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Cholecystokinin, acting through the A receptor subtype, stimulates the proliferative activity of adrenocortical cells and thymocytes in the rat

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Summary. Cholecystokinin (CCK) is a multifunctional regulatory peptide, which acts through two main subtypes of receptors, named CCK-A and CCK-B. Evidence indicates that CCK modulates cell proliferation in various tissues in a paracrine manner, and proofs are available of the presence of CCK in both adrenal glands and thymus. Hence, we have investigated the possible mitogenic action of this peptide on these two tissues, by evaluating the ‰ of metaphase-arrested cells after vincristin injection (mitotic index). The systemic administration of CCK (three subcutaneous injections of 20 nmol/kg, 28, 16 and 4 h before the sacrifice) increased the mitotic index in both the outer adrenal and thymus cortexes of immature (20-day-old) rats and the enucleated adrenal gland of adult (2-month-old) animals at day 5 and 8 of regeneration. The simultaneous administration of equimolar doses of a selective CCK-A receptor antagonist blocked the effect of CCK, while a CCK-B antagonist was ineffective. These findings indicate that CCK exerts a marked CCK-A-mediated proliferogenic effect on both adrenal cortex and thymus in the rat, the physiological relevance of which, however, remains to be demonstrated. In fact, the administration of the CCK-A antagonist alone was ineffective, thereby casting doubts on the role played by endogenous CCK in the maintenance and stimulation of adrenal and thymus growth.

Key words: Cholecystokinin, Cholecystokinin receptors, Cell proliferation, Adrenal cortex, Thymus, Rat

Introduction

Cholecystokinin (CCK) is a 33-amino acid regulatory peptide originally discovered in the porcine gut, and subsequently localized in the central nervous system and several peripheral organs and tissues. CCK acts through two main subtypes of receptors, named CCK-A and CCK-B, which belong to the G protein-coupled receptor superfamily, and which are predominantly located in the periphery and central nervous system, respectively (for review, see Crawley and Corwin, 1994). CCK, in addition to controlling digestion and exerting many behavioral actions, also appears to regulate in a paracrine/autocrine manner cell proliferation in several normal and tumorous tissues (Crawley and Corwin, 1994; Forguet-Lafitte et al., 1996; Nagata et al., 1996; Xu et al., 1996; Todisco et al., 1997).

Evidence indicates that CCK and its receptors are contained in the adrenal glands. CCK-immunoreactivity is present in some substance P-positive nerve fibers of the human and guinea-pig adrenals (Heym et al., 1995a,b; Heym, 1997), as well as in epinephrine-containing cell of the chicken interrenals (Ohmori et al., 1997). Proof is available that cat adrenal medulla (Gaumann and Yaksh, 1988a,b) and human pheochromocytomas (Bardram et al., 1989) are able to synthesize CCK, and cultured bovine adrenomedullary cells are provided with CCK-A receptors (Aarnisalo et al., 1996). Likewise, CCK and its receptors are present in the lymphoid tissue, including thymus (Felten et al., 1985; Weinberg et al., 1997).

Therefore, it seemed worthwhile to investigate whether CCK is able to modulate adrenocortical-cell and thymocyte proliferation in the rat. Since the mitotic rate in adrenals and thymus of adult animals is very low or negligible, we used immature rats or animals bearing enucleated-regenerating adrenals.

Materials and methods

Animals and reagents

Adult (2-month-old) female Wistar rats and their offspring (20-day-old) were kept under a 12:12 h light-

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dark cycle (illumination onset at 8:00 a.m.) at 23±1 °C, and maintained on a standard diet and tap water *ad libitum*. CCK (octapeptide sulfated) was purchased from Bachem (Bubendorf, Switzerland), and the selective CCK-A and CCK-B receptor antagonists PD 140,548 and PD 135,158, respectively (Hughes et al., 1990; Higginbottom et al., 1993) were from Research Biochemicals International (Natick, MA, USA). Vincristin was obtained from Gedeon-Richter (Budapest, Hungary).

Experimental procedures

Under ether anaesthesia, the left adrenal gland of adult rats was enucleated, and the controlateral gland removed. Operated rats were given 0.9% NaCl to drink, and were killed 5 or 8 days after surgery. Groups of adult rats with adrenal regeneration and immature animals (n = 6) were given three subcutaneous injections (28, 16 and 4 h before the sacrifice) of the following chemicals dissolved in 0.2 ml 0.9% NaCl: (i) CCK (20 nmol/kg); (ii) CCK-A or CCK-B receptor antagonist (20 nmol/kg); and (iii) CCK plus CCK-A or CCK-B receptor antagonist. Control rats were injected with saline vehicle. All groups of animals received an intraperitoneal injection of vincristin (0.1 mg/100 g) 180 min before the autopsy. Rats were decapitated at 11:00 a.m.

Cell-proliferation assay

Thymuses were promptly removed from immature rats, and capsule-adjacent fragments were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Araldite; 0.5 μm-thick sections were cut, and stained with toluidine blue. Adrenal glands from immature rats and regenerating adrenals were fixed in Bouin's solution for 24 h, embedded in paraffin and sectioned at 5-6 μm; sections were stained with hematoxylin and eosin. The mitotic index (‰ of metaphase-arrested cells) was calculated at x400, by counting 5,000 cells in the subcapsular zone of each thymus (4-5 layers of cells) or adrenal cortex (7-9 layers of cells), and in the regenerating adrenal parenchyma.

Statistical analysis

Individual results were averaged per experimental group, and SEM was calculated. The statistical comparison of the data was done by ANOVA, followed by the Multiple Range Test of Duncan.

Results

CCK increased metaphase index by about 77% and 80% in the thymus cortex and adrenal subcapsular layers of immature rats (Fig. 1). As expected, the mitotic activity was higher at day 5 of adrenal regeneration than at day 8, and CCK significantly enhanced it at both experimental times (by about 66% and 196%, respectively) (Fig. 2). The simultaneous administration of the CCK-A antagonist annulled the effects of CCK, while the CCK-B antagonist was ineffective. The administration of either CCK-A or CCK-B antagonists alone did not significantly alter basal mitotic index of all tissues examined (Figs. 1, 2).

Discussion

Our present results provide clear-cut evidence that CCK administration markedly enhances the mitotic

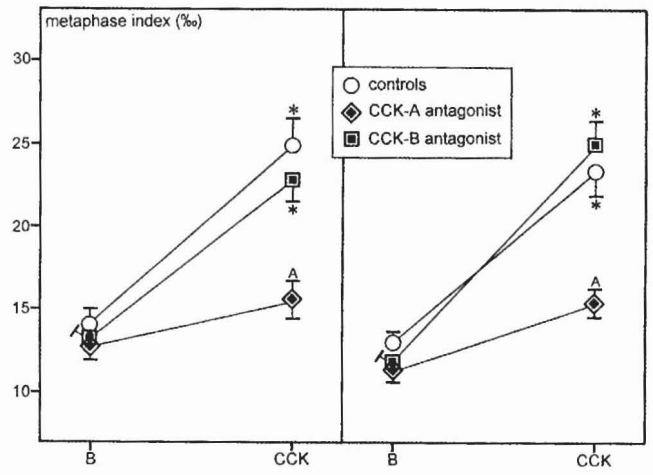

Fig. 1. Effects of CCK and CCK-receptor antagonists on thymocyte (left panel) and adrenocortical-cell (right panel) proliferation in immature rats (means±SEM; n = 6). *: p<0.01 from the respective basal (B) value; A: p<0.01 from the respective control group.

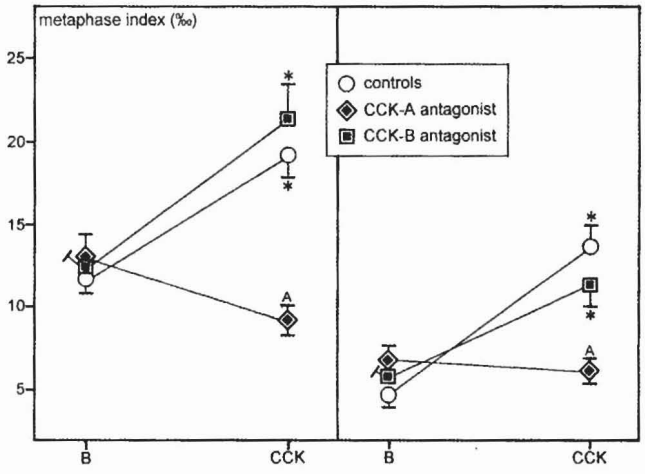

Fig. 2. Effects of CCK and CCK-receptor antagonists on adrenocortical-cell proliferation at day 5 (left panel) and day 8 (right panel) of rat adrenal regeneration after enucleation (means±SEM; n = 6). *: p<0.01 from the respective basal (B) value; A: p<0.01 from the respective control group.

index in both adrenal and thymus, thereby confirming the involvement of this peptide in the control of cell proliferation (see Introduction). They also indicate that this effect of CCK is mediated by the peripheral type CCK-A receptors, because it is annulled by the CCK-A receptor antagonist, and unaffected by the CCK-B receptor antagonist. The possibility of a non-specific toxic effect of the CCK-A receptor antagonist is ruled out by the demonstration that this chemical *per se* does not alter basal mitotic rate. However, our study cast doubts that CCK, although contained in both adrenal and thymus (see Introduction), acts on them in a paracrine/autocrine manner, as has been suggested to occur in other tissues (Xu et al., 1996; Todisco et al., 1997; and for review, Crawley and Corvin, 1994).

It is current knowledge that adrenal growth in immature animals and adrenal regeneration after enucleation are processes strictly dependent on the pituitary ACTH release (for review, see Nussdorfer, 1986), and compelling evidence indicates that CCK is able to activate the entire hypothalamo-pituitary-adrenal (HPA) axis. CCK was found to be co-localized with corticotropin-releasing hormone (CRH) and vasopressin in the paraventricular nucleus (Kiss et al., 1984; Mezey et al., 1986; Ceccatelli et al., 1989) and in the external layer of the median eminence adjacent to portal capillaries (Anhut et al., 1983). CCK protein and mRNA were detected in the anterior pituitary gland (Houben and Deneff, 1994), and CCK/gastrin binding sites were demonstrated in the *pars distalis* of the goldfish pituitary gland (Himmick et al., 1996). The systemic administration of CCK was reported to raise ACTH and glucocorticoid blood levels in rats, and to activate paraventricular nucleus, acting through the CCK-A receptor subtype (Itoh et al., 1979a,b, 1982; Kamilaris et al., 1992; Katsuura et al., 1992; Calogero et al., 1993; Monnikes et al., 1997). Hence, it seems reasonable to admit that the adrenocortical proliferogenic action of CCK may be, at least in part, indirect and mediated by the stimulation of pituitary ACTH release. Obviously, the synthesis of CCK in adrenal medulla and the presence of CCK-A receptors in medullary cells (see Introduction) makes the possibility that CCK may also activate adrenocortical-cell proliferation in a paracrine manner likely, at least when the integrity of the gland is preserved. In this connection, it is to be recalled that rat adrenal medulla contains an active CRH/ACTH system (Andreis et al., 1991, 1992; Mazzocchi et al., 1993), which is involved in the paracrine control not only of the cortical secretion (for review, see Nussdorfer, 1996; Mazzocchi et al., 1998), but also of adrenal growth (Mazzocchi et al., 1994). However, it is evident that this paracrine mechanism cannot underly the stimulating effect of CCK on adrenal regeneration, inasmuch as enucleation eliminates adrenal medulla.

In vivo studies suggest that CCK exerts an immunomodulatory effect, since it is able to restore thymus-dependent immune responses in thymectomized mice and to stimulate IgM-plaque forming cells (Belokrylov et al., 1990; Molchanova et al., 1992). Our present findings are in keeping with this contention. In fact, CCK-A receptor activation by CCK markedly enhances thymocyte proliferation, 25% of which takes place in the thymus cortex, and especially in the subcapsular zone, which is considered the region where thymopoiesis initiates (Scollay and Shortman, 1985; Boyd and Hugo, 1991; Scollay and Godfrey, 1995). Although CCK and its receptors are both present in the thymus (see Introduction), *in vitro* studies did not reveal any effect of this peptide or pentagastrin (a CCK-A receptor agonist) on the mitotic activity of cultured human thymocytes, guinea-pig T lymphocytes and rat B lymphocytes (Soder and Hellstrom, 1987), thereby making unlikely the possibility of a direct action of CCK on thymus. At present, only tentative hypotheses can be advanced to explain the indirect mechanism whereby CCK stimulates thymus growth. There is proof that glucocorticoids, in addition to enhancing lymphocytolysis, are also necessary for the intrathymic thymocyte survival and differentiation in young animals (Compton et al., 1987; Vacchio et al., 1994), and that the HPA axis is still hypofunctioning in immature rats (for review, see Nussdorfer, 1986). In light of these considerations, it seems not unreasonable to conceive that the CCK proliferogenic action on thymocytes may be consequent to its stimulatory effect on the HPA axis.

To summarize, we have demonstrated that CCK, via the CCK-A receptor subtype, stimulates the proliferation of (i) rat adrenocortical cells in experimental models in which adrenal gland undergoes a rapid growth, and (ii) thymocytes in immature rats. The hypothesis has been advanced that both effects are dependent on the CCK-induced central activation of the HPA axis. Before concluding, a few words of caution stressing that the physiological relevance of these proliferogenic actions of CCK remain to be demonstrated. In fact, the finding that the administration of the CCK-A receptor antagonist alone does not affect mitotic rate in both adrenal and thymus would suggest that endogenous CCK does not play a major role in either the maintenance of normal adrenal and thymus growth in immature rats or the stimulation of adrenal regeneration in adult animals. Further studies are needed, employing higher doses of CCK receptor antagonists and possibly more prolonged treatments also using continuous infusion with osmotic pumps.

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