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Cholecystokinin(CCK)-A and CCK-B/Gastrin Receptors in Human Tumors

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ABSTRACT

Cholecystokinin (CCK)-A and CCK-B/gastrin receptors were evaluated with *in vitro* receptor autoradiography in 406 human tumors of various origins using a sulfated ^{125}I-labeled CCK decapeptide analogue ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26-33 and ^{125}I-labeled Leu^{15}-gastrin as radioligands. CCK-B/gastrin receptors were found frequently in medullary thyroid carcinomas (92%), in small cell lung cancers (57%), in astrocytomas (65%), and in stromal ovarian cancers (100%). They were found occasionally in gastroenteropancreatic tumors, breast, endometrial, and ovarian adenocarcinomas. They were either not expressed or rarely expressed in colorectal cancers, differentiated thyroid cancers, non-small cell lung cancers, meningiomas, neuroblastomas, schwannomas, glioblastomas, lymphomas, renal cell cancers, prostate carcinomas, and the remaining neuroendocrine tumors (*i.e.*, pituitary adenomas, pheochromocytomas, paragangliomas, and parathyroid adenomas). CCK-A receptors were expressed rarely in tumors except in gastroenteropancreatic tumors (38%), meningiomas (30%), and some neuroblastomas (19%). The identified CCK-A and CCK-B receptors were specific and of high affinity in the subnanomolar range. The rank order of potency of various CCK analogues was: sulfated CCK-8 = L-364,718 ≫ nonsulfated CCK-8 = L-365,260 ≥ gastrin for CCK-A receptors and sulfated CCK-8 > gastrin = nonsulfated CCK-8 > L-365,260 > L-364,718 for CCK-B receptors. CCK-B receptors could also be selectively and specifically labeled with a newly designed nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26-33. Gastrin mRNA measured by *in situ* hybridization was present in most CCK-B receptor-positive small cell lung cancers, breast tumors, and ovarian tumors, representing the molecular basis of a possible autocrine growth regulation of these tumors. Gastrin and CCK mRNAs were lacking in medullary thyroid cancers. Thus, these results may have pathogenic, diagnostic, differential diagnostic, and therapeutic implications.

INTRODUCTION

There has been recently an increasing interest in the expression of peptide receptors by human tumors. The *in vitro* identification of these receptors in tumors was indeed found to be an important prerequisite for the evaluation of new potential clinical applications of the corresponding peptides (1–3). For example, the discovery of the high incidence of somatostatin receptors in several types of human cancers has led to the development of *in vivo* somatostatin receptor imaging of tumors, allowing the *in vivo* localization in patients of somatostatin receptor-positive tumors after injection of a labeled somatostatin analogue (4, 5). The same may be true for VIP[2] receptors as well (6). The presence of peptide receptors in tumors may also be of therapeutic interest. Tumoral somatostatin receptors were shown to mediate the successful symptomatic treatment of neuroendocrine tumors with somatostatin analogues (2), and the growth of VIP receptor-positive breast cancers can be inhibited by a synthetic VIP receptor antagonist (7). These examples suggest that the evaluation of the expression of other peptide receptors in tumors should be of considerable potential interest.

The gastrointestinal peptides gastrin and/or CCK have been implicated in various regulatory functions; as neurotransmitters in the brain; and in the regulation of various functions of the gastrointestinal tract, primarily at the level of the stomach, pancreas, and gallbladder (8). In addition, they can act as physiological growth factors in most parts of the gastrointestinal tract (9–11) and also as stimulatory growth factors in several neoplasms, such as colonic and gastric cancers (12–14). Gastrin and CCK possess the same terminal five amino acids at their COOH terminus, which is the biologically active site; their actions are mediated by two different receptor types, CCK-A and CCK-B receptors (15, 16), which can be distinguished pharmacologically by their low (CCK-A) *versus* high (CCK-B) affinity for gastrin.

CCK-A and CCK-B/gastrin receptors have been identified in several normal tissues; CCK-B/gastrin receptors are present in the gut mucosa and in the brain (8, 17, 18). CCK-A receptors are present in the gallbladder, pancreas, and brain (8, 19). The presence of receptors for gastrin and CCK in tumors has also been reported. It is well established that small cell lung cancers often express CCK-B/gastrin receptors, whereas non-small cell lung cancers do not express them (20, 21). However, the findings are more equivocal for gastrointestinal cancers. Whereas earlier studies have reported CCK-B/gastrin receptors in colon cancers and gastric cancers (22), more recent investigations failed to find high-affinity CCK-B/gastrin receptors in most of these tumors (23). Recently, an unexpected high incidence of CCK-B receptors was identified in medullary thyroid carcinomas, whereas differentiated thyroid cancers were not expressing them (24). Little information is available, however, about the CCK-B/gastrin receptor incidence in other human tumors.

The main goal of the present study was to investigate the CCK-B/gastrin and CCK-A receptor prevalence in a variety of human tumors, including a large group of neuroendocrine tumors. Receptors can be best evaluated in complex human tissues with receptor autoradiography, which preserves the morphological integrity of the tissues and allows the precise localization of the receptors. Adequate ligands have been shown previously to identify CCK-B and/or CCK-A receptors in animal and human tissues (17, 24–26). Therefore, the following two ligands were used routinely in all cases: ^{125}I-Leu^{15}-gastrin, which directly identifies gastrin receptors, and ^{125}I-CCK decapeptide, which identifies CCK-B/gastrin or CCK-A receptors based on whether the ligand is displaced by nanomolar concentrations of CCK and gastrin or of CCK only.

MATERIALS AND METHODS

Aliquots of surgically resected tumors or of biopsies submitted for diagnostic histopathology were frozen immediately after surgical resection and stored at −70°C. The specimens originated from several different clinical institutions, and some have been used previously for other purposes. The following tumors were investigated: neuroendocrine tumors, including small cell lung cancers, pituitary adenomas, endocrine gastroenteropancreatic tumors, medullary thyroid carcinomas, parathyroid adenomas, pheochromocytomas, paragangliomas, and neuroblastomas; non-small cell lung cancers; colorectal adenocarcinomas; papillary and follicular thyroid carcinomas (differentiated thyroid cancers); breast carcinomas; astrocytomas; glioblastomas; schwannomas; meningiomas; endometrial and prostate carcinomas; ovarian cancers (including adenocarcinomas and stromal tumors); renal cell tumors; and non-Hodgkin's lymphomas (Table 1).

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[2] The abbreviations used are: VIP, vasoactive intestinal peptide; CCK, cholecystokinin; ^{125}I-gastrin, ^{125}I-Leu^{15}-gastrin I; ^{125}I-CCK, ^{125}I-D-Tyr-Gly-Asp-Tyr(SO$_3$H)-Nle-Gly-Trp-Nle-Asp-Phe-amide, *i.e.*, ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26-33.

Table 1 *CCK receptor incidence in tumors*

Tumor type	CCK-B receptors[a]	CCK-A receptors[b]
Neuroendocrine tumors		
Medullary thyroid carcinomas	22 of 24 (92%)[c]	2 of 24 (8%)[c]
Small cell lung cancers	8 of 14 (57%)	0 of 14 (0%)
Gastroenteropancreatic tumors	7 of 32 (22%)	12 of 32 (38%)
Growth hormone pituitary adenomas	0 of 9 (0%)	0 of 9 (0%)
Inactive pituitary adenomas	0 of 10 (0%)	0 of 10 (0%)
Pheochromocytomas	0 of 10 (0%)	0 of 10 (0%)
Paragangliomas	0 of 10 (0%)	0 of 10 (0%)
Neuroblastomas	1 of 16 (6%)	3 of 16 (19%)
Parathyroid adenomas	0 of 4 (0%)	0 of 4 (0%)
Tumors of the nervous system		
Astrocytomas	11 of 17 (65%)	0 of 17 (0%)
Meningiomas	1 of 27 (4%)	8 of 27 (30%)
Schwannomas	0 of 13 (0%)	0 of 13 (0%)
Glioblastomas	0 of 10 (0%)	0 of 10 (0%)
Tumors of the reproductive system		
Breast carcinomas	5 of 65 (8%)	2 of 65 (3%)
Endometrial carcinomas	2 of 16 (13%)	0 of 16 (0%)
Ovarian cancers		
Epithelial tumors	4 of 28 (14%)	0 of 28 (0%)
Stromal tumors	3 of 3 (100%)	0 of 3 (0%)
Prostate carcinomas	1 of 15 (7%)	0 of 15 (0%)
Colorectal carcinomas	0 of 22 (0%)	0 of 22 (0%)
Lung cancers (nSCLCs)[d]	1 of 14 (7%)	0 of 14 (0%)
Differentiated thyroid cancers[e]	0 of 11 (0%)[c]	0 of 11 (0%)[c]
Renal cell tumors	0 of 14 (0%)	0 of 14 (0%)
Non-Hodgkin-lymphomas	0 of 22 (0%)	0 of 22 (0%)

[a] ^{125}I-gastrin as well as ^{125}I-CCK ligands were used and were displaced completely with 50 nM sulfated CCK-8 or gastrin.
[b] ^{125}I-CCK ligand was used and can be displaced completely with 50 nM sulfated CCK-8 but not displaced by gastrin; there was no binding of ^{125}I-gastrin.
[c] Data were taken in part from Ref. 24.
[d] nSCLCs, non-small cell lung cancers.
[e] Differentiated thyroid cancers included follicular thyroid carcinomas and papillary thyroid carcinomas.

The following synthetic peptides were used: human [Leu^{15}]-gastrin I, human gastrin I, sulfated CCK-8, nonsulfated CCK-8, somatostatin-14, all from Bachem (Bubendorf, Switzerland); (D-Tyr-Gly, Nle^{28,31})-CCK 26–33 (D-Tyr-Gly-Asp-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-amide) purchased from R Plus, Inc. (Bayonne, NJ). The nonsulfated form of (D-Tyr-Gly, Nle^{28,31})-CCK 26–33 was synthesized by Chiron (Clayton, Australia). The two CCK antagonists L-364,718 and L-365,260 were provided kindly by Dr. R. Freidinger (Merck and Co., West Point, PA).

Receptor Autoradiography

Receptor autoradiography was performed on 10- and 20-μm thick cryostat (Leitz 1720, Rockleigh, NJ) sections of the tissue samples, mounted on microscope slides, and then stored at −20°C for at least 3 days to improve adhesion of the tissue to the slide, as described previously (27). Each tissue underwent two different types of autoradiographic procedures with two different radioligands, ^{125}I-gastrin and ^{125}I-CCK.

^{125}I-Gastrin Receptor Autoradiography. The method used was based on the ^{125}I-gastrin receptor autoradiography of enterochromaffin-like tumors reported in detail previously (25), with minor modifications. Briefly, sections were preincubated in 50 mM Tris-HCl, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl, 1 mM ethyleneglycol bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid, and 0.5% BSA, pH 7.4 (preincubation solution) for 30 min at 25°C. The slides were then incubated in a solution containing the same medium as the preincubation solution except that the BSA was omitted, and the following compounds were added: 55 pM ^{125}I-gastrin (2000 Ci/mmol; Anawa, Wangen, Switzerland), 0.025% bacitracin, 1 mM DTT, 2 μg/ml chymostatin, and 4 μg/ml leupeptin, pH 6.5. The slides were incubated at room temperature with the radioligand for 150 min. Increasing amounts of nonradioactive CCK-8 or gastrin were added to the incubation medium to generate competitive inhibition curves. On completion of the incubation, the slides were washed six times for 15 min each in ice-cold preincubation solution (pH 7.4). The slides were rinsed twice in ice-cold distilled water for 5 s each. The slides were then dried under a stream of cold air at 4°C, apposed to ^{3}H-Hyperfilms (Amersham UK, Little Chalfont, UK) and exposed for 1–7 days in X-ray cassettes.

^{125}I-CCK Receptor Autoradiography. The method described by Mantyh *et al.* with the ^{125}I-CCK as radioligand was used in all assays (17). The sections were preincubated as described above and then incubated at room temperature for 150 min in a solution containing the same medium as for the preincubation except that BSA was omitted, and the following compounds were added: 45 pM ^{125}I-CCK (2000 Ci/mmol; Anawa), 0.025% bacitracin, 1 mM DTT, 2 μg/ml chymostatin, and 4 μg/ml leupeptin, pH 6.5. The slides were then treated as described above for ^{125}I-gastrin receptor autoradiography.

The autoradiograms were quantified using a computer-assisted image-processing system, as described previously (27, 28). Tissue standards for iodinated compounds (Amersham UK, Little Chalfont, United Kingdom) were used for this purpose. A tissue was defined as receptor positive when the absorbance measured over a tissue area in the total binding section was at least twice the absorbance of the nonspecific binding section.

Receptor Autoradiography Using Nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33. Nonsulfated (D-Tyr-Gly, Nle^{28,31})-CCK 26–33 was labeled with ^{125}I and purified by Anawa with a specific activity of 2000 Ci/mmol. Receptor autoradiography was performed on several types of tumors using the same methods as with the sulfated radioligand.

In all three types of receptor autoradiographic experiments, positive controls were included; samples including human tissues known to express CCK-A and CCK-B receptors such as gastric mucosa, gallbladder, or brain were added to each experiment. In addition, CCK receptor-positive tissues (*i.e.*, mucosa, smooth muscles, and neurons) adjacent to a tumor could be taken as positive, internal control for the quality of a surgical biopsy. As further proof of quality, most of the tumor samples, particularly those found to be CCK receptor negative, were evaluated for the expression of at least one other peptide receptor (*i.e.*, somatostatin, VIP, or substance P receptor; data not shown).

In Situ Hybridization of Gastrin and CCK mRNAs

Gastrin and/or CCK mRNAs were identified with *in situ* hybridization histochemistry on adjacent sections of medullary thyroid carcinomas; gastrinomas; breast, ovarian, and small cell lung cancers; as well as normal human brain, as described previously for somatostatin and somatostatin receptor mRNA (29). Oligonucleotide probes complementary to nucleotides coding for amino acids 51–70 (30) or 75–82 (31) of the human *gastrin* gene and for amino acids 1–11 or 80–95 (32) of the human *CCK* gene were synthesized and purified on a 20% polyacrylamide-8 M urea sequencing gel (Microsynth, Balgach, Switzerland). They were labeled at the 3′ end by using [α-^{32}P]dATP (>3000 Ci/mmol; Amersham UK) and terminal deoxynucleotidyltransferase (Boehringer, Mannheim, Germany) to specific activities of $0.9–2.0 \times 10^4$ Ci/mmol (33, 34).

Several control experiments were carried out with the probes used in the present study to determine the specificity of the hybridization signal obtained (33, 34). The hybridization pattern obtained with two oligonucleotides complementary to different regions of the same mRNA, when used independently as hybridization probes in consecutive sections of tumor tissue, was similar for both probes, with similar exposure times. Among the two gastrin probes, however, the one designed by Larsson and Hougaard (31) was the more sensitive. The thermal stability of the hybrids was close to the theoretical melting temperature. The various oligonucleotide probes were shown to hybridize in normal human tissue specimens known to express the different peptides. In all types of tumors tested, abolishment of the hybridization signal was observed when consecutive sections were hybridized with the radiolabeled probe in the presence of a 20-fold excess of the corresponding unlabeled probe, proving also the specificity of the signal obtained. This last control was particularly important when considering the heterogeneity of the tissue samples investigated. All experiments were done using each of the two oligonucleotides as the hybridization probe for each mRNA under study.

The absorbance was measured in the autoradiograms over the tumor area with a computer-assisted image-processing system, as described previously (27). A tumor was considered positive for the respective mRNA when the absorbance measured in a normally hybridized section was at least twice that in a parallel section in which hybridization was blocked with 20-fold excess of the corresponding probe. In a majority of cases, tissue sections were hybridized with an oligonucleotide complementary to bp 45–92 of the human β-actin mRNA (35) to confirm and normalize the presence of mRNA in the tissues analyzed; tumors lacking β-actin mRNA were excluded from the study.

RESULTS

Table 1 summarizes the results of the CCK receptor evaluation in various tumors. Among all the different tumor types tested, the medullary thyroid carcinomas, small cell lung cancers, astrocytomas, and stromal ovarian cancers showed a high incidence of CCK-B receptors. CCK-B receptors were found occasionally in gastroenteropancreatic tumors and in breast, endometrial, and ovarian adenocarcinomas. They were either not expressed or rarely expressed in colorectal carcinomas, differentiated thyroid cancers, non-small cell lung cancers, meningiomas, neuroblastomas, schwannomas, glioblastomas, non-Hodgkin's lymphomas, renal cell cancers, prostate carcinomas, and the remaining neuroendocrine tumors (pituitary adenomas, pheochromocytomas, paragangliomas, and parathyroid adenomas). CCK-A receptors were more rarely expressed than CCK-B receptors except for gastroenteropancreatic tumors (38%), meningiomas (30%), and neuroblastomas (19%; Table 1).

Congruent results were obtained with both radioligands in all the tumors; indeed, the tumors having CCK-B/gastrin receptors could be labeled on one hand with ^{125}I-gastrin, which was displaced completely by cold gastrin; on the other hand, these tumors were labeled similarly with the ^{125}I-CCK decapeptide, the ligand being displaced by nanomolar concentrations of both CCK and gastrin. Fig. 1 shows a typical example of a medullary thyroid carcinoma expressing CCK-B/gastrin receptors, and Fig. 2 shows an example of a small cell lung cancer expressing CCK-B/gastrin receptors. These tumors do not express CCK-A receptors, because all bound ^{125}I-CCK is displaced completely by 50 nM gastrin. Fig. 3 shows a typical example of a gastroenteropancreatic tumor expressing CCK-A receptors but no CCK-B/gastrin receptors. No binding with ^{125}I-gastrin can be observed; furthermore, a strong labeling is obtained with the ^{125}I-CCK analogue, which can be displaced fully by 50 nM sulfated CCK-8 but not by gastrin. In all these cases, the receptor autoradiographic technique shows the presence of the receptors located precisely on the tumor cells. As shown in Fig. 1, no receptors can be identified on the normal, adjacent thyroid gland.

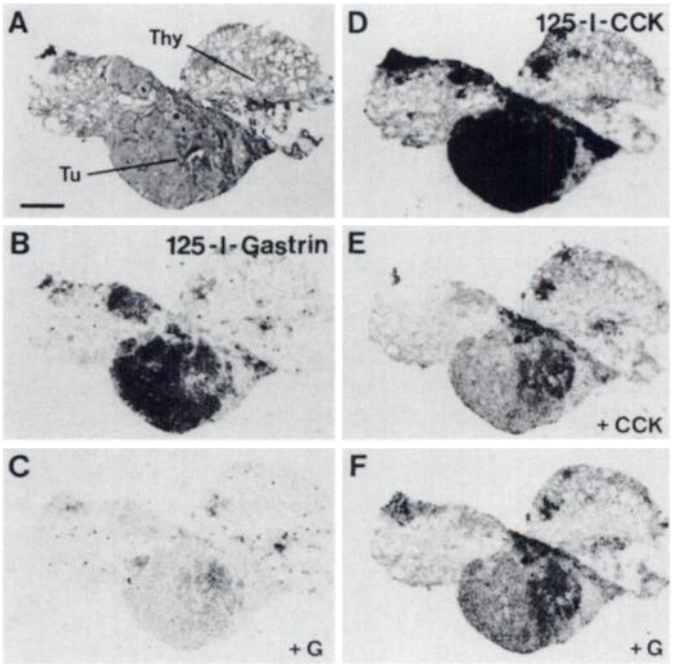

Fig. 1. *A–F, in vitro* receptor autoradiographic identification of CCK-B/gastrin receptors in a human medullary thyroid carcinoma. *A*, H&E-stained section showing the tumor tissue (*Tu*) and adjacent, normal glandular thyroid tissue (*Thy*). *Bar*, 1 mm. *B*, autoradiogram showing total binding of ^{125}I-gastrin. The tumor tissue is labeled (*black areas*), whereas normal thyroid is not. *C*, autoradiogram showing nonspecific binding of ^{125}I-gastrin (in the presence of 100 nM unlabeled gastrin). *D*, autoradiogram showing total binding of ^{125}I-CCK analogue. Tumor tissue is massively labeled. *E* and *F*, autoradiograms showing nonspecific binding of ^{125}I-CCK analogue in the presence of 50 nM sulfated CCK-8 (*E*) and 50 nM gastrin (*F*).

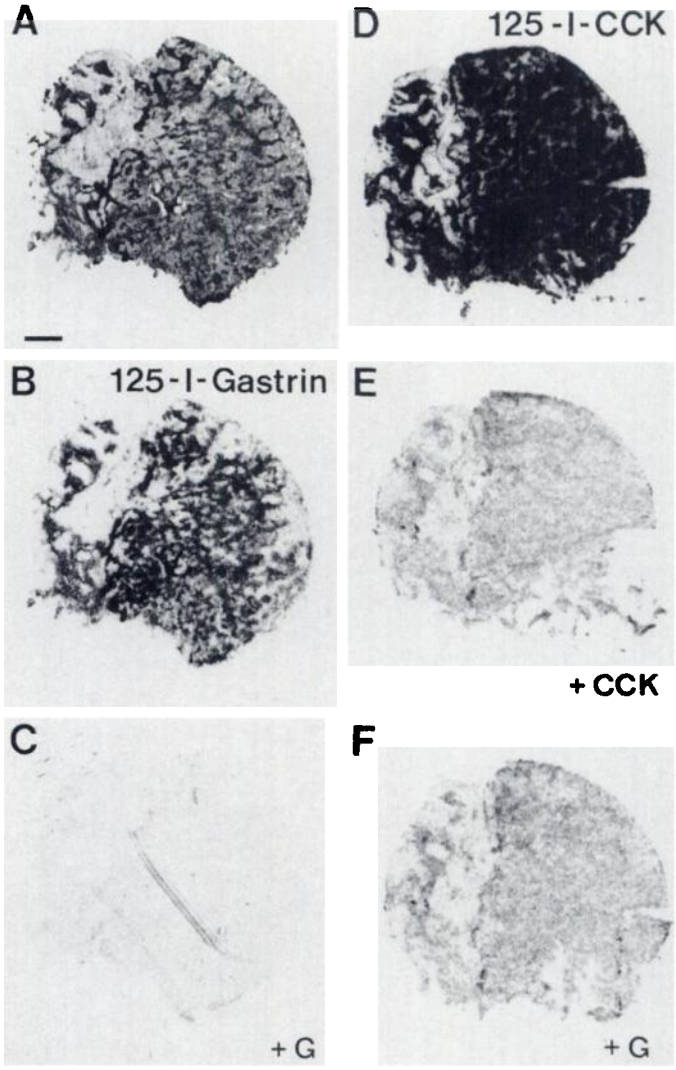

Fig. 2. *A–F, in vitro* receptor autoradiographic identification of CCK-B/gastrin receptors in a human small cell lung cancer. *A*, H&E-stained section showing the tumor tissue. *Bar*, 1 mm. *B*, autoradiogram showing total binding of ^{125}I-gastrin. The tumor tissue is labeled (*black areas*). *C*, autoradiogram showing nonspecific binding of ^{125}I-gastrin (in the presence of 100 nM unlabeled gastrin). *D*, autoradiogram showing total binding of ^{125}I-CCK analogue. Tumor tissue is massively labeled. *E* and *F*, autoradiograms showing nonspecific binding of ^{125}I-CCK analogue in the presence of 50 nM sulfated CCK-8 (*E*) and 50 nM gastrin (*F*).

Pharmacological characterization of the CCK-B/gastrin receptors in a small cell lung cancer by competition experiments is shown in Fig. 4. Gastrin and sulfated and nonsulfated CCK-8 displace completely in the nanomolar range the ^{125}I-gastrin ligand, whereas somatostatin-14 has no effect. In the same tumor, sulfated and nonsulfated CCK-8 and gastrin displace in the nanomolar range the ^{125}I-CCK decapeptide ligand, whereas somatostatin-14 is inactive. In these examples, the nonsulfated CCK-8 is approximately as potent as gastrin, both being slightly less potent than sulfated CCK-8.

Conversely, in a CCK-A receptor-expressing tumor, such as the gastroenteropancreatic tumor shown in Fig. 4, the competition experiment gives different results. Whereas sulfated CCK-8 can displace the ^{125}I-CCK decapeptide analogue in the nanomolar range, both gastrin and nonsulfated CCK-8 need a 1000 times higher concentration to displace the ligand. Somatostatin is inactive.

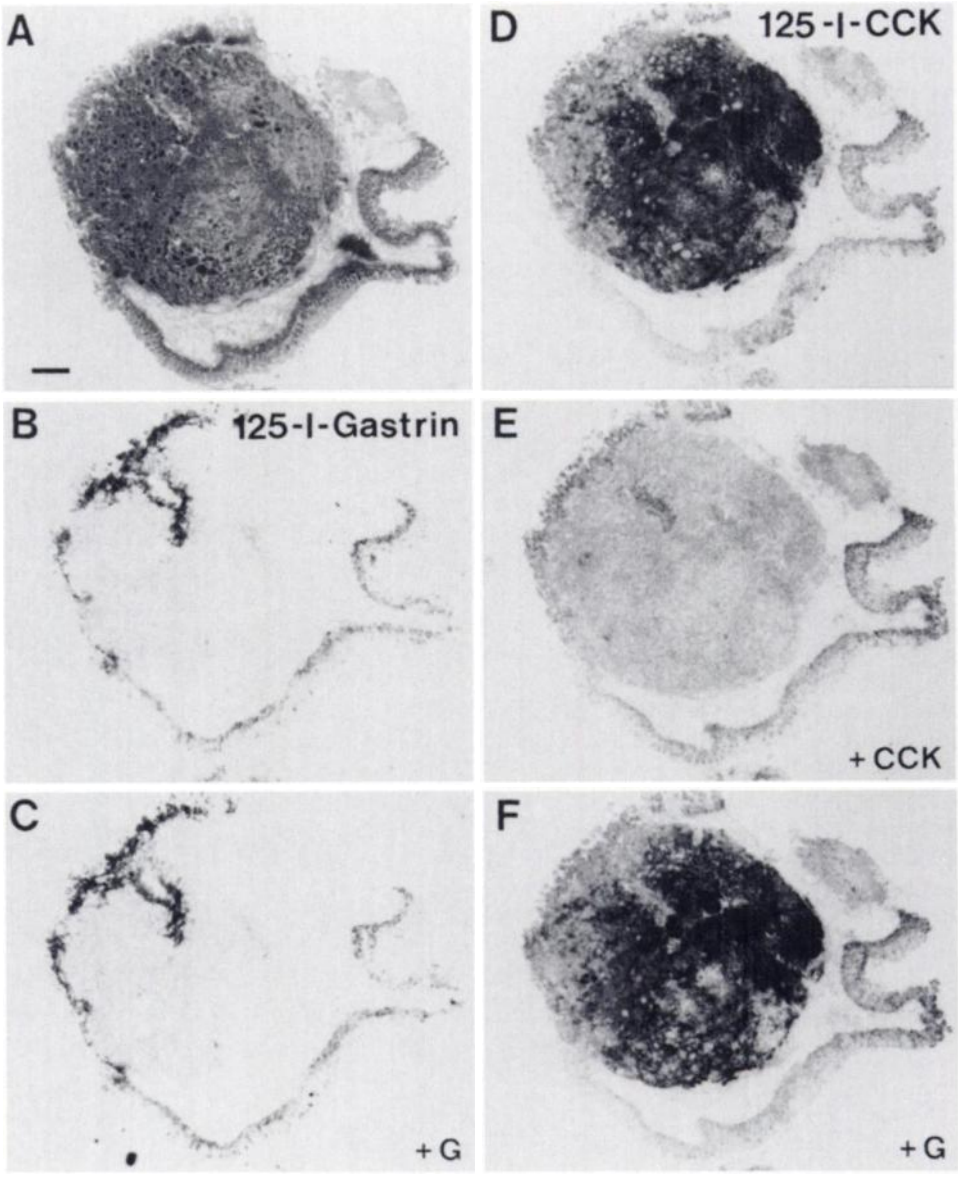

Fig. 3. *A–F, in vitro* receptor autoradiographic identification of CCK-A receptors in a human neuroendocrine gastroenteropancreatic tumor. *A*, H&E-stained section showing the tumor tissue. *Bar*, 1 mm. *B*, autoradiogram showing total binding of ^{125}I-gastrin. The tumor tissue is not labeled. *C*, autoradiogram showing nonspecific binding of ^{125}I-gastrin (in the presence of 100 nM unlabeled gastrin). *D*, autoradiogram showing total binding of ^{125}I-CCK analogue. Tumor tissue is massively labeled. *E* and *F*, autoradiograms showing nonspecific binding of ^{125}I-CCK analogue in the presence of 50 nM sulfated CCK-8 (*E*) and 50 nM gastrin (*F*). Because ^{125}I-CCK is displaced fully by CCK (*E*) but not by gastrin (*F*), all receptors belong to the CCK-A type.

Two nonpeptide CCK receptor antagonists were also tested in displacement experiments. As shown in Fig. 5, the CCK-A receptor-selective antagonist L-364,718 had a higher affinity (IC_{50}, 0.4 nM) in a CCK-A receptor expressing meningioma than L-365,260 (IC_{50}, 250 nM), a CCK-B receptor-selective antagonist, whereas the reverse was true in a CCK-B receptor-expressing breast tumor (IC_{50}, 20 nM for L-365,260; IC_{50}, 400 nM for L-364,718). The same rank order of potency of these antagonists had been found previously in the normal human gastrointestinal tract.[3]

On the basis of the above-mentioned results, we designed a CCK decapeptide analogue, the nonsulfated (D-Tyr-Gly, Nle^{28,31})-CCK 26–33, with the aim of having a short CCK analogue that would bind with high affinity and selectively to the CCK-B receptor, for future use as a specific radioligand for CCK-B receptors, as an alternative to the much longer gastrin ligand. As shown in a CCK-B receptor-expressing breast tumor (Fig. 5), this nonsulfated CCK-10 analogue revealed a high affinity (IC_{50}, 0.6 nM), close to that of sulfated CCK-8. Conversely, in the CCK-A receptor-expressing meningioma (Fig. 5), this CCK-10 analogue had a rather low affinity (IC_{50}, 210 nM), similar to that of the CCK-B receptor-selective antagonist L-365,260, approximately 3 orders of magnitude less potent than sulfated CCK-8. The subsequent use of nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33 as a radioligand confirmed its ability to label CCK-B receptors, because CCK-B receptor-positive tumors tested in *in vitro* autoradiography experiments were labeled by this ligand, whereas CCK-A receptor-positive or CCK-B receptor-negative tumors were not labeled. As seen in Fig. 6, the potencies of several analogues in a CCK-B receptor-positive medullary thyroid carcinoma to displace the nonsulfated ^{125}I-CCK decapeptide analogue corresponded to their potencies to displace ^{125}I-gastrin. The cellular localization was also the same for both ligands (Fig. 6). Radioligands with the iodination on either tyrosine in position 1 or 4 of the nonsulfated CCK-10 gave comparable results.

In gastroenteropancreatic tumors, both CCK-B and CCK-A receptors were found (Table 1), however, usually not concomitantly in the same tumor. In the 10 gastrin-producing tumors or gastrinomas of this group, no CCK-B/gastrin receptors could be identified, but CCK-A receptors were present in 4 of 10 cases. A prewashing step including 10^{-6} M GTP was added with the aim of removing all endogenous gastrin bound to the receptor. Despite this precaution, we cannot

[3] J. C. Reubi, unpublished data.

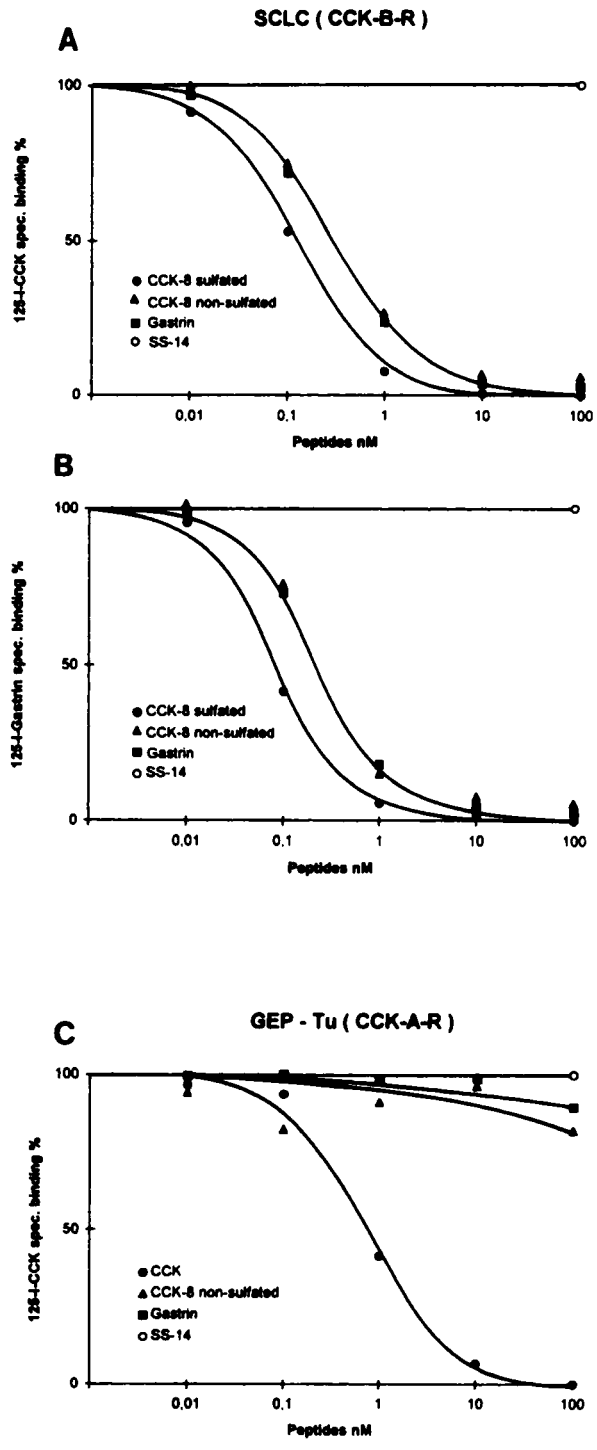

Fig. 4. CCK-B receptors in a small cell lung cancer (*A* and *B*) and CCK-A receptors in a gastroenteropancreatic tumor (*C*). *A*, a typical displacement experiment of ^{125}I-CCK analogue in tissue sections from a human small cell lung cancer. Tissue sections were incubated with 20,000 cpm/100 μl ^{125}I-CCK and increasing concentrations of unlabeled sulfated CCK-8 (●), nonsulfated CCK-8 (▲), and gastrin (■), and 100 nM somatostatin (○; *SS-14*). *B*, a typical displacement experiment of ^{125}I-gastrin in tissue sections from the same small cell lung cancer, incubated with 25,000 cpm/100 μl ^{125}I-gastrin and increasing concentrations of unlabeled sulfated CCK-8 (●), nonsulfated CCK-8 (▲), and gastrin (■), and 100 nM somatostatin (○). *Each point* represents the absorbance of binding measured in the tumor area. Nonspecific binding was subtracted from all values. The displacement of both ligands in the nanomolar range, not only by sulfated CCK-8 but also by gastrin and nonsulfated CCK-8, indicates the presence of CCK-B/gastrin receptors. *C*, a typical displacement experiment of ^{125}I-CCK analogue in tissue sections from a human CCK-A receptor-expressing gastroenteropancreatic tumor. Tissue sections were incubated with 20,000 cpm/100 μl ^{125}I-CCK and increasing concentrations of unlabeled sulfated CCK-8 (●), nonsulfated CCK-8 (▲), and gastrin (■), and 100 nM somatostatin (○; *SS-14*). *Each point* represents the absorbance of binding measured in the tumor area. Nonspecific binding was subtracted from all values. The low-affinity displacement by gastrin but high-affinity displacement by sulfated CCK-8 indicates CCK-A receptors in the tumor.

completely exclude the possibility that CCK-B/gastrin receptors are expressed in these tumors but remain masked in our experiments due to excessive endogenous gastrin. The 22 colorectal cancers, which are known to synthesize progastrin (14, 36), were all CCK receptor negative. However, small cell lung cancers, which are also known to synthesize gastrin in significant amounts (37), were found to express CCK-B receptors in high amounts, suggesting that a putative receptor occupancy by endogenous gastrin is not interfering significantly with the present CCK-B receptor identification in gastrin-producing tumors. In receptor-negative tumors, such as colorectal cancers, an internal positive control of the quality of the tissue was used: in almost all tumor samples, adjacent normal tissue consisting in smooth muscle layers and neural plexus were found to express significant amounts of

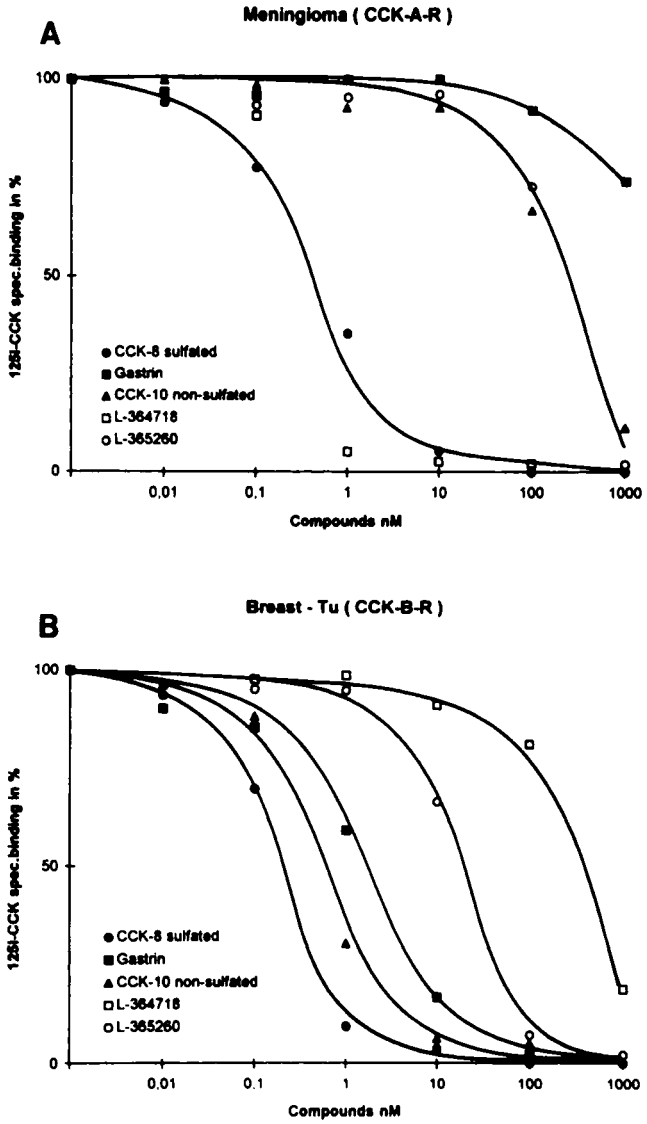

Fig. 5. Rank order of potencies of several CCK-A or CCK-B receptor selective analogues in a meningioma and a breast tumor. *A*, a typical displacement experiment of ^{125}I-CCK analogue in tissue sections from a human CCK-A receptor-expressing meningioma. Tissue sections were incubated with 20,000 cpm/100 μl ^{125}I-CCK and increasing concentrations of unlabeled sulfated CCK-8 (●), gastrin (■), nonsulfated CCK-10 analogue (▲), L364–718 (□), and L365–260 (○). The CCK-A-selective antagonist L364–718 displaces the ligand with high affinity but L365–260 or nonsulfated CCK-10 with low affinity only. *B*, a typical displacement experiment of ^{125}I-CCK analogue in tissue sections from a human CCK-B receptor-expressing breast tumor. Tissue sections were incubated with 20,000 cpm/100 μl ^{125}I-CCK and increasing concentrations of unlabeled sulfated CCK-8 (●), gastrin (■), nonsulfated CCK-10 analogue (▲), L364–718 (□), and L365–260 (○). The CCK-10 analogue and L365–260 displace the radioligand at lower concentrations than L364–718.

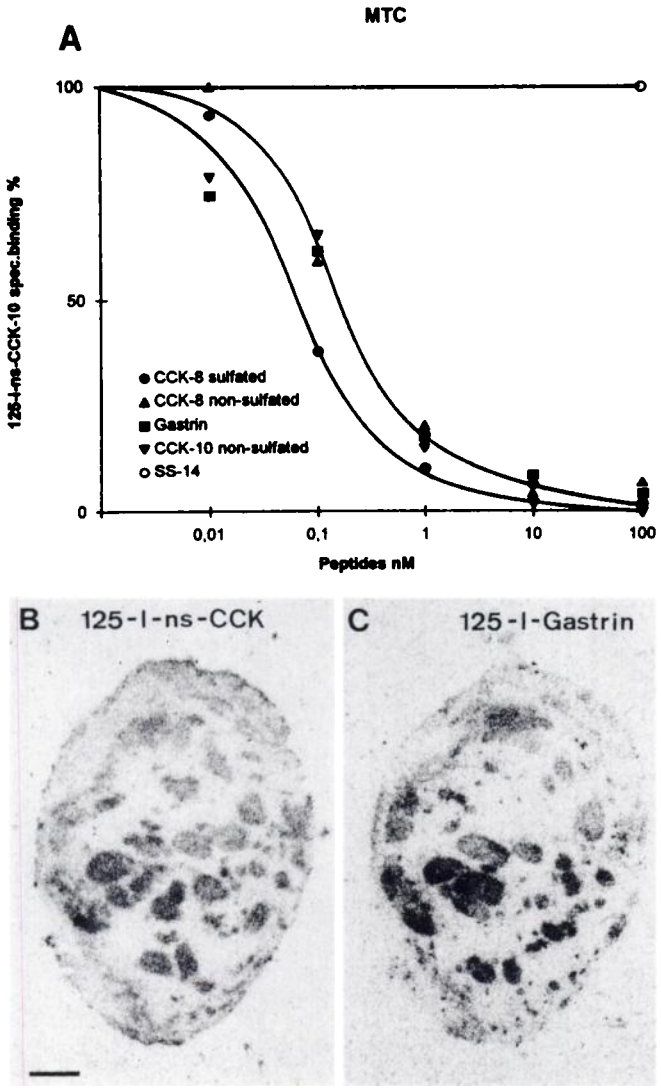

Fig. 6. Displacement curve of nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33 (*125I-ns-CCK-10*). *A*, curve represents a typical displacement experiment of nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33 analogue in tissue sections from a human CCK-B receptor-expressing medullary thyroid cancer. Tissue sections were incubated with 20,000 cpm/100 μl nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33 and increasing concentrations of unlabeled sulfated CCK-8 (●), nonsulfated CCK-8 (▲), gastrin (■), and nonsulfated CCK-10 (▼), and 100 nM somatostatin (○; *SS-14*). *B*, autoradiograms showing total binding of nonsulfated ^{125}I-(D-Tyr-Gly, Nle^{28,31})-CCK 26–33 (*left*) and ^{125}I-gastrin (*right*) in a CCK-B receptor-positive medullary thyroid cancer. Nonspecific binding was negligible in both cases. Both ligands label the same tumor structures.

CCK receptors, suggesting therefore that the negative receptor status found in these tumors was not simply due to receptor degradation.

Gastrin mRNA and CCK mRNA were measured with *in situ* hybridization histochemistry in several types of tissues. All the gastrinomas (5 of 5), all the small cell lung cancers (10 of 10), and a majority of ovarian cancers (5 of 10 adenocarcinomas and 3 of 3 stromal cancers), all tumors previously reported to synthesize gastrin (14, 37–39), were positive for gastrin mRNA, whereas the 24 medullary thyroid carcinomas were all negative. Examples of a gastrinoma, a small cell lung cancer, and a medullary thyroid carcinoma are shown in Fig. 7. CCK mRNA was not detected in medullary thyroid carcinomas, but was shown as positive control to be expressed in the human brain. This suggests that medullary thyroid carcinomas have no autocrine growth stimulation by gastrin or CCK. This is different from small cell lung cancers, which can have simultaneously CCK-B receptors and gastrin mRNA, as seen in an example in Fig. 8. Most interestingly, the basis for an autocrine feedback growth regulation by gastrin could also exist in the few CCK-B receptor-positive breast carcinomas, because many breast cancers (16 of 22), including all those expressing CCK-B receptors, were shown to have low but measurable amounts of gastrin mRNA in the tumor cells (Fig. 9). The same autocrine feedback growth regulation may possibly exist in ovarian cancers, especially the stromal type (Fig. 9), because all three cases expressed both CCK-B receptors and gastrin mRNA.

DISCUSSION

The present study shows with two different complementary *in vitro* binding methods that selected human tumor types can express CCK-B or CCK-A receptors. In particular, the medullary thyroid carcinomas, small cell lung cancers, astrocytomas, and stromal ovarian cancers (granulosa cell tumors) can frequently express CCK-B/gastrin receptors. Several other tumor types, such as gastroenteropancreatic tumors, meningiomas, endometrial and ovarian adenocarcinomas, and breast carcinomas occasionally express CCK-B receptors. CCK-A receptors are in general expressed rarely in human tumors and were found primarily in significant numbers in gastroenteropancreatic tumors, meningiomas, and neuroblastomas. The incidence of CCK receptor expression in human tumors is therefore considerably lower than that for other peptide receptors, such as VIP and somatostatin receptors (2, 3, 40). To our knowledge, this is the first study evaluating CCK receptors in large numbers of primary human cancers with *in vitro* receptor autoradiography.

For optimal proof of the receptor identity, the CCK receptors were evaluated with two different radioligands; identical results were obtained with both ^{125}I-gastrin and ^{125}I-CCK decapeptide analogue as radioligands in all CCK-B receptor-positive tumors. Indeed, the tumors having CCK-B/gastrin receptors could, as expected (25), be labeled with ^{125}I-gastrin, which was displaced completely by unlabeled gastrin; similarly, on adjacent tissue sections, these tumors were labeled with the ^{125}I-CCK ligand and characterized by a complete displacement of the ligand by nanomolar concentrations of CCK and gastrin, as shown previously for canine CCK-B receptors (17). The rank order of potency and the selectivity of several analogues further confirmed the identification as CCK-A or CCK-B receptors. Nonsulfated CCK-8 or CCK-10 analogues and the antagonist L-365,260 were more potent on CCK-B receptors, whereas the CCK-A-selective L365–718 was much more potent on CCK-A receptors.

A novel gastrin receptor, different from the established CCK-B/gastrin receptor, has been reported to be expressed by Swiss 3T3 fibroblasts (41). Although this novel receptor has a high affinity for gastrin, it has only a low affinity for CCK-8 (41). It is unlikely that the gastrin receptor found in the present study represents this novel gastrin receptor, because the former has, as the CCK-B/gastrin receptor, a high affinity both for gastrin and for CCK-8. It is also unlikely that the present study identifies CCK-C receptors, which have only a micromolar affinity for gastrin (42).

The present findings indicate indirectly that the role of gastrin in the body is wider than previously expected, not being restricted to gastrointestinal or neuronal tissues, but including diseased endocrine (thyroid, ovary, and endometrium) and breast tissues that had not been related to a gastrin action previously. The results of the stromal ovarian cancers are particularly intriguing and will require extensive additional investigations, once additional samples of this very rare tumor will have been collected.

A crucial question is whether these expressed CCK-B receptors will play a role in the development and growth of the receptor-positive medullary thyroid carcinomas, astrocytomas, breast, or ovarian tumors. Indeed, gastrin and CCK have an established role as growth

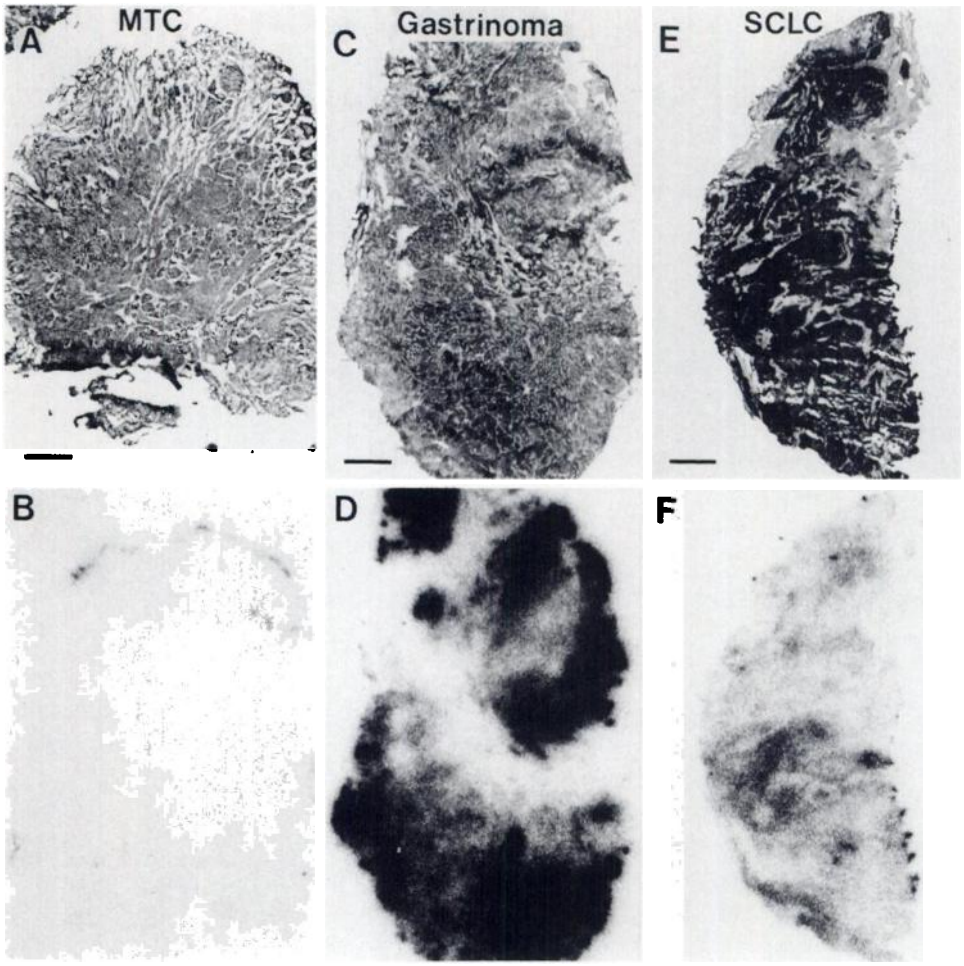

Fig. 7. Different gastrin mRNA expression in three different tumor types: medullary thyroid carcinoma (*A* and *B*), gastrinoma (*C* and *D*), and small cell lung cancer (*E* and *F*). *A*, *C*, and *E*, H&E-stained sections. *Bar*, 1 mm. *B*, *D*, and *F*, autoradiograms showing gastrin mRNA. No gastrin mRNA is found in medullary thyroid carcinoma, whereas high abundance is found in gastrinoma and moderate in small cell lung cancer.

factors in certain normal tissues and tumors such as small cell lung cancers and colon cancers (20). It has been questioned recently whether the growth stimulation of colorectal cancers by gastrin could be a consequence of tumorally synthesized and secreted gastrin (paracrine or autocrine action) or whether it was due to physiological, circulating gastrin originating from distant gastrointestinal tissue; in particular, the question whether drug-induced hypergastrinemia may be responsible for an accelerated growth of colon cancers has been raised (43). Although earlier studies (22) have suggested the presence of CCK-B receptors in colonic cancers, subsequent studies, including

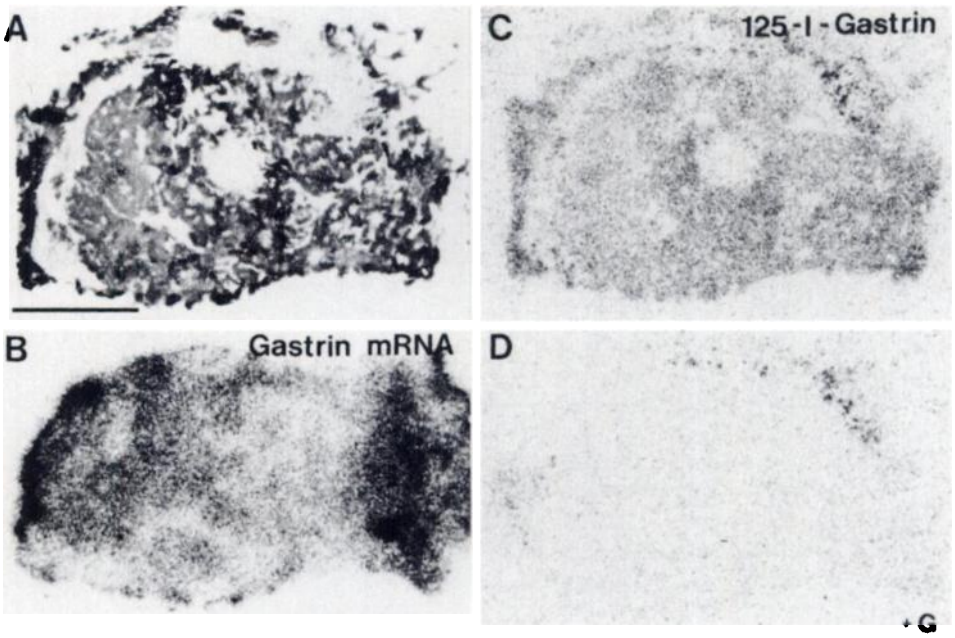

Fig. 8. CCK-B/gastrin receptors and gastrin mRNA in small cell lung cancer. *A*, H&E-stained section. *Bar*, 1 mm. *B*, autoradiogram showing gastrin mRNA. *C*, autoradiogram showing total binding of ^{125}I-gastrin. *D*, autoradiogram showing nonspecific binding of ^{125}I-gastrin. CCK-B receptors and gastrin mRNA are present in the same tumor.

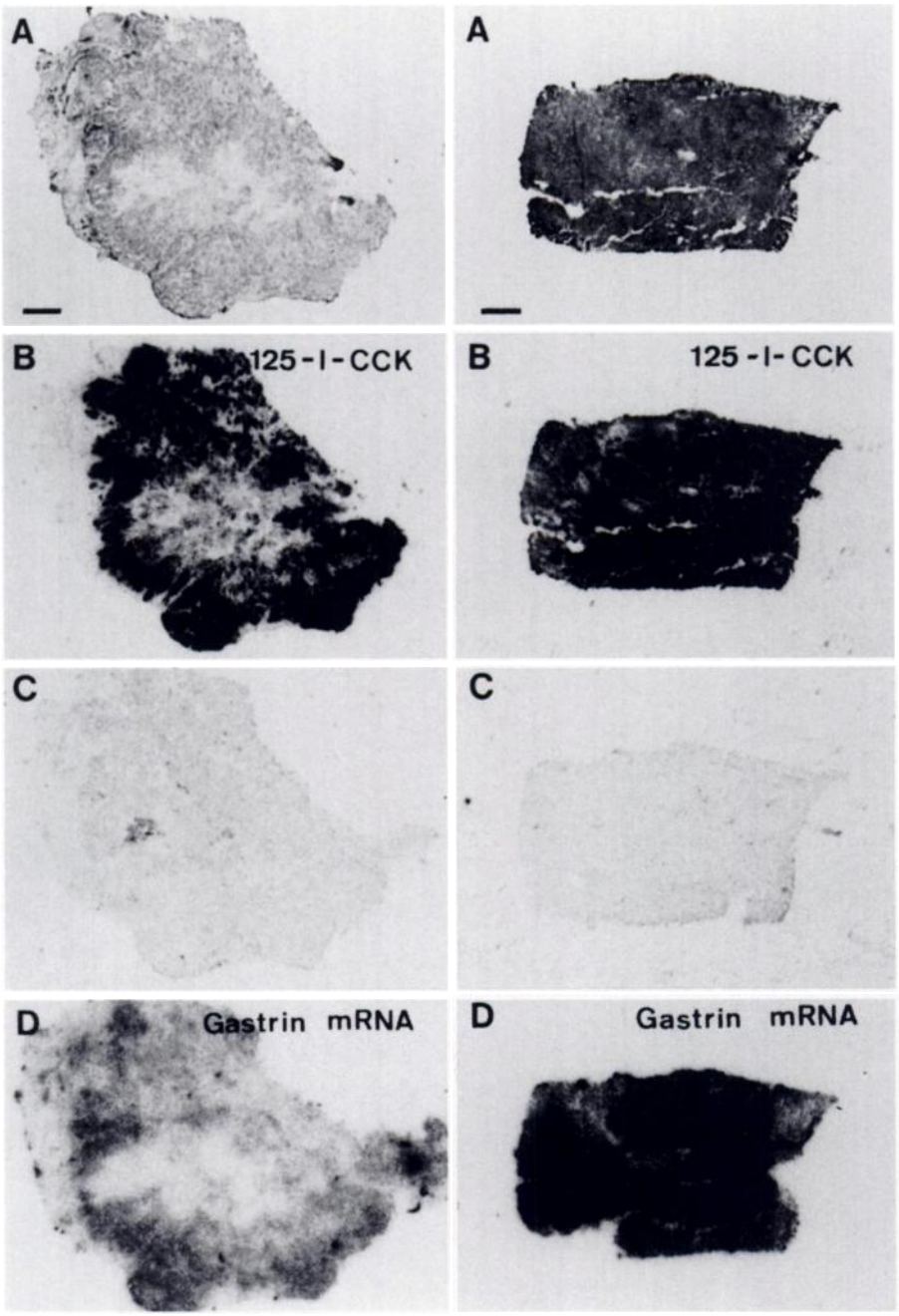

Fig. 9. CCK-B/gastrin receptors and gastrin mRNA in a breast tumor (*left*) and a stromal ovarian tumor (*right*). *A*, H&E-stained sections showing the tumor tissue. *Bar*, 1 mm. *B*, autoradiograms showing total binding of ^{125}I-CCK analogue. Tumor tissues are massively labeled. *C*, autoradiograms showing nonspecific binding of ^{125}I-CCK analogue in the presence of 50 nM gastrin. *D*, autoradiograms showing gastrin mRNA expressed in both tumors.

the present one, failed to identify high-affinity CCK-B receptors in these tumors (23). On the basis of the high amount and prevalence of CCK-B receptors in medullary thyroid carcinomas, small cell lung cancers, astrocytomas, and stromal ovarian cancers, the extent to which gastrin could affect the growth of these tumors can be expected to be larger than for colonic cancers.

The simultaneous expression of a peptide and its receptor in selected tumors has been shown, as in the case of bombesin/gastrin-releasing peptide in small cell lung cancers (44), to represent a potent autocrine feedback mechanism for tumor growth regulation. In medullary thyroid carcinomas, our *in situ* hybridization studies could not detect gastrin mRNA, whereas gastrinomas, as controls, showed abundant gastrin mRNA, as demonstrated previously (45, 46). In the same medullary thyroid carcinomas, we were also unable to identify CCK mRNA. However, a study by Rehfeld *et al.* (47) showed that medullary thyroid carcinomas can contain nonsulfated CCK, as measured with specific radioimmunoassays. Although specific, the *in situ* hybridization methods may not be sufficiently sensitive to identify small amounts of CCK mRNA, which could lead to small amounts of nonsulfated CCK. Because nonsulfated CCK binds to CCK-B receptors with high affinity, an autocrine feedback mechanism of growth control in medullary thyroid carcinomas is conceivable through tumorally produced nonsulfated CCK. Alternatively, the nonsulfated CCK found in medullary thyroid carcinomas may originate from a distant source. Furthermore, elevated circulating gastrin, in particular in conditions of drug-induced hypergastrinemia, could also reach the tumor and lead to gastrin-induced medullary thyroid carcinomas growth through its CCK-B receptors. Clinical investigations to evaluate this aspect should be started in the near future.

The molecular basis for an autocrine growth stimulation by gastrin, *i.e.*, the presence of gastrin and gastrin receptors, has been well established in various tumor models (20) and is confirmed by the

present results for human small cell lung cancers primary tumors. Moreover, this autocrine regulation by gastrin may also be present in stromal ovarian cancers and in the few cases of CCK-B receptor-positive ovarian adenocarcinomas and breast cancers, a finding that may be of considerable pathogenic importance for these tumors.

A potentially important clinical implication of the present results is the possible use of ^{123}I-labeled gastrin or CCK analogues, *e.g.*, nonsulfated iodinated (D-Tyr-Gly, Nle^{28,31})-CCK 26–33, to identify and localize *in vivo* in patients the CCK-B-receptor-positive tumors and their metastases. As shown previously with somatostatin receptor and VIP receptor scintigraphy, i.v. injected peptide radioligands are expected to bind rapidly and with high affinity to the respective tumoral receptors and can be then identified with gamma camera scanning techniques (5, 6). For medullary thyroid carcinomas, a high tumor:background ratio can be expected in the thyroid region, given that the normal thyroid gland does not express measurable amounts of CCK-B receptors. The homogeneous distribution of the receptors seen in all medullary thyroid carcinomas tested suggests that all tumor tissue grown in a patient, including metastases, is likely to be identified *in vivo*. The knowledge that nonmedullary thyroid tumors and parathyroid adenomas do not express measurable amounts of receptors suggests strongly that this diagnostic tool could have a differential diagnostic value, because a positive scan in the thyroid region may indicate solely the presence of a medullary thyroid carcinoma.

These differential diagnostic implications apply also to CCK-B receptor-expressing small cell lung cancers. Because small cell lung cancers but not non-small cell lung cancers express CCK-B receptors, labeled gastrin or CCK analogues could be used for the *in vivo* differential diagnosis of lung cancers. In this regard, the newly designed nonsulfated (D-Tyr-Gly, Nle^{28,31})-CCK 26–33 could represent a potentially useful tool as an iodinated radioligand to detect CCK-B receptors expressed by human tumors, *in vitro* and *in vivo*, especially given that iodination can occur on both tyrosines without affecting the binding properties of the molecule. For the future, however, CCK analogues linked to a chelator (*e.g.*, diethylenetriaminepentaacetic acid) would be preferable for practical reasons (5).

Finally, the presence of CCK-B receptors in several tumors may suggest the use of new generations of nonpeptide CCK-B receptor-selective antagonists to treat patients, once it has been demonstrated that gastrin has growth-stimulatory properties in all these CCK-B receptor-positive tumors, as it was shown in small cell lung cancers. Although L-365,260 is more potent than L-364,718 on CCK-B receptors, it is probably not sufficiently potent and selective to represent an adequate therapeutical tool; more potent CCK-B antagonists (48) are needed.

The present results clearly point toward medullary thyroid carcinomas, small cell lung cancers, astrocytomas, and stromal ovarian cancers as a major target of interest for gastrin research, based on the high CCK-B receptor content of these tumors.

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