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Effects of cholecystokinin octapeptide on the exocrine pancreas in a new rat model of type 2 diabetes

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Abstract

We investigated the effects of increasing concentrations of cholecystokinin octapeptide (CCK-8) on the exocrine pancreas of a new model of type 2 diabetic rats due to the partial protection exerted by nicotinamide against the β-cytotoxic effect of streptozotocin. CCK-8, administered for 8 successive days, exerted a biphasic action on the growth of the pancreas in non-diabetic and type 2 diabetic rats; however, the latter were less sensitive to CCK-8. Similar results were obtained in vitro by measuring the uptake of 5-bromo-2′-deoxyuridine (BrdU) in cultured isolated acinar cells. This effect was completely blocked by 3*S*(−)(*N*′-2,3-dihydro-1-methyl-2-oxo5-phenyl-1*H*-1,4-benzo-diazepin-3-yl)-1*H*-indole-2-carboxamide (L 364,718; a CCK_1 receptor antagonist) but not by (3*R*)-3[*N*′-(3-methylphenyl)ureido]-1,3-dihydro-1-methyl-5-phenyl-2*H*1,4-benzo-diazepin-2-one (L 365,260; a CCK_2 receptor antagonist), suggesting a direct effect via CCK_1 receptors. Binding studies showed that these effects were mediated by a single class of low-affinity CCK_1 receptors in diabetic rats and two classes of CCK-8 binding sites (with high and low affinity) in non-diabetic rats. Thus, in our new type 2 diabetes model, the loss of sensitivity of the pancreas to CCK-8 could be attributed to the loss of CCK_1 receptors of high affinity.

Keywords: Affinity binding site; Cholecystokinin; Diabetes; Pancreas

1. Introduction

Cholecystokinin (CCK) is a peptide found predominantly in the digestive tract and brain. It is secreted by endocrine cells in the duodenum and proximal jejunum in response to the absorption of digested food components such as amino acids and fat (Liddle et al., 1985).

CCK is involved in gall bladder contraction, gastric emptying, intestinal motility and causes the release of insulin and digestive enzymes from the pancreas (Crawley and Corwin, 1994; Liddle et al., 1985; Tachibana et al., 1996; Verspohl et al., 1986). The increase of plasma CCK either exogenously induced by long-term administration of CCK, or endogenously by feeding raw soya flour or fat, exerts a trophic effect on the exocrine pancreas (Axelson et al., 1990; Hajri et al., 1989). This effect was partly or completely suppressed by CCK receptor antagonists such as D,L-4-(3,4-Dichloro-benzoyl-amino)-5-(di-*n*-pentyl-amino)-5-oxo-pentanoic acid (CR 1409) or 3*S*(−)(*N*′-2,3-dihydro-1-methyl-2-oxo5-phenyl-1*H*-1,4-benzo-diazepin-3-yl)-1*H*-indole-2-carboxamide (L 364,718), suggesting a direct effect via CCK receptors on both acinar and duct cells of the pancreas (Axelson et al., 1990; Hajri et al., 1989; Hajri and Damgé, 1998; Wisner et al., 1988). In fact, CCK trophic effects were mediated via CCK_1 receptors in the rat (Povoski et al., 1994).

Type 2 diabetes or non-insulin-dependent diabetes is a chronic metabolic disease due to insulin resistance and/or defective insulin secretion, resulting in a hyperglycemic state under both fasted and fed conditions. The effects of CCK on the pancreas in type 2 diabetic models in rodents have been described in Zucker obese rats characterized by obesity, increased pancreatic insulin content, hyperinsulinemia and insulin resistance (Stern et al., 1975; Zucker, 1965) and in the genetically diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats characterized by hyperglycemia, obesity, hyperinsulinemia and insulin resistance (Kawano et al., 1992). The former model showed a reduced exocrine pancreatic response to CCK while the latter model was completely insensitive to exogenous and endogenous CCK stimulation, regarding exocrine and endocrine pancreatic

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secretions (Tachibana et al., 1996). In fact, these latter abnormalities can be explained by a defect in CCK_1 receptors reported for OLTEF rats (Moran et al., 1998). In type 2 diabetic subjects, CCK reduces exocrine pancreatic secretions (Kim et al., 2000).

However, the effects on the endocrine pancreas are controversial. According to Kim et al. (2000), CCK did not increase serum insulin levels in type 2 diabetics in response to intravenous glucose administration, when compared to those in healthy controls. According to Ahren et al. (2000), CCK-8 potentiated the increase in circulating insulin and reduced the increase in circulating glucose after meal ingestion, suggesting that CCK-8 exerts an antidiabetogenic effect. However, CCK could also improve the diabetic state by inhibiting food intake, this hormone being well recognized as the hormone of satiety (Degen et al., 2001).

Thus, the aim of this work was to analyse the effects of chronic administration of increasing concentrations of CCK octapeptide (CCK-8), which is the smallest form to retain a full range of biological activity (Crawley and Corwin, 1994) on rat pancreatic growth, feeding behaviour and CCK receptors in a new experimental model of type 2 diabetes in adult rats given streptozotocin and partially protected with a suitable dose of nicotinamide (Masiello et al., 1998). This model, which shares a number of similarities with human type 2 diabetes, is characterized by moderately stable hyperglycemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, responsiveness to tolbutamide and a reduction of pancreatic beta cell mass (Masiello et al., 1998; Novelli et al., 2001).

2. Materials and methods

2.1. Materials

Sulphated CCK-8 was obtained from Neosystem (Strasbourg, France). Streptozotocin, nicotinamide, hydrolysed gelatin, bovine serum albumin, EGTA (ethylene glycol-bis (β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), benzamidine, bacitracin, soybean trypsin inhibitor, type IV collagenase, ascorbic acid, isobutyl-1-methylxanthine, carbamylcholine and dexamethasone were from Sigma-Aldrich Chimie (L'Isle d'Abeau Chesnes, France). Sucrose was from Bio-Rad Laboratories (Ivry-sur-Seine, France), HEPES, Waymouth MB 752/1 medium, DMEM (Dulbecco's modified Eagle's medium), penicillin, streptomycin, fungizone, vitamins for Basal Medium of Eagle (BME) and ITS (insulin–transferrin–selenium) from Life Technologies (Cergy Pontoise, France). Protease inhibitor cocktail tablets, Complete™, was from Boehringer (Mannheim, Germany) and fetal calf serum from Eurobio (Les Ulis, France). [^{125}I]CCK-8 (sulphated) (2200 Ci/mmol) was purchased from NEN Life Science Products (Paris, France). Other chemicals were standard reagent grade. The glucometer (Prestige) and sticks were kindly provided by Chronolyss (Le Raincy, France). The CCK_1 and CCK_2 receptors antagonists, L 364,718 and (3*R*)-3[*N*′-(3-methylphenyl)ureido]-1,3-dihydro-1-methyl-5-phenyl-2*H*1,4-benzo-diazepin-2-one (L 365,260), were a generous gift from ML Laboratories (Liverpool, England). Male Wistar rats were from DEPRE (St. Doulchard, France).

2.2. Animals and treatments

Ten-week-old male Wistar rats, weighing 220–240 g, were randomly divided into two groups of 28 animals each. One group received, intraperitoneally, nicotinamide (260 mg/kg body weight) dissolved in saline 15 min before intravenous administration of streptozotocin (65 mg/kg body weight) in order to induce type 2 diabetes. The second group, considered as control, received injections of saline.

After 15 days, glycemia was measured in the tail vein of all animals, using a glucometer. Type 2 diabetes was confirmed by an increase of glycemia (140 to 200 mg/dl) without weight loss.

Then, the animals in each group were subdivided into four groups of seven animals each, given for 8 successive days either saline (controls) or CCK-8 at various concentrations: 1, 2 and 4 μg/kg body weight. Subcutaneous injections were performed three times daily in 15% hydrolysed gelatin. During the whole experiment, animals were weighed every day and fed ad libitum a chow diet (UAR, Villemoisson-sur-Orge, France). Food intake was assessed by giving a weighed amount of fresh food pellets daily, and the following day, food left in the containers was weighed. Food intake was calculated as the difference between both values each day of the 8-day experiment.

On the 8th day of injections, glycemia was measured in the tail vein of all animals. On day 9, the animals were killed after an overnight fast by exsanguination; blood was collected in heparinized tubes and the pancreas was quickly removed, carefully trimmed free of fat and lymph nodes, weighed and stored at −20 °C until analysis.

2.3. Plasmatic and pancreatic insulin analyses

Collected blood samples were immediately centrifuged for 10 min at 1000×*g* at 4 °C. The plasma was removed and stored at −20 °C for further insulin determinations. Plasma insulin was measured directly by radioimmunological assay (Insulin-CT kit from CIS Bio International, Gif-sur-Yvette, France).

For measurement of pancreatic insulin content, 100-mg aliquots of the pancreases were homogenized in 5 ml acid ethanol using a Polytron and samples were stored for 48 h at 4 °C. After centrifugation and separation of the supernatant, the pellet was extracted again with acidified ethanol for 24 h at 4 °C. The supernatant obtained after centrifugation was pooled with the previous one and kept at −20 °C until assayed. The pancreatic insulin content was measured by a

radioimmunological method using the same kit as for plasma insulin.

2.4. Growth parameters analyses

The pancreases were homogenized in ice-cold distilled water (200 mg/ml) in a Polytron set. Protein content was determined by the method of Bradford (1976). Amylase content was measured according to the method of Danielsson (1974), using maltose as a standard. Lipase activity was determined by a turbidimetric method (Verduin et al., 1973). After extraction, DNA was determined by the diphenylamine method using calf thymus DNA as a standard (Richards, 1974) and RNA by the orcinol method using yeast RNA as a standard (Schneider, 1957).

2.5. In vitro experiments

2.5.1. Preparation of acini

For in vitro studies, an additional group of eight normal rats and eight type 2 diabetic rats was used throughout the experiments. Isolated acini from normal and type 2 diabetic rats were prepared by the method of Williams at al. (1978). After laparotomy, the pancreases were distended by the injection of 10 ml of Hanks solution in the pancreatic duct. The pancreases were cut into 10–20-mm^2 pieces and incubated with 7.5 mg/ml collagenase for 17 min at 37 °C in a water bath shaking at 120 cycles/min. The acini were purified by filtration through 150-μm screen mesh and sedimentation through phosphate-buffered saline (PBS) containing 4% bovine serum albumin. Then, the acini were washed five times in PBS buffer.

2.5.2. Measurement of 5-bromo-2′-deoxyuridine (BrdU) uptake

The acini were seeded on 96-well collagen-coated plates at a density of 2×10^4 cells/well in an enriched medium which contained 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 amphotericin B, 0.5 mM isobutyl-1-methylxanthine, 0.25 mg/ml soybean trypsin inhibitor, 5 μM ascorbic acid, 10 μg/ml transferrin, 0.35 ng/ml sodium selenite, 1% vitamins for Basal Medium of Eagle, 1 μM carbachol, 1 μg/ml dexamethasone. The culture was maintained, to allow cell attachment overnight, at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Then, the cells were re-fed with medium containing only 2.5% fetal calf serum. After 24 h of serum starvation, the cells were incubated with the same medium containing various concentrations of CCK-8 (10^{-11} to 10^{-7} M), or 10^{-8} and 10^{-9} M CCK-8 alone or in combination with 10^{-6} M L 364,718 or 10^{-6} M L 365,260. The peptides were added three times daily for 24 h. Ten micromolars 5-bromo-2′-deoxyuridine (BrdU) was added to each well during the last 12 h. Incorporation of BrdU was determined using a cell proliferation enzyme-linked immunosorbent assay system (Amersham Pharmacia Biotech) according to the instructions of the manufacturer. Optical density was measured in a spectrophotometer plate reader at 450 nm within 5 min.

2.6. Receptor binding assays

Receptor binding assays were performed on plasma membranes isolated from pancreases from type 2 diabetic rats and non-diabetic rats. The pancreases, weighing approximately 1 g, were resuspended in 5 volumes of a lysis sucrose HEPES buffer (SHB) containing 0.25 M sucrose, 50 mM HEPES pH 7.4, 1 mM benzamidine, 0.1% bacitracin, 0.01% soybean trypsin inhibitor and one tablet of Complete™. The pancreases were homogenized with a Polytron and then with a Dounce homogenizer using 10 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 and S2) were centrifuged at $10\,000\times g$ for 30 min at 4 °C. The supernatant was 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, 0.01% soybean trypsin inhibitor, and one tablet of Complete™), 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. Membrane proteins (300 μg) 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 the total binding. The binding reaction was terminated after 60 min at 22 °C by the addition of 0.5 ml ice-cold HEMI buffer, centrifuged at $14\,000\times g$ for 10 min at 4 °C, then washed twice with ice-cold PBS. The radioactivity was counted with a γ-counter.

2.7. Statistical analysis

The results are expressed as means±standard error of the mean (S.E.M.). For comparisons between groups, a one-way analysis of variance followed by Student's unpaired *t*-test was applied.

3. Results

3.1. Effects of CCK-8 on body weight and feeding behaviour

At the beginning of the experiment, the body weight of non-diabetic rats and type 2 diabetic animals was, respectively, 349±4 and 347±5 g. During the 8-day treatment with saline, the two groups of animals gained approximately

12–14% of their initial weight. CCK-8 treatment did not change this profile (Table 1).

Food intake, measured over a 24-h period, was not significantly different in non-diabetic and diabetic rats; CCK-8 treatment, whatever its concentration, did not modify this parameter (Table 1).

3.2. Effects of CCK-8 on glycemia and plasma and pancreatic insulin concentrations

Eight days after the treatment with saline, the glycemia of control rats was 99±4 mg/dl (Table 1). In type 2 diabetic rats, the glycemia was significantly increased compared to the control rats (154±8 mg/dl, $P<0.001$). Chronic administration of CCK-8 (1 to 4 μg/kg) did not affect significantly the glycemia in non-diabetic rats but slightly decreased it in diabetic rats at 1 and 2 μg/kg and significantly at 4 μg/kg (Table 1).

As reported in Table 1, plasma insulin concentration was slightly but not significantly reduced in diabetic rats when compared to that in non-diabetic rats. CCK-8 treatment did not modify this parameter in either group of animals.

As illustrated in Table 1, pancreatic insulin content was significantly reduced (−56%, $P<0.01$) in diabetic rats when compared to that in non-diabetic rats. In diabetic rats, CCK-8 treatment increased this parameter significantly, with a maximal effect (+225%, $P<0.01$) at 4 μg/kg. However, in non-diabetic rats, CCK-8 treatment did not significantly alter the pancreatic insulin content.

3.3. Effects of CCK-8 on pancreatic growth

As illustrated in Fig. 1, CCK-8 administered for 8 successive days in non-diabetic rats increased pancreatic growth parameters with a maximal effect at 1 μg/kg concentration, this effect being less pronounced at the concentration of 4 μg/kg. The maximal increases observed for pancreatic weight, protein, RNA, amylase and lipase were, respectively, 31% ($P<0.001$), 74% ($P<0.001$), 45% ($P<0.001$), 57% ($P<0.001$), 46% ($P<0.001$).

In type 2 diabetic rats (Fig. 1), the profile of these growth parameters was quite similar but the maximal increase was generally observed at the CCK-8 concentration of 2 μg/kg, except for DNA where the maximal increase (116%, $P<0.01$) was observed at 4 μg/kg. The maximal increases for pancreatic weight, protein, RNA, amylase and lipase contents were, respectively, 49% ($P<0.01$), 49% ($P<0.001$), 38% ($P<0.01$), 192% ($P<0.001$) and 62% ($P<0.01$).

Thus, CCK-8 exerts a biphasic effect on pancreatic growth as a function of the dose administered, but the dose–response curve was shifted toward higher concentrations in diabetic rats by comparison with that in non-diabetic rats.

In order to determine if pancreatic growth was due to cellular hypertrophy, we calculated the corresponding indices, i.e. the ratios of pancreatic weight/DNA, protein/DNA and RNA/DNA expressed as a function of control values. Hypertrophy is considered when these ratios are greater than 1.00 and hypotrophy when they are lower than 1.00. As illustrated in Table 2, these indices were significantly increased in non-diabetic rats with 1 μg/kg CCK-8, suggesting cellular hypertrophy. The latter was not accompanied by cellular hyperplasia, according to the ratios of pancreatic DNA content in CCK-8-treated animals to that in saline-treated rats. However, since the pancreatic content of DNA was slightly but not significantly reduced at 4 μg/kg CCK-8, the three corresponding indices of cellular hypertrophy were significantly increased but this increase has to be interpreted with caution, being mainly due to the reduction in pancreatic DNA content.

In diabetic rats, the index of hyperplasia increased in parallel to the concentration of CCK-8, being maximal at 4 μg/kg. However, the indices of cellular hypertrophy were not different from 1.00 after treatment with CCK-8 at the concentrations of 1 and 2 μg/kg, indicating that hyperplasia was not accompanied by a change in cell size; in contrast, at the concentration of 4 μg/kg CCK-8, the increase of the index of hyperplasia was parallel to the indices of cellular hypertrophy significantly lower than 1.00, suggesting the presence of an increased number of

Table 1
Body weight, food intake over 24 h, blood glucose and insulin concentrations and pancreatic insulin content in non-diabetic and diabetic rats treated with saline or CCK-8 at various concentrations (n=7 per group). Comparisons between CCK-8- and saline-treated rats

	Non-diabetic rats				Type 2 diabetic rats			
	Saline	CCK (1 μg/kg)	CCK (2 μg/kg)	CCK (4 μg/kg)	Saline	CCK (1 μg/kg)	CCK (2 μg/kg)	CCK (4 μg/kg)
Body weight (g)	398±6	400±5	384±4	384±6	388±6	383±8	402±12	383±9
Food intake (g)	27±1	27±1	27±1	28±1	25±1	26±1	25±1	25±1
Blood glucose concentration (mg/dl)	99±4	95±4	97±5	104±6	154±8	128±11	139±15	125±3[a]
Plasma insulin concentration (μU/ml)	110±26	119±6	115±20	91±10	89±9	87±12	81±10	84±11
Pancreatic insulin content (mU)	360±49	405±56	440±33	436±52	157±36[a]	310±28[a]	346±36[a]	353±43[a]

[a] $P<0.01$.

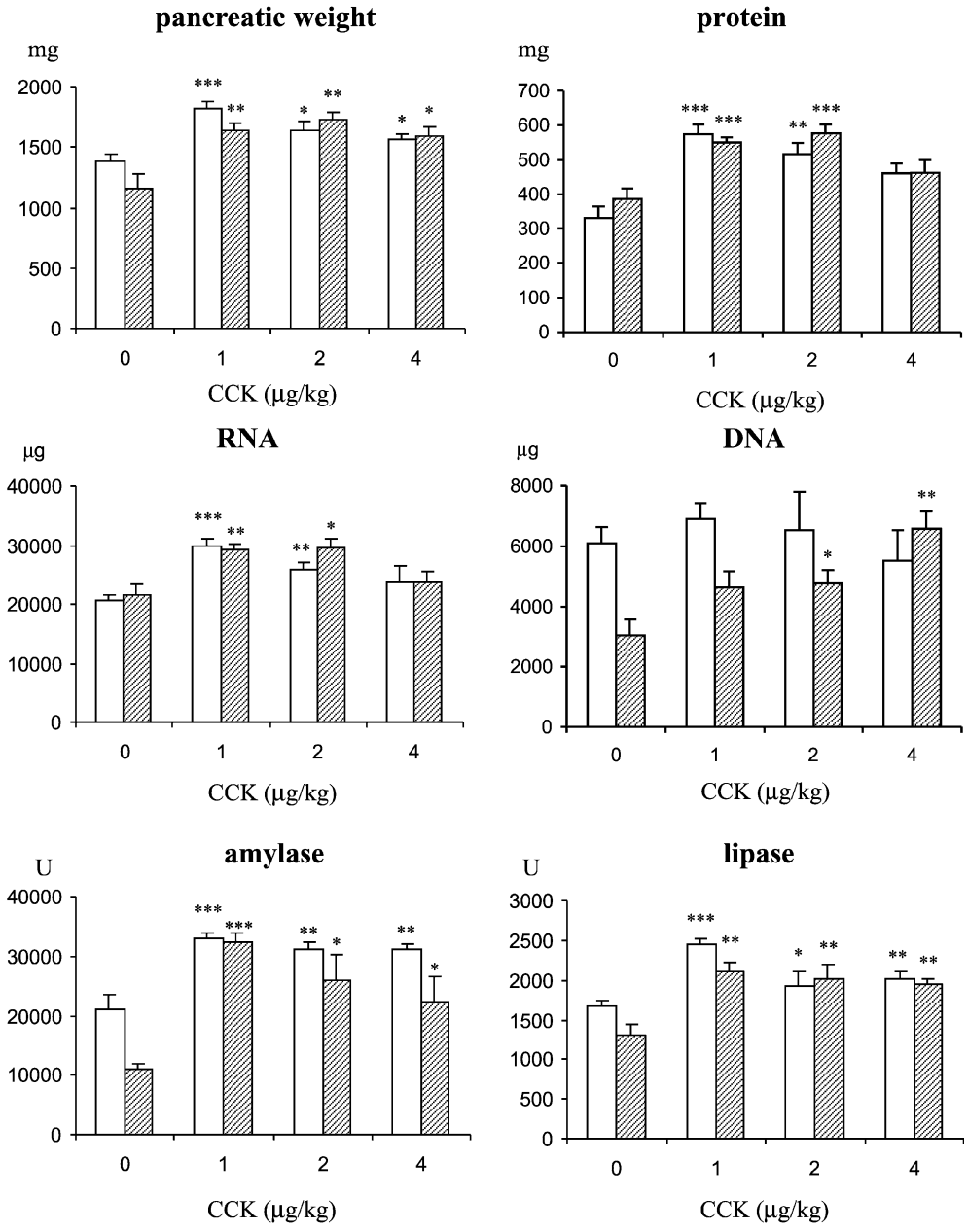

Fig. 1. Total pancreatic weight, protein, RNA, DNA, amylase and lipase contents in non-diabetic rats (white columns) and type 2 diabetic rats (hatched columns) treated with saline or CCK-8 (1, 2 and 4 µg/kg). Results are means±S.E.M. for seven animals. *P<0.05, **P<0.01, ***P<0.001 vs. saline-treated control.

smaller cells. Thus, in diabetic rats, hyperplasia was accompanied with cellular hypotrophy after treatment with CCK-8 at the highest dose.

3.4. Effects of CCK-8 on BrdU uptake in cultured acini

Cell proliferation was estimated by BrdU incorporation in primary cultured pancreatic acini isolated from diabetic and non-diabetic rats during the last 12 h of culture. As shown in Fig. 2, CCK-8 added to the culture medium at different concentrations (10^{-11} to 10^{-7} M) exerted a biphasic effect on BrdU incorporation into the cells from the pancreas of non-diabetic rats. It stimulated this parameter in a dose-dependent manner from 10^{-11} M, the maximal stimulation being observed with 10^{-9} M (+62%, P<0.01). However, this effect disappeared when the concentration of CCK-8 was increased to 10^{-8} and 10^{-7} M.

In the acini from type 2 diabetic rats (Fig. 2), BrdU uptake was quite similar, but the maximal effect was observed with 10^{-8} M CCK-8 and was lower than that in normal rats (+39%, P<0.01).

Table 2
Indices of cellular hypertrophy and hyperplasia in non-diabetic and diabetic rats treated with CCK-8 at various concentrations

		Non-diabetic rats			Type 2 diabetic rats		
		CCK (1 μg/kg)	CCK (2 μg/kg)	CCK (4 μg/kg)	CCK (1 μg/kg)	CCK (2 μg/kg)	CCK (4 μg/kg)
Indices of hypertrophy	Pancreatic weight/DNA	1.16	1.11	1.24[a]	0.93	0.95	0.64[b]
	Protein/DNA	1.53[c]	1.46[c]	1.54[c]	0.93	0.95	0.55[c]
	RNA/DNA	1.28[a]	1.17	1.28[a]	0.89	0.88	0.51[c]
Index of hyperplasia	DNA	1.13	1.07	0.90	1.52[c]	1.57[c]	2.16[c]

Values are ratios of treatment groups to control group. For individual rats, pancreatic weight, protein and total RNA in mg and total DNA in mg per mg of total DNA were calculated. These group means were computed and the ratios of these to the corresponding control means were calculated. Thus, values are ratios of CCK-treated groups to saline-treated group. Values significantly greater or lower than control (i.e. 1.00): [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$.

Thus, concerning the in vitro cell proliferation index, the dose–response curve to CCK-8 was also shifted toward higher concentrations in diabetic rats when compared to that for non-diabetic rats.

The addition of the CCK_1 receptor antagonist, L 364,718 (10^{-6} M), to pancreatic acini from non-diabetic and type 2 diabetic rats (Fig. 3), exposed to the most effective concentration of CCK-8, completely blocked the stimulated BrdU uptake. In contrast, the addition of the CCK_2 receptor antagonist, L 365,260, at the same concentration (10^{-6} M) did not affect the uptake of BrdU induced by CCK-8.

3.5. Effects of CCK-8 on [^{125}I]CCK-8 binding to pancreatic cell membranes in type 2 diabetic rats

Specific binding of [^{125} I]CCK-8 was analysed in crude membranes prepared from the pancreas of non-diabetic and type 2 diabetic rats.

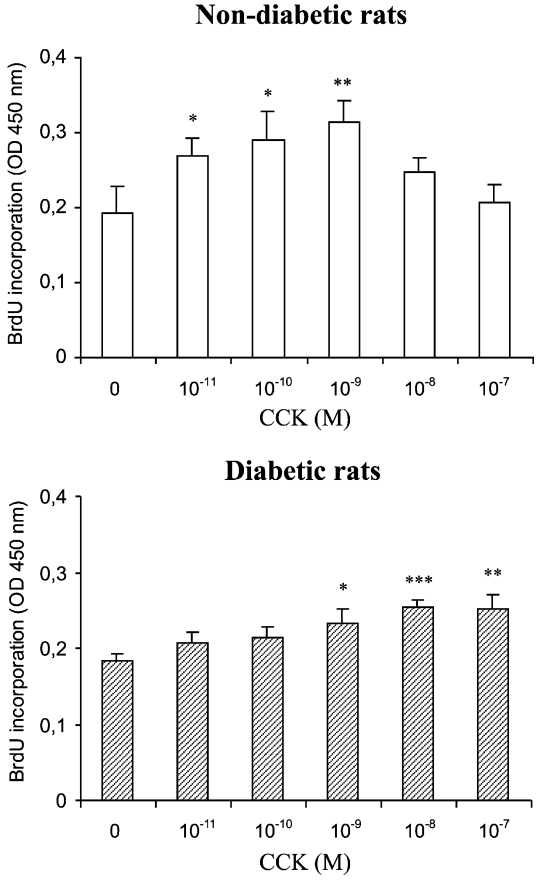

Fig. 2. BrdU uptake in pancreatic cultured acini from non-diabetic rats and type 2 diabetic rats, in the presence of various concentrations of CCK-8. Results, expressed as means±S.E.M. of 14 observations, pooled from at least three separate experiments, were compared with those for the untreated control group. *$P<0.05$; **$P<0.01$.

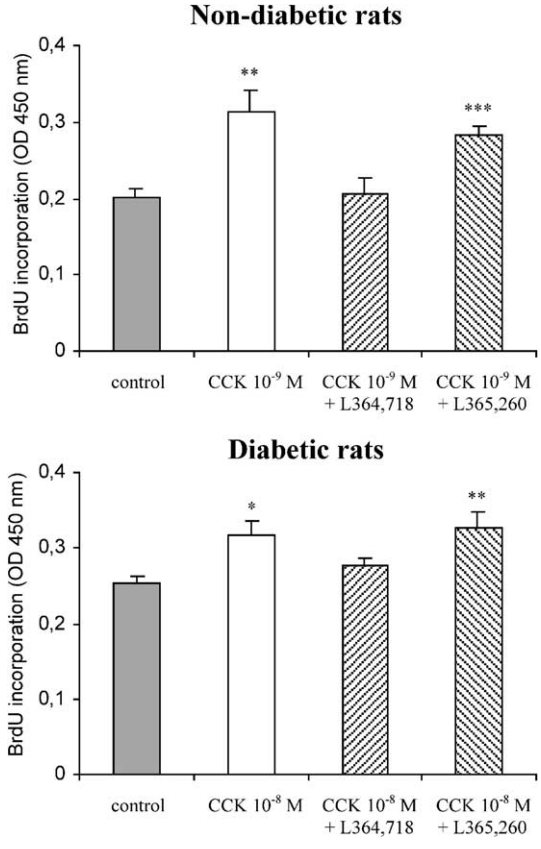

Fig. 3. BrdU uptake in pancreatic cultured acini from non-diabetic rats and type 2 diabetic rats, in the presence of CCK-8 combined or not with the CCK_1 and CCK_2 receptor antagonists L 364,718 or L 365,260 (10^{-6} M). Results, expressed as means±S.E.M. of 16 observations, pooled from at least two separate experiments, were compared with those for the untreated control group. *$P<0.05$; **$P<0.01$, ***$P<0.01$.

The optimal binding assay conditions for measuring the specific binding characteristics for CCK-8 were established in previous experiments (Hajri and Damgé, 1998). We have found that a 60-min incubation at 22 °C, pH 6.8, was satisfactory for CCK-8 binding in our experiments. The non-specific binding in these conditions was always less than 20% in pancreatic cell membranes from non-diabetic and diabetic rats.

To examine the affinity and capacity of CCK-8 binding to pancreatic cell membranes, unlabeled CCK-8 in graded concentrations was used to competitively inhibit the binding of [^{125}I]CCK-8. Fig. 4 shows the competition curves obtained with pancreatic cell membranes from both types of animals.

The Scatchard analysis of these data yielded a curvilinear concave upward plot, suggesting the presence of at least two classes of CCK-8 binding sites in non-diabetic rats with the following characteristics: a high-affinity site (K_{d1}=3.0±0.2 nM; B_{max1}=113.3±10.3 fmol/mg protein) and a low-affinity site (K_{d2}=90.4±9.4 nM; B_{max2}=1042.5±76.5 fmol/mg protein).

In type 2 diabetic rats, there was only one class of CCK-8 binding sites: K_d=103.9±18.4 nM; B_{max}=1455.8±100.3 fmol/mg protein.

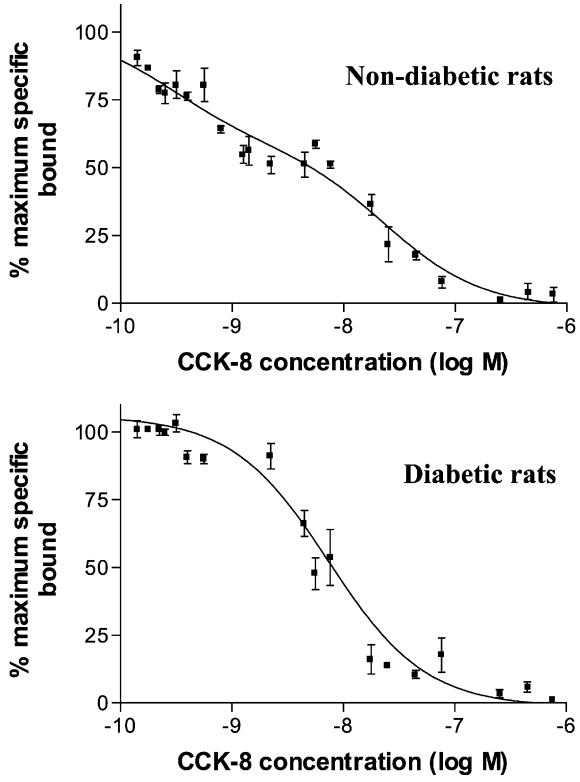

Fig. 4. Binding of [^{125}I]CCK-8 to pancreatic cell membranes from non-diabetic rats and type 2 diabetic rats. Competitive inhibition of specific [^{125}I]CCK-8 binding by increasing concentrations of unlabeled CCK-8 is presented. Membranes were incubated with 25 pM [^{125}I]CCK-8 for 60 min at 22 °C in the presence of various concentrations of unlabeled CCK-8. Specific saturable binding is expressed as a percentage of maximal specific binding. Each value represents the mean of six independent observations from three separate experiments.

Thus, the binding characteristics for CCK-8 were different in pancreatic cell membranes from non-diabetic and from diabetic rats. In the latter, only the low-affinity binding sites were well preserved and their number was significantly increased.

4. Discussion

The present findings clearly indicate that long-term administration of CCK-8 induces pancreatic growth in our new type 2 diabetic model in the rat. This effect, characterized by increases in pancreatic weight, protein, RNA and enzyme contents, was biphasic, with stimulation up to 2 μg/kg CCK-8, and lesser or no stimulation with the highest concentration of CCK-8 (4 μg/kg). Pancreatic growth generally results from increased cellular volume (hypertrophy) and/or increased number of cells (cellular hyperplasia). The DNA content of the pancreas increased significantly as a function of the concentration of CCK-8, indicating hyperplasia. According to our calculated indices of hypertrophy, this hyperplasia was not accompanied by a change in cell volume with the lowest concentrations of CCK-8 (1 and 2 μg/kg) but was accompanied by cellular hypotrophy (reduced volume of the cells) with the highest concentration of CCK-8 (4 μg/kg).

In non-diabetic rats, the dose–response curve to CCK-8 was shifted toward lower concentrations of CCK, indicating a higher sensitivity to the peptide. The maximal effect was observed with 1 μg/kg while in diabetic rats the most efficient concentration of CCK-8 was 2 μg/kg. In non-diabetic rats, growth was mainly characterized by cellular hypertrophy. These results confirm our previous data (Hajri et al., 1989). Indeed, after 4 days of treatment with CCK-8 in the rat, pancreatic growth was not accompanied by increased DNA contents. However, after 12 successive days of treatment with the octapeptide, pancreatic growth was accompanied by a significant increase in DNA content (Hajri and Damgé, 1998). Thus, CCK-8 administration in the rat is generally followed by cellular hypertrophy after a few days and hyperplasia after a longer time.

The results of this study indicate that long-term CCK-8 treatment induces the growth of the pancreas in both normal and diabetic rats, but the latter are less sensitive to CCK-8. In order to verify whether CCK-8 acts directly on the growth of the pancreas, we analysed the effect of increasing concentrations of this octapeptide on the proliferation of primary acinar cell cultures from the pancreas of non-diabetic and diabetic rats. Our results are based on the uptake of BrdU, a more sensitive test for cell proliferation than the direct measurement of pancreatic DNA content. They showed a biphasic effect on BrdU uptake in both groups of rats. However, in diabetic rats, the maximal effect was observed with 10^{-8} M CCK-8 while in non-diabetic rats it was observed with 10^{-9} M. In addition, the intensity of the response was less in diabetic than in non-diabetic rats

(+39% versus +62%, respectively). Thus, our in vitro results, in agreement with our in vivo results, confirm a lower sensitivity to CCK-8 in diabetic rats than in control rats.

The decreased sensitivity to CCK may be explained by a decreased ability of CCK-8 to bind to its receptor or by abnormal signal transduction mechanisms.

CCK interacts specifically with an 85- to 95-kDa glycoprotein on the cell membrane of rat pancreatic acini (Duong et al., 1989). In previous experiments (Hajri and Damgé, 1998), we have shown the presence of two classes of CCK receptors in normal rat pancreas, one with high affinity and one with low affinity. The present study confirmed these results and indicated that the latter are nine times more dense than the former. In order to explain the biphasic response to increasing concentrations of CCK-8, it could be hypothesized that the occupancy of high-affinity receptors stimulates cell proliferation, and that of low-affinity receptors inhibits it. Such a hypothesis was proposed for the biphasic secretion of amylase in response to CCK (Sankaran et al., 1982). However, in diabetic rats, we found only one class of CCK-8 receptors, these being of low affinity. Thus, in diabetes, more CCK-8 receptors need to be occupied in order to induce a maximal growth effect. Therefore, the loss of high-affinity receptors agrees well with the decreased sensitivity to CCK in the acini from diabetic rats.

Since CCK-8 interacts with approximately equal affinity with both CCK_1 and CCK_2 (gastrin) receptor subtypes (Yu et al., 1990), the question was to find which CCK-binding sites are involved in the biphasic growth response for our pancreatic acinar cells. Thus, in this study, we blocked CCK_1 and CCK_2 receptors with specific antagonists, respectively, L 364,718 and L 365,260, the latter being the most potent antagonists of pancreatic function and growth (Lotti and Chang, 1989; Louie et al., 1988). Our results showed that the proliferation of pancreatic acini was completely blocked by L 364,718 but not by L 365,260 in both non-diabetic and diabetic rats, suggesting that these growth effects were mediated by the occupancy of CCK_1 receptors. Thus, for this new type 2 diabetic model (Masiello et al., 1998), our present results indicate that these rats preserve a functional CCK_1 receptor. In comparison, Zucker obese rats, another model of type 2 diabetes, are also less responsive to the effects of CCK-8 on pancreatic size and secretory response (McLaughlin et al., 1982; Praissman and Izzo, 1986). This effect was related to a decreased number of pancreatic receptors for CCK rather than to changes in the affinity of CCK receptors (Praissman and Izzo, 1986). In contrast, the OLETF rats, a non-insulin-dependent rat strain, were found to be completely insensitive to both exogenous and endogenous CCK regarding pancreatic exocrine and endocrine secretions (Tachibana et al., 1996). These impaired pancreatic functions were attributed to disruption of the CCK_1 receptor gene in the pancreas (Funakoshi et al., 1995). In addition, the OLETF rats are characterized by the absence of insulin secretion in response to CCK though the pancreatic content of insulin was not altered; in addition, the pancreatic amylase content was not modified. In our type 2 diabetic model, the amylase content of the pancreas was reduced; these data are in agreement with those of Novelli et al. (2001). This effect could be due to a reduction in pancreatic insulin content since insulin is implicated in the regulation of amylase synthesis (Korc et al., 1981). However, we have noted that CCK-8 stimulates pancreatic insulin content and this could be in agreement with the results of Ahren et al. (2000) who reported that in type 2 diabetic humans, CCK exerted an antidiabetogenic effect. A possible improvement of the diabetic state cannot be due to a reduction in food intake since this parameter remained unchanged in both diabetic and non-diabetic rats, even if CCK is one component of the system controlling satiety (Moran, 2000).

In conclusion, our present study demonstrated that CCK-8 induced growth of the pancreas in both adult non-diabetic and diabetic rats. CCK-8 acts directly on the acinar cells by the occupancy of CCK_1 receptors. However, the pancreas of rats in our new type 2 diabetes model is less sensitive to this octapeptide and this may be related to the loss of high-affinity binding sites.

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References

Ahren, B., Holst, J.J., Efendic, S., 2000. Antidiabetogenic action of cholecystokinin-8 in type 2 diabetes. J. Clin. Endocrinol. Metab. 85, 1043–1048.

Axelson, J., Hakanson, R., Ihse, I., Lilja, I., Rehfeld, J.F., Sundler, F., 1990. Effects of endogenous and exogenous cholecystokinin and of infusion with the cholecystokinin antagonist L-364,718 on pancreatic and gastrointestinal growth. Scand. J. Gastroenterol. 25, 471–480.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Crawley, J.N., Corwin, R.L., 1994. Biological actions of cholecystokinin. Peptides 15, 731–755.

Danielsson, A., 1974. Techniques for measuring amylase secretion from pieces of mouse pancreas. Anal. Biochem. 59, 220–234.

Degen, L., Matzinger, D., Drewe, J., Beglinger, C., 2001. The effect of cholecystokinin in controlling appetite and food intake in humans. Peptides 22, 1265–1269.

Duong, L.T., Hadac, E.M., Miller, L.J., Vlasuk, G.P., 1989. Purification and characterization of the rat pancreatic cholecystokinin receptor. J. Biol. Chem. 264, 17990–17996.

Funakoshi, A., Miyasaka, K., Shinozaki, H., Masuda, M., Kawanami, T., Takata, Y., Kono, A., 1995. An animal model of congenital defect of gene expression of cholecystokinin (CCK)-A receptor. Biochem. Biophys. Res. Commun. 210, 787–796.

Hajri, A., Damgé, C., 1998. Effects of cholecystokinin octapeptide on a pancreatic acinar carcinoma in the rat. Pharm. Res. 15, 1767–1774.

Hajri, A., Aprahamian, M., Damgé, C., 1989. Effect of a new CCK-receptor antagonist, CR 1409, on pancreatic growth induced by caerulein, CCK-8, bombesin and gastrin-releasing peptide in the rat. Digestion 43, 66–72.

Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M., Natori, T., 1992. Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes 41, 1422–1428.

Kim, K.H., Lee, H.S., Kim, C.D., Chun, H.J., Song, C.W., Um, S.H., Ryu, H.S., Hyun, J.H., 2000. Evaluation of pancreatic exocrine function using pure pancreatic juice in noninsulin-dependent diabetes mellitus. J. Clin. Gastroenterol. 31, 51–54.

Korc, M., Owerbach, D., Quinto, C., Rutter, W.J., 1981. Pancreatic islet–acinar cell interaction: amylase messenger RNA levels are determined by insulin. Science 213, 351–353.

Liddle, R.A., Goldfine, I.D., Rosen, M.S., Taplitz, R.A., Williams, J.A., 1985. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. J. Clin. Invest. 75, 1144–1152.

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

Louie, D.S., Liang, J.P., Owyang, C., 1988. Characterization of a new CCK antagonist, L364,718: in vitro and in vivo studies. Am. J. Physiol. 255, G261–G266.

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G., 1998. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 47, 224–229.

McLaughlin, C.L., Peikin, S.R., Baile, C.A., 1982. Decreased pancreatic exocrine response to cholecystokinin in Zucker obese rats. Am. J. Physiol. 242, G612–G619.

Moran, T.H., 2000. Cholecystokinin and satiety: current perspectives. Nutrition 16, 858–865.

Moran, T.H., Katz, L.F., Plata-Salaman, C.R., Schwartz, G.J., 1998. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. Am. J. Physiol. 274, R618–R625.

Novelli, M., Fabregat, M.E., Fernandez-Alvarez, J., Gomis, R., Masiello, P., 2001. Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. Mol. Cell. Endocrinol. 175, 57–66.

Povoski, S.P., Zhou, W., Longnecker, D.S., Jensen, R.T., Mantey, S.A., Bell Jr., R.H., 1994. Stimulation of in vivo pancreatic growth in the rat is mediated specifically by way of cholecystokinin-A receptors. Gastroenterology 107, 1135–1146.

Praissman, M., Izzo, R.S., 1986. A reduced pancreatic protein secretion in response to cholecystokinin (CCK) in the obese Zucker rat correlates with a reduced receptor capacity for CCK. Endocrinology 119, 546–553.

Richards, G.M., 1974. Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem. 57, 369–376.

Sankaran, H., Goldfine, I.D., Bailey, A., Licko, V., Williams, J.A., 1982. Relationship of cholecystokinin receptor binding to regulation of biological functions in pancreatic acini. Am. J. Physiol. 242, G250–G257.

Schneider, W.C., 1957. Determination of nucleic acids in tissues by pentose analysis. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods of Enzymology. Academic Press, New York, pp. 680–684.

Stern, J.S., Johnson, P.R., Batchelor, B.R., Zucker, L.M., Hirsch, J., 1975. Pancreatic insulin release and peripheral tissue resistance in Zucker obese rats fed high- and low-carbohydrate diets. Am. J. Physiol. 228, 543–548.

Tachibana, I., Akiyama, T., Kanagawa, K., Shiohara, H., Furumi, K., Watanabe, N., Otsuki, M., 1996. Defect in pancreatic exocrine and endocrine response to CCK in genetically diabetic OLETF rats. Am. J. Physiol. 270, G730–G737.

Verduin, P.A., Punt, J.M., Kreutzer, H.H., 1973. Studies on the determination of lipase activity. Clin. Chim. Acta. 46, 11–19.

Verspohl, E.J., Ammon, H.P., Williams, J.A., Goldfine, I.D., 1986. Evidence that cholecystokinin interacts with specific receptors and regulates insulin release in isolated rat islets of Langerhans. Diabetes 35, 38–43.

Williams, J.A., Korc, M., Dormer, R.L., 1978. Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am. J. Physiol. 235, 517–524.

Wisner Jr., J.R., McLaughlin, R.E., Rich, K.A., Ozawa, S., Renner, I.G., 1988. Effects of L-364,718, a new cholecystokinin receptor antagonist, on camostate-induced growth of the rat pancreas. Gastroenterology 94, 109–113.

Yu, D.H., Huang, S.C., Wank, S.A., Mantey, S., Gardner, J.D., Jensen, R.T., 1990. Pancreatic receptors for cholecystokinin: evidence for three receptor classes. Am. J. Physiol. 258, G86–G95.

Zucker, L.M., 1965. Hereditary obesity in the rat associated with hyperlipemia. Ann. N. Y. Acad. Sci. 131, 447–458.