Pancreatic CCK$_B$ receptors: their potential roles in somatostatin release and $\delta$-cell proliferation

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Morisset, Jean, Helen Wong, John H. Walsh, J. Lainé, and Judith Bourassa. Pancreatic CCK$_B$ receptors: their potential roles in somatostatin release and $\delta$-cell proliferation. Am J Physiol Gastrointest Liver Physiol 279: G148–G156, 2000.—In rodents, cholecystokinin (CCK) induces pancreatic enzyme secretion and pancreas growth through its CCK$_A$ receptors. It is unknown whether occupation of the CCK$_B$ receptors present in pig and human pancreas can cause the same effects. This study evaluates CCK$_B$ receptor expression in rat, mouse, pig, and fetal human pancreata using Northern blot, Western blot, and immunofluorescence techniques. The reported 2.7-kb CCK$_B$ receptor mRNA transcript in the rat brain and gastric fundus is absent in pancreas; the message was, however, detected by RT-PCR and by a CCK$_B$ receptor antibody as an 80-kDa protein present uniquely in islet $\delta$-cells. Proteins of 50 and 80 kDa appear in mouse pancreas, and proteins of 50 and 115 kDa appear in pig and human pancreas, respectively, all localized in islet $\delta$-cells. Gastrin mRNAs are strongly present in fetal rat pancreas, and the hormone is localized in islets; both are repressed 10 days after birth. In conclusion, the CCK$_B$ receptors are present in pancreas of four species with exclusive location in islet $\delta$-cells. In such a location, they could be indirectly involved in the control of enzyme secretion.

The physiology and pharmacology of the CCK$_B$/gastrin receptor seem to be dependent on the species, and, so far, no clear biological functions have been assigned to its occupation. This receptor subtype has been identified in human (49), calf (26), dog (8, 16), guinea pig (56), and most recently in pig pancreas (36). Zhou et al. (57) indicated that the CCK$_B$ receptor could not be detected in the rat pancreas by Northern blot analysis, a finding confirmed later by RT-PCR (34).

No clear biological functions have been assigned to the pancreatic CCK$_B$/gastrin receptors, although gastric enterochromaffin-like (ECL) cell receptors do have clear functions. Their occupation was associated with ECL cell growth (7), histamine release (20), and mucosal growth (53). In CCK$_B$/gastrin receptor-deficient mice, parietal and ECL cells were decreased, along with a reduction in somatostatin cell density and an increase in antral gastrin cell number (18). Overexpression of this CCK$_B$/gastrin receptor in the stomach of naturally occurring CCK$_A$ receptor gene knockout rats led to thickening of the fundic mucosa and hyperplasia and hypertrophy of the parietal cells (32).

Although the presence of the CCK$_B$/gastrin receptors in the pancreas of different species is not contested, the physiological responses resulting from their occupation raise questions and present controversial results. In CCK$_B$ receptor gene knockout mice, Miyasaka et al. (33) recently indicated that the CCK$_B$ receptor has no role in pancreatic growth, exocrine secretion, or bile secretion. These results are in total opposition to those of Saillan-Barreau et al. (42), who demonstrated in a transgenic mouse strain expressing the human CCK$_B$/gastrin receptor that CCK and sulfated gastrin stimulated enzyme secretion with identical potencies and efficacies, and with those of Detjen et al. (6), who showed in CCK$_B$ receptor-transfected human pancreatic cancer cells that their occupation by CCK led to inhibition of their growth. In the pig, whose pancreas possesses predominantly CCK$_B$ receptors rather than CCK$_A$ receptors (36), CCK is a rather weak stimulant of pancreatic enzyme secretion in live animals (5) and in freshly isolated acini (36). Such insensitivity to CCK...
was also observed in newborn rat pancreata (55), suggesting a paracrine effect of gastrin found in large quantity in pancreata of fetal and newborn rats (30). Such a hypothesis is supported by our recent findings that treatment of pregnant female rats during their entire pregnancy with either gastrin or the CCKB receptor antagonist L-365260 resulted in fetal pancreas hypertrophy and atrophy, respectively (35), suggesting a role for the CCKB receptors and gastrin at least in pancreas differentiation early in life.

In view of the great uncertainty regarding the physiological roles of CCKB receptor occupation in pancreatic physiology, this study was undertaken 1) to document the ontogeny of expression of the rat pancreatic CCKB receptor and pancreatic gastrin mRNA, its specific agonist; 2) to confirm the CCKB receptor mRNA expression by evaluating the CCKB receptor protein by Western blot and immunofluorescence; and 3) to establish the presence and localization of the CCKB Receptor protein in the pancreas of three different species other than the rat: the mouse, the pig, and the human. We expect that these new data will clarify the importance and the role of the CCKB receptors in pancreatic physiolo-

MATERIALS AND METHODS

Animals. These studies were performed on male (180–200 g) and female (250–300 g) Sprague-Dawley rats from Charles River (St-Constant, QC, Canada) and their pups after birth. Fetal rat pancreata were excised from 21-day-old fetuses, neonatal pancreata came from newborn rats, and other pancreata came from rats 3–35 days after birth and adult animals (60 days). Pieces of pancreas from suckling and adult mice and pig and from 18-wk human fetuses were taken for evaluation. Human fetal pancreatic tissues were obtained from normal elective pregnancy terminations. No tissue was collected from cases associated with known fetal abnormality or fetal death. These studies were approved by the Institutional Human Subject Review Board.

Tissue preparation. Once excised, tissues were 1) quickly frozen in liquid nitrogen and processed later for RNA extraction and Northern blot analysis; 2) embedded in Tissue-Tek OCT 4583 compound (Sakuta Fine Tek, Torrance, CA) and frozen in liquid nitrogen and processed later for RNA extraction and probe preparation; or 3) homogenized in a specific buffer for Western blot analysis.

Membrane preparation. All procedures were carried out at 4°C. Freshly removed tissues were minced and disrupted in 10 mM HEPES, pH 7.5, 250 mM sucrose, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 21 μM leupeptin, and 1.5 μM aprotinin with the use of five passes of a Potter-Elvehjem homogenizer. Unbroken cells and nuclei were removed by centrifugation at 500 g for 5 min. Membranes were collected by centrifugation at 30,000 g for 30 min using a TLS-55 rotor. The supernatants were removed, and membranes were suspended to a concentration of 15–30 mg/ml in homogenization buffer and stored at −80°C until used. Protein concentrations were determined using a bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL).

Gel electrophoresis and immunoblotting. Membrane proteins (30 μg) were boiled 5 min in 62.5 mM Tris·HCl, pH 6.8, 10% glycerol, 2% SDS, and 5% β-mercaptoethanol and separated by SDS-PAGE using 10% polyacrylamide gels. After transfer to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Mississauga, ON, Canada), the membranes were blocked for 2 h with 6% nonfat dry milk in TBST (20 mM Tris·HCl, pH 7.4, 0.15 M NaCl, and 0.1% Tween 20) and incubated overnight at 4°C with polyclonal antibodies corresponding to different areas of the canine or rat CCKB/gastrin receptor molecule (10). These antibodies were raised in rabbit using the following sequences: antibody 9262 (amino acids 42–55) from the extracellular part of the canine receptor and antibody 9252 (amino acids 253–264) from the intracellular part of the canine receptor. We also used antibody 9413 (amino acids 252–266) from the intracellular part of the rat receptor. Characterization of these antibodies has been previously described (10). The blots were then washed 5 times for 5 min in TBST and incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG (Amersham Pharmacia Biotech, Baie d’Urfé, QC, Canada) at 1:8,000 in TBST-3% nonfat dry milk. After membranes were washed six times for 5 min, they were developed with Lumi-Light Plus chemiluminescence substrate (Roche Diagnostics, Montreal, QC, Canada). Immunoneutralization was performed by preincubating the primary antibody with its specific peptide antigen (10 μg/ml) for 1 h.

Indirect immunofluorescence. Tissues embedded in TissueTek OCT 4583 compound were handled as previously described (1). For this specific technique, the CCKB/gastrin antibody 8007 (canine) was used at a 1:500 dilution. The gastrin antibody 8007, a generous gift from Dr. Rehfeld (Copenhagen, Denmark) was used at a 1:100 dilution. The antibody raised against insulin, used at a 1:50 dilution, was a gift from Dr. Bendayan (University of Montreal, Montreal, QC, Canada). The somatostatin antibody used at a 1:2,000 dilution, glucagon (1:50), and pancreatic polypeptide (1:2,000) were generous gifts from Dr. Lebel (Department of Biology, Université de Sherbrooke).

RNA extraction and probe preparation. Total RNAs from rat pancreata and gastric fundus were isolated according to a modification of the procedure of Chirgwin et al. (4) as previously described (3). The gastrin open reading frame with 5’- and 3’-flanking sequences (nucleotides 96–376) was cloned by PCR using the following sense and antisense primers, respectively, composed with Kpn I and Sac I restriction sites: 5’-TGCTGGCTAGTTACCTTCTCGG-3’ (nucleotides 80–103) and 5’-GATGGCTGAGCTCCTGAAGAC-3’ (complementary to nucleotides 365–386). Single-stranded cDNA reverse transcribed from rat brain mRNA served as the DNA template, and this amplification product was used for sequencing and transcription of cDNA probe with a specific RNA polymerase, as described by the Riboprobe System (Promega, Madison, WI). A fragment of 490 bp (nucleotides 207–697) subcloned from the rat CCKB receptor cDNA (54) was also used.

Northern blot analysis. Total RNA (20 μg, quantified by measuring absorbance at 260 nm) was size fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to nylon membranes (Nytran Plus; Schleicher & Schuell, Keene, NH) as described by Sambrook et al. (33). The remainder of the technique was as described earlier (3). The autoradiograms were quantified by scanning densitometry, and values were normalized against the corresponding 18S RNA levels.

DNA amplification. For amplification starting from 5 μg of RNA as template, single-stranded cDNA was synthesized from total RNA using an oligo(dT) primer and first-strand cDNA kit (Pharmacia Biotech). For direct amplification, 0.5 μg of total RNA that had been reverse transcribed was used for each reaction. Reactions were performed using 2 units of
Taq polymerase (Pharmacia Biotech) and the CCK_B receptor primers: sense 5’-CTCTATCCGGGTGTGTATGCG-3’ (corresponding to nucleotides 816–840) and antisense: 5’-CCCCAGGTGCTGATGGTGGTATAGC-3’ (nucleotides 1460–1485) at 2.5 mM each in 50 μl of 67 mM Tris · HCl, pH 8.8, 16 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, deoxyribonucleotides at 0.25 mM each, and Tween 20 (0.01% vol/vol). Parameters for DNA amplification were 94°C for 30 s, annealing temperature (57°C) for 30 s, and 72°C for 30 s. Oligonucleotide primers used for DNA amplification were synthesized by Gibco (Life Technologies, Burlington, ON, Canada). DNA amplification products were analyzed by gel electrophoresis on a polyacrylamide gel stained with ethidium bromide.

RESULTS

Ontogeny of CCK_B/gastrin receptor mRNA in rat pancreas. As shown in Fig. 1A, we were unable to detect any mRNA coding for the CCK_B receptor in the rat pancreas; this absence of receptor messenger was evident at all ages, including the fetus. This lack of detection did not result from a probe problem, since a strong message of 2.7 kb was detected in the rat brain and gastric fundus, our positive controls (54). With the use of RT-PCR, the CCK_B receptor mRNAs were, however, detected in rat fetal and adult pancreata and in rat brain and gastric fundus, but the messenger was not amplified in the gastric antrum sample, our negative control (12) (Fig. 1B).

Ontogeny of CCK_B/gastrin receptor protein in rat pancreas. Using a specific antibody raised against the intracellular part of the canine CCK_B receptor, we were able to detect the CCK_B receptor protein in the rat pancreatic membranes, as shown in Fig. 2A. We were surprised to see that its concentration remained unaffected with age. The receptor protein was absent in the gastric antrum but present in the gastric fundus, our respective negative (12) and positive (54) controls.

The receptor protein was visualized as an 80-kDa protein in adult pancreas, and its specificity was ensured by the observation that the 80-kDa band disappeared after preabsorption of the antibody with the corresponding antigen, as shown in Fig. 2B.

Ontogeny of the pancreatic rat CCK_B/gastrin receptor visualized by indirect immunofluorescence. By immunofluorescence (Fig. 3), the presence of the CCK_B receptor protein can be ascertained in the rat pancreas at all ages examined. Even if labeling is less intense in the fetal and newborn organs, it is surprisingly located at the level of the islets of Langerhans at all ages, from fetal life up to adulthood, with no labeling at all on the acinar and ductal cells. Specificity of the labeling was confirmed with tissue exposition to preimmune serum.

Pancreatic and gastric fundus cellular localization of the CCK_B/gastrin receptor by indirect immunofluorescence. Because of the exclusive location of the CCK_B receptor protein in the pancreatic islet of Langerhans anatomic structure, the specific cellular location within the islet was investigated. As shown in Fig. 4, serial sections of pancreatic tissue clearly indicate that the CCK_B/gastrin receptor protein colocalizes with somatostatin in the islet’s δ-cells; indeed, there is no match with the insulin, glucagon, or pancreatic polypeptide cells. In the gastric fundus, the CCK_B receptor protein also clearly colocalizes with the somatostatin δ-cells. The receptor was also detected on the parietal cells with much less intensity. It is interesting to visualize the somatostatin δ-cells closely associated with the parietal cells.

Identification and localization of the CCK_B/gastrin receptor and somatostatin in mouse, pig, and human fetal pancreas. As shown in Fig. 5A, the adult mouse pancreas exhibits two CCK_B/gastrin receptor proteins with a major band of ~80 kDa and a minor one around 50 kDa. In the 8- and 16-day suckling mice, the 80-kDa
protein is present, whereas we were unable to detect the 50 kDa protein. Preabsorption of antibody 9262 with its corresponding antigen prevented recognition of both proteins in the samples (only the adult was presented), thus confirming the specificity of the antibody. As in the rat pancreas, the mouse CCKβ/gastrin receptor observed in the adult pancreas was also localized in the islets of Langerhans, more specifically in the β-cells surrounding the islets, as shown by its colocalization with somatostatin.

Ontogeny of the pancreatic gastrin mRNA. Since gastrin has been reported in human fetal and neonatal rat pancreas (19, 30) and the hormone binds specifically to its CCKβ/gastrin receptor, we evaluated whether there was a correlation between pancreatic gastrin expression and its receptor in the rat. As shown in Fig. 6, the gastrin mRNA is strongly expressed in fetal and newborn rat pancreas. This expression, although still quite intense in the early days of life, exhibited a strong decrease at day 10, with the message almost absent later on. With a specific gastrin antibody, we were able to localize the rat gastrin protein in the G cells of the gastric antrum (Fig. 7A), our positive control. In support of the data obtained with the gastrin mRNA in the rat pancreas, the gastrin protein is intensely visible in the fetal and newborn rat pancreas and undetectable in 10-day-old and adult rat pancreas (Fig. 7B). These data suggest that expression of the CCKβ/gastrin receptor protein is not under the direct control of pancreatic gastrin, at least after 10 days of life.

DISCUSSION

The major objective of this study was to ascertain the status of the pancreatic CCKβ/gastrin receptor with regard to its presence and cellular localization in different species. Our data clearly indicate that 1) the CCKβ/gastrin receptor mRNA could not be identified in the rat pancreas by Northern blot but was detectable by RT-PCR; 2) the CCKβ/gastrin receptor was present in rat pancreas at all ages studied and in equal proportions as an 80-kDa protein and was localized exclusively in the somatostatin β-cells of the islets of Langerhans; 3) the CCKβ/gastrin receptor protein was also identified in mouse, pig, and human fetal pancreas, with its specific location also in the somatostatin β-cells of the islets of Langerhans; and 4) the presence of the CCKβ/gastrin receptor in the rat pancreas does not correlate with that of gastrin throughout the gland’s development.

Identification of the CCKβ/gastrin receptor in the rat pancreas has been a difficult task in recent years. Indeed, the receptor mRNA remained undetectable by means of Northern blot (54, 57) and RT-PCR techniques (34), whereas its message has been detected in fetal pancreas by Northern blot analysis and in adult pancreas only by RT-PCR (9). Our data confirm results from investigators unsuccessful in showing CCKβ/gas-
trin mRNAs by Northern blots (54, 57) and those who were successful by RT-PCR (9). We cannot explain, however, why Monstein et al. (34) and Zhou et al. (57) failed to detect this receptor mRNA by RT-PCR, although we and Funakoshi et al. (9) succeeded. The detection of the CCKB/gastrin receptor protein with a specific antibody for the first time in the rat pancreas indicates undoubtedly that the message has been translated.

Demonstration of the CCKB/gastrin receptor protein in pig and human pancreas also confirms for the first time that the CCKB/gastrin receptor mRNAs previously detected in these two species (24, 36) are indeed translated. The mouse CCKA receptor gene was recently cloned (17). From the sequence of the CCKβ receptor gene present in GenBank but not yet published, we established that the 14-amino acid sequence of the dog CCKβ receptor chosen to develop the antibody has 78.6% homology with that of the rat and mouse and 92.9% homology with that of the human CCKB receptor. Similarities are of 87.9%, 82.9%, and 97.1% with those of the rat, mouse, and human CCKβ receptor, respectively. Our data indicate that the receptor protein is indeed also present in the pancreas of suckling and adult mice.

By the different techniques of SDS-PAGE, affinity labeling, and radiolabeling, studies agreed on the various molecular masses of the rat CCK receptors between 26 and 200 kDa or greater (37); a predicted relative molecular mass of 48,954 (which can reach 90 kDa when the protein is glycosylated) was established by molecular biology (54). The estimated relative molecular mass of 80,000 determined with our specific CCKB/gastrin receptor antibody agrees with the previously published data. In the mouse pancreas, nothing is available for comparison with the size of its CCKB/gastrin receptor. Our results, however, present two proteins of ~50 and ~80 kDa recognized by our specific antibody; the smaller faint protein band could represent the core protein and the larger band the glycosylated receptor. This pancreatic mouse CCKB/gastrin receptor protein is quite comparable to that present in the rat pancreas and in the isolated ECL cells from the African mastomys (50), two other rodents.

Fig. 4. Indirect immunofluorescence of the rat pancreatic (A) and gastric fundus (B) CCKB/gastrin receptor and markers of the endocrine pancreas and gastric fundus. Serial sections of an adult rat pancreas and gastric fundus were stained with the CCKB/gastrin receptor antibody 9262 (1:500), somatostatin (SS), glucagon, insulin, and pancreatic polypeptide (PP) antibodies as described in MATERIALS AND METHODS. Data indicate colocalization of the CCKB/gastrin receptor with the somatostatin δ-cells in the pancreas and gastric fundus; the gastric parietal cells also express the CCKB receptor but with much less intensity than the δ-cells. Numbers beneath images indicate magnification. SPI, preimmune serum.
finity labeling, the pig pancreatic CCKB receptor was recently described as a 48- to 52-kDa protein (38), which corresponds quite accurately with the 50-kDa protein identified with our specific antibody. The size of the human CCKB/gastrin receptor core protein was estimated at 48.5 kDa (25, 39); the ~115 kDa protein identified with our specific antibody probably represents the glycosylated form of the receptor, which was recently recognized with a specific antibody as a 105-kDa protein following transfection of the CCKB/gastrin gene in mouse (42).

Localization of the CCKB/gastrin receptor has been performed by storage phosphor autoradiography in human (49) and in transgenic mouse (42) pancreata; in both species, binding of $^{125}$I-labeled Bolton-Hunter (BH)-CCK-8 and $^{125}$I-BH-sulfated gastrin, respectively, was diffusely and homogeneously distributed in the exocrine component of the gland. In the dog and guinea pig gastric mucosae (10), staining with the same CCKB/gastrin receptor antibodies used in our study revealed the receptor exclusively on the gastric somatostatin δ-cells. Our data confirm this location on the δ-cells, but, contrary to Helander et al. (10), we were able to demonstrate the presence of the CCKB receptor protein also on the parietal cells, although with much less intensity than on the δ-cells. The difference in the two studies may reside in the tissue preparation. Helander et al. (10) fixed their tissues in 4% formaldehyde and embedded them in paraffin, whereas ours were embedded in OCT. Our technical approach seems more sensitive, because we used the somatostatin antibody at a 1:2,000 dilution, whereas Helander et al. used theirs at a 1:30 dilution; their CCKB receptor antibody was diluted 1:100, whereas ours, the same antibody, was at 1:500.

Even though it was previously stated that no CCKB/gastrin mRNA transcripts nor receptors were present on pancreatic islets (15, 52), our data clearly and convincingly show the contrary in the rat, mouse, pig, and human pancreas. Indeed, serial sections stained with specific CCKB/gastrin and somatostatin antibodies reveal the presence of the CCKB/gastrin receptor in the somatostatin δ-cells, in agreement with a recent study performed in rabbit fundic mucosa with specific CCK receptor agonists (41). This finding was quite unexpected because the receptors were anticipated mostly on acinar cells of the human and pig pancreas for secretion of their enzyme content in response to CCK,
whose receptors were described on acinar cells either by autoradiography (49) or by physiological secretory studies (36), respectively. As indicated by Helander et al. (10), this absence of immunofluorescence on pancreatic acinar cells does not exclude the presence of the CCKB/gastrin receptors on these cells; it has been postulated that the receptor density could be too low to allow detection by the antibodies used. This hypothesis is plausible and is supported by our data in the gastric fundus, in which labeling of the CCKB receptor on the δ-cells was much more intense than on the parietal cells.

A constant low level of CCKB/gastrin receptor mRNA undetected by Northern blot but present under RT-PCR may reflect a transcription rate leading to the synthesis of a certain amount of receptors that accumulate at the cell surface in the pancreas. When this receptor type development is finished, a lower level of receptor mRNA would be required because it serves uniquely to maintain the receptor number. Our data on failure to detect the CCKB/gastrin receptor mRNA by Northern blot and on our success by RT-PCR, combined with the constant concentration of CCKB/gastrin receptor protein observed by Western blot during pancreas development, support this hypothesis. The few data indicating that the half-life for receptor proteins is much longer than that of the corresponding mRNA also support this hypothesis (29). Moreover, a recent study by Sanchez et al. (45) indicated no progressive increase or decrease in the pattern of gene expression of the four endocrine pancreatic hormones during human fetal pancreas development. Furthermore, no increase in immunostaining of the four hormones was observed and a dispersion of endocrine cells within the exocrine tissue was noticed. It is highly plausible that a similar phenomenon proceeds after birth involving the CCKB/gastrin receptors present on the rat pancreatic δ-cells.

If, as suggested by Helander et al. (10), the CCKB/gastrin receptor present on pig and human pancreatic acinar cells are undetectable by immunofluorescence because of their low density, it would indicate in these two species that CCK via the occupation of its CCKB/gastrin receptor can control the secretion of the pancreatic enzymes from the acinar cells and that of somatostatin from the endocrine δ-cells. In the rat and mouse pancreas, however, the occupation of the CCKB/gastrin receptors would be uniquely involved in soma-
tostatin release because the CCKA receptors were located on acinar and endocrine β-cells in these two species (1).

Assuming that the CCKRAP/gastrin receptors are absent from the pig and human pancreatic acinar cells but present only on δ-cells as shown in this study, how can we then envisage the control of the secretory effects of CCK on pancreatic enzyme secretion from pig (36) and human (49) acinar cells? It was recently reported that endogenous insulin is a major player regulating the postprandial pancreatic enzyme secretion in rats (24) and dogs (22). Indeed, immunoneutralization of circulating insulin following a meal or the infusion of secretin-CKK totally inhibited pancreatic enzyme secretion in response to these two stimuli, accompanied by a significant increase in somatostatin in portal venous effluent (23).

The following mechanism may operate to explain the control of pancreatic enzyme secretion in pig and human pancreas in the absence of any CCK receptor subtypes on their acinar cells. In response to a meal, the CCK-immunoreactive nerve fibers demonstrated in the pancreas of several species (20) would release CCK early, which in turn can induce insulin secretion from the β-cells through occupation of the CCKA receptor subtype present on these cells (1, 14). Since this physiological step involves the CCKA receptors with high affinity for CCK within the pimoclar range, CCK could come either from the gut and/or the pancreatic nerve terminals. It is also expected that intrapancreatic local concentrations of CCK released from the nerves are much higher than in the plasma and reach the nanomolar range, sufficient to occupy the less-sensitive CCKB/gastrin receptors present on the somatostatin-δ-cells and release their hormonal content (51). Levels of pancreatic nanomolar concentrations of CCK could be reached late in response to meal consumption, causing a delayed release of somatostatin involved in the control of insulin (27) and enzyme (47) secretions.

Finally, in addition to its potential control of insulin and somatostatin secretion via occupation of the CCKA and CCKB receptors, respectively, CCK may also be important in maintaining the pancreatic δ-cell population at a normal density. Indeed, in CCKAP/gastrin-deficient mice, the δ-cell population was reduced by 43% in the gastric antrum (18); unfortunately, the pancreatic δ-cell population was not looked at in these animals.

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