Evidence for a Functional Role of the Cholecystokinin-B/Gastrin Receptor in the Human Fetal and Adult Pancreas

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Gastrin (G) and cholecystokinin (CCK) are gastrointestinal neuropeptides that are released into circulation during a meal. G is also transiently expressed during embryogenic and early ontogenic development of the pancreas and is believed to act on islet-cell development. Both peptides act on pancreatic endocrine function; however, the effects are dependent on the species and on cellular and molecular underlying mechanisms that remain poorly characterized. Since CCK-B/G subtype receptor is predominant over the CCK-A subtype in the human pancreas, we hypothesized that it could be expressed by islet cells. Here we present reverse transcription–polymerase chain reaction and immunohistochemistry data demonstrating that the CCK-B/G receptor is expressed in islet cells and that islet glucagon-producing cells are the major site of CCK-B/G receptor expression in adult and fetal pancreas. Moreover, G immunoreactivity was detected in the fetal human pancreas at embryogenic week 22. G- and CCK-stimulated glucagon are released from purified human islets. Concentration of CCK and G eliciting a half-maximal level of glucagon secretion were 13 ± 6 and 8 ± 5 pmol/l, respectively. Maximal glucagon secretion was achieved in the presence of 30 pmol/l peptides and was similar to that obtained in the presence of 10 mmol/l L-arginine (1.6 pmol · ml^{-1} · 90 min^{-1}). The nonpeptide antagonist of the CCK-B/G receptor, RPR-101048, fully inhibited CCK- and G-stimulated glucagon secretion at 100 nmol/l concentration. These data are consistent with the view that the CCK-B/G receptor is involved in glucose homeostasis in adult humans and mediates the autocrine effects of G on islet differentiation and growth in the fetal pancreas. *Diabetes* 48:2015–2021, 1999

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BSA, bovine serum albumin; CCK, cholecystokinin; FITC, fluorescein isothiocyanate; G, gastrin; PCR, polymerase chain reaction; RT, reverse transcription; TGF, transforming growth factor; TRITC, rhodamin.

Cholecystokinin (CCK) and gastrin (G) are structurally related regulatory peptides distributed in the gastrointestinal tract and the central and peripheral nervous systems. They notably possess five common COOH-terminal amino acid residues (Gly-Trp-Met-Asp-Phe-NH_2) that are crucial for biological activity (1). Two distinct GTP-binding protein–coupled receptors for this family of peptides have been cloned, the CCK-A and CCK-B/G receptors (1,2). The CCK-A receptor is highly selective for sulfated CCK because it has a 500- to 10,000-fold higher affinity for CCK than for G and the common COOH-terminal tetrapeptide to CCK and G, G/CCK-4. In contrast, the CCK-B/G receptor binds G and CCK with almost the same affinity. Peptide and nonpeptide agonists and antagonists of therapeutic potential for both receptors are presently available (1,2).

Similar to the exocrine pancreas, the endocrine pancreas could be a biological target for CCK and G actions, as demonstrated in several species (3–6). Studies performed in rats and mice in vivo or on isolated pancreas have demonstrated the insulinotropic action of CCK (4). In rats, this action was correlated with autoradiographic identification of CCK-A receptors to β-cells (7). Of interest, in this species, the CCK-A receptor antagonist L364,718 was shown to inhibit the secretion of insulin by ~50% in response to ingestion of a mixture of glucose and caseine (8). In pigs, G/CCK-4, and to a lesser extent CCK and G, were shown to stimulate the secretion of insulin, glucagon, somatostatin, and pancreatic polypeptide from isolated pancreas (3). The fact that G was as potent as CCK in stimulating pancreatic hormone secretion suggests that CCK-B/G receptors rather than CCK-A receptors were involved (3).

Several studies in humans have indicated that CCK might be a physiological regulator of insulin secretion and glucose homeostasis, whereas others have led to the conclusion that, different from glucagon-like peptide-1 (GLP-1) and gastrointestinal polypeptide (GIP), CCK is not an incretin (6,9–11). In addition, the involvement of the CCK-A receptor in insulin secretion was questioned on the basis of results indicating that the nonpeptide antagonist L364,718 was without effect on postprandial increase in plasma insulin levels (12). Thus, although experimental data suggest that CCK may play a role in the regulation of the endocrine pancreas, the receptor that mediates this action as well as its physiological rele-

vance need to be further investigated in humans. Furthermore, in mammals, G might be important for the development of the endocrine pancreas. Indeed, G, which is secreted by G-cells in the antrum in adults, is expressed exclusively in the pancreas during embryogenic development and right after birth, and is believed to act in synergy with transforming growth factor-α (TGF-α) to promote growth and differentiation of pancreatic islet cells (13–15).

We have demonstrated previously a predominant expression of the CCK-B/G receptor mRNA or binding sites in the pancreas of higher mammals, including bovine, pig, and human (16–18). These findings, together with data regarding the action of CCK and G peptides on the endocrine pancreas of humans, led us to hypothesize that the CCK-B/G receptor could be expressed in the human endocrine pancreas. Here, we demonstrate that the CCK-B/G receptor is expressed by glucagon-producing cells in the pancreas of fetal and adult humans and confirm the presence of G immunoreactivity in human fetal pancreas. Moreover, we show that the CCK-B/G receptor mediated G- and CCK-stimulated secretion of glucagon in purified human pancreatic islets.

RESEARCH DESIGN AND METHODS

Tissue preparation. Surgical samples of human pancreas (three specimens), gallbladder (one specimen), and fundus (one specimen) were obtained from the Department of Anatomy and Pathology from the CHU Rangueil, Toulouse, France. Tissues from adult humans were fixed at room temperature in Bouin's solution, then dehydrated through a graded ethanol series, embedded in paraffin, and cut into 5-µm sections. Pancreatic fetal tissues were collected from two fetuses at embryogenic stages ranging from 17 to 28 weeks after spontaneous or induced terminations. Fetal pancreatic fragments were immersed for 4 h in Baker's formalin at 4°C, dehydrated, and embedded in cytoparaffin. According to macroscopic and microscopic examinations, all tissue fragments used for polymerase chain reaction (PCR) and histochemistry experiments were normal. The study on fetus was performed as part of the teratovigilance network, the latter having been approved by the ethical committee.

Reverse transcription–PCR. RNA from human islets were a gift of Dr. Noel Morgan, Keele University, U.K., or were prepared from isolated human islets. RNA preparation was performed as follows: isolated cells or small pieces of fresh tissue were homogenized with a polytron tissue homogenizer in RNAzol B (Bioprobe, Montreuil-sous-Bois, France). Homogenized material was then incubated in RNAzol for 10 min at 4°C, and RNA was extracted. Some 3–10 µg of total RNA was heated for 10 min at 70°C in the presence of an oligodT primer (1 mmol/l) (Promega, Charbonnieres, France) and then incubated at 37°C in a 20-µl reaction volume consisting of reverse transcriptase buffer (50 mmol/l Tris-HCl, pH 8.3, 75 mmol/l KCl, 3 mmol/l $MgCl_2$, 10 mmol/l dithiothreitol), 20 U RNAsine (Gibco-BRL, Cergy Pontoise, France), and 200 U Superscript reverse transcriptase (Gibco-BRL). The single-stranded cDNA obtained from 1 µg of reverse-transcribed total RNA was amplified using specific primers for the human CCK-B/G receptor, glucagon, and amylase. For the CCK-B/G receptor, sense and antisense primers were analogous to nucleotides 140–163, and the complement to nucleotides 734–758. PCR was carried out in a total volume of 100 µl that was composed of PCR buffer (50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 9.0, 0.01% Triton X-100), 0.25 mmol/l dNTPs, 2 µmol/l primers, and 2.5 U Taq polymerase (Promega). The amplification reaction involved denaturation at 94°C for 5 min, followed by 32 cycles as follows: 94°C for 1 min, primer annealing at 65°C for 1 min, and extension at 72°C for 1.5 min. After cycling, a terminal elongation of 10 min at 72°C was performed. For the identification of glucagon mRNA, sense and antisense primers were analogous to nucleotides 58–81, and the complement to nucleotides 473–496. The PCR conditions were identical to those used for the CCK-B/G receptor mRNA, except that primer annealing was performed at 62°C and 20 cycles were used. For reverse transcription (RT)-PCR of amylase mRNA, sense and antisense primers were analogous to nucleotides 524–543, and the complement to nucleotides 1468–1491; annealing temperature was 55°C, and 15 cycles were used. RT-PCR conditions used for the determination of CCK-B/G receptor mRNA levels allowed for quantitative measurements (18). For the measurement of glucagon and amylase mRNA levels, RT-PCR conditions were determined in pilot experiments with increasing cycle numbers. The numbers of PCR cycles that produced amplicon bands in a phase of nearly linear DNA production below the maximal possible yields were determined.

Immunohistochemistry. Antibodies to the human CCK-B/G receptor were obtained by immunization of rabbits using a synthetic replicate of the third extracellular loop of the receptor as previously described (19). Monoclonal antibodies to porcine glucagon (mouse IgG1 isotype) were from Sigma (Saint Quentin Fallavier, France). Guinea pig antibodies to porcine insulin were a gift of Dr. Hollande, Toulouse, France. Rat polyclonal antibodies to somatostatin were from Tebu (Le Perray en Yvelines, France). Rabbit antibodies to human pancreatic polypeptide were from Dako (Trappes, France). Rabbit polyclonal antibodies to the COOH-terminal and NH_2-terminal regions (fragment 1–13) of human G-17 were from Dako and Sigma, respectively.

For indirect immunoperoxidase assays, endogenous peroxidase activity of deparaffined rehydrated sections was blocked with 1% (vol/vol) hydrogen peroxide for 20 min. Antibodies to CCK-B/G receptor, G, or normal serum in the negative control were applied to the sections (dilutions 1:2,000 for the CCK-B/G receptor antibody and 1:500 and 1:100 for COOH-terminus and NH_2-terminus directed G antibodies, respectively) and incubated overnight at 4°C in a humid chamber. After three washes, the sections were successively incubated at room temperature for 30 min with the peroxidase-labeled IgG fraction of swine anti-rabbit Ig and with rabbit anti-swine Ig, and were exposed to a solution of diaminobenzidine in 0.03% hydrogen peroxide for 3 min. Sections were counterstained with hematoxylin, dehydrated through a graded series of ethanol to toluene washes, and coverslipped with Eukitt (Prolabo, Fontenay sous Bois, France). All dilutions and washings were done with phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA). The stained sections were observed under an Optiphot II Nikon light microscope (Champigny sur Marne, France).

For indirect immunofluorescence assays, the staining was carried out as described above, except that fluorescein isothiocyanate (FITC)- or rhodamin (TRITC)-labeled IgG adsorbed with human serum was used in place of peroxidase-labeled IgG, and sections were coverslipped with fluorescent mounting medium. For double-label immunofluorescence, one of the two primary antibodies was applied and revealed using FITC-labeled IgG, then the second primary antibody was applied and revealed by TRITC-labeled IgG. Antibodies to insulin, glucagon, somatostatin, and pancreatic polypeptide were used at dilutions of 1:2,000, 1:2,000, 1:500, and 1:1,400, respectively. Sections were examined under a Zeiss laser scanning confocal microscope (model LSM 410; Göttingen, Germany), using argon and helium-neon laser beams and appropriate filters. Stained cells were counted using the LSM 410 software.

Isolation and purification of human islets. Human pancreases were obtained from beating-heart cadaveric multiorgan donors. Tissue procurement and all experimental protocols with human islets were approved by the Human Ethics Committee of the Hospital Clinic of Barcelona.

The islets were isolated using a modification of the automated method for human islet isolation (20,21). Briefly, the pancreatic duct of the pancreas was cannulated. Then, the pancreas was distended with a collagenase solution (3.5 mg/ml, type P; Boehringer-Mannheim Biochemica, Mannheim, Germany) in Hank's balanced salt solution supplemented with 1% fetal calf serum (Gibco-BRL) and deoxyribonuclease I (0.04 mg/ml, DNase I; Sigma, St. Louis, MO) at a ratio of 2 ml/g tissue. After digestion at 37°C, the dispersed tissue was washed three times in a total volume of 5 L of Hank's balanced salt solution, and islets were purified from the exocrine tissue by centrifugation through a discontinuous density gradient using BSA (BSA 300 mOS; Applied Protein Products, Brierly Hill, U.K.) as described (22). After purification, free islets were washed at least three times in Hank's solution and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. Culture medium was RPMI 1640 containing 5.5 mmol/l glucose (Gibco Laboratories, Grand Island, NY), supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (Flow Laboratories, Ayrshire, U.K.), penicillin (100 U/ml), gentamycin (100 mg/ml), and fungizone (1 mg/ml).

Glucagon secretion of human islets. Stimulation of human islet glucagon secretion was carried out in a static incubation at 3 mmol/l glucose concentration. Stimulation by 10 mmol/l L-Arg was performed as positive control. Series of eight hand-picked human islets were incubated in a shaking water bath for 90 min at 37°C in 1 ml Krebs-Ringer bicarbonate–buffered medium containing BSA (5 mg/ml, fraction V; Sigma), 0.03% of soybean trypsin inhibitor, and either of the tested compounds. For the first 10 min of incubation, the vials containing the incubation medium with the islets were gassed with O_2:CO_2 (95:5%). Several concentrations of G and CCK in the presence of an antagonist of the CCK-A receptor (1-[2-(4-[2-chlorophenyl]thiazol-2-yl) aminocarbonyl indoyl] acetic acid, SR 27897) (22) were tested. Stimulations by 10 mmol/l L-Arg and inhibition of CCK- and G-induced secretion by the CCK-B/G receptor antagonist ([(N-[methoxy-3 phenyl] N-[N-methyl N-phenyl carbamoylmethyl], carbomoylmethyl)-3 ureido]-3-phenyl)2-propionic acid RPR-101048 (23) were performed as controls. At the end of the incubation period, supernatants were aliquoted and stored at –20°C until use. The secretion of glucagon in the incubation medium was assayed by radioimmunoassay using a commercial kit from Behring Diagnostic (Rueil-Malmaison, France) according to the manufacturer's recommendations.

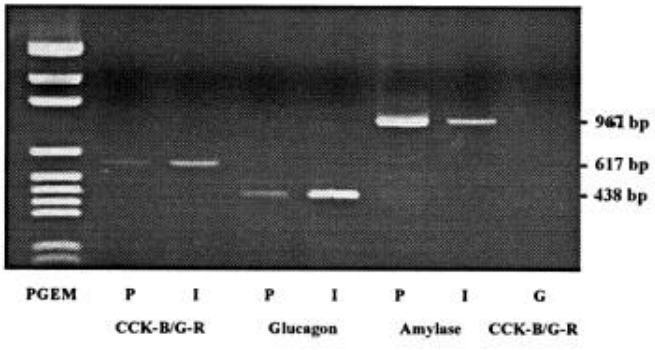

FIG. 1. Expression of CCK-B/G receptor (R) mRNA in human pancreatic tissues and purified pancreatic islets detected by RT-PCR. Total RNA isolated from human pancreas fragments (*lanes P*) or from purified pancreatic islets (*lanes I*) were RT-PCR amplified using specific primers for the CCK-B/G receptor, glucagon, or amylase in the conditions described in METHODS. *Lane G* shows the result of RT-PCR amplification of human gallbladder RNA using primers for the CCK-B/G receptor.

RESULTS

The CCK-B/G receptor gene is expressed in human pancreatic islets. Cloning of the human CCK-B/G receptor cDNA allowed for demonstration of an expression of RNA messenger coding for this receptor in several anatomical regions of the human body, including the pancreas (24–26). To determine CCK-B/G receptor localization in the pancreas, we first amplified reversed-transcribed RNA that was extracted either from surgical human pancreatic fragments or from purified islets. As illustrated in Fig. 1, an amplicon of the expected size (617 bp) was yielded by RT-PCR amplification from both RNA extracts. However, the signal was more intense in RNA extracted from isolated islets, as was that of glucagon mRNA. Conversely, the signal from amylase mRNA was much more intense in RNA extracted from a pancreatic fragment than from isolated cells. In contrast, no detectable signal was obtained from human gallbladder RNA extracts. Thus, although purity of the islet preparation could not ensure absence of any contaminating cells, these first results are suggestive of an expression of the CCK-B/G receptor gene in human pancreatic islets. However, because the 617-bp amplicon represents a partial mRNA product, we verified that full-length CCK-B/G receptor transcripts were also present in the human pancreatic islets and that they were correctly spliced. We amplified, cloned, and then sequenced total cDNA coding region using RT-PCR. The isolated cDNA had an open reading frame of 1,331 bp and was 100% identical to the CCK-B/G cDNA cloned from the human brain and stomach (not shown) (24–26). This first set of experiments indicates that correctly spliced mRNA coding for the CCK-B/G receptor are expressed in the human pancreas, most likely in pancreatic islets.

The CCK-B/G receptor protein is strongly expressed in human pancreatic islets. To verify that the CCK-B/G mRNA identified in human pancreatic islets was translated, human pancreatic sections were stained using an antibody directed against the third extracellular loop of the receptor protein (19). As shown in Fig. 2*A*, immunostaining was highly positive in the islets of Langerhans cells. It was not uniform within the islets and was almost entirely found in a subset of cells forming strings. To assess the specificity of the immunostaining reaction, we applied the CCK-B/G receptor antibody to human fundic glands (Fig. 2*B*) and gallbladder (Fig. 2*C*), and also performed identical experiments using a preimmune antiserum (Fig. 2, *lower panels*). Application of the anti-CCK-B/G receptor antibody produced an intense staining of parietal cells seen within a region from the middle to the bottom part of fundic glands. In contrast, the gallbladder muscle layer was not labeled. These results are concordant with pharmacological and functional studies demonstrating that the CCK-B/G receptor is involved in the function of the human parietal cells but not in that of the human gallbladder (2,3). None of these tissues were stained when preimmune serum was used, confirming specificity of the detection of the CCK-B/G receptor.

The CCK-B/G receptor is expressed in pancreatic α-cells from adult humans. To identify the endocrine cell type(s) that expressed the CCK-B/G receptor, human pancreatic sections were double-stained using the antibody to the CCK-B/G receptor together with an antibody to either glucagon, insulin, somatostatin, or pancreatic polypeptide to visualize α-, β-, δ-, and pancreatic polypeptide cells, respectively. Examination of pancreatic islets by confocal microscopy clearly showed colocalization of CCK-B/G receptor immunoreactivity with glucagon (Fig. 3*A*). In contrast, insulin-, somatostatin-, and pancreatic polypeptide–producing cells did not express any significant CCK-B/G receptor immunoreactivity. Some islet cells that were stained with the antibody to the CCK-B/G receptor were not positive for any of the pancreatic islet hormones used (not shown). Such cells might correspond to premature islet cells or to an unidentified type of endocrine cells. Quantification of the CCK-B/G receptor distribution within human pancreatic islets indicated that at least 95% of cells expressing CCK-B/G receptor were glucagon-producing cells.

The CCK-B/G receptor mRNA and protein and G are expressed in the human fetal pancreas. G, one of the two endogenous agonists of the CCK-B/G receptor, was demonstrated to be present in the pancreas of newborn humans (14). Moreover, glucagon-producing cells were identified at fetal stages as early as 10 weeks (27,28). These data raise the question of whether the CCK-B/G receptor is also present in the human pancreas at fetal stages of development. Since we had pancreatic sections from human fetus at embryogenic week 22, we performed an immunoperoxidase staining of these sections. They revealed expression of the CCK-B/G receptor by islet cells (data not shown). Double-immunofluorescent staining using the antibody to the CCK-B/G receptor together with antibodies to pancreatic hormones indicated that glucagon-producing α-cells strongly expressed CCK-B/G receptor immunoreactivity (illustrated for embryogenic week 22 in Fig. 3*B*). A fraction of cells that expressed the CCK-B/G receptor did not express glucagon or the other pancreatic hormones. Noteworthy, immunoperoxidase staining of pancreatic sections from human fetus at embryogenic week 22 using G-directed antibodies confirmed expression of G in the human pancreas during fetal life (Fig.3*B*, *lower panel*). The staining was observed with both the antibody directed against the NN_2-terminal region of G, which did not cross-react with CCK, and with the antibody directed against the COOH-terminal region of G, which did recognize CCK. Numerous groups of cells scattered in the growing organ were labeled with these antibodies, whereas they were not

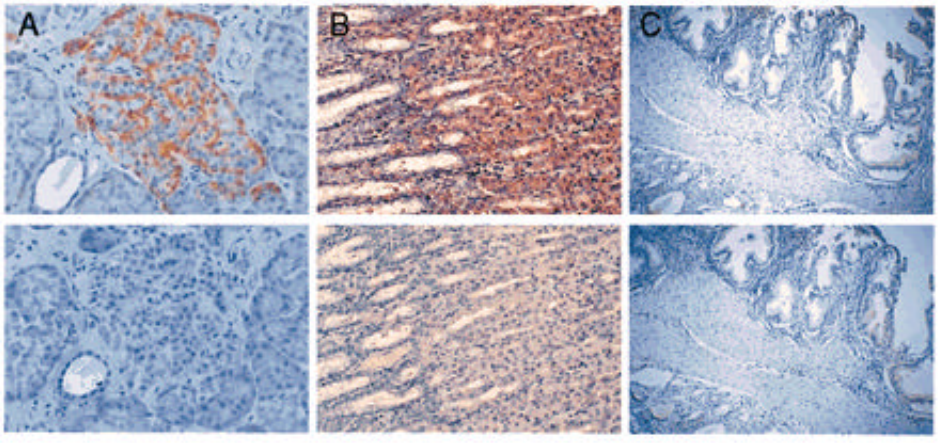

FIG. 2. Immunoreactive CCK-B/G receptor cells in the human pancreas. Immunoperoxidase staining of sections from human pancreas (*A*), gastric fundus (*B*), and gallbladder (*C*) using an antibody against the human CCK-B/G receptor. Specificity of the immune reaction was assessed using preimmune serum instead of antiserum to the CCK-B/G receptor (*lower panels*). Original magnification ×25.

stained when preimmune antiserum was applied. G immunoreactivity could not be detected in pancreatic sections from adult humans (not shown).

In addition, RT-PCR amplification of RNA extracted from fetal pancreas corresponding to embryogenic stages from 17 to 28 weeks indicated that mRNA encoding the CCK-B/G receptor was detected at all stages of fetal development studied (Fig. 4). The average level of mRNA coding for the CCK-B/G receptor during fetal life between 17 and 22 weeks was three- to fourfold higher than that observed in adults; then it decreased to a similar level. Nevertheless, we could not state whether this decrease of receptor mRNA levels reflects a decrease of gene expression in glucagon-producing cells or whether it was due to a decrease of the ratio between the number of glucagon-producing cells and the whole population of pancreatic cells within the studied period of development.

The CCK-B/G receptor mediates G- and CCK-stimulated glucagon secretion in isolated human islets. To examine whether the CCK-B/G receptor could be involved in glucagon secretion, we tested the ability of CCK and G, which have very close affinities for the CCK-B/G receptor, to stimulate glucagon secretion from hand-picked human islets. As shown in Fig. 5, both agonists stimulated the release of glucagon in a dose-dependent manner and with close potencies and efficacies. Concentration of CCK and G eliciting half-maximal level of glucagon secretion were 13 ± 6 and 8 ± 5 pmol/l, respectively, and maximal secretion was achieved in the presence of ~30 pmol/l peptides. It is noteworthy that the

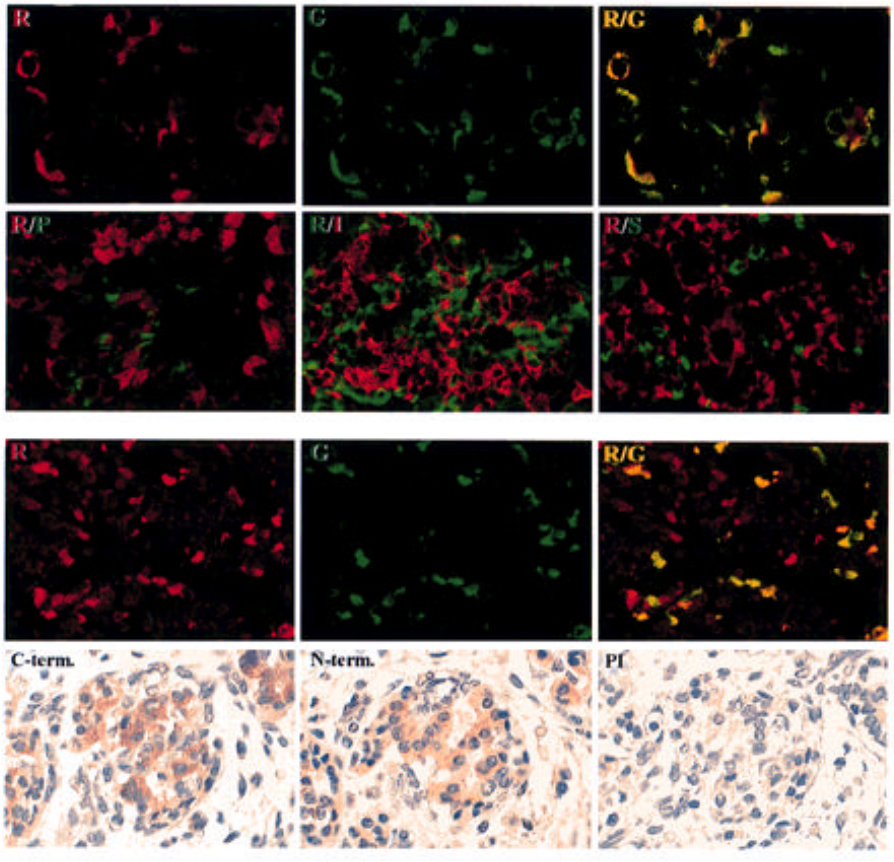

FIG. 3. Immunohistochemical localization of the CCK-B/G receptor and G in human pancreatic islets by confocal microscopy. *A*: Confocal images of an immunofluorescent staining of a human pancreatic islet for the CCK-B/G receptor (*R*), glucagon (*G*), insulin (*I*), somatostatin (*S*), and pancreatic polypeptide (*P*). Double staining for both the receptor and the hormones seen in yellow shows that CCK-B/G receptors co-localized with glucagon-producing cells in adult pancreas (R/G), whereas it did not co-localize with pancreatic polypeptide (R/P), insulin (R/I), or somatostatin (R/S). Original magnification ×60. *B*: Immunofluorescent staining of a human fetal pancreatic islet (embryogenic stage: 22 weeks) for the CCK-B/G receptor (*R*) and glucagon (*G*). Positive cells for both the receptor and the hormone are seen in yellow on the double-staining picture (R/G). Original magnification ×60. Photographs at the bottom of the figure show an immunoperoxidase staining of pancreatic sections (embryogenic stage: 22 weeks) using G antibodies that recognize the NH_2-terminal (N-term) and COOH-terminal (C-term) portions of G. No staining was observed using preimmune antiserum (PI). Original magnification ×100.

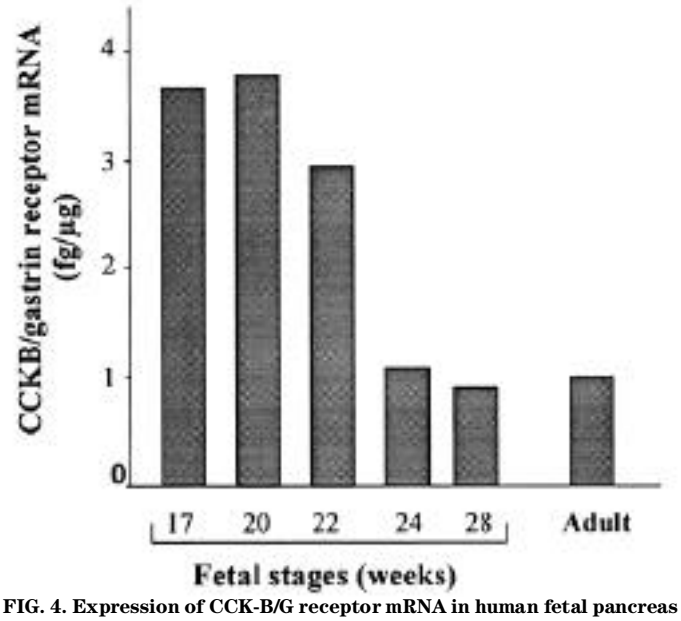

FIG. 4. Expression of CCK-B/G receptor mRNA in human fetal pancreas detected by RT-PCR. Total RNA from isolated human fetal pancreas were RT-PCR amplified using specific primers allowing quantification of mRNA levels as described in METHODS. Results are the mean of two determinations performed on two separate organ specimens.

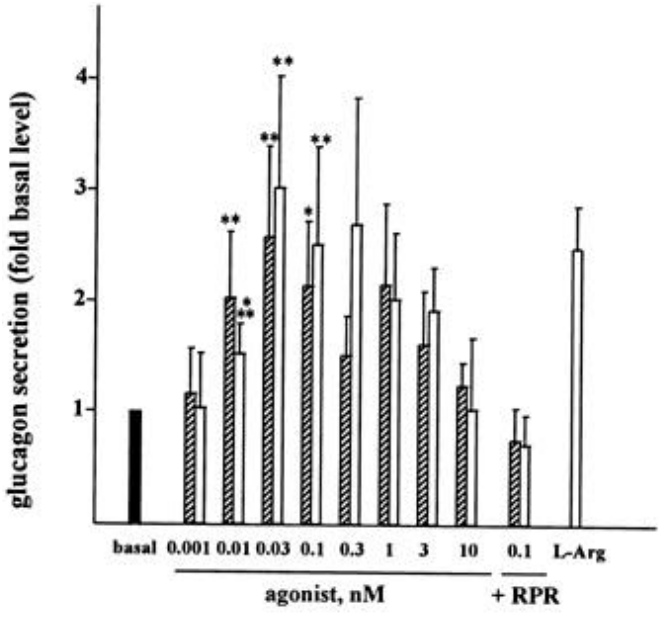

FIG. 5. Glucagon secretion by purified human pancreatic islets. Eight hand-picked human islets were incubated for 90 min at 37°C in the presence of different concentrations of G (▨), CCK (□), or 10 mmol/l L-arginine as described in METHODS. The glucose concentration was 3 mmol/l, and 1.8 µmol/l SR27,897 (a CCK-A receptor antagonist) was included in all assays. RPR-101048, a specific CCK-B/G antagonist, was used at a concentration of 100 nmol/l in combination with 0.1 nmol/l CCK or G. Results are the means ± SE of two glucagon determinations on six stimulations using separated islet preparations from three healthy donors. *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$ compared with the basal secretion in absence of agonist. P values were determined using the paired Student's t test.

maximal level of glucagon secretion following CCK and G stimulation (1.6 pmol · ml^{-1} · 90 min^{-1}) was identical to that obtained in the presence of 10 mmol/l L-arginine. Supramaximal concentrations of CCK and G ranging from 1 to 10 nmol/l elicited decreased glucagon secretion levels relative to that with 0.1 nmol/l peptides. Finally, the nonpeptide antagonist of the CCK-B/G receptor RPR-101048 fully inhibited CCK- and G-stimulated glucagon secretion at 100 nmol/l concentration.

DISCUSSION

In the current study, we show that the gene encoding the CCK-B/G receptor is expressed in human pancreatic islets, and, using antibodies against the CCK-B/G receptor and pancreatic hormones, we demonstrate that the CCK-B/G receptor protein is localized almost exclusively to islet glucagon-producing cells during both fetal and adult life. The expression of the CCK-B/G receptor in human islets of Langerhans was not previously reported. Indeed, the only report available on the localization of CCK-B/G receptors in the human pancreas was a binding study on pancreatic sections using the technique of storage phosphor autoradiography, which does not have adequate resolution to identify binding sites at the cellular level (29).

Another new finding is the demonstration that the CCK-B/G receptor mediates CCK- and G-induced secretion of glucagon from isolated human islets. Results showing that the stimulatory effect of the CCK-B/G receptor on glucagon secretion occurs on concentrations of agonists in the range of plasma concentrations, together with the localization of the receptor, provide strong evidence for a direct regulation of glucagon-producing cell activity by the gastrointestinal peptides CCK and G. Glucagon is a counter-regulatory hormone critical for the maintenance of glucose homeostasis. It stimulates glycogenolysis and gluconeogenesis in the liver and improves insulin secretion both by a direct action on β-cells and indirectly through hepatic release of glucose (30–33). As a consequence, the insulinotropic effect of CCK in humans, which was previously shown to be insensitive to a CCK-A receptor antagonist, could result in part from CCK-B/G receptor–mediated glucagon release (12). G and CCK are released from endocrine cells of the gastrointestinal mucosae to circulation in response to food ingestion as well as from nerve endings. One may suggest that these peptides contribute directly to glucose homeostasis by means of glucagon-induced glycogenolysis and/or via insulin secretion that is facilitated by paracrine and endocrine glucagon (31). These hypotheses are in agreement with data showing that glucagonemia is rapidly increased in response to an ordinary, as well as to a protein-rich, meal (10,34). Recent advances in the understanding of insulin secretion physiology showed that β-cell responsiveness to glucose is in part controlled by enteral hormonal stimuli, including CCK, and that the timing and duration of hormonal stimulation are important for correct pattern of insulin secretion (35). CCK-B/G receptor–mediated action of G in the human endocrine pancreas should contribute to correct timing of islet cell activation, due to the facts that G secretion precedes CCK secretion during a meal and that postprandial plasma concentrations of G are 5- to 10-fold higher than that of CCK (36). Furthermore, results from the current study may explain why elevated Gemia and elevated glucagonemia in newborn humans are linked (37). In the same way, a relationship could exist between tissular expression of G by pancreatic adenocarcinomas and increased glucagon levels seen in pancreatic cancer patients (38). On the other hand, the fact that glucagon release from isolated islets upon stimulation by supramaximal concentrations of CCK and G decreases to

basal values agrees with an absence of elevated glucagonemia in patients presenting a hyperGemia caused by Zollinger-Ellison syndrome (39).

Finally, the current study demonstrates expression of the CCK-B/G receptor in glucagon-producing cells of human pancreas during the fetal life. It also confirms the expression of pancreatic G in human fetus. Although the function of pancreatic CCK-B/G receptors during embryogenic development remains to be fully understood, this last finding is of particular potential interest because of the exclusive expression of pancreatic G during fetal life and right after birth in humans (14). Thus, an additional experimental support, i.e., presence of the CCK-B/G receptor, for a physiological role of pancreatic G at an early stage of development now exists. It has also been shown that transgenic mice overexpressing G and TGF-α in the pancreas have an increase mass of their islets (15). Additionally, G and TGF-α have been shown to be overexpressed during duct–to–islet cell differentiation in the pancreas of duct-ligated rats (40). From these different data, one can now envisage an involvement of the CCK-B/G receptor in the neogenesis, proliferation, and differentiation of pancreatic endocrine cells.

From a pathological point of view, glucagon seems to be the most important hormone that exacerbates the metabolic consequences of insulin deficiency in diabetes (41). Therefore, the finding that the CCK-B/G receptor is expressed by islet α-cells and its role in the adult, and possibly in the fetus, suggest that the gene encoding this G-protein–coupled receptor may be a candidate for the pathogenesis of certain forms of diabetes. The finding of a genetic linkage between a severe form of non-insulin-dependent diabetes diagnosed before 45 years of age and the CCK-B/G locus (11p15.4) represents a basis on which to test our hypothesis (42). Future studies will need to address this important question and whether a therapeutic benefit could result from the use of CCK-B/G receptor ligands in diabetes.

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