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Detection of Somatostatin Receptors in Surgical and Percutaneous Needle Biopsy Samples of Carcinoids and Islet Cell Carcinomas

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ABSTRACT

Somatostatin (SS) receptor status was investigated in the tumor tissues from 62 patients with carcinoid tumors and 15 patients with islet cell carcinomas using receptor autoradiography techniques with two different iodinated somatostatin analogues as radioligands, a [Leu^8, DTrp^{22}, Tyr^{25}]somatostatin-28 and a somatostatin octapeptide, Tyr^3-octreotide. The carcinoid tumors were either primaries (n = 32) or metastases (n = 43), sampled as surgical specimens or as small needle liver biopsies. Fifty-four of 62 carcinoid patients had SS receptor-positive tumors (87%). All 15 islet cell carcinoma patients had positive tumors (4 primaries, 11 metastases), *i.e.*, 3 vipomas, 3 insulinomas, 2 glucagonomas, 1 gastrinoma, 2 polyfunctional tumors, and 4 nonfunctioning tumors. Saturation and competition experiments on tissue sections revealed saturable, high affinity binding sites pharmacologically specific for bioactive SS analogues. In a majority of the tumors, the receptors were densely distributed and were always homogeneously found in the whole tumor. All except two tumors were labeled with both radioligands. Multiple liver metastases (n = 16) from three different patients were all shown to contain a comparable amount of receptors. SS receptors could be demonstrated even in very small tissue samples of liver metastases obtained by percutaneous liver biopsies (mean weight, 6.8 mg). The majority of the eight SS receptor-negative carcinoids were mainly bronchial carcinoids (n = 5), usually poorly differentiated. On the contrary, SS receptor-positive cases were never found to be anaplastic. All tumors except one from patients pretreated with octreotide (3 days to 3.8 years) were SS receptor positive. In the majority of carcinoids or islet cell carcinomas, the SS receptor status correlated with the *in vivo* biochemical response (hormone inhibition) to octreotide. These data demonstrate (*a*) the high prevalence of SS receptors in the primary tumors of both carcinoids and islet cell carcinomas, (*b*) their presence in metastases as well, (*c*) their continuous expression even during long term octreotide therapy, (*d*) the possibility of measuring SS receptors in percutaneous needle liver biopsies, and (*e*) the evidence of their functionality. This study therefore suggests that tumoral SS receptors may be the likely molecular basis for octreotide action and may be an important parameter for predicting the therapeutic efficacy of SS analogues in carcinoids and islet cell carcinomas.

INTRODUCTION

The variable symptom complexes associated with malignant carcinoid tumors and the peptide-producing metastatic islet cell carcinomas (1, 2) have posed therapeutic challenges to clinicians since they were first discussed in the medical literature more than three decades ago. These malignant diseases produce a constellation of clinical problems which create unique difficulties in management for the surgical and medical oncologist (3).

Recently, however, an analogue of the neuropeptide somatostatin, octreotide (Sandostatin, SMS 201-995), has been synthesized and shown to be therapeutically beneficial in reducing most of the symptomatology in both islet cell carcinoma and carcinoid patients (4–9).

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The exact mechanism of action of the SS[2] analogue in these tumors is not fully understood (10). We have recently shown in a small study that a high percentage of hormone-producing islet cell carcinomas such as vipomas, gastrinomas, insulinomas, or GRFomas do possess specific SS receptors (11–14). This suggests that SS analogues such as octreotide may have a direct effect on the tumor tissue itself, by regulation of the hormone secretion and/or by regulating tumor growth (10, 15, 16). However, because of the numerous actions of SS in the body (17), an indirect effect through a physiological SS target still remains conceivable (15, 16). At the present time, information on SS receptor prevalence is completely lacking for carcinoids, which represent the largest group of tumors among the gastroenteropancreatic tumors. Carcinoid tumors arise most often in small intestine and metastasize early into the liver; less frequently they arise from other organs such as the lung, thymus, pancreas, or stomach (18). In all of these cases, episodic flushing and diarrhea are the most common initial symptoms, whereas biochemically an increase in serotonin (in the form of urinary 5-hydroxyindoleacetic acid) and sometimes substance P can be measured (2, 18).

In the present study, we evaluated for the first time the SS receptor status of carcinoid tumors in a large number of cases. We tested additional islet cell carcinomas, including glucagonomas and nonfunctioning tumors, two types of tumors never tested for SS receptors before. Furthermore, we evaluated the feasibility of measuring SS receptors in nonsurgically removed tumors, *i.e.*, in ultrasound-directed percutaneous needle biopsies for liver metastases, as an alternative to the SS receptor measurement in surgical samples. This method permits correlation of SS receptor status and the *in vivo* effect of octreotide on hormone levels in these tumors. This may ultimately provide information about the functionality of these receptors and the possible predictive value of SS receptor measurements for future octreotide therapy.

MATERIALS AND METHODS

Tumor samples from 62 carcinoid patients, including 32 primaries and 43 metastases, were investigated (Table 1). Sixty samples were obtained after surgical removal; in the remaining cases (n = 15) they were obtained through percutaneous needle biopsies of the liver metastases.

The islet cell carcinomas investigated included three vipomas, three insulinomas, two glucagonomas, one gastrinoma, as well as two polyfunctional and four nonfunctioning tumors. Nine of these cases were liver biopsies. The biopsies were performed under local anesthesia. A Bard Biopty instrument with an 18-gauge Biopty-Cut biopsy needle that had a sampling notch of 17 mm was used with ultrasound guidance (19, 20).

All tumor tissue from surgical and needle biopsy sampling was immediately frozen and processed later for autoradiography. The cases in which needle biopsies of the liver were performed were subsequently treated with octreotide therapy and had monitoring of hormone levels. In three cases of carcinoid, multiple liver metastases (nine, four, and

[2] The abbreviations used are: SS, somatostatin; GRFoma, a carcinoma that secretes growth hormone-releasing factor; ACTH, adrenocorticotropic hormone.

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Table 1 *Incidence of SS receptors in carcinoids*

Case no.	Tumor and site[a]	Sample[b]	Receptor autoradiography[c,d] SS-28 R	Receptor autoradiography[c,d] 204-090 R	*In vivo* response to Sandostatin[c]	Sandostatin pretreatment
1 MC	Small intest. carcinoid	m Liver biopsy (n)	+	+	Yes	No
2 MR	Small intest. carcinoid	m Liver biopsy (n)	++	++	NT	No
3 BY	Small intest. carcinoid	m Liver met.	++	++	NT	No
4 MO	Thymic ACTH-prod. carcinoid	m Lymph node met.	++	−	No	
5 HA	Small intest. carcinoid	m Lymph node met.	+	++	Yes	No
6 KZ	Small intest. carcinoid	m Liver biopsy (n)	+	+	Yes	No
7 AT	Small intest. carcinoid	m Liver biopsy (n)	++	++	Yes	No
8 BX	Bronch. carcinoid	p	+	+	Yes	No
9 KS	Small intest. carcinoid	m Breast met.	++	++	Yes	No
10 RY	Pancreatic carcinoid	m Liver met.	++	++	NT	No
11 PH	Small intest. carcinoid	p	+	++	NT	No
12 GI	Small intest. carcinoid	p	++	++	Yes	Yes (13 mo)
13 SK	Small intest. carcinoid	p	+	+	NT	Yes (3 days)
14 BR	Carcinoid	m Liver biopsy (n)	+	+	Yes	No
15 JP	Small intest. carcinoid		NT	++	NT	No
16 MU	Gastric carcinoid	m Liver met.	+	+	NT	No
17 BL	Small intest. carcinoid	m Liver met.	++	++	Yes	Yes (1 mo)
18 HE	Small intest. carcinoid	p	++	++	NT	No
19 MG	Carcinoid	m Liver met.	++	++	NT	No
20 BU	Bronch. carcinoid	m Liver met.	++	++	Yes	Yes (3.8 yr)
21 HI	Bronch. carcinoid	p	+	+	NT	No
22 HM	Small intest. carcinoid	p	++	++	NT	No
23 ST	Bronch. carcinoid	p	++	++	NT	No
24 MK	Small intest. carcinoid	p	++	++	NT	No
25 FR	Small intest. carcinoid	p	+	+	NT	No
26 LK	Small intest. carcinoid	p	+	+	Yes	Yes (5 mo)
27 TI	Bronch. carcinoid	m Liver biopsy (n)	+	+	Yes	No
28 EN	Carcinoid	m Liver biopsy (n)	++	++	Yes	No
29 KE	Anapl. bronch. carcinoid	m Lymph node met.	−	−	NT	No
30 GN	Bronch. ACTH-prod. carcinoid	m Liver biopsy (n)	−	−	No	No
31 KN	Thymic carcinoid	p	+	+	NT	No
32 LA	Bronch. carcinoid	p	+	−	NT	No
33 RO	Bronch. carcinoid	p	+	++	NT	No
34 BT	Small intest. carcinoid	p	+	++	NT	No
35 BE	Bronch. carcinoid	p	−	−	NT	No
36 ML	Bronch. carcinoid	p	++	++	NT	No
37 MA	Bronch. carcinoid	p	−	−	NT	No
38 WL	Bronch. ACTH-prod. carcinoid	m Lymph node met.	++	++	NT	No
39 WI	Small intest. carcinoid	p	++	++	NT	No
40 BI	Small intest. carcinoid	p	++	++	NT	No
41 WA	Small intest. carcinoid	m Liver biopsy (n)	+	+	NT	No
42 EC	Small intest. carcinoid	m Liver met.	++	++	NT	No
43 TW	Bronch. carcinoid		++	++/−	NT	No
44 ST	Carcinoid	m Lymph node met.	NT	++	NT	No
45 TG	Small intest. carcinoid	p	NT	++	NT	No
46 BA	Small intest. carcinoid	m Liver biopsy (n)	NT	+	Yes	No
47 PA	Small intest. carcinoid	p	NT	++/−	No	Yes (3 mo)
48 LE	Bronch. carcinoid	p	NT	−	NT	No
49 WH	Small intest. carcinoid	p	NT	+	NT	No
50 HM	Small intest. carcinoid	m Liver biopsy (n)	NT	+	Yes	No
51 RO	Carcinoid	m Liver biopsy (n)	NT	−	Yes	No
52 ME	Small intest. carcinoid	m Liver biopsy (n)	NT	+	Yes	No
53 CO	Small intest. carcinoid	p	NT	++	NT	No
54 RT	Carcinoid	m	NT	++	NT	Yes (3 days)
55 MD	Small intest. carcinoid	p	NT	−	Yes	Yes (2 mo)
56 MJ	Small intest. carcinoid	p	NT	++	NT	No
57 CA	Anapl. rectal carcinoid	m Liver biopsy (n)	NT	−	NT	No
58 PT	Small intest. carcinoid	m Liver biopsy (n)	NT	+	No	Yes (10 mo)
59 BE	Small intest. carcinoid	m	NT	++	NT	No
60 PE	Bronch. carcinoid		NT	+	NT	No
61 YK	Bronch. carcinoid	p	NT	+	NT	No
62 SW	Bronch. carcinoid	p	NT	+	NT	No

[a] Intest., intestine; prod., producing; bronch., bronchial; anapl., anaplastic.
[b] p, primary tumor; m, metastases; (n), small needle biopsy; met., metastases.
[c] R, receptors; NT, not tested.
[d] SS receptors: +, low to moderate density; ++, high density; −, absence of receptors; ++/−, receptors are nonhomogeneously distributed.

three metastases, respectively) of various size were taken from different regions of the liver. The study also includes tumor material from nine patients pretreated with octreotide for periods ranging from 3 days to 3.8 years. All other cases were not pretreated with somatostatin analogues.

SS Receptor Measurements. All tumor tissue was frozen within 15 min after removal. The biopsies were then embedded immediately to prevent dehydration of the small samples. They were kept frozen at −70°C until they were used for receptor autoradiography.

Receptor Autoradiography. SS receptors were visualized by autoradiography as described previously (13, 21) using the stable octapeptide ^{125}I-204-090, a Tyr^3 analogue of octreotide (22), or the somatostatin-28 analogue ^{125}I-[Leu^8, DTrp^{22}, Tyr^{25}]-SS-28. Both ligands were iodinated and purified as described previously (13, 23) and characterized in standard binding assays. For autoradiography, the tumors were cut on a cryostat (Leitz 1720) in 10-μm sections, mounted on precleaned microscope slides, and stored at −20°C for at least 3 days to improve adhesion of the tissue to the slide. The sections were incubated with iodinated ligand (0.16×10^6 dpm/ml) for 2 h at ambient temperature in 170 mmol/liter Tris-HCl buffer, pH 7.4, containing 10 g/liter bovine serum albumin, 40 mg/liter bacitracin, and 5 mmol/liter $MgCl_2$ to inhibit endogenous proteases. Nonspecific binding was determined by

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adding unlabeled 204-090 or SS-28, depending on the radioligand used, at a concentration of 1 μmol/liter. The incubated sections were washed twice for 5 min in cold incubation buffer containing 2.5 g/liter bovine serum albumin. After a brief dip in distilled water to remove excess salts, the sections were dried quickly, apposed to ^{3}H-labeled LKB films, and exposed for 1 week in X-ray cassettes. In addition to this procedure, several cases (including all octreotide-pretreated cases and all cases found to be SS receptor negative) underwent a prewash procedure in order to wash out putative residual tissue octreotide or SS. Prior to incubation with radioligands, therefore, the sections were preincubated twice for 10 min at ambient temperature in a 170-mmol/liter Tris-HCl buffer solution, pH 7.4.

In a limited number of cases, in particular in needle biopsies, displacement experiments using successive sections of a tumor were performed with increasing concentrations of various biologically active or inactive peptides. Furthermore, saturation experiments using increasing amounts of radioligand were performed in successive tumor tissue sections as well. The autoradiograms were quantified using a computer-assisted image processing system, as previously described (21). Tissue standards for iodinated compounds (Amersham Laboratories, Little Chalfront, England) were used for this purpose. The number of SS receptors represents a mean of the density measured over the whole tumor sample.

Some of the patients who had SS receptors measured in their tumors were studied, in the course of another investigation (20), for their responsiveness to somatostatin. Inhibition of excess hormone release by octreotide was measured over several months; patients were classified as responders and nonresponders according to the degree of inhibition.

RESULTS

Table 1 shows the SS receptor status in all of the studied carcinoids, and Table 2 demonstrates SS receptor incidence in islet cell carcinomas. Among the carcinoids, 54 of 62 were SS receptor positive, which represents an 87% incidence. In addition, in the three patients from whom multiple specimens were available from varying metastatic lesions (cases 20, 42, and 43), all 9, 4, and 3 liver metastases, respectively, were positive. All islet cell carcinomas, including 3 vipomas, 3 insulinomas, 2 glucagonomas, 1 gastrinoma, as well as 2 polyfunctional and 4 nonfunctioning tumors were positive. All except 2 samples among the 55 tumors tested with both radioligands showed labeling in both cases. The 2 others (both carcinoids) were labeled preferentially with SS-28 ligand. In the majority of cases, the labeling of receptors was homogeneous; moreover, a majority of cases showed a high density of SS receptors. An example is shown in Fig. 1, in which both tracers label densely the whole carcinoid tissue. Fig. 2 shows an example of the high density of receptors in another carcinoid tumor as compared to a low density of receptors in a region of normal gastrointestinal mucosa. Fig. 3 shows a unique tumor (case 43) in which a single carcinoid shows two histopathologically indistinguishable parts which each contain an SS receptor subtype: one part is labeled with both ligands; the other part is labeled only with the SS-28 analogue, similar to what is found in the brain substantia nigra (24). Fig. 4, *A* and *B*, demonstrates that receptors measured by autoradiography also show similar saturation curves and pharmacological specificity for SS analogues as found in binding experiments with homogenates (13, 21). Binding is saturable, of high affinity (Fig. 4*A*), and specific for bioactive SS analogues (Fig. 4*B*). Furthermore, the time course of association in a carcinoid was approximately 2 h on tissue sections. Fig. 5 shows that all four of the metastases in a single patient's liver contain a comparable density of receptors. Interestingly, the four metastases from a patient treated for 3.8 years with octreotide were all also found to be strongly positive (Fig. 6). All except one of the cases pretreated with octreotide had significant amounts of SS receptors in tumor tissue. In most of these cases, extensive prewashing revealed a higher receptor density than without prewashing, suggesting the presence of residual octreotide competing with the radioligand. In octreotide-free patients, prewashing procedures did not affect the SS receptor determination. Careful histopathological examination of all octreotide-treated cases did not reveal any unique features with regard to fibrosis, necrosis, or cell pleomorphism, which might have been a consequence of the long term octreotide therapy. SS receptor-negative cases were found only among the carcinoids: five of them were bronchial carcinoids, one ileal, one rectal, and one of unknown origin. One of them was an ACTH-producing tumor (Table 1). These SS receptor-negative tumors all belong to the group of atypical carcinoids (25), often being relatively undifferentiated ($n = 6$) or even anaplastic ($n = 1$). None of the SS receptor-positive cases was anaplastic. Fig. 7 shows the histopathological picture of two SS receptor-positive and two receptor-negative cases.

Small needle biopsies of liver metastases were performed in 15 carcinoids and 9 islet cell carcinoma cases. Fig. 8 shows a SS receptor-positive carcinoid metastasis together with a biopsy of normal liver which has only nonspecific binding sites. Fig. 9

Table 2 *Incidence of SS receptors in islet cell carcinomas*

Case no.	Tumor and site[a]	Sample[b]	Receptor autoradiography[c,d]		*In vivo* response to Sandostatin[c]
			SS-28 R	204-090 R	
1 BI	Poly-fx ICC	m Liver met.	++	++	Yes
2 KN	Insulinoma	m Liver biopsy (n)	+	+	No
3 AN	Insulinoma	p	++	++	NT
4 KL	Non-fx ICC	m Liver biopsy (n)	+	+	Yes
5 ST	Vipoma	m Liver biopsy (n)	++	++	Yes
6 WI	Insulinoma	p	++	++	NT
7 MA	Gastrinoma	m Liver biopsy (n)	++	++	Yes
8 GW	Poly-fx ICC	m Liver biopsy (n)	++	++	Yes
9 SM	Vipoma	m Liver biopsy (n)	NT	++	Yes
10 GE	Vipoma	m Liver biopsy (n)	NT	++	Yes
11 WA	Non-fx ICC	m Liver biopsy (n)	NT	++	NT
12 LY	Glucagonoma	p	NT	++	NT
13 RO	Glucagonoma	m Liver biopsy (n)	NT	++	Yes
14 HA	Non-fx ICC	p	NT	++	NT
15 SA	Non-fx ICC	m Lymph node met.	NT	++	NT

[a] Poly-fx ICC, polyfunctional islet cell carcinoma; non-fx ICC, nonfunctioning islet cell carcinoma.
[b] p, primary tumor; m, metastases; (n), small needle biopsy; met., metastases.
[c] R, receptors; NT, not tested.
[d] SS receptors: +, low to moderate density; ++, high density.

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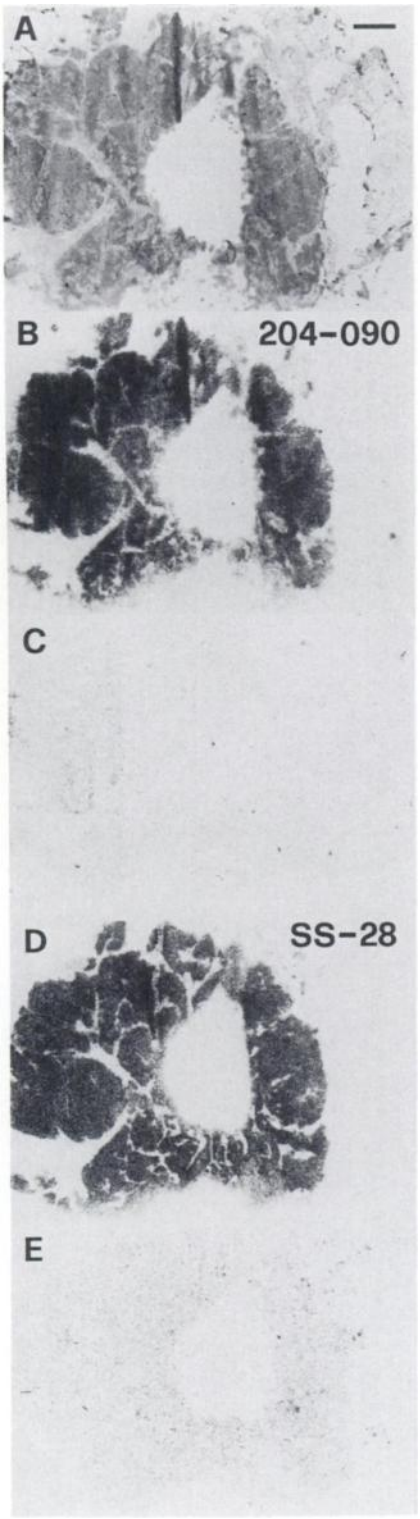

Fig. 1. Somatostatin receptors in an ileal carcinoid (case 9, KS). *A*, hematoxylin-eosin-stained section. *B*, and *C*, autoradiograms showing (*B*) total and (*C*) nonspecific (in presence of 10^{-6} M 204-090) binding of ^{125}I-204-090. *D* and *E*, autoradiograms showing (*D*) total and (*E*) nonspecific (in presence of 10^{-6} M SS-28) binding of ^{125}I-[Leu^{8}, DTrp^{22}, Tyr^{25}]-SS-28. Both tracers label homogeneously the whole tumor tissue. *Bar* = 1 mm.

DISCUSSION

The present study demonstrates the high prevalence of SS receptors in a large series of carcinoids and various islet cell carcinomas. Whereas the study shows for the first time SS receptors in carcinoids as well as in glucagonomas and in nonfunctioning islet cell carcinomas, it confirms in addition the results from previous studies reporting the presence of receptors in vipomas, gastrinomas, GRFomas, and insulinomas (11–13). It also confirms that, in the majority of the cases, the SS receptors are densely and homogeneously distributed throughout the whole tumor. This strong receptor homogeneity in carcinoids is worth mentioning since we have shown previously that some SS receptor-positive pituitary adenomas and several breast tumors sometimes have a nonhomogeneous distribution of receptors (26–28).

Our previous study showed that SS receptor-positive primary islet cell carcinomas also have SS receptor-positive metastases (11, 13). The high number of SS receptor-positive metastases is another example from biopsy material showing two rare tumors which have not been described previously, *i.e.*, a nonfunctioning islet cell carcinoma and a glucagonoma, with a high density of SS receptors.

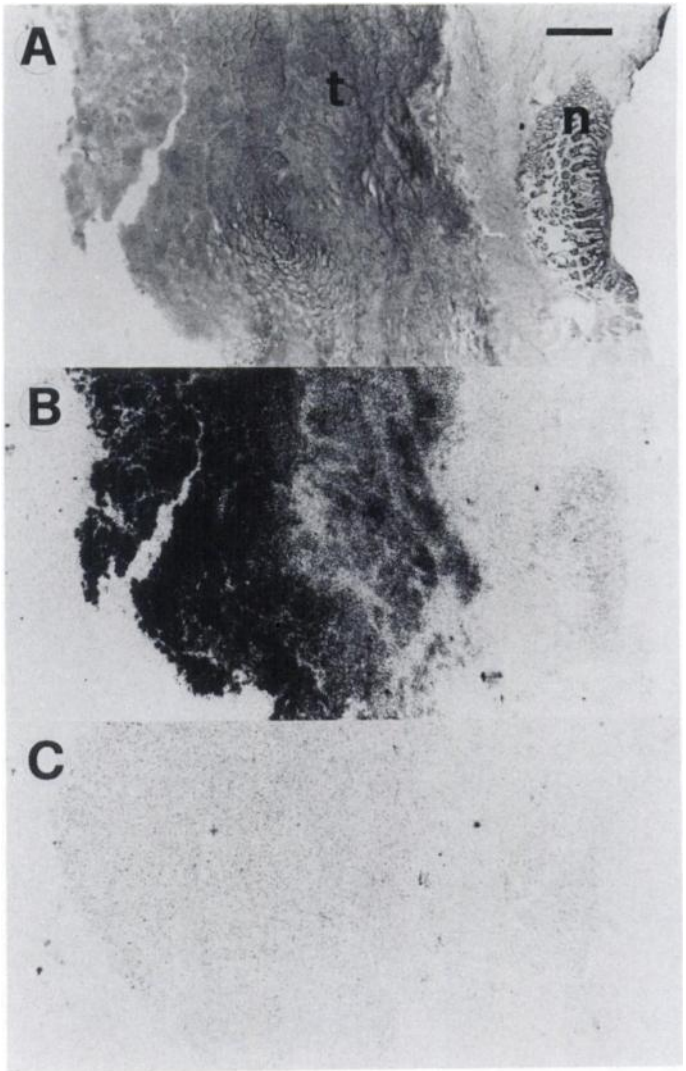

Fig. 2. Somatostatin receptors in an ileal carcinoid (*t*) as well as in the mucosa of adjacent healthy tissue (*n*) (case 45, TG). *A*, hematoxylin-eosin-stained section. *B*, total binding of ^{125}I-204-090. *C*, nonspecific binding (in presence of 10^{-6} M 204-090). Healthy mucosa has a considerably lower density of receptors than tumor. *Bar* = 1 mm.

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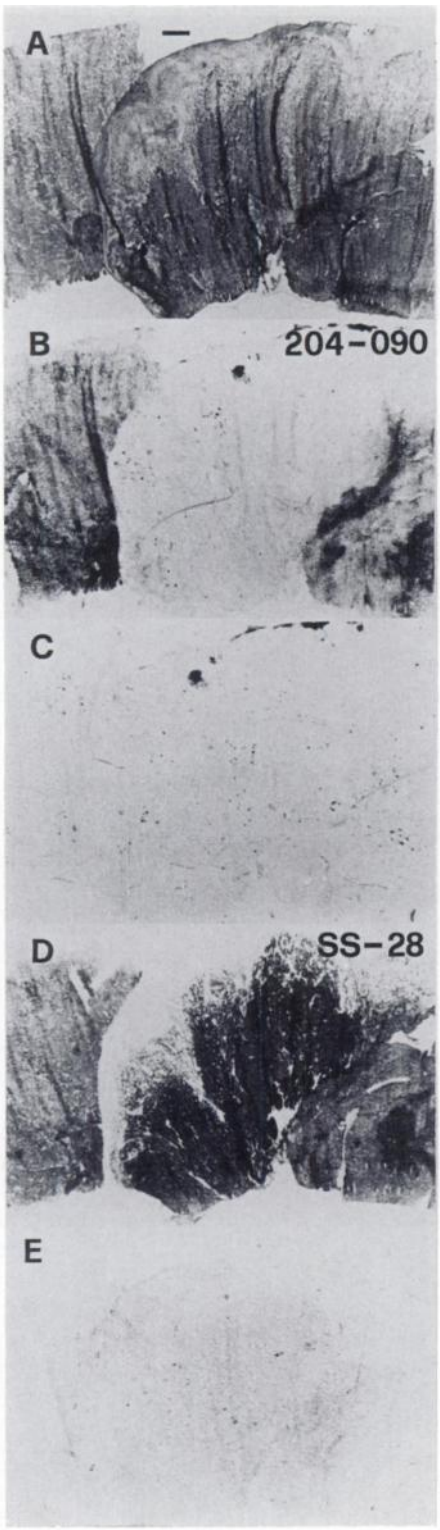

Fig. 3. Somatostatin receptor subtypes in a single case of bronchial carcinoid (case 43, TW). *A*, hematoxylin-eosin-stained section. *B* and *C*, autoradiograms from sections incubated with ^{125}I-204-090. *B*, total binding; *C*, nonspecific binding (in presence of 10^{-6} M 204-090). *D* and *E*, autoradiograms from sections incubated with ^{125}I-[Leu^{8}, DTrp^{22}, Tyr^{25}]-SS-28. *D*, total binding; *E*, nonspecific binding (in presence of 10^{-6} M SS-28). The *left* and *right parts* of the tumor have SS receptors with similar affinity for 204-090 or SS-28 whereas the *middle part* has only SS receptors with high affinity for SS-28. *Bar* = 1 mm.

seen in the present study therefore confirms our findings and extends it to carcinoids. Moreover, there is good evidence from the present study that all metastases in a single patient's liver contain a similar amount of SS receptors.

The above mentioned results, *i.e.*, the high prevalence of SS receptors in liver metastases of hormone-producing gastrointestinal tumors and their homogeneous distribution over the whole tumor, allow the study of SS receptor status in a patient's tumor by evaluating a small specimen of tumor obtained by percutaneous needle biopsy of the liver metastasis. The data indicate that we can extrapolate for the receptor status in the primary tumor and possibly in other metastases of the patient. Receptor autoradiography has the advantage over receptor measurements on homogenates of allowing an evaluation of SS receptors in very small tissue samples of a few mg and at the same time identifying whether the receptors are on tumor tissue (28). A false negative result on an accidentally false biopsy of normal liver (see Fig. 8) is therefore excluded. Furthermore, the biochemical and pharmacological characteristics of the SS receptors as measured on sections from such needle biopsies are similar to those found previously with homogenate binding of surgical specimens (13). The good correlation between the

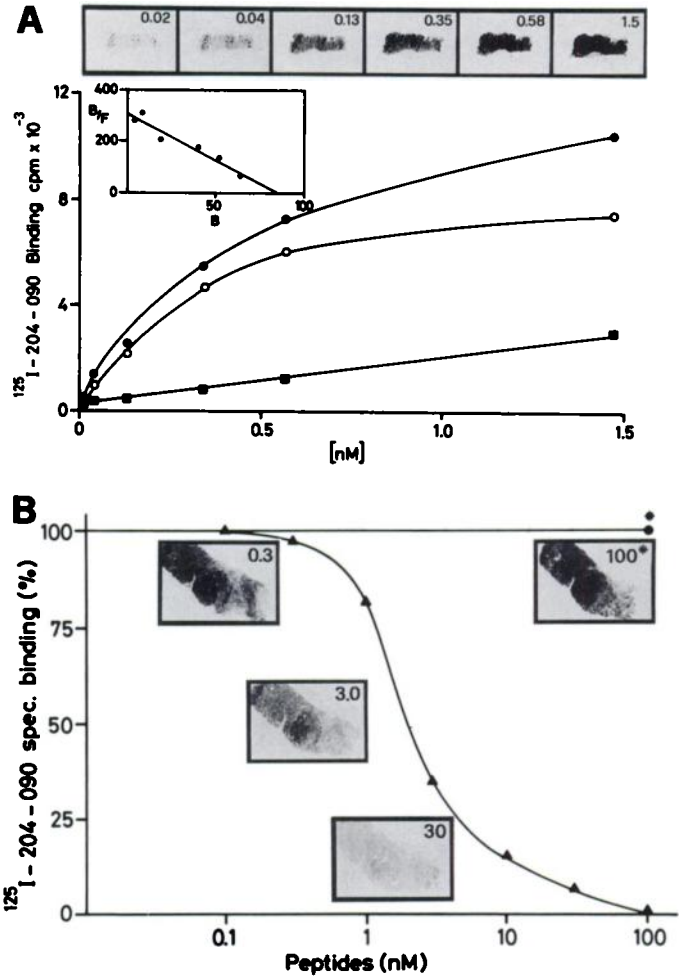

Fig. 4. *A*, saturation experiment using tissue sections of a carcinoid tumor incubated with increasing concentrations of ^{125}I-204-090. ●, total binding; ○, specific binding; ■, nonspecific binding (in presence of 10^{-6} M 204-090). Photographs represent autoradiograms from sections incubated with corresponding concentrations of ^{125}I-204-090 (nM), from which saturation curves were drawn. *Inset*, Scatchard analysis of the data: *B* (fmol/mg protein), *B/F* (fmol/mg protein − nM). K_D = 0.4 nM, B_{max} = 85 fmol/mg protein. *B*, displacement curve of ^{125}I-204-090 in tissue sections from a liver metastasis of a carcinoid from patient 42, EC. Tissue sections were incubated with 23,000 cpm/100 μl ^{125}I-204-090 and increasing concentrations of unlabeled 204-090 (▲), 100 nM luteinizing hormone-releasing hormone (*), or 100 nM SS-28 (1–12) (●). Photographs represent autoradiograms from sections incubated with ^{125}I-204-090 as well as 0.3, 3, or 30 nM 204-090 or 100 nM luteinizing hormone-releasing hormone (*), respectively.

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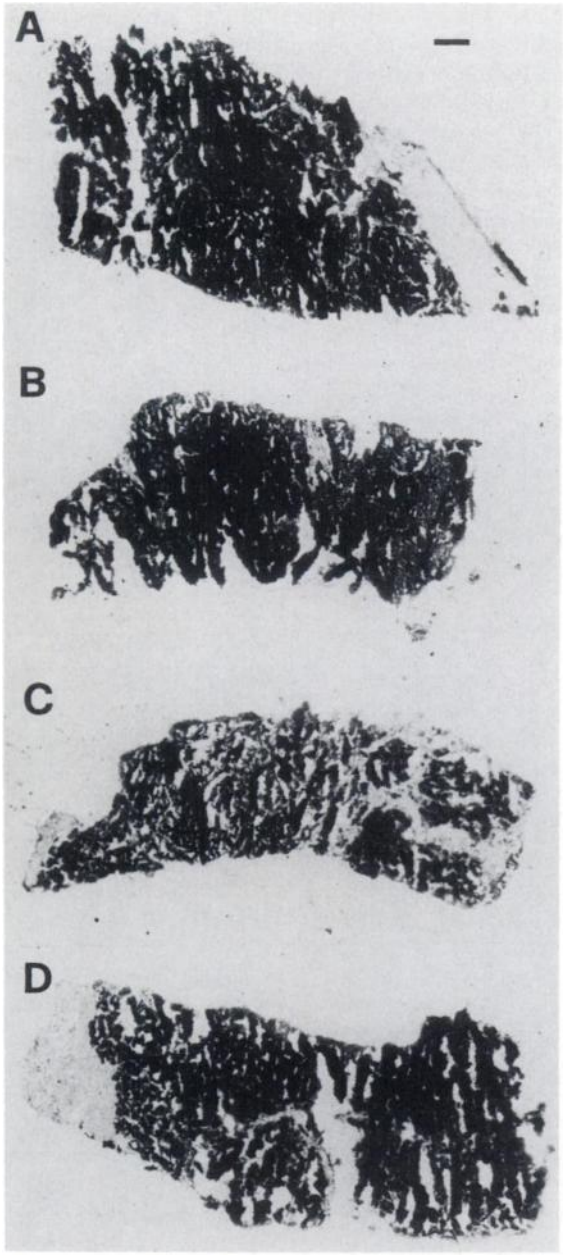

Fig. 5. Somatostatin receptors in four different liver metastases from patient 42, EC. *A–D*, autoradiograms showing total binding of ^{125}I-204-090. Nonspecific binding is negligible. *Bar* = 1 mm.

SS receptor status in the tumor and the *in vivo* biochemical response in the patient measured as the percentage of hormone secretion inhibition by octreotide *in vivo* (20), which will be presented and discussed in detail in a companion paper, is excellent evidence that the measured SS receptors are functional. Similar evidence has recently been given in Rin M5F insulinoma cells (29), as well as in human pituitary adenomas (30, 31).

Ultimately, the SS receptor status measured with needle biopsy of liver metastases should allow prediction of the *in vivo* efficacy of octreotide in treating a particular tumor. This approach may be particularly valuable in nonfunctioning islet cell carcinomas, where no biochemical response can be measured *in vivo* to assess efficacy of octreotide. Moreover, it permits investigation of various biological facts, such as regulation and influence of the various hormones produced by the tumors, the heterogeneity within the tumor, and the heterogeneity from primary to secondary deposits.

The generally good correlation of labeling between both radioligands, the SS-28 analogue and the Tyr^3-octreotide, is in agreement with our previous studies (13, 31, 32). Interestingly, however, we find a small number of cases preferentially labeled with SS-28 ligand (13, 31). In the present study, this peculiarity is restricted to 2 carcinoids; in earlier studies, single cases of growth hormone-producing pituitary adenomas (31) and insulinomas (13) had this characteristic. We suggested previously (13) that insulinomas possessed a SS receptor subtype, analogous to the one found in some regions of the normal brain (23, 24). The present study, however, seems to suggest that insulinomas are only occasionally heterogeneous in terms of their SS receptor type. However, most tumor types might, for an unknown reason, occasionally express another SS receptor subtype. A particularly interesting case is no. 43, which shows two receptor subtypes in the same tumor.

The presence of SS receptors in octreotide-pretreated patients suggests that even a chronic therapy did not abolish the SS receptor content of the tumor in these patients. Since we do not have a control sample of tumor on these patients before therapy, we cannot exclude the possibility that a regulation of receptor content takes place to a limited extent. However, the fact that strongly receptor-positive tumors are still observed among these cases seems to exclude a massive down-regulation of SS receptors during therapy. At the moment, such a statement is restricted to hormone-producing gastroenteropancreatic tumors, which have been shown to be sensitive to octreotide *in vivo*. We do not know whether it applies to endocrine gastroenteropancreatic tumors becoming resistant to octreotide (33); we have no data yet on SS receptors in others, *i.e.*, pituitary, pretreated tumors. Furthermore, a careful examination of histological sections of the long term octreotide-treated tumors did not reveal any peculiarities in regard to incidence of necrosis, fibrosis, or cell pleomorphism, which might have occurred under such a treatment. Recently, indeed, one case of octreotide-treated pituitary adenoma was shown to have increased fibrotic areas (34).

A low percentage of carcinoids has been shown to be receptor negative. It is intriguing to note that most of the negative cases were bronchial carcinoids, whereas only one ileal carcinoid was negative. Of further interest is the fact that some of these cases were ACTH producing. It is tempting to speculate that the down-regulation of SS receptors by corticosteroids described recently (35, 36) might be responsible for the observed losses of receptors. Another characteristic of SS receptor-negative carcinoids is their higher grade of dedifferentiation or even anaplastic appearance. This would suggest that only well differentiated tumors retain the capacity to express SS receptors. This observation is in keeping with previous results that we obtained with central nervous system tumors, breast tumors, or exocrine pancreatic tumors (32, 37–39).

In summary, this study demonstrates the very high incidence of SS receptor-positive carcinoids and islet cell carcinomas, primaries as well as their metastases. Since the SS receptors are usually very homogeneously distributed in the whole tumor sample, it was possible to develop a technique for SS receptor detection in small needle biopsies of the liver metastases of such tumors. The fact that SS receptors in these tumors were shown to be functional makes such an SS receptor test of predictive value for the therapeutic efficacy of octreotide.

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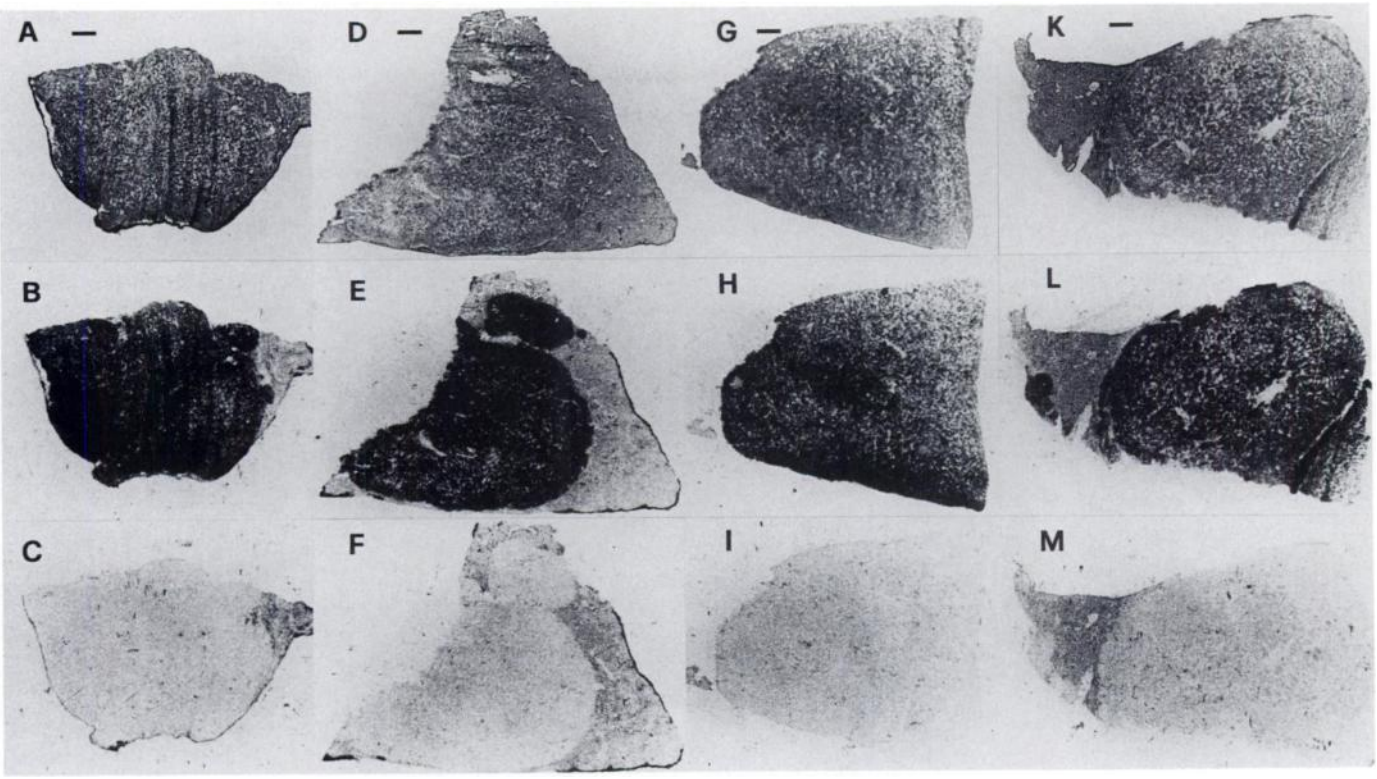

Fig. 6. Somatostatin receptors in four different liver metastases (*A*, *D*, *G*, *K*) from a patient with bronchial carcinoid pretreated with octreotide for 3.8 years (case 20, BU). *A*, *D*, *G*, *K*, hematoxylin-eosin-stained sections. A small piece of normal liver is seen on the border of each metastasis. *B*, *E*, *H*, *L*, total binding of ^{125}I-204-090. *C*, *F*, *I*, *M*, nonspecific binding (in presence of 10^{-6} M 204-090). *Bars* = 1 mm. Note that all four tumors have a homogeneous distribution of SS receptors. Significant nonspecific binding is seen in the liver tissue.

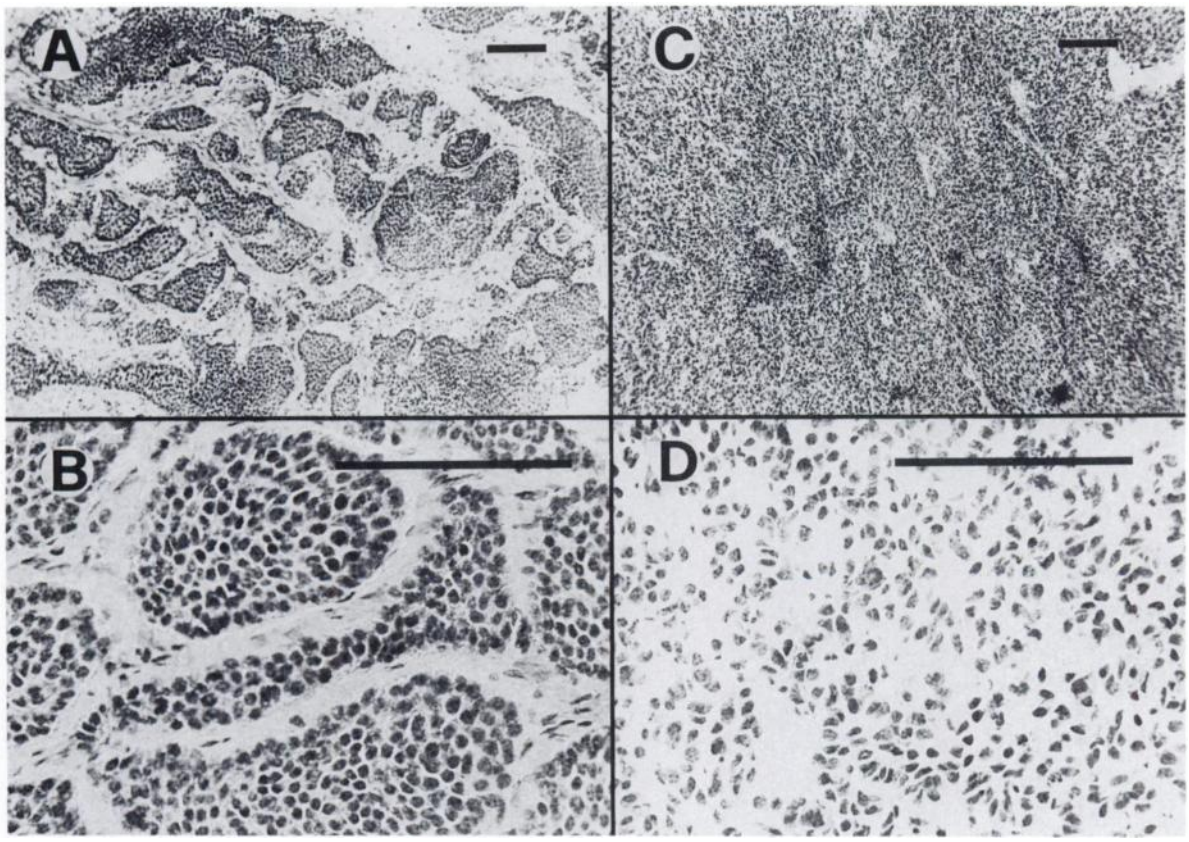

Fig. 7. Histological sections of two SS receptor-positive (*A* and *B*) and two SS receptor-negative (*C* and *D*) carcinoid tumors. Note that *C* and *D* are less differentiated than *A* and *B*. The highly differentiated carcinoids display individual groups or ribbons of tumor cells separated by septa of connective tissue (*A* and *B*). At higher power (*B*), a palisading of tumor cells is present at the periphery of cell clusters. The tumor cells and nuclei are rather monomorphous. The structure of less differentiated carcinoids is predominantly solid, *i.e.*, individual cell groups are absent (*C* and *D*). There is a prominent polymorphism of the tumor cells and their nuclei (*D*). *Bars* = 0.1 mm. *A*, case 25, FR; *B*, case 34, BT; *C*, case 35, BE; *D*, case 30, GN.

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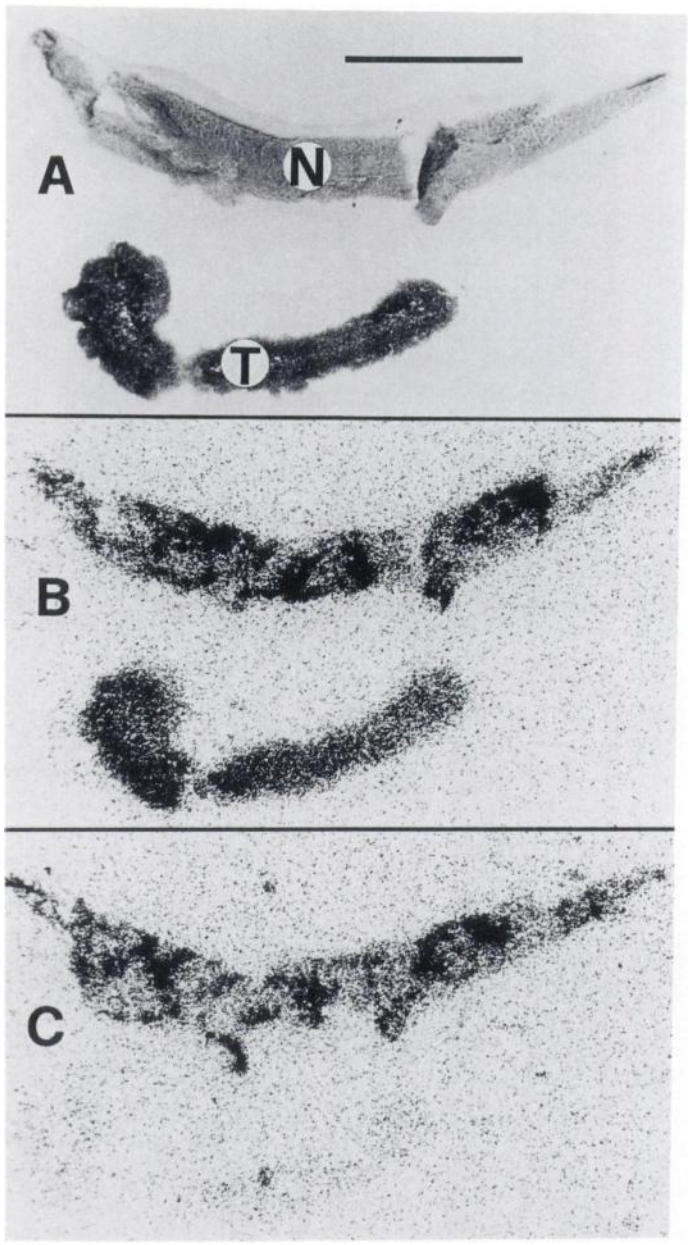

Fig. 8. Somatostatin receptors in a carcinoid tumor obtained from fine needle biopsy of the liver (case 1, MC). *A*, hematoxylin-eosin-stained sections. ***Upper tissue***, biopsy of normal liver tissue (*N*); ***lower piece***, liver biopsy of the carcinoid metastasis (*T*). *B*, autoradiograms showing total binding of ^{125}I-[Leu^{8}, DTrp^{22}, Tyr^{25}]-SS-28. *C*, autoradiogram showing nonspecific binding (in presence of 10^{-6} M SS-28). Note that only the carcinoid tumor (*T*) has specific SS receptors, whereas liver tissue (*N*) has only nonspecific binding. Exposure time, 1 week. *Bar* = 1 mm.

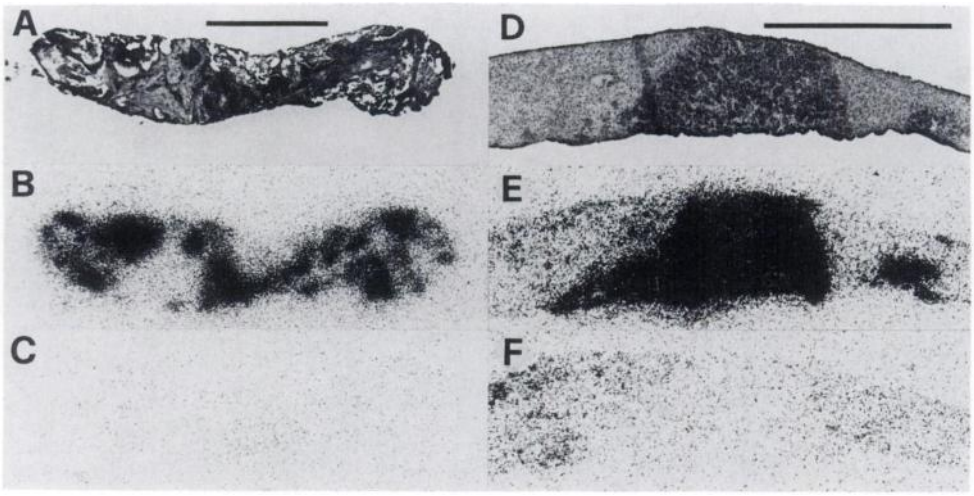

Fig. 9. Somatostatin receptors in small needle biopsies from liver metastasis of nonfunctioning islet cell carcinoma (case 11, WA) (*A–C*) and a glucagonoma (case 13, RO) (*D–F*). *A* and *D*, hematoxylin-eosin-stained sections. *B*, and *E*, total binding of ^{125}I-204-090. *C* and *F*, nonspecific binding (in presence of 10^{-6} M 204-090). *Bar* = 1 mm. Note that SS receptors are exclusively located on tumoral tissue. Biopsy tissue without labeling represents liver tissue.

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