Effects of Cholecystokinin Octapeptide on a Pancreatic Acinar Carcinoma in the Rat

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Purpose. To investigate the effects of increasing concentrations of cholecystokinin octapeptide (CCK-8) on a pancreatic acinar adenocarcinoma.

Methods. Growth of the tumour was estimated in vivo on rats bearing a subcutaneous pancreatic carcinoma, and in vitro on primary cultured tumour cells. CCK receptors were characterized by binding assays.

Results. CCK-8, administered for 12 successive days, exerted a biphasic action on tumour growth: a dose-dependent stimulation with low doses (0.1 and 0.5 μg/kg) and inhibition with high doses (2 and 4 μg/kg) as shown by respective increases and decreases in tumor volume, protein, RNA and amylase contents. In cell cultures, [^{3}H]thymidine incorporation was dose-dependently increased with 10^{-10} to 10^{-8} M CCK-8 and inhibited with 10^{-7} M. Both effects were completely suppressed by the CCK-receptor antagonists CR 1409 and L 364,718 (10^{-4} M). Binding studies showed the overexpression of two classes of CCK-A receptors of low and high affinity when compared to the normal pancreas which was less sensitive to CCK-8.

Conclusions. CCK-8 exerts a biphasic growth response on the acinar pancreatic carcinoma, mediated by two classes of CCK-A receptors overexpressed in the tumour.

KEY WORDS: affinity binding sites; cholecystokinin; cholecystokinin-antagonist; pancreatic cancer; exocrine pancreas.

INTRODUCTION

Several gastrointestinal hormones and neuropeptides have been shown to play a major role in the regulation of secretion, growth and differentiation of the normal pancreas. Among them, bombesin and its mammalian counterpart gastrin-releasing peptide as well as cholecystokinin and its structural analogue caerulein, as well as its synthetic C-terminal octapeptide (CCK-8) exert a trophic action on the pancreas after sustained exogenous administrations or endogenous elevation (1–5). In most cases, the trophic effects of CCK on the exocrine pancreas were partially or completely suppressed by the CCK antagonists CR 1409 (lorglumide) or L 364,718, suggesting a direct effect via CCK receptors in both acinar and ductular cells of the pancreas (1,5,6). More recently, it has been demonstrated that cholecystokinin trophic effects on the pancreas were mediated specifically by the way of cholecystokinin-A receptors (4).

CCK and its related peptides have also been implicated in the development and growth of human or experimental pancreatic tumours. Generally, experimental tumours induced in animals can provide from acinar cell origin; they were then induced by azaserine in the rat (7). They could also originate from ductular cells and were induced by nitrosamine in the hamster (3,8). In human, the major pancreatic tumours are of ductular origin.

The enhancement of plasma cholecystokinin levels, either by exogenous administrations of CCK, or by feeding raw soya flour or fat, exerted various effects on nitrosamine-induced pancreatic carcinogenesis. While some authors described a stimulation of preneoplastic ductular lesions induced by nitrosamine (6,8), others found an inhibitory effect on carcinogenesis of ductular cell phenotype (3,9), or no effect (10). The effects of CCK and its related peptides on growth of human and experimentally induced pancreatic cancers xenografted in animals were also conflicting. These peptides alone or in presence of secretin stimulated growth of pancreatic carcinoma cell lines transplanted in nude mice or in hamsters (11,12), or had no effect (13,14).

Hypercholecystokininemia also promoted azaserine-induced premalignant pancreatic nodules in the rat (15–18) but inhibited growth of an acinar cell carcinoma transplanted in the rat (2). These opposite effects should be explained in part by a difference in experimental procedures including the duration of the treatment and concentrations of peptides used.

Thus, the present study was designed to analyse in vivo and in vitro the effects of CCK-8 at low and high concentrations and to evaluate the CCK receptor binding capacities in an azaserine-induced pancreatic acinar cell carcinoma growing in the rat and to compare these effects to those on the normal pancreas in the same animals.

MATERIALS AND METHODS

Materials

The original pancreatic tumor was kindly obtained from D. S. Longnecker (Lebanon, NH, U.S.A.) and maintained in our laboratory by successive subcutaneous implantations in the interscapular region of Lewis rats (CNRS, Orléans, France). Originally, this tumor model was induced by azaserine in the rat and characterized as an acinar adenocarcinoma phenotype (7).

Sulphated CCK-8 was from Bachem (Bubendorf, Switzerland). CR 1409 was kindly given by Rotta Research Laboratories (Milano, Italy). L 364,718 was from Merck Sharp & Dohme Research Laboratories (Rahway, NJ). EGF (epidermal growth factor), BSA (bovine serum albumin), dexamethasone, charbamylcholine, isobutyl-1-methylxanthine, transferrin, ascorbic acid, selenium, EGTA (ethylene glycol-bis (β-aminoethylether)-N, N, N′, N′-tetraacetic acid), leupeptin, aprotinin, phenylmethylsulfonyl fluoride and SBTI (soybean trypsin inhibitor) were obtained from Sigma (La Verpillière, France). [^{3}H]thymidine (40–60 Ci/mmol) and [^{125}I]CCK-8 (sulphated) (2000 Ci/mmol) were purchased from Amersham France (Les Ulis, France). Waymouth's 752/1 medium, Dulbecco's modified Eagle's medium (DMEM) supplemented with antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml), fungizone 0.25 μg/ml, 10% fetal calf serum and EDTA (ethylenediamintetraacetic acid) were obtained from Gibco/BRL (Cergy-Pontoise, France). Other chemicals were standard reagent grade.

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In Vivo Experiments

Tumour Transplantation

The tumours were removed from donor rats, washed in ice-cold DMEM supplemented with antibiotics and chopped into small fragments which were passed through an 18 gauge needle. The so-obtained slurry was incubated for 10 min in Hank's solution containing 2.5 mmol/l EDTA (lacking Ca^{2+} and Mg^{2+}), centrifuged at 500 g for 5 min and whashed twice with cold medium. The pellet was resuspended in DMEM supplemented with antibiotics and 10% fetal calf serum. Aliquots (500 µl) of tumour tissue, corresponding approximately to 10^6 cells, were injected subcutaneously in the scapular region of male adult Lewis rats weighing 200–220 g. Palpable tumours appeared in 95–100% of rats, after 10–15 days.

Experimental Procedures

For in vivo studies, 40 Lewis rats bearing a palpable tumour were randomly divided into five groups of 8 rats each subjected for 12 successive days either to saline (control) or to CCK-8 at various concentrations: 0.1, 0.5, 2 and 4 µg/kg. Injections were performed three times daily in 15% hydrolysed gelatin in order to prolong the absorption of CCK-8. During this period, tumours were measured three times per week with calipers and tumour volume was calculated by the formula $\pi/6\times$ maximal length $\times$ maximal height $\times$ maximal width assuming an ellipsoid shape. On day 13, the animals were sacrificed after an overnight fast. Tumours and pancreases were quickly removed and carefully trimmed, weighed, and stored at −20°C until analyses.

Growth Parameters Analyses

The tumours and the pancreases were homogenized in ice-cold distilled water (100 mg/ml) in a Polytron set (medium speed). Protein content was determined by the method of Lowry et al. (19). Amylase content was measured according to the method of Danielsson (20), using maltose as a standard. After extraction, DNA was determined by the diphenylamine method using calf thymus DNA as standard (21) and RNA by the orcinol method using yeast RNA as a standard (22).

In Vitro Experiments

[^{3}H]thymidine Incorporation in Primary Cell Cultures

Tumour cells prepared as described above were dispersed in an enriched medium which consisted of Dulbecco's modified Eagle's and Waymouth media (v/v) supplemented with 15% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 10 µg/ml streptomycin, 0.25 µg/ml fungizone, 0.5 mM isobutyl-1-methylxanthine, 0.25 mg/ml soybean trypsin inhibitor (SBTI), 5 µM ascorbic acid, 10 µg/ml transferrin, 0.35 ng/ml sodium selenite, 1% (v/v) MEM essential vitamins, 1 µM carbachol, 1 µg/ml dexamethasone, 5 µg/ml insulin and 10 ng/ml EGF. For experimental purposes, $2–5\times 10^5$ cells in 2 ml of enriched medium were seeded into individual collagen-coated wells of 6-cell plates, and allowed to attach overnight at 37°C. The culture was maintained in a humidified atmosphere of 5% CO_2 / 95% air at 37°C, and the medium was changed every day.

After cell attachment, the enriched medium was aspirated and the cultures were washed twice with fetal calf serum free-DME medium to remove residual serum. After 24 h of serum starvation, the culture medium was supplemented with 2.5% fetal calf serum alone or with various concentrations of CCK-8 (10^{-11} to 10^{-7} M), or 10^{-8} and 10^{-7} M CCK-8 alone and in combination with 10^{-4} M CR 1409 or 10^{-6} M L 364,718. These peptides (200 µl/well) were added three times daily, from a concentrated stock solution in DMEM/Waymouth (v/v) for 24 hours. 0.5 µCi/ml [^{3}H]thymidine was added to each dish during the last 12 hours of culture. Then, cell cultures were rinsed with PBS at 4°C, harvested and centrifuged for 3 min at $300\times g$ at 4°C. The cells were then washed twice with ice-cold PBS, and sonicated in 1 ml of water with a probe-type sonicator. Aliquots were removed for determinations of protein content and 0.5 ml of each sonicated sample were precipitated with TCA (final concentration 10%) at 4°C for 20 min. The precipitates were washed twice with cold 5% TCA and dissolved in 1 ml of 0.2 N NaOH/0.1% SDS, neutralized with 1 N HCl and counted in a beta liquid scintillation counter.

Receptor Binding Assays

Receptor binding assays were performed on plasma membranes isolated from freshly excised tumour and normal pancreatic cells. Briefly, tumour and normal pancreatic cells were resuspended in 5 volumes of a lysis sucrose HEPES buffer (SHB) containing 0.25 M sucrose, 50 mM HEPES pH 7.4, 10 µg/ml leupeptin, 5 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 0.1% bacitracin and 0.01% soybean trypsin inhibitor. Cells were first homogenized with a Polytron for 30–45 s at setting 6, and then with a Dounce homogenizer using ten strokes with a tight-fitting pestle. This homogenate was centrifuged at $600\times g$, for 10 min at 4°C (S1). The pellet was resuspended in 2.5 volume SHB buffer, homogenized again and centrifuged (S2). The pooled supernatants (S1 + S2) were centrifuged at $10{,}000\times g$ for 30 min at 4°C. The supernatant solution was then made up to 0.1 M NaCl and 0.2 mM $MgSO_4$ by the addition of concentrated solutions of these salts and then centrifuged at $48{,}000\times g$ for 45 min at 4°C. The resulting pellet was washed in HEMGI buffer (50 mM HEPES pH 7.2, 2 mM EGTA, 5 mM $MgCl_2$, 120 mM NaCl, 4.7 mM KCl, 10% (v/v) glycerol, 40 µg/ml bacitracin, 1 mM PMSF, 0.01% SBTI, 10 µg/ml leupeptin and 5 µg/ml aprotinin), then centrifuged for 30 min at 4°C at $48{,}000\times g$. The pellet was resuspended in HEMGI (30% glycerol final concentration) and stored at −80°C.

Specific binding of [^{125}I]CCK-8 to plasma membranes was assayed in HEMI buffer, pH 6.8, containing 0.5% bovine serum albumin. Approximately 250–350 µg of membrane proteins were incubated with 25 pM [^{125}I]CCK-8 in the presence or absence of 0.1 µM unlabeled CCK-8 to determine the non-specific and total binding, respectively. The binding reaction was terminated after 60 min at 22°C by the addition of 0.5 ml of ice-cold HEMI buffer, centrifuged at $14{,}000\times g$ for 10 min at 4°C, then washed twice with ice-cold phosphate buffered saline (PBS). The radioactivity was counted with a γ-counter.

Histology

Samples of normal and tumour pancreatic tissues were fixed at 4°C in 2% glutaraldehyde buffered with 0.2 M sodium

cacodylate, pH 7.4 and post-fixed in 1% buffered osmium tetroxide. They were then dehydrated in a graded sequence of alcohols and embedded in araldite. Semi-thin sections were stained with toluidine blue and examined by photonic microscopy.

Statistical Analysis

In in vivo and in vitro experiments, the mean and standard error of the mean were calculated for each parameter. For group comparisons, a one way analysis of variance followed by a Newmann-Keuls test was applied.

RESULTS

Effects of CCK-8 on Tumor Growth In Vivo

Chronic administration of CCK-8 (0.1–4 μg/kg) did not influence the growth of the animals during the 12 days experiments and there was no significant difference in body weight gain between the CCK-8 treated groups and the saline treated control group. In addition, there was no adverse effects noted in rats treated with high doses of CCK-8 and no macroscopic evidence of peritoneal, liver or pulmonary metastasis.

As illustrated in Fig. 1, tumours implanted in control animals grew regularly until 10 days, then they tended to stabilize. The doubling time, measured during this first period, should be estimated as approximately 72 hours. Low doses of CCK-8 (0.1 and 0.5 μg/kg) administered three times daily, significantly increased tumour volume from the 2nd or 4th day of treatment. Thus, after a 12 days treatment, this parameter increased by 16% ($p < 0.05$) and 87% ($p < 0.001$) with 0.1 and 0.5 μg/kg treatments respectively. In contrast, higher doses of CCK-8 (2 and 4 μg/kg) significantly reduced tumour volume from the 4th day, this reduction being more marked after 12 days of treatment (−32% and −42% respectively, $p < 0.001$).

Tumour weight, protein, DNA, RNA and enzyme contents, evaluated at the 13th day of treatment, confirmed these data (Fig. 2). CCK-8 at the dose of 0.1 μg/kg, significantly stimulated tumour weight (32%, $p < 0.01$), its protein content (38%, $p < 0.01$), DNA (56%, $p < 0.001$), RNA (51%, $p < 0.001$) and amylase contents (31%, $p < 0.01$). These effects were more obvious with 0.5 μg/kg CCK-8. However, the 2 μg/kg CCK-8 treatment completely depressed these growth parameters by 35% ($p < 0.05$), 50 and 53% ($p < 0.01$) respectively for tumour weight, protein and amylase contents, and by 58 and 62% ($p < 0.001$) respectively for DNA and RNA contents. These effects were more obvious with 4 μg/kg CCK-8 treatment.

In agreement with our previous observations (23), histological examination of tumour fragments showed a disorganized tissue in which the ratio of nuclei to cytoplasm was markedly increased. In some areas, cells were arranged in acinar structures, with zymogen granules located around more or less dilated lumina. CCK-8 treatment did not change this structural aspect of the tumour.

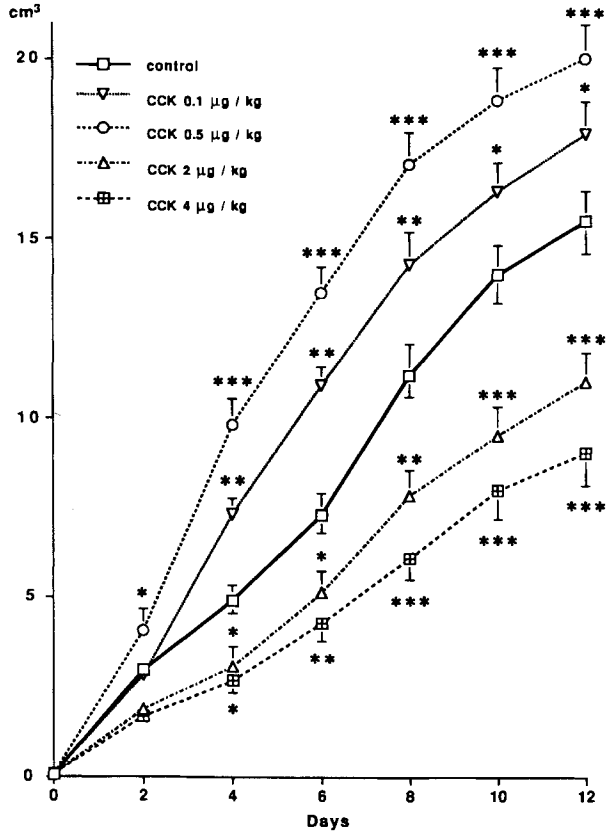

Fig. 1. Tumor volume in rats bearing a pancreatic carcinoma during chronic treatment with cholecystokinin octapeptide. Results are expressed as means ±S.E.M. of 8 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* control.

Effects of CCK-8 on [^{3}H]thymidine Incorporation in Cultured Pancreatic Tumour Cells

Cell proliferation was estimated by [^{3}H]thymidine incorporation in primary cultured pancreatic tumour cells during the last 12 hours of culture.

As represented in Fig. 3, CCK-8 added to the culture medium at different concentrations (10^{-11} to 10^{-7} M) exerted a biphasic effect on [^{3}H]thymidine incorporation into tumour cells. It stimulated this parameter in a dose-dependent manner from 10^{-10} M, the maximal stimulation being noted with 10^{-8} M (218%, $p < 0.001$). However, 10^{-7} M CCK-8 reduced [^{3}H]thymidine incorporation by −74% ($p < 0.001$).

The addition of CCK-receptor antagonists CR 1409 (10^{-4} M) or L 364,718 (10^{-6} M) completely suppressed both cell proliferative and antiproliferative effects of CCK-8 respectively obtained with 10^{-8} M and 10^{-7} M (Fig. 4).

Effects of CCK-8 on [^{125}I]CCK-8 Binding to Tumour and Normal Cell Membranes

Specific binding of [^{125}I]CCK-8 was analysed in crude membranes prepared from normal and cancerous pancreatic acinar cells.

In initial experiments, we established the optimal binding assay conditions for measuring the specific binding characteristics for CCK-8. We have found that a 60 min incubation at 22°C, pH 6.8 was satisfactory for CCK-8 binding in normal and cancerous pancreatic tissues. The non specific binding in these conditions was always less than 10% with tumour cells and 20% with normal pancreatic acinar cells.

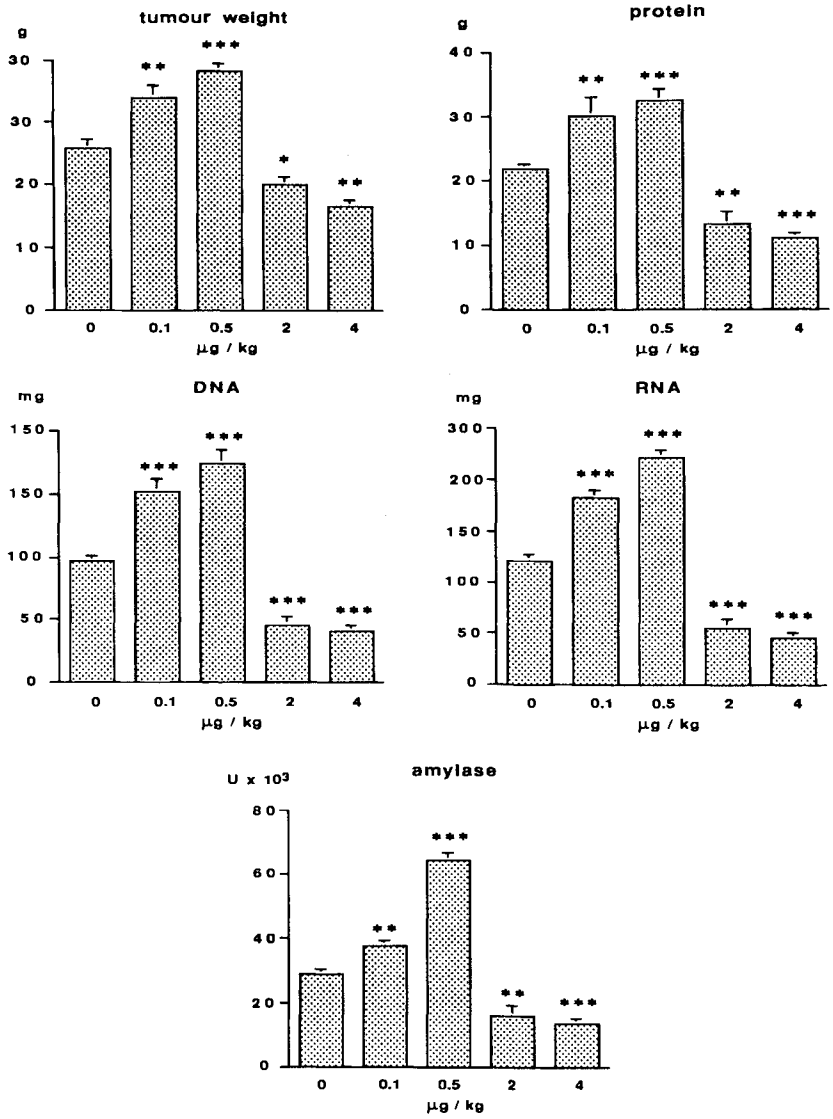

Fig. 2. Tumour weight and protein, DNA, RNA and amylase contents in animals chronically treated with saline (control group) or CCK-8 (0.1, 0.5, 2 and 4 µg/kg, three times daily). Results were expressed as means ±S.E.M. of 8 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *vs.* saline-treated control.

To examine the affinity and capacity of CCK-8 binding to pancreatic tumour and normal cell membranes, unlabeled CCK-8 in graded concentrations was used to competitively inhibit binding of [^{125}I]CCK-8. Figure 5 showed the competitive curve (Fig. 5A) and Scatchard plot (Fig. 5B) obtained with tumour cell membranes. The Scatchard analysis of these data displayed a curvilinear concave upward plot, suggesting the presence of at least two classes of CCK-8 binding sites: K_{d1} = 23.50 ± 1.25 nM; B_{max1} = 63 000 ± 7080 fmol/mg protein; K_{d2} = 0.20 ± 0.05 nM; B_{max2} = 770 ± 125 fmol/mg protein. We have also examined the CCK receptor binding characteristics of normal pancreatic cell membranes using the same experimental conditions and the same amount of membrane proteins. Our results indicated two classes of CCK-8 binding sites with following characteristics: K_{d1} = 79 ± 20 nM; B_{max1} = 842 ± 157 fmol/mg protein; K_{d2} = 0.26 ± 0.08 nM; B_{max2} = 35.8 ± 7.82 fmol/mg protein. Thus binding characteristics for CCK-8 were different in pancreatic tumour cells and normal cells.

Effects of CCK-8 on Normal Pancreatic Growth In Vivo

As represented in Fig. 6, CCK-8 administered for 12 successive days in rats increased pancreatic weight, protein, RNA and amylase contents in a dose-dependent manner from 0.1 up to 2 µg/kg. This effect, observed with 0.5 µg/kg CCK-8 was optimal with 2 µg/kg CCK-8. Pancreatic weight, protein and RNA contents were increased by 47%, 56% and 50% respectively ($p < 0.01$), amylase content by 106% ($p < 0.001$) and DNA content by 14% ($p < 0.05$). However, these pancreatic growth effects disappeared with the highest dose of CCK-8 (4 µg/kg), regarding all parameters.

As already reported by us (24), histological examination of the pancreases of CCK-8 treated animals showed no marked

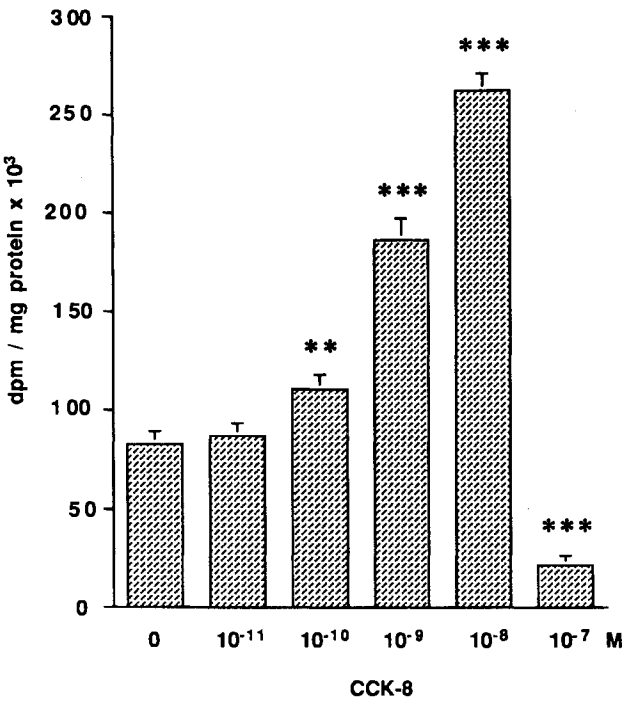

Fig. 3. [^{3}H]thymidine incorporation into pancreatic tumor cells cultured in presence of increasing concentrations of CCK-8 (10^{-11} to 10^{-7} M). Results, expressed as means ±S.E.M. of 3 experiments, were compared with untreated control group (**$p < 0.01$, ***$p < 0.001$).

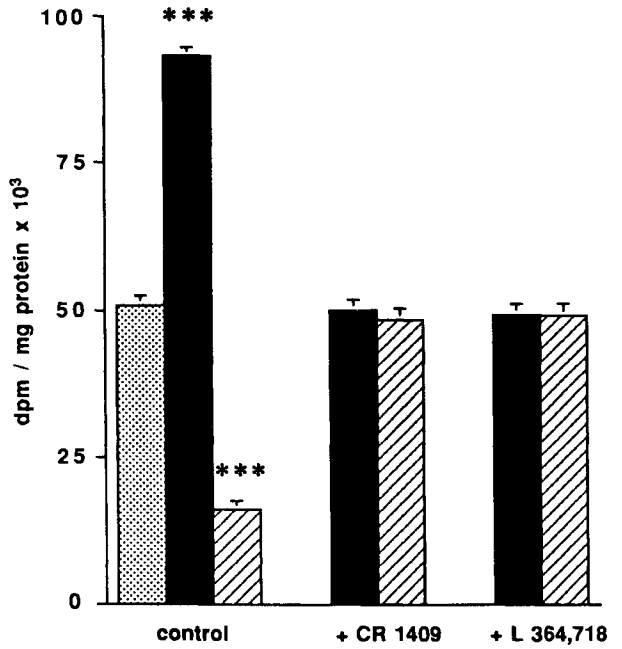

Fig. 4. [^{3}H]thymidine incorporation into cultured pancreatic tumor cells treated with CCK-8 10^{-8} M (black columns) or 10^{-7} M (hatched columns) alone or in association with 10^{-4} M CR 1409 or 10^{-6} M L 364,718. Results, expressed as means ±S.E.M. of 3 experiments, were compared with untreated control group (dotted column) (***$p < 0.001$).

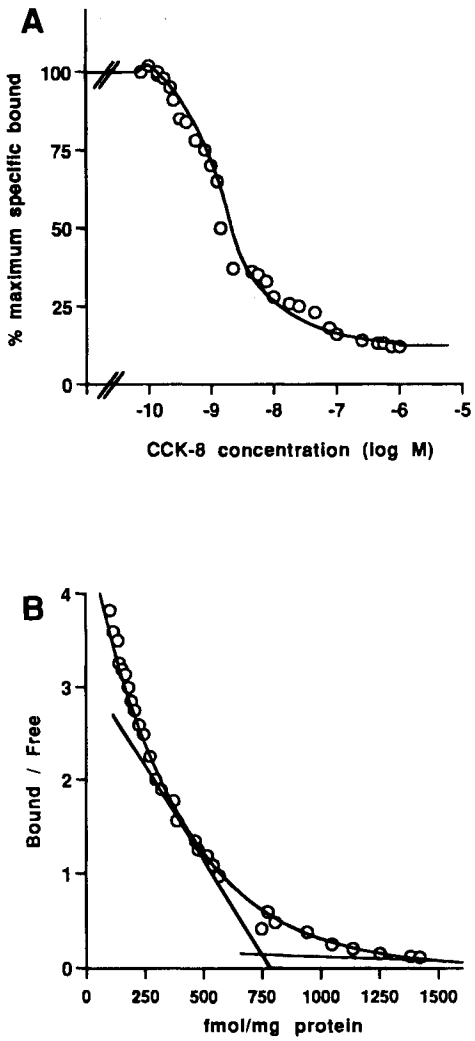

Fig. 5. Binding of [^{125}I]CCK-8 to the pancreatic tumour cells. (A) Competitive inhibition of specific [^{125}I]CCK-8 binding by increasing concentrations of unlabeled CCK-8. (B) Scatchard plot of the inhibition curve with unlabeled CCK-8. Cells were incubated with 25 pM [^{125}I]CCK-8 for 60 min at 22°C in the presence of varying concentrations of unlabeled CCK-8. Specific saturable binding is expressed as the percentage of maximal specific binding. Each value represents the mean of six independent observations from three separate experiments.

change in the general aspect of the pancreas when compared to saline treated animals. However, the group treated with a high concentration of CCK-8 (2 μg/kg) showed numerous zymogen granules in acinar cells while mitotic figures were often observed in both acinar and ductular.

DISCUSSION

In a previous study (2), we have shown that cholecystokinin octapeptide, administered three times daily at concentrations ranging from 1 to 4 μg/kg, reduced in a dose-dependent manner, growth of a pancreatic acinar carcinoma transplanted in the scapular region of rats. This tumor is a well differentiated carcinoma in which the cells are arranged in acini filled with zymogen granules and possess secretory enzyme activities such as amylase, lipase and chymotrypsin (23).

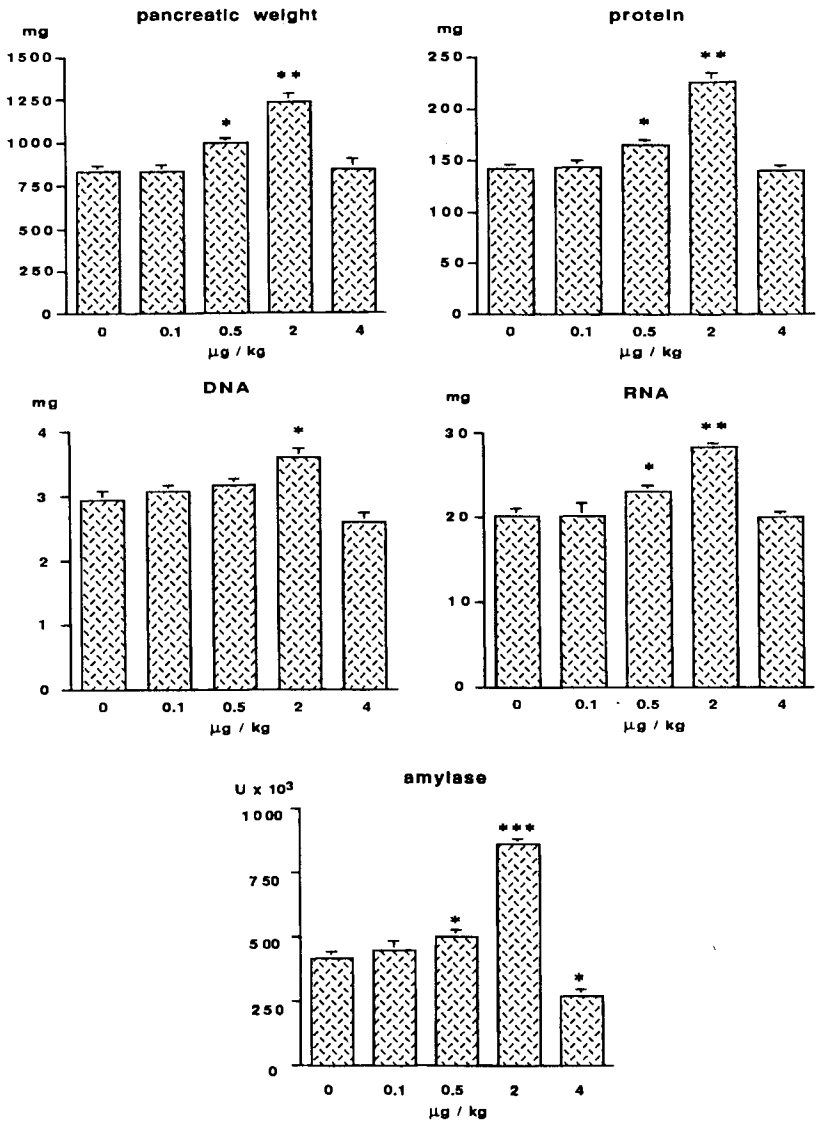

Fig. 6. Pancreatic weight and protein, RNA, DNA and amylase contents in animals chronically treated with saline (control group) or CCK-8 (0.1, 0.5, 2 and 4 µg/kg, three times daily). Results were expressed as means ±S.E.M. of 8 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *vs.* saline-treated control.

In the present investigation, we have enlarged the dosage of CCK-8 in the same experiment and used 0.1, 0.5, 2 and 4 µg/kg CCK-8 administered s.c. three times daily for 12 successive days. Our results showed a biphasic response of CCK-8 on the growth of the acinar cell carcinoma transplanted to the rat: stimulation with low doses (0.1 and 0.5 µg/kg) with an optimal effect at 0.5 µg/kg, and inhibition with higher doses (2 and 4 µg/kg) with an optimal effect at 4 µg/kg. Tumour growth was estimated by the evolution of its volume during 12 days and by its weight, protein, RNA, DNA and amylase contents determined on the 13th day of treatment. Such results have never been observed by others. Indeed, CCK or caerulein have been shown to exert a stimulatory effect (11,12) or no effect (13,14) on the growth of a pancreatic ductular carcinoma growing in animals. However, at first, these cancers were of ductular origin instead of acinar origin and secondly, in the experimental model the authors never used a wide range of peptide concentration.

The inhibition of tumour growth observed by us with high doses of CCK-8 should be attributed to a toxic effect of the peptide. However, our histological examinations of both tumour and normal pancreases are against this hypothesis since no structural alterations were noted after the sustained treatment with high doses of CCK-8.

In order to verify whether CCK-8 acts directly on tumor growth, we have analyzed the effect of increasing concentrations of this octapeptide on the proliferation of primary cell cultures of isolated acinar carcinoma cells. Our results, based on [^{3}H]thymidine incorporation, indicated that concentrations up to 10^{-8} M increased cell proliferation in a dose-dependent manner, while a higher concentration (10^{-7} M) strongly inhibited it. Thus, it was suggested that CCK octapeptide exerts a

direct biphasic effect on the proliferation of pancreatic acinar carcinoma cells.

These effects could be directly mediated by the occupancy of specific CCK receptors on tumour cells. In a previous work (2), we have demonstrated, with autoradiographic visualization, that these cells possessed a great density of CCK specific receptors. The present study confirms these results and shows an overexpression of two classes of CCK receptors in tumour cells when compared to the normal pancreas of the same animals: low affinity receptors (K_{d1} = 23.50 ± 1.25 nM) and high affinity receptors (K_{d2} = 0.20 ± 0.05 nM), the former being 82 times more dense than the latter (B_{max1} = 63 000 ± 7080 fmol/mg protein *versus* B_{max2} = 770 ± 125 fmol/mg protein). These results are in agreement with those of Bell et al. (25) who demonstrated the overexpression of two classes of CCK-A receptors on an azaserine-induced acinar cell carcinoma, DSL6, and on premalignant nodules of the same tumour. However, in our pancreatic carcinoma, low and high affinity CCK receptors were respectively 150 and 6 times more dense than in the DSL 6 pancreatic carcinoma described by Bell et al. (25). In addittion, these latter found, in this carcinoma, the presence of gastrin (CCK B) receptors, which constitute about one third of the total high affinity CCK receptors (26). Likewise, the pancreatic cancer cell line AR-42J, also derived from an azaserine-induced pancreatic carcinoma, possessed both CCK A and CCK B receptor subtypes (27) but CCK-9 did not stimulate DNA synthesis, though ornithine decarboxylase was increased (28). In contrast, premalignant nodules induced in the rat by azaserine, only expressed CCK-A receptors but not CCK B receptors (29,30).

Since CCK-8 interacts with approximately equal affinity with both CCK-A and CCK- B (gastrin) receptor subtype (31), the question was to know which CCK binding sites specifically mediate the hormonally induced biphasic growth effects on our acinar carcinoma cells. Thus, in the present study, we have blocked CCK receptors by specific CCK antagonists such as CR 1409 (lorglumide) and L 364,718 (devazepide). This latter is considered as the most potent CCK A antagonist, being 600 times more potent than CR 1409 on pancreatic secretory function and growth (32). Our results showed that both proliferative effect induced by 10^{-8} M CCK-8 and antiproliferative effect induced by 10^{-7} M were completely suppressed by both antagonists, suggesting that these effects were mediated by the occupancy of CCK-A receptors. Thus, low concentrations of CCK-8 probably interact with high affinity CCK-A binding sites while high concentrations should interact with low affinity CCK A binding sites.

The present study also demonstrated that the normal pancreas possesses two subtypes of CCK receptors: low affinity sites (K_{d1} = 79 ± 20 nM) and high affinity sites (K_{d2} = 0.26 ± 0.08 nM), the density of low affinity sites being about 24 times higher than that of high affinity sites (B_{max1} = 842 ± 157 fmol/mg protein *versus* B_{max2} = 35.8 ± 7.82 fmol/mg protein). Two CCK A binding sites have also been described in the pancreas by others (25), but CCK B (gastrin) receptors were absent (27,28). As in the pancreatic carcinoma, these two CCK A binding sites could be related to the biphasic effect of CCK octapeptide on the growth of the normal pancreas. However, the dose-response curve was shifted to the right, indicating that the normal pancreas was less sensitive to the peptide. These effects were partly or completely suppressed by the CCK antagonist CR 1409 (1,2) suggesting that CCK responses are mediated by the occupancy of specific receptors. Thus, our results are consistent with the occupancy of both CCK high and low affinity binding sites.

In conclusion, our present study clearly demonstrates that the growth of both pancreatic acinar carcinoma and normal pancreas can be modulated by CCK-8. However, the carcinoma was more sensitive than the normal pancreas. In addition, both proliferative and antiproliferative effects induced respectively by low and high concentrations of CCK are mediated by specific CCK A receptors. These effects merit to be investigated in other tumour cells in order to understand the postreceptor mechanism leading to the inhibitory effect.

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