbalis *et al.*, 1986b) and lesions of the NTS abolish the behavioural effects of CCK on food intake (Crawley & Schwaber, 1984). Thus, peripherally administered CCK may act via the ascending gastric vagus, and influence the magnocellular oxytocin system via a direct projection from the region of the NTS. Alternatively, peripheral CCK may be acting at a circumventricular organ that lies outside the blood-brain barrier. Lesions of the organum vasculosum of the lamina terminalis, a forebrain structure that has a strong neuronal input to the supraoptic and paraventricular nuclei, do not impair the oxytocin release following CCK injection (Blackburn & Leng, 1990). However, lesions of the area postrema do attenuate the effects of CCK on oxytocin release and behaviour (Van der Kooy, 1984; Carter & Lightman, 1987). The NTS and the area postrema are heavily interconnected, and both areas are activated by peripheral administration of CCK (Luckman, 1992). Unlike the area postrema, the NTS is the source of a direct afferent input to the magnocellular oxytocin neurones (Day & Sibbald, 1988a, b; Raby & Renaud, 1989), part of which comprises noradrenergic neurones of the A2 cell group. Peripheral administration of CCK has been shown to activate catecholaminergic neurones in the A2 group and to result in noradrenaline release in both the paraventricular nucleus and in the dorsal region of the supraoptic nucleus (Kendrick *et al.*, 1991; Luckman, 1992). In the present study, no increase in Fos-LI was seen in the ventrolateral medulla, the region of the locus coeruleus or parabrachial nucleus, all of which are possible relay sites on the pathway between the brainstem and the hypothalamus. However, Fos-LI is present in these areas in all animals and therefore it is possible that changes in the activation of specific neuronal populations, for example noradrenergic neurones in the ventrolateral medulla or locus coeruleus, may be masked by absolute cell counts. It should also be noted that many of the neurones activated in the NTS are non-catecholaminergic (Luckman, 1992), and thus a recently described peptidergic innervation of oxytocin neurones from the NTS (Sawchenko *et al.*, 1988) may also be involved.

An additional target for peripheral CCK is believed to be the neural lobe of the pituitary, since CCK induces oxytocin and vasopressin release from the neural lobe *in vitro* (Bondy *et al.*, 1989). This *in vitro* finding has many unusual and intriguing aspects. The release occurs only after a long latency, and is independent of extracellular calcium entry. As yet, it appears to have no parallel *in vivo*, where observed release is immediate and selective for oxytocin.

Since CCK is present in afferent vagal neurones, in neurones of the NTS, and in magnocellular oxytocin neurones (Vanderhaeghen *et al.*, 1980), it is possible that centrally released CCK participates in coding the neuronal responses to peripheral CCK injection. The apparent effect of the CCK_B receptor antagonist ($100 \mu\text{g kg}^{-1}$) on Fos-LI in the hypothalamus would suggest a role for central CCK receptors in the mediation of the effects of CCK. This is surprising since this and higher concentrations of the CCK_B antagonist were ineffective at inhibiting the electrical and secretory activity of oxytocin neurones. It remains possible that several neurotransmitters are involved in these central pathways, and that the expression of Fos in these neurones is more sensitive to antagonism than is their electrical activity.

The physiological role of the pathway activated by CCK is not known. One of the postulated functions of oxytocin in the rat is to promote sodium excretion (Balment *et al.*, 1986; Verbalis *et al.*, 1991a), hence the pathway may initiate a reflex natriuresis following feeding. Since there is little evidence of significant oxytocin release following feeding, this

hypothesis is so far unsupported. An alternative interpretation comes from the evidence that in the rat, oxytocin release is activated by diverse acute stressors, including immobilization, ether inhalation and fear-related emotional stress (Lang *et al.*, 1983; Gibbs, 1986; Onaka & Yagi, 1990). Link *et al.* (1992) have shown a synergistic effect of oxytocin and CRF on the release of ACTH at the level of the pituitary, though it is more likely that oxytocin released from the median eminence is involved, rather than that released by the magnocellular system into the general circulation. It is clearly possible, however, that the activation of oxytocin and CRF neurones following CCK administration may be via a common 'stress' pathway.

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