Distribution of CCK1 and CCK2 Receptors in Normal and Diseased Human Pancreatic Tissue

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Background & Aims: **The localization and functional role of cholecystokinin (CCK) receptor proteins in normal and diseased human pancreas, particularly in ductal pancreatic carcinomas, remain unclear.** ***Methods:*** **Tissue samples of normal human pancreas, chronic pancreatitis, and ductal pancreatic carcinomas were investigated under carefully controlled conditions for expression of CCK1 and CCK2 receptor messenger RNA (mRNA) and proteins using in situ hybridization and in vitro CCK receptor autoradiography by means of subtype-selective analogues. Synaptophysin immunohistochemistry was used concomitantly for optimal identification of islets, nerves, and tumor areas with neuroendocrine features.** ***Results:*** **CCK2 receptor mRNA and proteins were found abundantly in human pancreatic islets in normal pancreas and chronic pancreatitis. CCK1 receptor proteins were found occasionally in small-sized pancreatic nerves, whereas acini expressed a low density of CCK2 receptors in a few cases of chronic pancreatitis. Ductal pancreatic carcinomas rarely expressed CCK receptors; a few receptor-positive tumors, often characterized by neuroendocrine differentiation, expressed the CCK2 receptor at the mRNA or protein level. However, the main source of CCK receptors in the pancreatic tumor samples consisted of CCK2-expressing islets and/or CCK1-expressing nerves rather than neoplastic tissue.** ***Conclusions:*** **These data indicate that the presence of CCK receptors in human ductal pancreatic tumor samples is mainly due to CCK2 expression in residual pancreatic islets and CCK1 in pancreatic nerves. Pancreatic acini and ductal pancreatic tumor cells very rarely express CCK2 receptors. These observations suggest that CCK analogues may not be of clinical use to target most of these cancers.**

A role for cholecystokinin (CCK) and gastrin in the normal and diseased pancreas, particularly in pancreatic cancer, has been claimed for many years.[1–6] In humans, however, in contrast to rodents, there are numerous conflicting data on CCK1 and CCK2 expression in normal and diseased human pancreas[1,7–15]; more recently, unexpected negative functional data obtained in human pancreatic acinar cells[16] have addressed the mechanism of action of gastrin and CCK in the pancreas. Although many studies have identified messenger RNA (mRNA) for CCK1 and CCK2[8,12,13,16] in human pancreatic acinar cells, sometimes at low levels only,[16] most reports have failed to identify and localize the CCK receptor protein in these cells[10,16] (but see Tang et al.[9]). Moreover, Ji et al.[16] have challenged the concept that CCK stimulation of pancreatic enzyme secretion in humans is based on a direct action mediated through CCK receptors on acinar cells,[1] because they found that human pancreatic acinar cells do not respond to physiologic concentrations of CCK receptor agonists and express only very low levels of CCK receptors.[16] In ductal pancreatic cancers, CCK receptors have been identified by various groups.[6,11,12,15] However, these studies have been performed using nonmorphologic methods of analysis unable to identify the cellular site expressing the receptor (phosphor-storage autoradiography, reverse-transcription polymerase chain reaction); moreover, nonquantitative reverse-transcription polymerase chain reaction can detect extremely low mRNA message levels, and these may not translate into the expression of functional receptors. Therefore, further studies using morphologic methods measuring the CCK receptor protein need to be performed to address the role of CCK receptors in the normal human pancreas and in pancreatic cancers.

The aim of the present study was to evaluate tissue samples of normal and diseased human pancreas (including chronic pancreatitis and exocrine pancreatic carcinoma) for expression of CCK1 and CCK2 receptor mRNA and proteins measured with morphologic methods such as in situ hybridization and in vitro receptor autoradiography using subtype-selective analogues.

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Materials and Methods

Tissues

Available tissues for this study included 10 samples of normal pancreas (mostly from donors), 16 samples of chronic pancreatitis, and 32 samples of ductal pancreatic carcinomas. All tissues were immediately frozen in liquid nitrogen after resection and stored at −80°C. In addition, 5 samples of rat pancreas were used for control purposes.

CCK1 and CCK2 Receptor Autoradiography

Receptor autoradiography was performed as reported previously[17,18] using a sulfated decapeptide analogue ^{125}I-[DTyr-Gly, Nle^{28,31}]-CCK(26-33) as radioligand and measuring its displacement by increasing concentrations of CCK-8 or gastrin as well as the CCK1-selective analogue L-364,718[19] and the CCK2-selective analogue YF476.[20] Sections were not only stained with H&E for histopathologic identification of the tissue but also immunohistochemically with a specific antibody raised against synaptophysin (Dako, Carpinteria, CA) to more accurately identify residual pancreatic islets and nerves. Moreover, because pancreatic tissues are extremely sensitive to degradation, we also measured both vasoactive intestinal polypeptide receptors[21] and neurotensin receptors[22] in the same human tumor samples (in sections adjacent to those used for CCK receptors) as a positive control for the quality of the pancreatic tissue samples; these tumors very often express vasoactive intestinal polypeptide and neurotensin receptors.[21,22]

The autoradiograms were quantified using a computer-assisted image processing system as described previously.[17] Tissue standards for iodinated compounds (Amersham, Aylesbury, England) were used for this purpose. A tissue was defined as receptor positive when the absorbance measured in the total binding section was at least twice that of the nonspecific binding section.

CCK1 and CCK2 Receptor In Situ Hybridization

Specific human CCK1 and CCK2 riboprobes were generated by reverse-transcription polymerase chain reaction using the following primer pairs: CCK1: sense, 5′-CAC CTA CTT CAT GGG CAC CT-3′; antisense, 5′-CGC GGT CTG GTT GTT ATT TT-3′; CCK2: sense, 5′-TGA AGG TGA GGG TGA GAA GG-3′; antisense, 5′-TGG CAA CCA ACA CAG AAA AA-3′. The resulting polymerase chain reaction products were subcloned into the pGEM-T easy vector (Promega GmbH, Mannheim, Germany) containing promoters for DNA-dependent SP6 and T7 RNA polymerases. The authenticity of the subcloned 230–base pair CCK1 and the 356–base pair CCK2 fragment was confirmed by sequencing (Qiagen GmbH, Hilden, Germany). Plasmids were linearized using *Spe*I and *Nco*I restriction enzymes. T7 and SP6 RNA polymerases were used to construct sense and antisense complementary RNA riboprobes. Biotin complementary RNA labeling was performed using the biotin RNA labeling kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany).

Tissue sections (3 μm) were deparaffinized, rehydrated with 1× phosphate-buffered saline, and incubated in 0.2 mol/L HCl for 20 minutes at room temperature. After rinsing the slides in 2× standard saline citrate, sections were treated with proteinase K (Roche Diagnostics) at a concentration of 25 μg/mL for 15 minutes at 37°C. After postfixation with 4% paraformaldehyde in phosphate-buffered saline for 5 minutes and washing in 2× standard saline citrate, samples were acetylated in 2.5% acetic anhydride and 1.5% triethanolamine for 10 minutes. Subsequently, sections were prehybridized at 78°C for 2 hours in 50% formamide, 4× standard saline citrate, 2× Denhardt's reagent, and 250 μg RNA/mL. Hybridization was performed overnight at 78°C in 50% formamide, 4× standard saline citrate, 2× Denhardt's reagent, 500 μg RNA/mL, and 10% dextran sulfate. The final concentration of the biotin-labeled probes was 0.8 ng/μL. After hybridization, excess probe was removed by washing the slides 3 times in Dako stringent wash solution (Dako) at 78°C for 15 minutes. The samples were then incubated with streptavidin alkaline phosphatase conjugate (Dako) for 30 minutes at room temperature. For the color reaction, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate (Dako) was used.

Results

Tables 1–3 summarize the CCK receptor protein data in the various pancreatic tissues as measured with receptor autoradiography. In the normal pancreas (Table 1), it was striking to see that all but one of the 10 samples expressed CCK2 receptors in the pancreatic islets. The receptor density was generally low. Moreover, in one case, the pancreatic nerves were expressing CCK1 receptors; however, in none of the samples were acini or ducts expressing CCK receptors. In the samples of chronic pancreatitis (Table 2), the islets were CCK2 receptor positive in most cases. The islets in this disease are often more numerous and larger than in normal pancreas[23,24]; in several samples, the CCK receptors in the islets were expressed at higher density than in normal pancreas. About one third of the chronic pancreatitis samples had nerves expressing CCK1 receptors, whereas only a few cases showed acini expressing CCK2 receptors (in low density). Figure 1 is a typical example of CCK receptor distribution in chronic pancreatitis. Most of the pancreatic islets, identified with synaptophysin immunostaining, express CCK2 receptors; CCK2 receptors are defined as those receptors labeled with the ^{125}I-CCK-10 analogue and displaced by 50 nmol/L CCK-8 as well as 50 nmol/L gastrin. Moreover, they could also be displaced by nanomolar concentrations of the CCK2 receptor–selective analogue YF476 but not by the CCK1

Table 1. Incidence and Density of CCK1 and CCK2 Receptor Proteins in Samples of Normal Pancreas as Measured with In Vitro Receptor Autoradiography

			Receptor content (*dpm/mg tissue*)			
Case no.	Age (*yr*)	Sex	Islets (CCK2)	Nerves (CCK1)	Acini (CCK2)	Origin of tissue
BP 195	1.5	M	624	0	0	Organ donor
BP 232	24	F	325	0	0	Organ donor
BP 81	68	M	386	0	0	Normal pancreas around cancer
594	64	M	493	2012	0	Organ donor
591	40	F	507	0	0	Organ donor
BP 117	42	F	289	0	0	Organ donor
BL 201	65	M	350	0	0	Organ donor
BP 278	35	F	551	0	0	Organ donor
BP 286	8	F	0	0	0	Organ donor
BP 326	37	M	369	0	0	Organ donor

0, no CCK receptors were found in the corresponding tissue.

receptor–selective L-364,718. Also, 2 small nerves were found labeled with ^{125}I-CCK-10 that were displaced by 50 nmol/L CCK-8 but not by gastrin, implying the presence of CCK1 receptors (Figure 1). No significant labeling was seen in the acini. In comparison, an example of a rat pancreas shows the strongly labeled acini, where the predominant receptor subtype was CCK1 (Fig. 1). Figure 2 is an example of human tissue showing at high magnification CCK2 receptor–expressing islets and CCK1 receptor–expressing nerves, all identified as immunohistochemically positive for synaptophysin. In general, small-sized pancreatic nerves were more readily labeled than large-sized nerves. Figure 3 is a representative displacement experiment obtained with ^{125}I-CCK-10 autoradiography on successive tissue sections containing pancreatic islets. The high-affinity displacement by CCK-8, gastrin, and the CCK2 receptor–selective YF476 is indicative of CCK2 receptors.

Similar results could be obtained in the 32 samples containing ductal carcinomas surrounded by residual pancreatic tissue usually showing signs of chronic inflammation. There, pancreatic islets were found to express CCK2 receptors in most cases. A few cases also showed small nerves expressing CCK1 receptors (Table 3). Conversely, tumor cells in the 32 samples were extremely rarely found to express CCK receptors; in this series, this was found in 3 tumors only. Interestingly, these 3 tumors all had a neuroendocrine differentiation determined by the positive synaptophysin immunostaining of tumor cells. Figure 4 is an example of a tumor sample with a synaptophysin-positive tumor area expressing CCK2 receptors, whereas a synaptophysin-neg-

Table 2. Incidence and Density of CCK1 and CCK2 Receptor Proteins in Samples of Chronic Pancreatitis as Measured With In Vitro Receptor Autoradiography

			Receptor content (*dpm/mg tissue*)			
Case no.	Age (*yr*)	Sex	Islets (CCK2)	Nerves (CCK1)	Acini (CCK2)	Etiology, duration of disease (*yr*)
BP 50	44	M	0	0	0	Ethyl alcohol, 4
BP 1	42	M	434	0	368	Idiopathic, 1.5
BP 41	49	F	776	1314	501	Idiopathic, 20
BP 7	51	M	386	0	0	Ethyl alcohol, 2
BP 25	37	M	0	0	346	Ethyl alcohol, 2
BP 9	47	M	910	302	0	Ethyl alcohol, 9
BP 117	35	M	1124	1632	0	Ethyl alcohol, 3
BP 1147	42	M	1944	0	0	Ethyl alcohol, 5
BP 1098	43	M	460	1647	0	Ethyl alcohol, 4.5
BP 1066	38	F	0	0	0	Ethyl alcohol, 3
BP 1051	38	M	0	0	0	Ethyl alcohol, 1
BP 824	45	M	0	0	0	Ethyl alcohol, 7
BP 778	49	F	863	0	0	Ethyl alcohol, 3
BP 700	53	M	904	0	0	Ethyl alcohol, 1
BP 654	46	M	1078	0	0	Ethyl alcohol, 4
BP 629	49	M	740	833	0	Ethyl alcohol, 1

0, no CCK receptors were found in the corresponding tissue.

Table 3. Incidence and Density of CCK1 and CCK2 Receptor Proteins in Samples of Ductal Pancreatic Carcinomas as Measured With In Vitro Receptor Autoradiography

			Receptor content (*dpm/mg tissue*)			
Case no.	Age (*yr*)	Sex	Islets (CCK2)	Nerves (CCK1)	Tumor cells (CCK2)	Tumor pathology, grade, stage
BP 178	60	M	1000	0	0	Adenoca, II, T3N1M0
BP 404	70	F	380	0	0	Adenoca, II, T3N0M0
BP 350	73	F			0	Adenoca, II, T3N0M0
BP 463R	62	M	495	0	0	Adenoca, III, T3N1M0
BP 447	72	M	326	0	0	Adenoca, II, T2N1M0
590	63	F	558		0	Adenoca, II, T3N0M0
541	53	M			0	Adenoca, III, T3N1M0
581	72	F		0	0	Adenoca, II-III, T3N1M0
BP 206	76	F	604	0	0	Adenoca, I, T2N1M0
BP 774	78	F	722	881	0	Adenoca, I-II, T1N0M0
544	72	M	1402		0	Adenoca, II, T3N0M0
559	62	F	1072		0	Adenoca, II, T2N1M0
BP 112	70	M			0	Adenoca, II, T3N1M1
592	55	F			0	Adenoca, II-III, T2N1M0
BP 32	75	M	1241	0	0	Adenoca, T2N1M0
BP 131	75	M		512	0	Adenoca, II, T3N1M0
BP 208	61	F	1891		0	Adenoca, II, T2N1M0
BP 425	75	F	1472	0	0	Adenoca, I, T3N0M0
BP 1104	63	M	1074		0	Adenoca, III, T3N1M0
BP 1095	72	F	1354	0	210	Adenoca, II, T2N1M0
BP 1052	64	M			0	Adenoca, II, T2N0M0
569	59	M	1179	0	0	Adenoca, III, T2N0M0
BP 347	65	M	510		0	Adenoca, II, T3N1M0
BP 805	49	M	650		266	Adenoca, II, T3N1M0
BP 12	49	M			0	Adenoca, II, T2N1M0
563	69	M	958		0	Adenoca, II-III, T2N1M0
BP 221	75	M	1911		0	Adenoca, I-II, T2N1M0
BP 1161	66	M	1725	556	0	Adenoca, III, T2N0M0
BP 1048	57	M	1861		0	Adenoca, T2N0M0
BP 267	77	M			0	Endocrine carcinoma, T2N1M0
BP 1154	74	F		0	597	Adenoca, II-III, T3N1M0
550	70	M		2734	0	Adenoca, II, T2N1M0

0, no CCK receptors were found in the corresponding tissue; empty area, the corresponding tissue was not present in that particular sample; Adenoca, adenocarcinoma.

ative area of the ductal pancreatic carcinoma was CCK receptor negative. Figures 5 and 6 show 2 examples of tumors lacking CCK receptors, whereas the adjacent pancreatic tissue, considered as positive control for the respective samples, frequently expressed CCK receptors (islets with CCK2 receptors in Figure 5 and nerves with CCK1 receptors in Figure 6).

As additional positive control for the good quality of the tumor samples, we could show that 17 of the tumor samples expressed vasoactive intestinal polypeptide receptors in tumor cells and that 21 tumors expressed neurotensin receptors; these incidence numbers correspond to earlier reports.[21,22]

In situ hybridization studies for CCK1 receptor mRNA were used to investigate 4 samples of normal pancreas, 4 samples of carcinomas, and 13 samples of chronic pancreatitis. No positive signal was seen in these samples, notably not in the nerve fibers. It should be remembered that the presence of mRNA in nerves is restricted to their cell bodies, which are located outside the pancreas.

CCK2 mRNA in situ hybridization was performed in 8 samples of normal pancreas, 12 samples of carcinomas, and 10 samples of chronic pancreatitis. Strong signals were found in the pancreatic islets of 6 samples, either originating from carcinoma or chronic pancreatitis tissue. Focal, weak staining was found occasionally in some acini. Figure 7 shows an example of in situ hybridization with a strong CCK2 mRNA signal in pancreatic islets and a very weak signal in the surrounding acini. Furthermore, CCK2 mRNA was detected in 4 of 12 carcinoma samples in the neoplastic cells.

Discussion

The present CCK receptor study shows unequivocally that the normal human pancreas frequently ex-

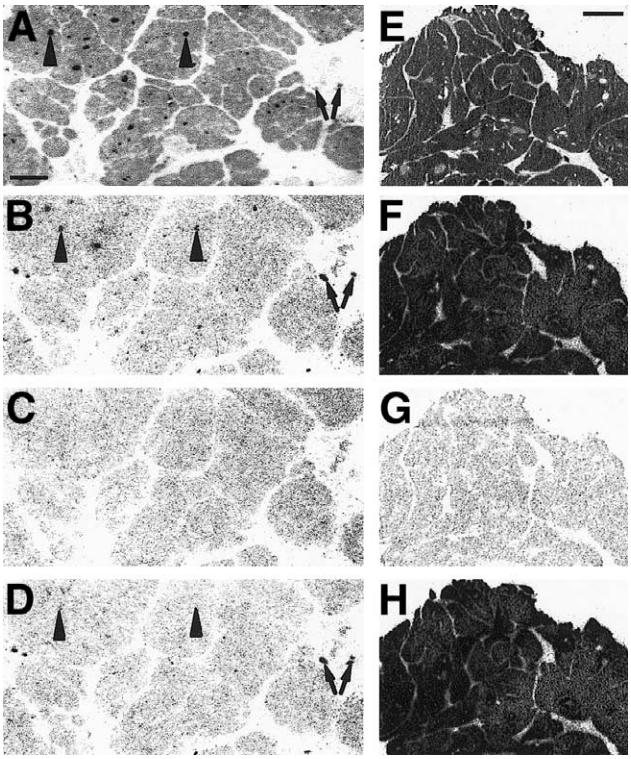

Figure 1. CCK receptors in pancreatic islets and nerves in a sample of (*A–D*) human chronic pancreatitis and a sample of (*E–H*) normal rat pancreas. (*A*) Synaptophysin-stained section showing pancreatic tissue with islets (*black dots*, 2 with *arrowheads*) and nerves (*arrows*). *Bar* = 1 mm. (*B*) Autoradiogram showing total binding of ^{125}I-CCK-10. The islets (*arrowheads*) and nerves (*arrows*) are labeled but not the rest of the parenchyma. (*C*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. The radioligand is completely displaced in islets and nerves. (*D*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is displaced in islets but not in nerves. This indicates that islets express CCK2, whereas nerves express CCK1 receptors. (*E*) H&E-stained section of rat pancreas. *Bar* = 1 mm. (*F*) Autoradiogram showing total binding of ^{125}I-CCK-10. The pancreatic acinar parenchyma is strongly labeled. (*G*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. (*H*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is displaced by CCK but not by gastrin, indicating the presence of CCK1 receptors in the rat pancreas.

presses CCK2 receptors in the islets, occasionally CCK1 receptors in the nerves, and extremely rarely CCK receptors in acini or ducts. This is not only observed in the normal pancreas but also in chronic pancreatitis, where in general a similar receptor distribution is found. Here, however, the CCK receptor–positive structures are more prominent than in the normal pancreas because chronic pancreatitis is a disease in which the acini disappear with disease progression and are replaced by fibrous tissue, whereas the islets are relatively resistant to that process and may even proliferate, as well as the nerves, which may increase in number and in diameter.[23–26] This pathologic condition may reflect a relatively higher density of CCK2 receptors (originating in islets) and CCK1 receptors (originating in nerves) per tissue volume in this disease. Finally, as expected, the same pattern of receptor distribution is also found in the residual pancreas remaining around ductal carcinomas. Indeed, this residual pancreas is very often altered by chronic inflammation. In situ hybridization studies largely confirm the receptor autoradiographic data. Pancreatic islets have measurable amounts of CCK2 mRNA, whereas most of the acini are devoid of CCK2 or CCK1 mRNA. Conversely, the absence of CCK1 mRNA in pancreatic nerves can easily be explained by the fact that the cell bodies containing

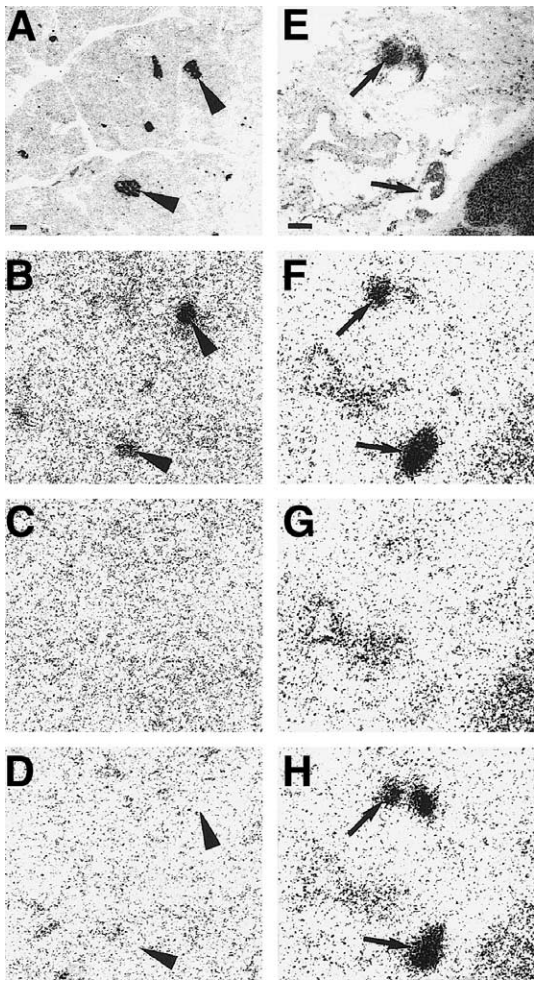

Figure 2. CCK2 receptors in (*A–D*) islets and (*E–H*) CCK1 receptors in nerves at high magnification. (*A* and *E*) Synaptophysin-stained sections showing pancreatic tissue with (*A*, *arrowheads*) islets and (*E*, *arrows*) nerves. *Bars* = 1 mm. (*B* and *F*) Autoradiograms showing total binding of ^{125}I-CCK-10. The islets (*arrowheads*) and nerves (*arrows*) are strongly labeled. (*C* and *G*) Autoradiograms showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. The radioligand is displaced in islets and nerves. (*D* and *H*) Autoradiograms showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is displaced in (*D*, *arrowheads*) islets but not in (*H*, *arrows*) nerves. This shows that islets express CCK2 and nerves express CCK1 receptors.

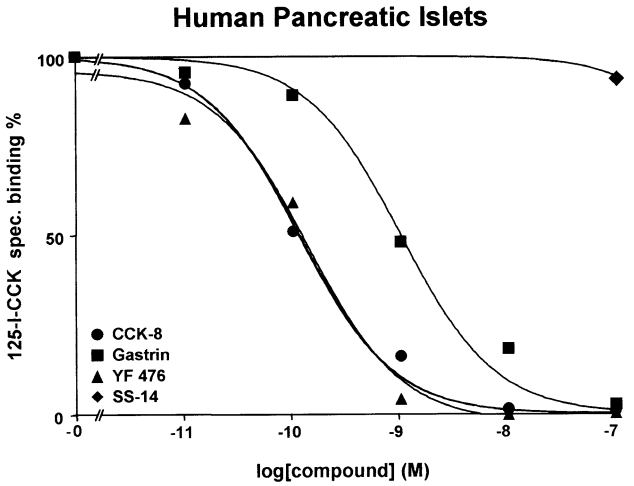

Figure 3. Representative displacement experiment showing CCK2 receptors in human pancreatic islets. ^{125}I-CCK-10 was displaced by increasing concentrations of CCK-8, YF476, and gastrin, but not 100 nmol/L somatostatin (SS-14) in successive sections of pancreatic tissue containing pancreatic islets. The high-affinity displacement of the radioligand by CCK-8 as well as YF476 and gastrin suggests the presence of CCK2 receptors.

mRNA are usually located outside the pancreas. Similar observations have been made previously with substance P mRNA in those nerves.[27]

Another important message of the present study is that only very few ductal pancreatic cancers were shown to express CCK receptor proteins. The few receptor-positive carcinomas were characterized by a neuroendocrine differentiation. It is known that single normal pancreatic duct cells as well as some ductal pancreatic cancers can show a neuroendocrine differentiation.[28,29] The present data now suggest that this subgroup of ductal carcinomas with neuroendocrine differentiation may be further distinguished from the other pancreatic carcinomas by its CCK2 receptor expression. In situ hybridization studies confirmed that the CCK2 but not CCK1 receptor could be expressed in a subgroup of ductal pancreatic carcinomas. Although the presence of CCK2 receptors in a small subgroup of tumors is interesting, it must be emphasized that the general lack of CCK receptors in most of the ductal pancreatic carcinomas suggests a very limited clinical impact for CCK analogues in this group of tumors. Although pancreatic carcinomas are unlikely to represent an important target for CCK-related tumor diagnosis and/or therapy, such a clinical application has recently been established for CCK receptor–expressing medullary thyroid carcinomas.[30]

The pancreas is very susceptible to degradation and rapid digestion by enzymes. Therefore, the cell membrane receptor proteins that are present in this tissue may be rapidly destroyed and therefore not measurable if the tissue is not processed adequately. For that reason, we considered it important to have several controls in the receptor autoradiographic experiments, indicating that

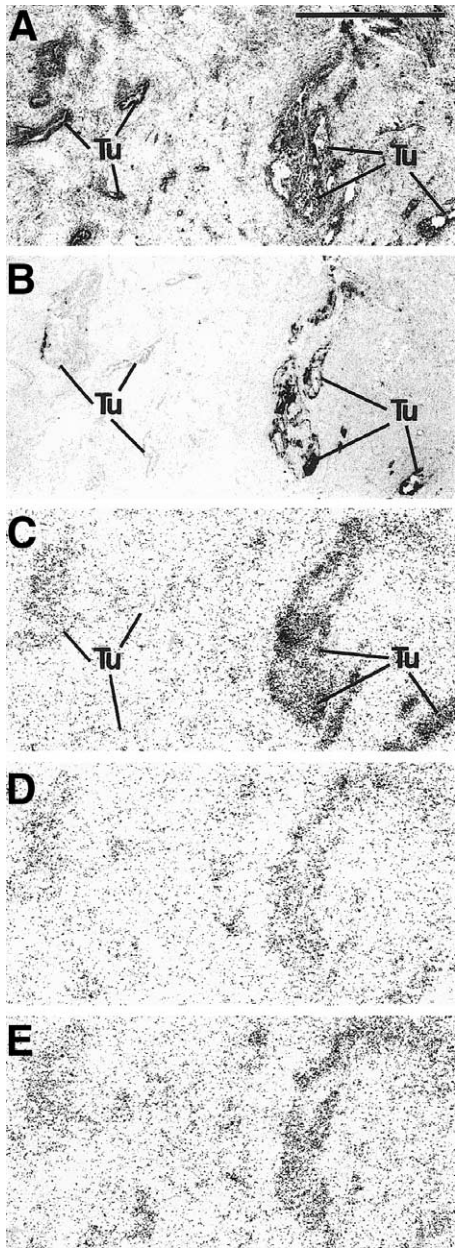

Figure 4. CCK2 receptors in a ductal pancreatic adenocarcinoma. (*A*) H&E-stained section showing tumor tissue (Tu) on the left and on the right. *Bar* = 1 mm. (*B*) Synaptophysin immunostaining of an adjacent section showing a strong immunostaining of the right part of the tumor, whereas the left part was almost devoid of immunoreactivity. (*C*) Autoradiogram showing total binding of ^{125}I-CCK-10. The tumor on the right is strongly labeled, whereas the left part of the tumor is virtually not labeled. (*D*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. The radioligand is displaced in the right part of the tumor. (*E*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is also displaced here, suggesting that the tumor on the right expresses CCK2 receptors.

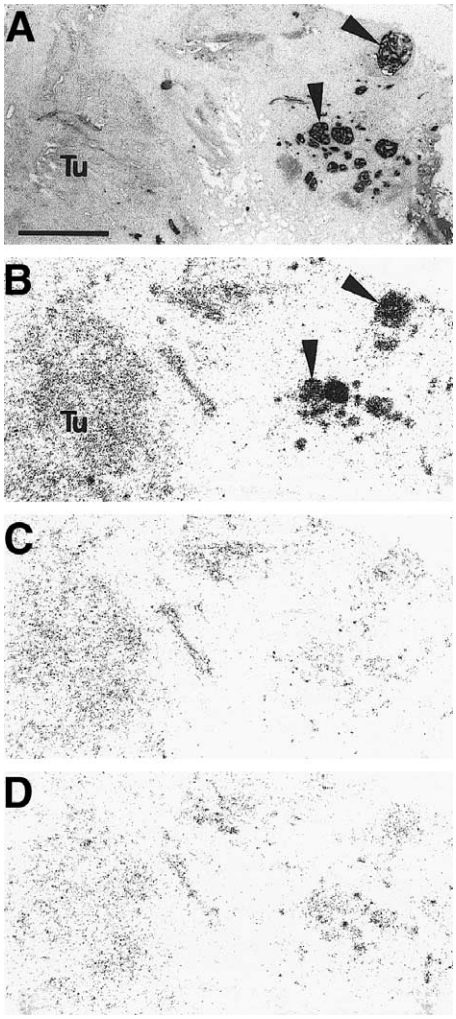

Figure 5. Expression of CCK2 receptors in the islets but not in the adjacent ductal pancreatic carcinoma. (*A*) Synaptophysin-stained section showing pancreatic carcinoma (Tu) as well as pancreatic islets (*arrowheads*). *Bar* = 1 mm. (*B*) Autoradiogram showing total binding of ^{125}I-CCK-10. The islets (*arrowheads*) are labeled but not the carcinoma. (*C*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. The radioligand is displaced in islets. (*D*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is displaced in islets, indicating that islets express CCK2.

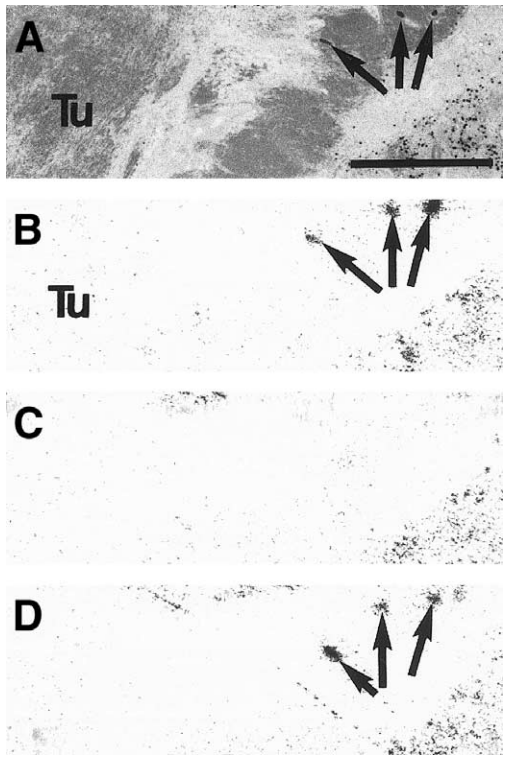

Figure 6. Expression of CCK1 receptors in pancreatic nerves but not in the adjacent ductal pancreatic carcinoma. (*A*) Synaptophysin-stained section showing pancreatic carcinoma (Tu) and pancreatic nerves (*arrows*). *Bar* = 1 mm. (*B*) Autoradiogram showing total binding of ^{125}I-CCK-10. The nerves (*arrows*) are labeled but not the carcinoma. (*C*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L CCK-8. The radioligand is displaced in nerves. (*D*) Autoradiogram showing binding of ^{125}I-CCK-10 in the presence of 50 nmol/L gastrin. The radioligand is not displaced in nerves, indicating that nerves express CCK1 receptors.

the CCK receptor–negative tumors tested in the present study were truly negative. First, in many samples with a receptor-negative tumor, we had an internal positive control, namely the identification of CCK receptor–positive islets or nerves in pancreatic tissue surrounding the tumor. Second, in most of the tumor samples, we could identify the expression of other G-protein–coupled peptide receptors, either neurotensin receptors or vasoactive intestinal polypeptide receptors, suggesting that the conditions of the tested tumors were good enough to retain measurable amounts of G-protein–coupled membrane receptors. Finally, we have been able to identify successfully, using the same methodology, a high density of

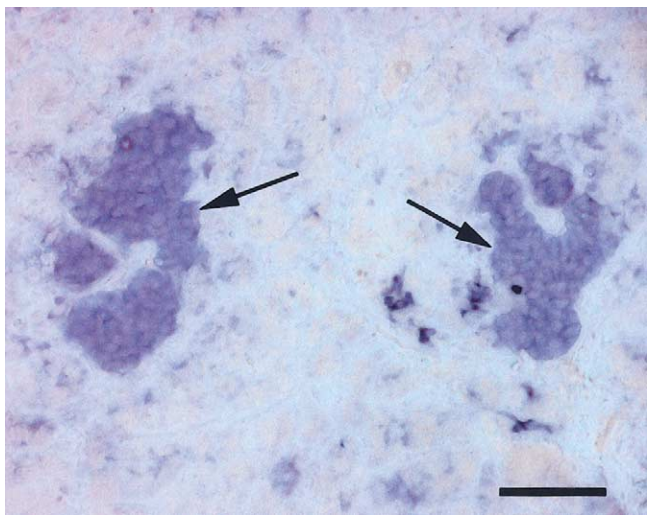

Figure 7. In situ hybridization of CCK2 receptor mRNA showing 2 strongly positive (*blue*) pancreatic islets (*arrows*), whereas the surrounding acini are largely negative except for a few (*pale blue*) foci. *Bar* = 50 μm.

CCK1 receptors in the rat pancreas, confirming prior reports.[14] The discrepancy between the high expression of CCK1 receptors in the rat pancreas and the absence of such receptors in normal human acinar cells[16] (present study) points once more toward the considerable species variability of peptide receptor expression. The low amount of CCK2 receptor proteins present in the acini of a few samples of chronic pancreatitis is intriguing in regard to the absence of such receptors in normal acini. At least it indicates that human acini have the potential to express CCK2 receptors under certain conditions. However, their biological relevance in pancreatitis is unknown. They may be expressed as a pathophysiologic reaction related to the progression of the disease. It is also worth mentioning that gastrin-releasing peptide receptor proteins were recently found in pancreatic acini of chronic pancreatitis samples, whereas they were undetectable in normal pancreas.[31]

This study may give an explanation for the numerous conflicting results reported on the expression of CCK receptors in normal and especially in tumoral human pancreatic tissues. Although the studies that have identified CCK receptors in the normal human and diseased pancreas were technically adequate, not all may have been able to identify the CCK receptors in the stipulated cell type, particularly those studies using reverse-transcription polymerase chain reaction methods measuring the receptors in pancreatic homogenates.[6,8,12,13] Because it seems that "contamination" of pancreatic cancer samples by pancreas (often in the form of pathologically altered pancreas such as in chronic pancreatitis) is frequent (two thirds of cancer samples in this study contained islets, and one half of the samples contained nerves), it may well be that receptor investigations not based on morphologic identification methods may have falsely interpreted the receptor expression as being of tumoral origin, while it was in fact from islets and nerves. It should be noticed that the number of islets and nerves could vary in individual pancreas and also be more abundant in certain areas of the pancreas than in others. Moreover, chronic pancreatitis, as a single pathologic entity or when in the vicinity of a pancreatic cancer, has relatively more CCK receptor–positive elements (islets and nerves) than normal pancreas. This fact may explain why it has been suggested, using nonmorphologic methods, that pancreatic tumor samples (which often include chronic pancreatitis) have more receptors than normal pancreas. The present in vitro data are very much in line with the report by Ji et al.[16] describing the lack of functional CCK receptors in the human pancreatic acini and listing the consequences thereof.

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