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Peptide Receptors as Molecular Targets for Cancer Diagnosis and Therapy

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During the past decade, proof of the principle that peptide receptors can be used successfully for *in vivo* targeting of human cancers has been provided. The molecular basis for targeting rests on the *in vitro* observation that peptide receptors can be expressed in large quantities in certain tumors. The clinical impact is at the diagnostic level: *in vivo* receptor scintigraphy uses radiolabeled peptides for the localization of tumors and their metastases. It is also at the therapeutic level: peptide receptor radiotherapy of tumors emerges as a serious treatment option. Peptides linked to cytotoxic agents are also considered for therapeutic applications. The use of nonradiolabeled, noncytotoxic peptide analogs for long-term antiproliferative treatment of tumors appears promising for only a few tumor types, whereas the symptomatic treatment of neuroendocrine tumors by somatostatin analogs is clearly successful. The present review summarizes and critically evaluates the *in vitro* data on peptide and peptide receptor expression in human cancers. These data are considered to be the molecular basis for peptide receptor targeting of tumors. The paradigmatic peptide somatostatin and its receptors are extensively reviewed in the light of *in vivo* targeting of neuroendocrine tumors. The role of the more recently described targeting peptides vasoactive intestinal peptide, gastrin-releasing peptide, and cholecystokinin/gastrin is discussed. Other emerging and promising peptides and their respective receptors, including neurotensin, substance P, and neuropeptide Y, are introduced. This information relates to established and potential clinical applications in oncology. (*Endocrine Reviews* 24: 389–427, 2003)

Abbreviations: ANP, Atrial natriuretic peptide; BB_1, BB_2, BB_3, and BB_4, bombesin receptor subtypes 1, 2, 3, and 4; CCK_1 and CCK_2, cholecystokinin receptor subtypes 1 and 2; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, diethylenetriaminopentaacetic acid; GLP-1, glucagon-like-peptide-1; GRP, gastrin-releasing peptide; KRL-VIP/GRF, [Lys^{15},Arg^{16}, Leu^{27}]VIP(1–7)/GRF(8–27); α-MSH, α-melanocyte-stimulating hormone; NK_1, NK_2, and NK_3, neurokinin receptor subtypes 1, 2, and 3; NPY, neuropeptide Y; NTR1, NTR2, and NTR3, neurotensin receptor subtypes 1, 2, and 3; PAC_1, PACAP receptor subtype 1; PACAP, pituitary adenylate cyclase activating peptide; sst_1, sst_2, sst_3, sst_4, and sst_5, somatostatin receptor subtypes 1, 2, 3, 4, and 5; VIP, vasoactive intestinal peptide; $VPAC_1$ and $VPAC_2$, VIP receptor subtype 1 and 2; Y_1, Y_2, Y_4, and Y_5, NPY receptor subtypes 1, 2, 4, and 5; ^{90}Y-DOTATOC, ^{90}Y-DOTA-Tyr^3-octreotide.

I. Introduction

IT HAS BEEN a challenge for physicians, in particular for oncologists, to identify a simple tool that has the potential to localize and treat human neoplasms at an early stage of development. About 20 yr ago, monoclonal antibodies became very popular as potential magic bullets to be used in cancer (1); however, this fascinating and simple principle turned out to be much more difficult to transpose into reality than expected, mainly because of the excessive molecular mass (~150 kDa) of antibodies (2, 3). It is only in the past few years that adequate drugs based on antibody or antibody fragments have become commercially available for diagnosis and therapy of cancer, in particular of hematological neoplasias (4). About 15 yr ago, an alternative to radiolabeled antibodies appeared in the form of a small (1.5 kDa) radiolabeled peptide, a somatostatin analog, which led to a major breakthrough in this field. On the basis of the discovery that most human neuroendocrine tumors express a high density of somatostatin receptors (5), it has been possible to develop a method for localizing these tumors and their metastases by *in vivo* somatostatin receptor scintigraphy (6), using iv injection of a radiolabeled somatostatin analog (7). The tumors, after radioligand binding to their receptors and internaliza-

tion of the ligand-receptor complex, could thus be identified as hot spots on γ-camera scans (Fig. 1) (7). This sensitive procedure is superior to all standard diagnostic tools available today for the detection of specific neuroendocrine tumors, such as gastrinomas (8). From a therapeutic point of view, recent pilot studies using high doses of somatostatin analogs radiolabeled with ^{90}Y have shown a reduction or at least a stabilization of the tumor growth (9–12). Another successful clinical application has been the long-term use of nonradioactive somatostatin analogs as symptomatic treatment of hormone-secreting neuroendocrine tumors. On the basis of the strong inhibitory effect of somatostatin on hormone secretion, it usually results in a remarkable improvement of life quality, predominantly because of normalization of hormone secretion.

The molecular basis for such clinical applications is the presence of a high density of somatostatin receptors in these tumors. The *in vitro* detection of somatostatin receptors in human tumor samples, using methods such as *in vitro* autoradiography, has therefore, in parallel to clinical applications, been of prime interest during the past decade. *In vitro* receptor data have predicted the outcome of somatostatin receptor scintigraphy (5, 13) or octreotide therapy (14) and have been used to select tumor types suitable for those clinical applications (7).

Although the clinical use of somatostatin has been refined during the past decade, it remained limited to tumor categories that express somatostatin receptors in sufficiently large quantities, *i.e.*, mainly to neuroendocrine tumors. Therefore, it has been of increasing interest to investigate whether receptors for other regulatory peptides are overexpressed in more common human cancers (*i.e.*, in lung, prostate, colon, or pancreatic carcinomas) to apply a strategy similar to that used with somatostatin. This field of investigation, which appears to be a small niche in the very large oncology field, has gained increasing interest in the past decade. The targeting of overexpressed peptide receptors in tumors by small peptides has become a very strong focus of interest for nuclear medicine. Henry Wagner, at the 100-yr anniversary of nuclear medicine, named the peptide approach in nuclear oncology as one of the most promising fields for the next decade (15); gastroenterologists and endocrinologists are also attracted by the concept of peptide receptor targeting (16, 17).

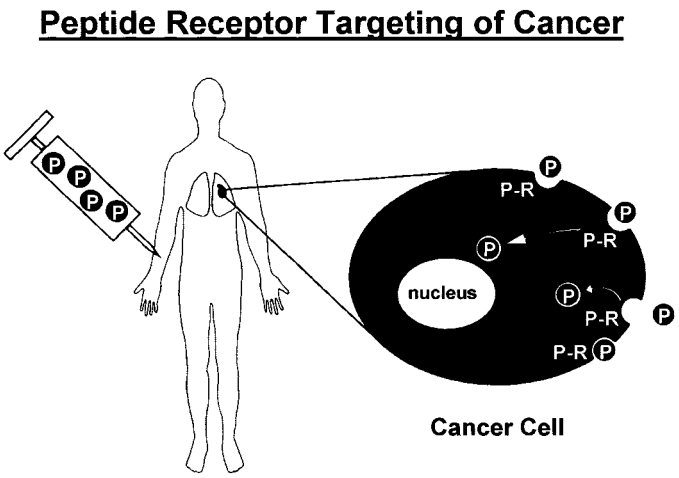

FIG. 1. Principle of *in vivo* peptide receptor targeting of cancer. The radiolabeled peptide (P) is injected iv into the patient and distributed in the whole body. If the patient has a tumor with cancer cells expressing the corresponding peptide receptor (P-R), the radiopeptide will bind to it and internalize with the receptor into the cell (*arrows*) where the radioactivity will accumulate. Whole body γ-camera scan will detect the radioactivity accumulated in the tumor, whereas the remaining radioactivity in the body will rapidly be cleared through the kidneys.

For a better understanding of these clinical applications, it appears therefore timely to review our current knowledge about peptides and peptide receptors, in particular their tissue expression, their role, or potential applications, in cancer pathogenesis, diagnosis, and treatment.

II. Definitions

Peptides are molecules consisting of several amino acids linked together with peptide bonds. The size of peptides can vary from molecules with only two amino acids to as many as 50. In contrast to proteins, they generally do not possess a well-defined three-dimensional (tertiary) structure. Moreover, peptides do not only exist in natural form but also can be designed synthetically as novel molecules. Thus, their actual number is presently very large. This review will be restricted to physiologically occurring peptides and, within this large group, will focus on the so-called regulatory peptides that include the neuropeptides present in the brain, the gut peptide hormones, as well as peptides present in the vasculature (vasoactive peptides) and peptides of the endocrine system. A list of such regulatory peptides with a link to cancer is found in Table 1. Particular attention will be given to somatostatin, vasoactive intestinal peptide (VIP), cholecystokinin (CCK), gastrin-releasing peptide (GRP), and neurotensin.

In general terms, these regulatory peptides represent a group of different families of molecules known to act on multiple targets in the human body at extremely low concentrations (5). Targets of these peptides are not only the brain and the gastrointestinal tract, but also the endocrine system, the kidneys, the lungs, and the immune, vascular, and peripheral nervous systems. Therefore, regulatory peptides control and modulate the function of almost all key organs and metabolic processes. Their action is mediated through specific membrane-bound receptors; almost all belong to the group of G protein-coupled receptors. They can influence many intracellular effector systems; for instance, the emerging role of peptides in MAPK pathways, known to play an important role in cell proliferation, or in apoptosis, may contribute to the current interest for peptides in cancer research (18, 19). Receptor subtypes with their own ligand specificity and second messenger systems exist for almost all regulatory peptides, thus increasing the diversity of their mode of action (Table 1). These peptides may play prominent roles in not only normal conditions but also pathological processes. They may be factors involved in inflammation, but may also play a receptor-mediated role in cancer and cancer progression (Table 1).

TABLE 1. General characteristics of selected peptides

Peptide	No. of amino acids	Receptors (including subtypes nomenclature)	Effects on tumor growth[a]
Somatostatin	14 or 28	sst_1, sst_{2A}, sst_{2B}, sst_3, sst_4, sst_5	↓
VIP	28	$VPAC_1$, $VPAC_2$	↑
PACAP	27 or 38	PAC_1	↑
CCK	8, 33, 39, or 58	CCK_1, CCK_2	↑
Gastrin	17 or 34	CCK_2	↑
Bombesin	14	BB_1 (NMB-R), BB_2 (GRP-R), BB_3, BB_4	↑
GRP	27	BB_2 (GRP-R)	↑
Neurotensin	13	NTR1, NTR2, NTR3	↑
NPY	36	Y_1, Y_2, Y_4, Y_5	?
Substance P	11	NK_1, NK_2, NK_3	↑
Oxytocin	9	OT-R	?
LHRH	10	LHRH-R	↓
GLP-1	36	GLP-1-R	?
Calcitonin	32	Calcitonin-R	?
Endothelin	21	ET_A, ET_B	↑
Atrial natriuretic factor	28	ANP_A, ANP_B	?
α-MSH	13	α-MSH-R	?

[a] Animal studies or cell lines; R, receptor.

III. Significance of Peptides and Peptide Receptors in Cancer

A. Overexpressed receptors as molecular targets

One of the main reasons for the increasing interest for peptides and peptide receptors in cancer is the possibility of receptor targeting, because the peptide receptors are often expressed in many primary human cancers. In several instances, it can even be demonstrated that these peptide receptors are overexpressed in cancer, in comparison to their expression in normal tissue adjacent to the neoplasm and/or in its normal tissue of origin. This aspect will be covered as a main topic of this review in *Section V*. Basically, one may use these receptors as molecular targets in two ways:

1. Binding sites for radioligands. The receptors are used primarily as binding sites for a peptide analog, little consideration being given to their biological function. Accordingly, this strategy relies primarily on the presence of tumoral receptors able to bind with high affinity the peptide analogs, and not on a receptor-mediated physiological or pathophysiological action of the peptide. Successful clinical applications of this principle are the diagnostic and radiotherapeutic targeting of peptide receptors with radiolabeled peptides. This procedure takes advantage of one important characteristic of many G protein-coupled peptide receptors, namely that they can internalize into the cell together with their ligand (usually agonists) after receptor-ligand interaction at the cell membrane (20–25). The remarkable paper by Mantyh *et al*. (21) in the substance P receptor field should be mentioned as an example; they have demonstrated, with a specific antibody to the neurokinin 1 (NK_1) receptor, that the NK_1 receptor protein, normally confined to the cell membrane of a neuron population in the dorsal horn, was massively internalized after somatosensory stimulation. Furthermore, they used the internalization mechanism to selectively ablate neurons in the dorsal horn that expressed NK_1 receptors (26). Substance P, conjugated to a cytotoxic compound, was infused into the spinal cord. The conjugate, after its internalization in neurons expressing the NK_1 receptors, killed them. Somatostatin receptors have also been shown to be internalized to various degrees, depending on the receptor subtype involved; for instance, the commercially available targeting agent ^{111}In-DTPA-[D-Phe^1]-octreotide (Octreoscan, Mallinckrodt, Inc., St. Louis, MO) was rapidly internalized in a receptor-specific and temperature-dependent manner (22). The internalized receptor-radioligand complex provides an important and useful accumulation of radiotracer in the cell, thus increasing the radioactive signal at the target site (27). This basic strategy can use either peptides linked to cytotoxic drugs (26, 28) or peptides linked to radioactive isotopes (22). In both cases, the internalized ligand may be able to selectively destroy the targeted cell.

2. Targets mediating functional responses. Alternatively, one can take advantage of the functional receptors as targets to elicit a particular biological response, using unlabeled, nontoxic peptide analogs over a long period of time. The best example of a peptide receptor-mediated biological response is the inhibition of hormone secretion by somatostatin and its analog octreotide (14, 17, 29). In addition, various peptides have been shown *in vitro* to play an active role in the growth regulation of many types of tumor cells (30) (Table 1), suggesting that long-term treatment with adequate peptide analogs may be able to reduce or stop tumor growth *in vivo*. In a large variety of animal tumor models expressing various peptide receptors, Schally *et al.* (31, 32) and Moody *et al.* (30) were indeed able to demonstrate significant tumor growth inhibition or even to stop the growth by use of specific, nonradioactive, and noncytotoxic peptide analogs.

B. Peptides and radiopeptides as targeting agents

The nature of the peptide itself, in particular its molecular structure and behavior, makes it an attractive compound to act as a bullet targeted at the corresponding peptide receptors (Table 2).

1. Tissue permeability. As small and usually hydrophilic molecules, peptides are characterized by an excellent permeability that permits an easy and rapid access to the tumor site

TABLE 2. Regulatory peptides as targeting agents

Advantages	Disadvantages
• Small molecules	• Rapidly degraded by peptidases
• Excellent permeability	
• No antigenicity	
• Minimal side effects	
• Easy to synthesize and modify chemically	
• Easy to link to chelators	
• Easy to radiolabel	
• High-affinity receptor binding	
• Rapid clearance from the body	
• No brain targeting due to inability to cross the blood-brain barrier	

after systemic injection. There is one exception: peptides will usually not cross a normal blood-brain barrier and will not enter the brain in significant amounts (<0.1% of total peptide injected) after systemic injection. This is a clear advantage when peripheral organs or tumors are the intended targets; because the brain expresses a high density of most of the peptide receptors, brain targeting could be at the origin of numerous central nervous system side effects. However, peptides may be able to penetrate through the blood-brain barrier when the latter is disturbed, as seen in undifferentiated glial tumors such as glioblastomas. The peptide will then be able to reach the tumor site and remain there (33).

Peptides are usually rapidly excreted from the body. This will occur through renal or hepatobiliary excretion or both, depending on the peptide and the type of structural modifications performed on it.

2. Side effects. Peptides are physiological compounds and, as such, intrinsically nontoxic, as compared with current chemotherapeutic drugs. Side effects, if they occur, may primarily be due to the physiological actions of the peptides (34, 35) and may be expected after administration of pharmacological doses of the nonradioactive compounds, for instance during long-term treatment of tumors. Conversely, side effects at physiological receptor sites are expected to be negligible if radiolabeled peptides are given for *in vivo* diagnosis or radiotherapy, because very low peptide doses need to be applied for this purpose (7, 36). Furthermore, because peptides usually play a modulatory role in various biological systems, their actions will often be counterbalanced and possibly annihilated by other hormones, growth factors, or neurotransmitters acting in these same systems. Another important characteristic of regulatory peptides is their usual lack of antigenicity, because their size is small. Their analogs are usually not antigenic either.

3. Stability. Peptides are quite easily synthesized and modified. They withstand the rather harsh conditions for modification or labeling. However, their natural structural conformation makes them extremely sensitive to peptidases; they are rapidly broken down due to cleavage of peptide bonds by several types of peptidases present in most tissues. Thus, metabolically stable analogs must be developed as a prerequisite for successful clinical applications, in particular for long-term treatments. The best example is the development of the somatostatin analogs octreotide, lanreotide, and vapreotide, that have, compared with natural somatostatin, a much prolonged half-life in plasma and tissue and a longer action (Fig. 2). This has only been possible through a long and considerable effort of development (31, 37, 38). For many of the other peptides, only limited efforts have been made to develop peptide analogs having an improved stability in the order of hours, although the proteolytic enzymes as well as the precise peptide bonds being cleaved are well known for many of these peptides. For instance, most of the proteolytic enzymes that have been reported to cleave intact neurotensin are known. This allows a more rational design of suitable analogs with stabilized bonds against metabolic deactivation (39). Furthermore, because regulatory peptides and their receptors are physiological entities that have usually been well characterized a long time ago, it is a great advantage to be able to use previous knowledge of synthesis and structure-activity relationship for a corresponding peptide to design, synthesize, and develop novel peptide analogs that may become useful for clinical applications. The design may involve a higher stability, coupling to radioactive isotopes or to toxic moieties such as doxorubicin (28, 40).

4. Radiolabeling. Small (8–20 amino acids) peptides are usually large enough to provide an adequate attachment site for a chelator molecule that is sufficiently distant from the binding area to prevent a complete loss of the binding affinity of the peptide to the receptor (Fig. 2). Chelators such as diethylenetriaminopentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) are indispensable molecules to accept certain types of metallic radioisotopes (40). However, those relatively large molecules may affect the binding properties of the compound to which they are attached. Peptidic analogs of regulatory peptides are, due to their size, usually more suitable for chelator attachment than the smaller nonpeptidic analogs.

IV. Critical Evaluation of Tissues and Methods Used for Peptide Receptor Detection *in Vitro*

In vitro evaluation of peptide receptor expression, in particular of their incidence and density in human tumors and their metastases, is critical for identifying and defining 1) potential peptide receptors that may be of interest for clinical applications, and 2) tumor types that are particularly suitable to be targeted with a given peptide. It has been demonstrated that extensive *in vitro* information about the receptor incidence and density in a given tumor is required before *in vivo* investigations can be performed in humans. However, the various *in vitro* methods available for peptide receptor identification and for the prediction of suitable targets can provide variable information (Table 3). Therefore, it is important to critically review and evaluate those methods to understand their respective value.

A. Tissue type

To predict the *in vivo* peptide receptor status of a tumor as closely as possible, the *in vitro* method used should evaluate the peptide receptors in primary human tumors and their metastases. Such tissues are usually available after surgical

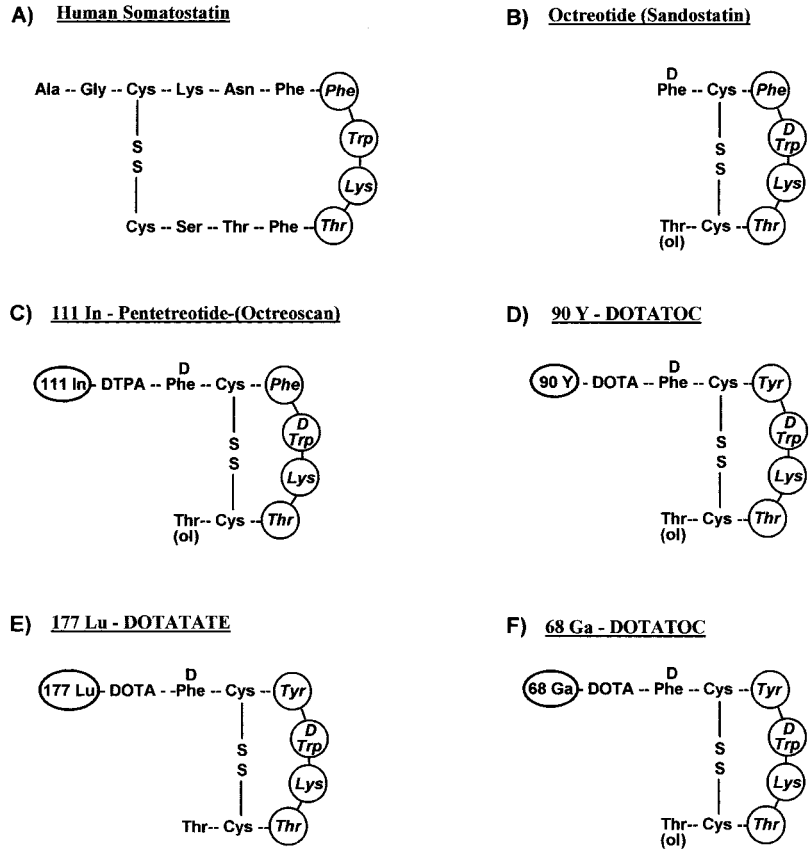

FIG. 2. Development of somatostatin analogs for various clinical applications. A, Human somatostatin. B, Octreotide (Sandostatin) for symptomatic long-term treatment of neuroendocrine tumors. C–F, Octreotide linked to various chelators plus radioisotopes. C, ^{111}In-pentetreotide [^{111}In-DTPA-[D-Phe^1]-octreotide (Octreoscan)] for tumor scintigraphy. D, ^{90}Y-DOTATOC (^{90}Y-DOTA-[D-Phe^1, Tyr^3]octreotide) for radiotherapy. E and F, Second-generation somatostatin radiopeptides, ^{177}Lu-DOTATATE and ^{68}Ga-DOTATOC. DOTATATE has a DOTA-[D-Phe^1, Tyr^3]-octreotide structure, but with a threonine instead of threoninol.

TABLE 3. *In vitro* evaluation of peptide receptor expression

A. Required material and parameters
Use of primary human material (preferable to cell lines)
Neoplastic as well as non-neoplastic tissue
Detection of receptor protein (preferable to mRNA)
Density of receptors
Incidence of receptors
Receptor subtype
Morphological methods

B. Available methods

	Characteristics		
	Identifies	Quantification	Morphology-associated
Receptor autoradiography	Protein	Yes	Yes
In vitro binding assay	Protein	Yes	No
In situ hybridization	mRNA	No	Yes
Real time PCR	mRNA	Yes	No
Immunohistochemistry	Protein	No	Yes

C. Problems and pitfalls associated with human tissues
Nonstandardized tissue sampling and processing (size, time interval, procedures)
Biopsy/surgical resection/postmortem tissue
Fresh/frozen/formalin-fixed tissue
Histopathological diagnosis
Multiple concomitant pathologies/topographical variability/ "normal" tissues may be pathologically altered
Variability of sample contents
Diseased: inflammation/adaptive-degenerative changes/neoplasia
Non-diseased: epithelia/vessels/nerves/immune cells/glands/ connective and adipose tissue
Sample size
Not always representative for the whole tumor

resection. There is increasing evidence to suggest that it is not sufficient to have identified receptors in tumor cell lines or in tumors grown in animals because there might be profound differences in receptor expression, not only between species but also between cultured tumor cells or tumor cells growing *in situ* (41–44). Peptide receptor expression in cell lines does not necessarily reflect the peptide receptor situation in the corresponding human primary tumors (41, 45, 46). Igarashi *et al.* (44) recently showed not only that species differences exist for the VIP receptor subtype 1 ($VPAC_1$) pharmacophore but, more importantly, that human $VPAC_1$ expressed in Chinese hamster ovary cells differed markedly from the native human $VPAC_1$. It is therefore essential not only to be aware of possible species differences but also to investigate systems expressing the native receptor, in particular when searching for new peptide analogs for clinical use.

B. Receptor protein or mRNA

Because it is the receptor protein that is ultimately targeted *in vivo*, it should also be the protein that is investigated *in vitro*, rather than the receptor mRNA. In human tumors, the mRNA and the protein levels for selected peptide receptors may differ (47, 48). Therefore, it is particularly relevant, as first priority, to gain information about receptor protein expression. Receptor binding methodologies (binding assay; receptor autoradiography) or receptor immunohistochemistry provides such basic information on receptor protein. The first of these methods is particularly suitable because it represents the closest correlation to *in vivo* binding as shown by

in vivo receptor scintigraphy; all clinical applications presently available are based on binding of a peptide analog to the receptor binding site. Receptor immunohistochemistry, on the other hand, has an excellent cellular resolution, but may, however, identify an epitope that is not identical with the binding site and may therefore not tell much about the binding capability of a tumoral receptor. The information about the mRNA abundance is important when the methods measuring receptor proteins are unable to give enough clues, for instance about receptor subtypes.

C. Quantification

It is necessary to evaluate not only the incidence of a peptide receptor in a given tumor type, but also its density. In particular, radiotherapeutic implications will rely mainly on tumors with a high receptor density (11, 12, 49). Thus, an *in vitro* method that quantifies the amount of peptide receptors is preferable. Because peptide receptors may exist in the form of multiple subtypes, it is important to use an *in vitro* method that is specific enough to detect these subtypes.

D. Morphological identification

To assess the tumor to background ratio of peptide receptors present in a given tissue, the receptors should be evaluated not only in tumor material but also in normal tissue, preferably tissue adjacent to the tumor and containing the tissue of origin of these tumors. We must remember that samples of human tissues, for instance surgically resected tumors, are morphologically complex: the tumor is often intermingled with a variety of nonneoplastic tissue, such as leukocytes, vessels (including newly formed vessels), reactive components (fibrosis, necrosis), as well as adjacent normal host tissue elements, such as epithelia (*e.g.*, in the mucosa of the gastrointestinal tract), smooth muscle, nerve, immune cells, or stroma that may in many instances express the corresponding peptide receptors. These facts imply that it is mandatory to evaluate the receptor protein of human tissue samples with morphological methods to identify precisely which tissue elements express the receptors of interest. Those elements cannot be identified in tissue homogenates.

E. Choice of methods

One of the methods that fulfills most of the abovementioned requirements is *in vitro* receptor autoradiography (Table 3). It localizes and quantitates the peptide receptor. It identifies the receptor protein through its binding site. Pharmacological experiments using subtype-selective analogs allow the gross identification of peptide receptor subtypes by their rank order of potencies in displacement experiments (50–53). The method has a high sensitivity when ^{125}I-labeled peptides are used. One of the disadvantages of the method is, however, its limited cellular resolution. An accumulation of several cells of the same type is necessary to generate a radioactive signal that can then be attributed to those cells expressing the peptide receptor. Such an accumulation of similar cells is usually not a problem in a solid tumor, but it may be difficult to find in a normal tissue with a complex histological pattern.

Binding sites can also be determined with the less time-consuming *in vitro* binding assays. In contrast to receptor autoradiography, these are performed on homogenates, lack the morphological correlates that are necessary in this type of investigation, and are therefore less adequate for the study of surgically resected material. In human tumor samples, receptor quantification with binding methods will usually express the receptor density per milligram of tissue or protein, in contrast to cell cultures in which the number of receptors can be expressed per cell. Receptor immunohistochemistry identifies the receptor protein with a much better cellular resolution than autoradiography. However, the proteins cannot be reliably quantified, and the assay may recognize proteins unrelated to the receptor binding site (54). Moreover, specific receptor antibodies are not available for each of the peptide receptor subtypes under investigation.

In situ hybridization, Northern blots, RNase protection assays, RT-PCR, and real-time-PCR all identify mRNA. PCR and RNase protection assays are methods with a very high sensitivity, but without morphological correlates. *In situ* hybridization, on the other hand, is a morphological method, however, with a lower sensitivity than RT-PCR. Recently, first reports on measurement of sst_2 receptor by real-time PCR indicate (55) that mRNA could be quantified precisely. It should be remembered that RT-PCR identifies extremely low message levels that may not translate into the expression of functional levels of receptors.

F. Pitfalls

In all investigations of human material, one deals with considerable individual variations in the measured parameters from one individual to the other, not only in tumors but also in normal tissues. Such a variability is usually not observed in studies using laboratory animals or tumor cell lines cultured *in vitro*. Awareness of the potential problems associated with human tissue sampling is indispensable (Table 3). It is difficult to standardize tissue sampling and processing: the sample size (biopsy, surgical resection), the delay after sampling (surgical, postmortem), and the sample processing (fresh, frozen, fixed; method of fixation) are variable parameters (Table 3). Inadequate storage (resected material not frozen immediately) and/or processing (thawing of tissue) may easily lead to false-negative receptor data. A resected tissue sample often displays multiple concomitant pathological changes (neoplasia, inflammation, adaptive changes) in a heterogeneous topographical distribution; furthermore, adjacent normal tissues may also reveal pathological features. Such constituents may be receptor-positive. These facts further emphasize the need for a morphological evaluation of the receptor distribution, if possible in cooperation with an experienced pathologist.

V. *In Vitro* Peptide Receptor Expression in Normal Tissues and Tumors

This section summarizes the peptide receptor characteristics of human tissues, in particular tumors, based on current *in vitro* information. Subsections have been dedicated to receptors for somatostatin, VIP, CCK, GRP, and neurotensin,

because those receptors have been extensively studied and because all of them have been targeted *in vivo* in patients. Another subsection will deal with additional peptide receptors, for which less information is available or which recently began to be evaluated.

A. Somatostatin receptors

1. General background. Somatostatin consists of a family of a 14-amino-acid (somatostatin-14) and a 28-amino-acid (somatostatin-28) peptide (Table 1 and Fig. 2). It appears in several organ systems, such as the central nervous system, the hypothalamopituitary system, the gastrointestinal tract, the exocrine and endocrine pancreas, and the immune system. It inhibits a wide spectrum of physiological functions, including peptide hormone secretion. In these different organ systems, somatostatin can be considered to be a neurotransmitter, a neurohormone, or a local hormone acting via autocrine or paracrine mechanisms (56). Moreover, somatostatin plays a role in cancer: in many animal tumor models and cultured tumor cell lines, somatostatin and somatostatin analogs inhibit tumor growth (30, 31).

Somatostatin and octreotide actions are mediated by specific, high-affinity somatostatin receptors located on the plasma membrane of the target cells. To date, five human somatostatin receptor subtypes (sst_1, sst_2, sst_3, sst_4, and sst_5) have been cloned and partially characterized (57, 58) (Table 1). They have distinct, often overlapping patterns of expression in human organs. These subtypes belong to a superfamily of G protein-coupled receptors that can functionally couple to various intracellular effector systems. One of the most widely studied systems is the adenylylcyclase-cAMP-protein kinase A pathway that can be inhibited by somatostatin in numerous cell types (56); furthermore, the modulation of potassium channels by somatostatin has been extensively documented (56). Other relevant signaling pathways regulated by somatostatin include the somatostatin-induced stimulation of phospholipase A2 (59) and the activation of phosphotyrosine phosphatases (60). Particular interest has been devoted recently to the inhibitory action of somatostatin through MAPK pathways, probably an important facet of somatostatin signaling (19). Because MAPK activation plays an important role in cell proliferation (61), inhibition of MAPK pathways is likely to contribute to the antiproliferative effect of somatostatin. The role of somatostatin in stimulating apoptotic mechanisms in sst_2- or sst_3-expressing cells (62, 63) is another notable antiproliferative mechanism. Remarkably, somatostatin receptors have recently been shown to form homo- and heterodimers (64–66) and to physically interact with a class of proteins displaying anchoring and scaffolding functions (67–69). Pharmacological studies revealed that all five human subtypes bind somatostatin-14 and somatostatin-28 with a high affinity. However, there are differences in the binding affinities of the structural analogs of somatostatin; for instance, octreotide is bound with high affinity by the sst_2 and sst_5 receptor subtypes and with a moderate affinity by sst_3, but not by subtypes sst_1 and sst_4 (58). There are also differences in the cell trafficking and internalization capabilities of the different receptor subtypes (20, 56); upon ligand binding, sst_3 and sst_2 internalize much better than sst_1 (20). sst_5 Not only internalizes after ligand binding but can additionally trigger a massive recruitment of sst_5 receptors from intracellular stores to the membrane (70). Presently, several groups in academia and in the pharmaceutical industry are searching for somatostatin analogs with binding profiles selective for specific subtypes.

2. In vitro detection of somatostatin receptor in normal human tissues. The information on somatostatin receptor and receptor subtype distribution in normal human tissues is still incomplete, mainly because of limited access to normal human tissues. Unfortunately, it is not possible to simply extrapolate animal data to humans because of species differences (71, 72). Receptor binding studies, mRNA determination, and/or receptor immunohistochemistry have identified somatostatin receptors in human brain (for review, see Ref. 73) as well as in numerous peripheral tissues, including pituitary, pancreas, gut, thyroid, adrenal, kidney, and the immune system; a complex pattern of somatostatin receptor subtype expression has been observed, including coexpression of multiple subtypes in a tissue-specific pattern (74–76). The subtype most frequently expressed is usually sst_{2A}, as shown in recent immunohistochemical and receptor autoradiographical studies using subtype-selective antibodies and somatostatin analogs; abundant sst_{2A} is found in pancreatic islets (72), in specific regions of the human brain (77), and in the peripheral nervous system (plexus myentericus and submucosus) (54). It shows up in the immune system, *i.e.*, in the germinal centers of lymphoid follicles and in human peripheral blood lymphocytes (54, 78). sst_2 is also present in the human adrenal gland (79) as well as in the kidneys (80, 81). The precise localization of the other ssts in human tissues is not yet fully established. sst_3 and sst_5 have been identified in T lymphocytes (82–84). The human placenta (85) as well as the fetal and adult lung display predominantly sst_4 (86, 87).

3. In vitro detection of somatostatin receptors in human tumors. Somatostatin receptors are not only expressed in physiological conditions. Indeed, a wide variety of human tumors express somatostatin receptors, which can be detected *in vitro* (Table 4).

a. Receptor binding. Early studies using *in vitro* receptor autoradiography have provided the identification of human tumors expressing somatostatin receptors, but without giving, at that time, information about subtypes, which had not yet been discovered. A very high incidence and often a high density of somatostatin receptors have been found in neuroendocrine tumors, in particular in GH-secreting pituitary adenomas (88–90) and gastroenteropancreatic tumors (91, 92), pheochromocytomas (79, 93), neuroblastomas (94, 95), and to a lesser extent in medullary thyroid cancers (96) and small cell lung cancers (97). Nonneuroendocrine tumors including brain tumors such as meningiomas, medulloblastomas, or gliomas (98–100), but also breast carcinomas (101, 102), lymphomas (103), renal cell cancers (104), mesenchymal tumors (105), prostatic (106–108), ovarian (109, 110), gastric (45, 111), hepatocellular (112, 113) and nasopharyngeal carcinomas (114) were also shown to express somatostatin re-

TABLE 4. Peptide receptor expression at the protein level in human tumors

Tumor type	Somatostatin-R[a]	VIP/PACAP-R[a]	GRP/bombesin-R[a]	NTR1	CCK_1/CCK_2[a]	NK_1	NPY-R[a]
GH-producing pituitary adenoma	**+** (sst_2, sst_5)	+ (PAC_1)		−	−		
Nonfunctioning pituitary adenoma	+ ($sst_3 > sst_2$)	+ (PAC_1)		−	−		
Gut carcinoid	**+** ($sst_2 > sst_1$, sst_5)	+	+ (NMB-R)	−	+ (CCK_1)		
Gastrinoma	**+** (sst_2)	+	**+** (GRP-R)	−			
Insulinoma	+	+		−	+ (CCK_2)		
Paraganglioma	**+** (sst_2)	+ (PAC_1)		−	−		
Pheochromocytoma	**+** (sst_2)	+ (PAC_1)			−		
Medullary thyroid carcinoma	+	−		+	**+** (CCK_2)	+	
Small cell lung cancer	+ (sst_2)	+	+ (BB_3)	+	+ (CCK_2)	+	
Non-small cell lung cancer	−	+ ($VPAC_1$)		−	−	−	−
Meningioma	**+** (sst_2)	+		**+**	+ (CCK_1)		
Neuroblastoma	**+** (sst_2)	+ (PAC_1)		−	+ (CCK_1)	−	
Medulloblastoma	**+** (sst_2)	+		+			
Astrocytoma	+	+ (PAC_1)		+	+ (CCK_2)	**+**	
Glioblastoma	−	+ (PAC_1)				**+**	
Exocrine pancreatic tumor	−	+ ($VPAC_1$)	−	**+**	−	−	
Colorectal carcinoma	−	+ ($VPAC_1$)		−	−	−	−
Gastric carcinoma	+	+ ($VPAC_1$)		−	−		
Hepatocellular carcinoma	+	+ ($VPAC_1$)		−	−		
Esophageal carcinoma	−	+ ($VPAC_1$)					
Renal cell carcinoma	+	+ ($VPAC_1$)	+ (GRP-R)	−	−		
Prostate carcinoma	+ (sst_1)	+ ($VPAC_1$)	**+** (GRP-R)	−	−		−
Urinary bladder carcinoma	−	+ ($VPAC_1$)					
Breast carcinoma	+	+ ($VPAC_1$)	**+** (GRP-R)	−	−	+	**+** (Y_1)
Endometrial carcinoma	−	+					
Ovarian carcinoma	+	+ ($VPAC_1$)		−	−		
Lymphoma	+	+		−	−	−	
Ewing sarcoma	−	−		**+**			
Leiomyoma	+	+ ($VPAC_2$)					

Bold +, receptors with particularly high density and incidence.

[a] Subtype preferentially expressed is listed in parentheses, only when compelling evidence is available (immunohistochemistry or autoradiography).

ceptors. The main classes of tumors that express somatostatin receptors are listed in Table 4. Various radioligands were used in binding studies, either natural somatostatin, *e.g.*, ^{125}I-analogs of somatostatin-14 or somatostatin-28 as universal radioligands, or synthetic, small-sized analogs, such as ^{125}I-[Tyr^3]octreotide, ^{125}I-MK-678, or ^{125}I-RC-160, which label only selected somatostatin receptor subtypes (115). In general, these studies revealed that somatostatin receptor expression was highly variable from one individual to another and from one tumor type to another. Whereas some tumors are characterized by a high density of receptors, such as meningiomas or medulloblastomas, others, such as lymphomas, have a much lower density. Some tumors have a rather homogeneous somatostatin receptor distribution, *e.g.*, most neuroendocrine tumors, in particular gastroenteropancreatic tumors, as shown by a somatostatin receptor-positive carcinoid in Fig. 3. Other tumors, such as breast carcinomas, are characterized, however, by a highly heterogeneous somatostatin receptor distribution, with regions of high density next to regions lacking the receptor (101), a pattern that reflects the marked polyclonality of this type of tumor. The determination of a precise value of receptor density is therefore hardly possible in such tumors, and only of relative significance. Receptor homogeneity is very important with regard to potential targeting of these somatostatin receptors for diagnosis or therapy.

As stated in *Section IV*, a crucial problem of *in vitro* analysis of somatostatin receptors in tumor samples is the incidence of false-positive results due to amalgamation of the tumor samples with nonneoplastic, somatostatin receptor-expressing tissues. For instance, most of the colorectal cancers minimally express octreotide binding sites (116, 117), whereas the vessels located around the tumors (peritumoral vessels) have a high density of such sites (116). In glial tumors, even morphological methods such as receptor autoradiography may not be able to adequately distinguish between somatostatin receptors present in tumor cells and those present in residual nerve fibers (99, 118). These glial tumors are known, indeed, to heavily infiltrate the normal brain, which itself strongly expresses somatostatin receptors. The high proportion of glial tumor samples contaminated by somatostatin receptor-positive nervous tissues makes it difficult to assess precisely the degree of somatostatin receptor expression by these tumors; although both Dutour *et al.* (119) and Held-Feindt *et al.* (120) could identify glial tumor cells expressing sst_2 with high resolution techniques, such findings were questioned in a more recent study (118).

Most of the receptor autoradiography studies done in the 1980s and early 1990s still epitomize the basic, still valid information on somatostatin receptor expression in tumors although somatostatin receptor subtypes could not precisely be identified at that time. Analysis of mRNA, immunohistochemistry with selective antibodies, and autoradiography with subtype-selective ligands have recently been introduced and have been able in most instances to confirm the previous data and extend them by determining the subtypes involved. However, by far not in all tumor types were these

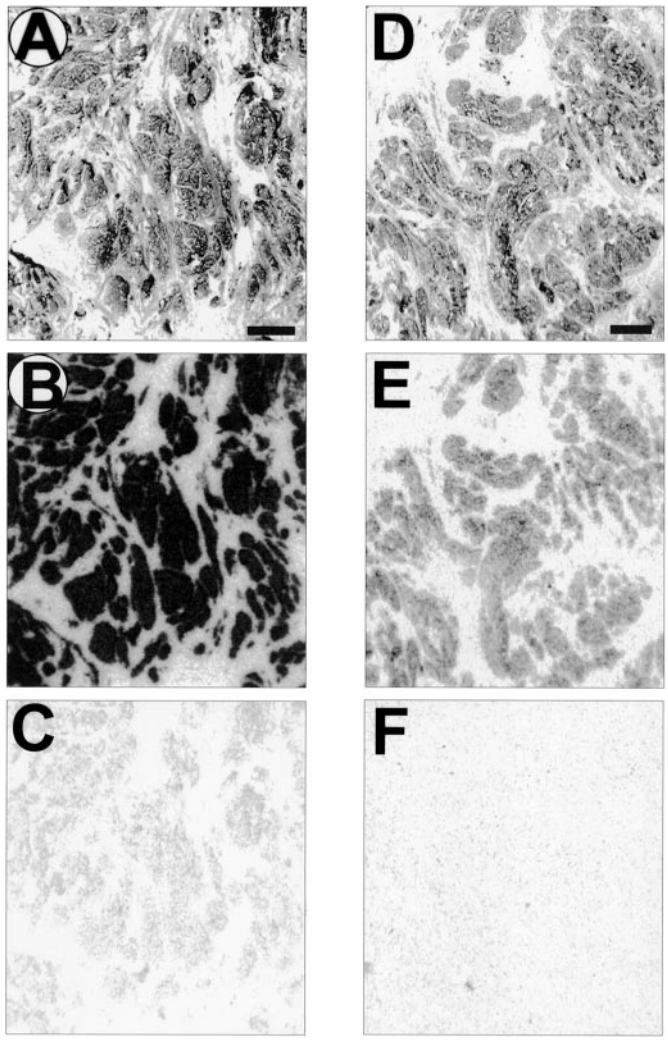

FIG. 3. *In vitro* detection of somatostatin receptors in carcinoids. A–C, Receptor autoradiographical analysis. A, Hematoxylin-eosin-stained section. *Scale bar*, 1 mm. B, Autoradiogram showing a high density of somatostatin receptors in the whole tumor (total binding of ^{125}I-[Leu^8, D-Trp^{22}, Tyr^{25}]-somatostatin-28). C, Autoradiogram showing nonspecific binding (in presence of 10^{-6} M somatostatin-28). D–F, *In situ* hybridization of sst_2 mRNA in the same carcinoid. D, Hematoxylin-eosin-stained section. *Scale bar*, 1 mm. E, Autoradiogram showing sst_2 mRNA in the tumor by use of a ^{33}P-labeled probe. F, Control section, in presence of excess of unlabeled probe.

methods able to bring clarity in terms of the sst subtype protein involved.

b. Receptor mRNA. Human tumors often express multiple somatostatin receptor subtype mRNAs, as reported first in pituitary adenomas (121–125) and gastroenteropancreatic tumors (125–127). A carcinoid with abundant sst_2 mRNA is shown in Fig. 3. In the past few years, a profusion of papers have appeared identifying mRNA for the various somatostatin receptor subtypes in a large variety of other human cancers (79, 108, 109, 119, 125, 128–138), confirming and extending the results of previous binding studies. In many of the RT-PCR-based investigations, the incidence of the various receptor mRNAs in tumors appears, however, to be higher than that detected by receptor binding or immunohistochemical studies (41, 48, 101, 116, 139); moreover, mRNAs for several somatostatin receptor subtypes appear to be frequently expressed concomitantly in individual tumors. It is presently not clear whether 1) these RT-PCR data reflect an overestimation of the real contribution of the various somatostatin receptor mRNAs due to the outstanding sensitivity of the method, 2) these mRNAs originate in part from nontumoral adjacent tissues, or 3) these mRNAs are not always translated into significant amounts of the respective receptor subtype proteins. Thus, one may caution against an overestimation of mRNA data obtained by ultrasensitive methods such as RT-PCR. We should not rely exclusively on mRNA determinations to assess the tumoral receptor status; the main target for the current clinical applications of somatostatin ligands is the receptor protein located on the cell membrane, not the mRNA.

c. Receptor immunohistochemistry. An emerging new technique to detect somatostatin receptor subtypes is immunohistochemistry, which has the advantage of a high cellular resolution. The results, however, depend clearly on the quality, selectivity, and specificity of the antibodies, several of which directed against somatostatin receptors are currently available. Even highly specific antibodies for sst_{2A}, such as R2-88, can weakly cross-react with unrelated proteins (54). Up to now, only a few, carefully controlled immunohistochemical studies have been performed in cancer, primarily with sst_2 antibodies. A high density of sst_{2A} was found in neuroblastomas (140), medulloblastomas (137, 140), paragangliomas (140), and small cell lung cancers (140) as well as in meningiomas (141) and breast cancers (142, 143). Most neuroendocrine lung and gastroenteropancreatic tumors were shown to have preferentially membrane-bound sst_2 with immunohistochemical methods (144–148). An example of an sst_{2A}-expressing carcinoid with strong membrane-bound receptor localization using R2-88 is shown in Fig. 4. There have been few reports investigating other somatostatin receptor subtypes with immunohistochemistry, such as sst_1, sst_3, and sst_5 in gastroenteropancreatic tumors (147, 149) and sst_3 in breast cancers (142). The observation of an intracellular location of some of these receptors (147, 149) is intriguing and not fully understood. It is, however, worth noticing that, both in rat brain and human tumors, a correlation between the local presence of endogenous somatostatin and an increased internalization of sst_2 receptors could be observed (140, 150).

Analysis of somatostatin receptors in tumors by immunohistochemistry may become an additional, useful parameter for the clinician to evaluate the biology of a tumor and choose therapeutic options, in particular because standard formalin-fixed material is sufficient for this type of investigation (145).

d. Binding studies with subtype-selective analogs. The recent development of somatostatin receptor subtype-selective analogs, both as peptides and nonpeptides (83, 151–153), is an important advance permitting 1) evaluation of the distribution of various receptor subtype proteins in tissue, 2) determination of the specific biological effects mediated by the various subtypes, and 3) the design of new drugs for specific therapeutic strategies. Some of these analogs were already

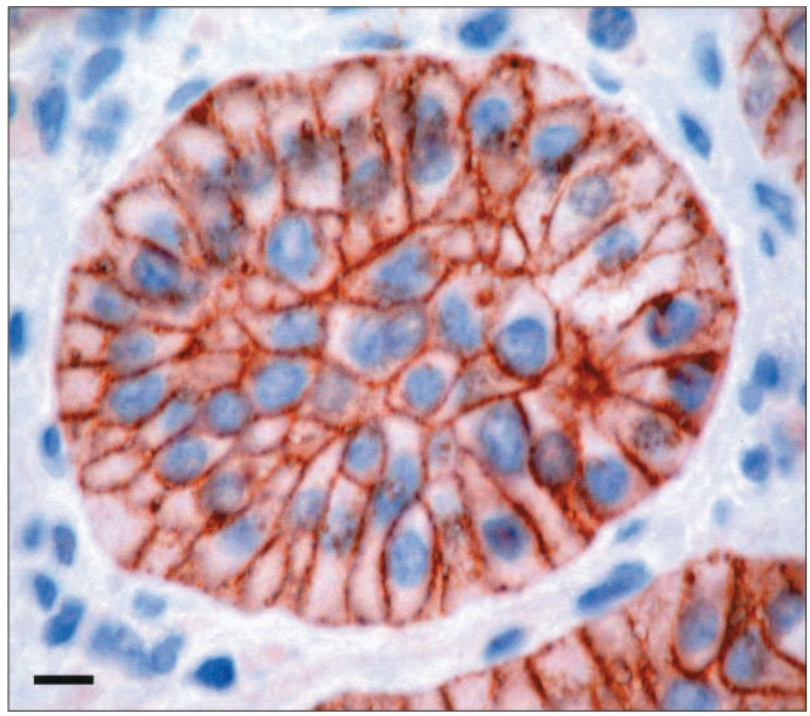

FIG. 4. sst_{2A} In a carcinoid tumor. Immunohistochemical detection of membrane-bound sst_{2A} receptors with R2-88 antibody. The strong *red-brownish* membrane-bound immunostaining on each of the tumor cells reflects the abundance of somatostatin receptors of the sst_{2A} subtype in this ileal carcinoid. (R2-88 antibody was generously provided by Dr. A. Schonbrunn, Houston.) *Scale bar*, 0.1 mm.

used to refine somatostatin receptor binding studies to detect receptor subtypes in tissues (154, 155). Moreover, in a study using receptor autoradiography with five different subtype-selective analogs, we evaluated somatostatin receptor subtypes expressed in cancers (50, 156); these data suggest that in many somatostatin receptor-positive tumors there is a predominance of the proteins for one or two somatostatin receptors. A preponderance of sst_2 binding sites is seen in the majority of neuroblastomas, medulloblastomas, breast cancers, meningiomas, paragangliomas, renal cell carcinomas, lymphomas, hepatocellular carcinomas, and small cell lung cancers (50). Conversely, sst_1 is frequent in prostate cancers and in many sarcomas, whereas sst_3 occurs frequently in inactive pituitary adenomas (50, 83). A larger subtype variability with several ssts expressed concomitantly is seen, among others, in GH-producing pituitary adenomas (especially sst_2 and sst_5), pheochromocytomas, hormone-producing gastroenteropancreatic tumors, and gastric cancers (50, 156, 157). Interestingly, sst_4 is not often expressed in the human cancers tested.

4. Somatostatin receptors in peritumoral vessels. Recently, the peritumoral vascular system of the host has emerged as a possible target of somatostatin action in tumor development. In a series of human colonic carcinomas, a high density of vascular somatostatin receptors was observed in vessels in the immediate vicinity of the tumors; the receptor density decreased continuously with increased distance of the vessels from the carcinomas, suggesting a local phenomenon related to the presence of the tumor (116). The presence of vascular somatostatin receptors seemed to be independent of the presence or absence of somatostatin receptors in the tumor itself. More recently, a study including a large number of different types of human neoplasms has suggested that the expression of somatostatin receptors in peritumoral veins is a general phenomenon (158). For instance, all medullary thyroid carcinomas, colonic, and gastric cancers express somatostatin receptors in peritumoral veins; a majority of parathyroid adenomas, renal cell cancers, melanomas, sarcomas, breast cancers, and prostate cancers have somatostatin receptors in peritumoral veins, whereas gastroenteropancreatic tumors or ovarian cancers rarely do. Recent studies demonstrated that angiogenic vessels as well as peritumoral vessels expressed predominantly sst_2 (119, 159). In some tumors, such as melanomas, the somatostatin receptors are expressed not only in peritumoral but also in intratumoral veins. This may be the reason why it is possible to successfully visualize this type of tumor *in vivo* with Octreoscan, although melanoma cells do not express significant levels of somatostatin receptors.

The function of somatostatin in the peritumoral vasculature, mediated by a high density of somatostatin receptors in the smooth muscle cells and possibly in the endothelium (158, 159), may be primarily vasoconstrictive, as shown in particular in the gut (160). Therefore, an increased somatostatin receptor density may allow a strong and rapid local vasoconstriction, possibly resulting in local hypoxia and necrosis of the tumor, or a more prolonged vasoconstriction, directed against metastatic tumor dissemination. Whether this mechanism is responsible for the occasional clinical observation of a decrease in tumor size during long-term octreotide therapy in some patients (161) is unknown. Despite a very broad interest in tumor angiogenesis in general, progress in understanding the role of peritumoral somatostatin receptors has been very slow (162). In neoplasms, somatostatin may act locally on tumor growth through two different mechanisms dependent on local somatostatin receptor expression: through direct action on tumor cells or through action on peritumoral vessels, which may alter the

dynamics of the tumoral blood circulation and/or inhibit angiogenesis (162).

5. Somatostatin receptors in nonneoplastic diseases. There is strong evidence that selected nontumoral lesions may also express somatostatin receptors. For instance, active granulomas in sarcoidosis express somatostatin receptors on the epithelioid cells (163). Inactive or successfully treated fibrosing granulomas devoid of epithelioid cells lack somatostatin receptors. Inflamed joints in active rheumatoid arthritis express somatostatin receptors, preferentially located in the proliferating synovial vessels (164). Furthermore, inflammatory bowel disease is characterized by an overexpression of somatostatin receptors in the vascular system (165) of the altered parts of the gastrointestinal tract. The expression of somatostatin receptors is therefore not specific for tumoral pathologies.

B. Vasoactive intestinal peptide (VIP) receptors

1. VIP/pituitary adenylate cyclase activating peptide (PACAP) and their receptor subtypes. VIP is a 28-amino-acid-long neuropeptide isolated from the small intestine. It is a member of the group of secretin-like peptides (166). Together with PACAP, a structurally similar, 27- or 38-amino-acid-long peptide, it is one of the important neurotransmitters in the gut. VIP and PACAP both play a neuromodulatory role in the central nervous system, at both the neuronal and glial levels (167). Furthermore, extensive immunomodulatory properties have been reported for these peptides (168–171). Their actions are mediated by specific G protein-coupled receptors that can be internalized after ligand binding (25). One of the most prominent signaling pathways of VIP/PACAP is the stimulation of adenylate cyclase activity, as seen impressively in nonfunctioning pituitary adenomas (172). In the last few years, molecular biology has provided evidence for the existence of several receptor subtypes within the VIP/PACAP family (173, 174). There are two VIP receptors, $VPAC_1$ and $VPAC_2$, both with high affinity for VIP and PACAP (Table 1). They can be distinguished pharmacologically by the $VPAC_1$-selective analog [Lys^{15},Arg^{16},Leu^{27}]-VIP(1–7)/GRF(8–27) (KRL-VIP/GRF) and the $VPAC_2$-selective RO 25-1553 (175, 176). There is at least one PACAP receptor, named PAC_1, that is characterized by high affinity for PACAP but by a low affinity for VIP (173, 174, 177); recently, several PAC_1 splice variants with distinct pharmacological behaviors have, however, been identified (178, 179).

2. VIP/PACAP receptors in normal human tissues. VIP/PACAP receptors are found not only in the brain (167), but ubiquitously in the majority of the human epithelial tissues (180). In most of these tissues, the VIP/PACAP receptor subtype preferentially expressed is the $VPAC_1$ receptor, for instance in hepatocytes, gastrointestinal mucosa, lobules and ducts of the breast, thyroid follicles, prostatic glands, urothelium of bladder and ureter, and acini of the lung and pancreatic ducts (52, 180, 181). Some other tissues, however, predominantly express the PAC_1 receptor, *e.g.*, the adrenal medulla, several brain areas, and the pituitary (52). Conversely, smooth muscle in various locations preferentially expresses $VPAC_2$ receptors, as documented by ^{125}I-VIP binding displaced by nanomolar concentrations of the $VPAC_2$-selective RO 25-1553, but not of the $VPAC_1$-selective analog KRL-VIP/GRF (52). Such $VPAC_2$ receptors in smooth muscles are present in locations as different as the gastrointestinal tract (stomach) or the seminal vesicle. Furthermore, blood vessels, arteries more than veins, express VIP receptors of the $VPAC_2$ subtype located primarily in the smooth muscle layers. Moreover, the wall, *i.e.*, most likely the smooth muscle, of the uterus and also of the prostate are primarily $VPAC_2$ receptor-expressing tissues. The peripheral nervous system, for instance the myenteric plexus in the colon wall, shows a predominance of PAC_1 receptors, whereas the Cajal cells may express $VPAC_2$ (182). A great majority of the human solid lymphoid tissues, including spleen, thymus, lymph nodes, and Peyer's patches express VIP/PACAP receptors at high density (169, 183). This presence of VIP/PACAP receptors in most normal human tissues points to multiple and complex biological actions of VIP/PACAP in the human body.

3. VIP/PACAP receptors in human tumors and their metastases. Human tumors derived from normal tissues expressing VIP/PACAP receptors frequently also express the same receptors. While the great majority of tumors analyzed earlier for VIP/PACAP receptors were actually tumor cell lines (Refs. 184 and 185; for review, see Ref. 174), very few authors have investigated primary human cancers. A few years ago, it was shown that most primary human tumors express VIP/PACAP receptors at high incidence (105, 186). In these studies, ^{125}I-VIP binding displaced by VIP or PACAP was investigated. More recently, a follow-up study tried to discriminate the subtype expressed by these tumors using receptor subtype-selective analogs (52).Tumors expressing $VPAC_1$ receptors include the most frequently occurring malignant epithelial neoplasms, such as cancers of the lung, stomach, colon, rectum, breast, prostate, pancreatic ducts, liver, and urinary bladder (52) (Table 4). In contrast to this ubiquitous expression of $VPAC_1$ receptors in most human tumors, a predominance of $VPAC_2$ receptors was found in only few tumors. The only example of a consistent and predominant $VPAC_2$ receptor expression, among the tumors tested, is that of the benign smooth muscle tumors, the leiomyomas (Table 4). They exhibit a strong ^{125}I-VIP binding displaced with nanomolar concentrations of RO 25-1553 but not of KRL-VIP/GRF; they can also be labeled directly by ^{125}I-RO 25-1553 (52). In contrast, several different human tumor types express predominantly PAC_1 receptors, in particular tumors originating from the neuronal and endocrine systems; this includes glial tumors (astrocytomas, glioblastomas, oligodendrogliomas), neuroblastomas, as well as various pituitary adenomas (especially GH-secreting and nonfunctioning adenomas, but not prolactinomas), as described previously by the group of Robberecht (177, 187, 188) and by Oka *et al.* (189). More recent data have shown that most catecholamine-secreting tumors, including both pheochromocytomas and paragangliomas, appear to express predominantly PAC_1 receptors (52). Moreover, many endometrial carcinomas also have PAC_1 receptors (52). Interestingly, medullary thyroid cancers are among the rare tumors that do not express VIP/PACAP receptors (186). Although $VPAC_1$ mRNA was identified in many tumor cell lines, such as lung

and breast cancers (Refs. 190 and 191; for review, see Ref. 174), immunohistochemical data showing VIP/PACAP receptor expression in human tumors are not yet available.

C. Cholecystokinin (CCK) receptors

1. CCK/gastrin and their receptor subtypes. The gastrointestinal peptides gastrin and CCK exist in different molecular forms (Table 1). Pro-gastrin and pro-CCK can be processed to peptides of variable length but, as biologically active peptides, they have the same five terminal amino acids at their carboxy terminus. They act as neurotransmitters in the brain, as regulators of various functions of the gastrointestinal tract, primarily at the level of the stomach, pancreas, and gallbladder (192). In addition, they can act as physiological growth factors in most parts of the gastrointestinal tract (193, 194) and also as growth factors in several neoplasms, such as in colonic, gastric, and brain cancers (195–198). CCK and gastrin actions are mediated by several receptor subtypes, the best characterized being CCK_1 (formerly CCK-A) and CCK_2 (formerly CCK-B) receptors (199, 200) (Table 1). They can be distinguished pharmacologically by their low (CCK_1) *vs.* high (CCK_2) affinity for gastrin, or by their different affinity for nonpeptidic selective CCK antagonists. Recently, additional CCK receptors have been described, such as a CCK-C or a gastrin receptor in Swiss 3T3 fibroblasts (201–203). Extensive information on CCK and gastrin signaling, recently reviewed (204), has been obtained over the past several years. CCK_1 and CCK_2 receptors have been identified in several normal tissues (for review, see Ref.205). CCK_2 receptors are present predominantly in the gut mucosa, in the endocrine pancreas, and in the brain (206–208); CCK_1 receptors in the gallbladder, in gastric smooth muscles (208, 209), and in the peripheral nervous system, for instance in afferent vagal neurons (210) or in the myenteric plexus (182). As reported recently (211), human pancreatic acinar cells do not express a significant amount of CCK receptors in contrast to rat pancreatic acinar cells. This is a further example of the wide species variability of peptide receptor expression. CCK receptors, as most peptide receptors, can be rapidly internalized (23).

2. CCK_1 and CCK_2 receptors in cancer. It has been established for a long time that small cell lung cancers often express CCK_2 receptors, whereas non-small cell lung cancers do not (51, 212, 213). The findings are more equivocal for gastrointestinal cancers (214). Whereas earlier studies have reported the presence of CCK_2 receptors in carcinomas of colon and stomach (215), more recent investigations have failed to find high-affinity CCK_2 receptor proteins in most of these tumors (45, 216) although their mRNA is usually identified (217). The same may also be true for exocrine pancreatic carcinomas; although CCK_1 and CCK_2 receptor mRNA were identified in most tumors (218, 219), the receptor protein is difficult to detect in tumor cells themselves. The most frequently identified CCK receptor-expressing tissue elements in pancreatic cancer samples are nerves (CCK_1) and islets (CCK_2), but not tumor cells (219a). A possible explanation for some of these discrepancies may be the existence of CCK_2 receptor mutations in pancreatic, colorectal, and gastric cancers (220–222). A misspliced form of the CCK_2 receptor that was detected in these tumors has constitutive activity and trophic effects (220, 221). Such mutated receptors may have altered binding characteristics. Recently, however, a high incidence of the regular CCK_2 receptor protein was identified in medullary thyroid carcinomas (92%), whereas it was absent in differentiated thyroid cancers (223–225). CCK_2 receptors were also found frequently in astrocytomas (65%) and in sex cord-stromal ovarian cancers (100%) (51) (Table 4); in some of the neuroendocrine gastroenteropancreatic tumors (in particular insulinomas) (156); in breast and endometrial adenocarcinomas; and in several soft tissue tumors, in particular in leiomyosarcomas (226). They were either not expressed or rarely expressed in meningiomas, neuroblastomas, schwannomas, glioblastomas, lymphomas, renal cell cancers, prostate carcinomas, hepatocellular carcinomas, and neuroendocrine tumors such as pituitary adenomas, pheochromocytomas, paragangliomas, or parathyroid adenomas (Table 4). CCK_1 receptors were expressed in neuroendocrine lung and gastroenteropancreatic tumors, meningiomas, and some neuroblastomas (51, 156, 227). Immunohistochemical detection of CCK_1 or CCK_2 receptors in tumors has not yet been reported.

Gastrin mRNA measured by *in situ* hybridization was found to be present in some CCK_2 receptor-positive small cell lung cancers, breast tumors, ovarian tumors, and stem cell tumors of various origins, possibly as indicators of an autocrine growth regulation of these tumors (51, 226). Conversely, gastrin and CCK mRNAs were absent in CCK_2-expressing medullary thyroid cancers.

D. Bombesin/gastrin-releasing peptide (GRP) receptors

1. Bombesin/GRP and their receptor subtypes. Bombesin and GRP, members of a family of brain-gut peptides, play an important role in cancer (228–230), in addition to their physiological function. Bombesin is a 14-amino-acid peptide present in amphibian tissues, whereas GRP, its human counterpart, consists of 27 amino acids. GRP and bombesin differ by only one of the 10 carboxy-terminal residues. This explains the similar biological activity of the two peptides. GRP acts primarily in the central and enteric nervous systems where it regulates several physiological processes including satiety, thermoregulation, circadian rhythm, smooth muscle contraction, immune function, as well as the release of other peptide hormones (229, 230). However, of all of the effects of GRP, the most studied is the one related to cancer. It was observed several years ago that cancer cell lines as well as primary human tumors can synthesize bombesin and GRP (231). Cuttitta *et al.* (228) showed that bombesin and GRP can stimulate small cell lung cancer growth and that this action is part of an autocrine feedback mechanism involving the expression of these peptides and that of their receptors in the tumor cells (232, 233). More recently, GRP and bombesin were deemed to play a role in other cancers as well. Stimulation of proliferation by bombesin was reported for lung, breast and pancreatic cancers (234–236). Moreover, GRP can promote cell proliferation in neuroblastoma cell lines (237) or in the androgen-independent human prostatic carcinoma cell line PC3; antagonists to the GRP receptor inhibit the growth of human prostatic carcinoma or of glioblastoma

xenografts in nude mice (238, 239). Bombesin and GRP mediate their actions through membrane-bound, G protein-coupled receptors, which include at least four different subtypes, namely the neuromedin B receptor subtype (BB_1), the GRP receptor subtype (BB_2), the BB_3 and BB_4 subtypes (240–243). With the exception of the GRP receptor (182, 244), these subtypes have been poorly characterized in regard to their distribution and function in human tissues.

2. Bombesin/GRP receptors in human tumors. Although GRP receptors have been readily detected in various types of tumor cell lines (245–247), it has been more difficult to identify them in primary human cancers. GRP receptor mRNA could well be measured in various human neoplasms, including cancers of the gastrointestinal tract, lung, prostate, and breast (248–250). Recently, GRP receptors have also been detected in neuroblastomas by immunohistochemistry (237). The GRP receptor proteins have been difficult to detect with binding methods in gastrointestinal cancers (251, 252). Results have been controversial in exocrine pancreatic carcinomas: whereas one study found GRP-receptor expression in these cancers (253), more recent investigations identified these receptors extremely rarely (254, 255); however, peritumoral vessels surrounding exocrine pancreatic carcinomas clearly express GRP receptors (254). GRP receptor proteins have been more easily identified in renal cell, breast, and prostate carcinomas (248, 251, 256–258). Interestingly, some tissues of origin of these cancers express the GRP receptor (*e.g.*, breast; Ref. 256), whereas others do not (*e.g.*, prostate; Ref. 257).

Two possible reasons have been put forward to explain the difficulty of detecting GRP receptor proteins in some tumors: some authors have suggested that the potent neutral endopeptidase EC 3.4.24.11 (259) rapidly degrades the bombesin ligands (251); others have proposed that aberrant and possibly mutated GRP receptors (249, 260) with altered pharmacological characteristics (261) account for this difficulty.

Because of the potential clinical impact, it is worth emphasizing the strong GRP receptor expression in two tumoral conditions. GRP receptors were detected, often in high density: 1) in 30 of 30 invasive prostatic carcinomas and in 26 of 26 cases of prostatic intraepithelial proliferative lesions, mostly prostatic intraepithelial neoplasias (257). Bone metastases of androgen-independent prostate cancers were also GRP receptor-positive in four of seven cases. Conversely, GRP receptors, absent in normal prostate, were identified in only a few hyperplastic prostates; they were localized in very low density in glandular tissue and, focally, in some stromal tissue (257). The massive GRP receptor expression in prostate tissues that are in the process of malignant transformation (*e.g.*, in prostatic intraepithelial neoplasias) or that are completely neoplastically transformed suggests that GRP receptors may be markers for early molecular events in prostate carcinogenesis and useful in differentiating prostate hyperplasia from prostate neoplasia. 2) GRP receptors were also detected in neoplastic epithelial mammary cells in two thirds of invasive ductal carcinomas and ductal carcinomas *in situ* (256). The lymph node metastases from those primary carcinomas expressing GRP receptors were all positive, whereas surrounding lymphoreticular tissue was GRP receptor-negative. Although these receptors were also present in ducts and lobules from nonneoplastic breast tissue samples, the strong GRP receptor expression in breast carcinomas suggests that these tumors may be a consequential target for GRP and bombesin analogs (256).

Recently, a very potent ligand, the [D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]bombesin(6–14), shown to be bound by all four bombesin receptors, has been developed by the Jensen group (262, 263). This compound, iodinated at the D-Tyr^6 residue, yields a useful radioligand able to distinguish the various bombesin receptor subtypes on the basis of the rank order of their affinity for GRP, neuromedin B, [D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]bombesin (6–14), or bombesin. Using this approach, we could specifically detect BB_3 receptor subtype expression in human pancreatic islets (254). More recently, we identified neuroendocrine tumors with a differentiated profile of receptor subtype expression: gastrinomas displayed preferentially GRP receptors, and ileal carcinoids expressed often neuromedin B receptors, whereas bronchial carcinoids and small cell lung carcinomas frequently had BB_3 receptors (156, 264).

E. Neurotensin receptors

1. Neurotensin and its receptor subtypes. Neurotensin is a tridecapeptide localized both in the central nervous system and in peripheral tissues, mainly in the gastrointestinal tract (265–267). In the central nervous system, neurotensin plays the role of neurotransmitter or neuromodulator of dopamine transmission and of anterior pituitary hormone secretion (267). It also shows potent hypothermic and analgesic effects in the brain. In the periphery, neurotensin acts as a local hormone exerting a paracrine and endocrine modulation of the digestive tract (267). It may also play an important role in gut mucosal immune responses (268, 269). Finally, it can stimulate growth in a variety of normal cells (270). The pharmacological effects of neurotensin result from the specific interaction of the peptide with cell-surface receptors. However, the pharmacology and mode of action of neurotensin receptors are not completely clear. The signaling pathway of one of the neurotensin receptor subtypes, the high-affinity NTR1 receptor, is well documented, including Ca^{2+} release after inositol 1,4,5-triphosphate stimulation (271), activation of MAPKs (272) via protein kinase C, leading to its role in cell proliferation. All of the effects mediated by the NTR1 are blocked by its selective nonpeptide antagonist SR 48692 (273). By contrast, the signaling pathway that governs the interaction of neurotensin with another neurotensin receptor subtype, the levocabastine-sensitive NTR2 receptor (274), is a matter of controversy. The complexity of neurotensin signaling has been recently emphasized by the molecular identification of a third membrane protein, non-G protein-coupled, capable of binding the peptide with a high affinity, the NTR3 receptor (275). This protein is identical to gp95 sortilin, a sorting protein originally identified by its ability to interact with a receptor-associated protein (276). Neurotensin receptors have been shown to be internalized after interaction with the peptide (24, 277). For instance, after interaction with neurotensin, 60–70% of the NTR1 receptor present in COS cells internalizes according to a temperature-

dependent process (271, 278). Neurotensin is rapidly degraded in blood plasma by endogenous peptidases and proteases. Several proteolytic enzymes including neutral endopeptidase EC 3.4.24.11, angiotensin-converting enzyme, and metalloendopeptidases EC 3.4.24.15 and EC 3.4.24.16 have been reported to cleave intact neurotensin.

2. Neurotensin receptors in cancer. Several lines of evidence suggest that neurotensin plays a role in cancer. It is known that neurotensin receptors are expressed in various tumor cell lines including small cell lung cancer, neuroblastoma, pancreatic, or colonic cancer (279, 280). Clinically more relevant, they can be overexpressed in primary human tumors, *e.g.*, in most meningiomas and Ewing's sarcomas (281, 282), more than three fourths of ductal pancreatic carcinomas (255, 283), and, in a somewhat lower incidence, in astrocytomas, medulloblastomas, medullary thyroid cancers, and small cell lung cancers (282). These neoplasms display NTR1 receptor proteins, characterized by their low affinity for levocabastine, as well as NTR1 mRNA (284, 285). NTR1 was rarely found in non-small cell lung cancers; in carcinomas of the breast, colon, rectum, prostate, ovary, renal, or hepatic cells; in neuroendocrine gut tumors; pituitary adenomas; schwannomas; neuroblastomas; and lymphomas (45, 282) (Table 4). An additional argument in favor of a role of neurotensin in cancer is that this peptide can stimulate the proliferation *in vitro* of tumor cell lines of various origins, including those originating in the pancreas, prostate, brain, and lung (30, 197, 286, 287). Conversely, the NTR1 receptor antagonist SR 48692 (273) inhibits tumor proliferation (30, 286–289). Also of further interest is NTR3, which has recently been shown to be involved in mediating neurotensin growth stimulation in cancer cell lines (290). The complex neurotensin-mediated signal transduction mechanisms are presently under investigation in cancer models (291, 292).

Interestingly, neurotensin itself appears to be expressed by numerous tumors or tumor cell lines. For instance, in some receptor-positive Ewing's sarcomas, neurotensin mRNA was detected by *in situ* hybridization techniques (282). The presence of neurotensin and of the neurotensin receptor in human neoplasia may therefore be an integrative part of an autocrine feedback mechanism of tumor growth stimulation (282), as shown previously for the GRP system.

F. Other peptide receptors

1. Substance P. Substance P is a neuropeptide involved in a variety of functions of the central and peripheral nervous systems, including pain perception and vasodilatation (293). One of the substance P receptor subtypes, the NK_1 receptor (Table 1), is quite frequently expressed in glial tumors, in particular in poorly differentiated glioblastomas, but can also be detected in medullary thyroid carcinomas, small cell lung cancers, pancreatic as well as breast cancers (294, 295); it is rarely found in gastrointestinal tumors or lymphomas. Interestingly, all tumor types, regardless of their histology, show high levels of NK_1 receptor expression in tumoral and peritumoral vessels. These vascular receptors may serve as the molecular basis for a substance P-mediated vasodilatation (294, 296). Substance P is able to stimulate the proliferation of malignant tumor cells (295). Accordingly, NK_1 receptor antagonists can inhibit the growth of human cancers, such as glioma U373 MG xenografts (297).

2. Neuropeptide Y (NPY). NPY is a member of a family of 36-amino-acid-long peptides including NPY, peptide YY, and pancreatic polypeptide. The main function of NPY is not that of an endocrine or gut hormone but that of a neurotransmitter; its best known actions are at the level of the central nervous system and include stimulation of feeding behavior and inhibition of anxiety (298–300). NPY actions mediated by the peripheral nervous system include vasoconstriction, as well as regulation of the gastrointestinal motility and secretion, insulin release, and renal function (298, 301–304). The effect of NPY can be mediated by several NPY receptor subtypes, named Y_1-Y_6, among which Y_1, Y_2, Y_4, and Y_5 have been well characterized and shown to be physiologically expressed (305). Several NPY analogs, in particular Y_1 and Y_2 antagonists, are being developed for potential clinical use to treat feeding disturbances and anxiety (306–308). Compared with other regulatory peptides, NPY has not often been associated with human cancer. A recent *in vitro* receptor autoradiography study (53), including more than 100 human breast cancer samples, reported however a NPY receptor incidence, predominantly of the Y_1 subtype, of 85% in primary human breast carcinomas and of 100% in lymph node metastases of receptor-positive breast cancer primaries. Y_1 receptors were not detected in primary human non-small cell lung cancers, colorectal cancers, or prostate carcinomas (J. C. Reubi *et al.*, unpublished data), whereas they have been identified in prostate cancer cell lines (309). In Y_1-expressing human SKN-MC tumor cells, a modest NPY-induced dose-dependent inhibition of tumor cell growth was observed (53), whereas NPY stimulated the growth of PC3 prostate cancer cells *in vitro* (309), suggesting a functional role of NPY in cancer via NPY receptors. The high incidence of Y_1 in *in situ*, invasive, and metastatic breast cancers allows for the possibility to target them for diagnosis and therapy with NPY analogs. Although Y_1-selective analogs are available (310), chelator-linked Y_1-analogs have not yet been developed for radioactive targeting in clinical settings. Conversely, Y_2-selective radiopharmaceuticals labeled with ^{99m}Tc have recently been described (311).

3. α-Melanocyte-stimulating hormone (α-MSH). α-MSH, a linear tridecapeptide produced in the pituitary gland from pro-opiomelanocortin, is primarily responsible for the regulation of skin pigmentation (312). α-MSH peptides bind their cognate receptors selectively with nanomolar affinities (312) and are rapidly internalized (313). Receptors for α-MSH have been demonstrated on the surface of human malignant melanomas (314); hence, these tumors were proposed as potential targets for the application of radiolabeled α-MSH peptides, albeit α-MSH receptors were usually expressed in low density (314).

4. LHRH. LHRH is a hypothalamic hormone acting primarily at the pituitary level to stimulate LH secretion. Early experimental studies performed predominantly by the Schally group (32, 315, 316) suggested that analogs of LHRH can be used for the treatment of estrogen-dependent breast cancer.

Regression of tumor mass and disappearance of metastases in premenopausal and postmenopausal women with breast cancer have been reported after treatment with D-Trp^6-LHRH, or with the analogs buserelin or leuprolide (316). Such inhibitory actions of LHRH analogs were originally thought to be mediated by LHRH receptors in pituitary gonadotrophs, inducing a suppression of the pituitary-gonadal axis with a resulting decrease in the circulating LH, FSH, estrogen, and prolactin levels (315). However, recent studies revealed that LHRH and some of its analogs can also exert a direct effect on rat and human breast cancers through specific LHRH receptors expressed in these tumors (317–319). More recently, such receptors were found in other tumors as well, including prostatic, endometrial, and ovarian carcinomas (320–323), and were characterized as belonging to the low-affinity GnRH-II subtype (324). The dual mechanism of action of LHRH analogs, direct on the tumor and indirect via the pituitary, makes it difficult to assess precisely the contribution of each of these mechanisms on tumor growth.

5. Calcitonin. Calcitonin is a 32-amino-acid neuropeptide involved in the regulation of calcium levels largely through its effects on osteoclasts and on the kidney (325). It is secreted by the C cells of the thyroid. Calcitonin receptors are present in increased numbers, *e.g.*, in osteolytic sites such as those occurring in metastatic bone cancers and Paget's disease (325). Very limited receptor information exists for primary human tumors, but giant-cell tumors of the bone (326) and medullary carcinomas of the thyroid (327) have been reported to express calcitonin receptors.

6. Atrial natriuretic peptide (ANP). ANP, a peptide hormone produced in the cardiac atrium, acts on the kidney, playing an important role in fluid, electrolyte, and blood pressure homeostasis (328). Three different ANP receptors have now been cloned: A and B receptors contain particulate guanylate cyclase in their intracellular domain, whereas the more abundant C receptor is not coupled to guanylate cyclase (328). *In vitro* studies have demonstrated a high density of specific ANP receptors in the kidney, adrenals, and lungs (329). Except for a recent report showing that neuroblastomas express ANP receptors of the A type in most cases (330), very little information exists on ANP receptor expression in human tumors.

7. Glucagon-like-peptide-1 (GLP-1). The basic role of the incretin hormone GLP-1 is the regulation of blood glucose levels. The postprandial stimulation of insulin secretion is mediated by a specific receptor, the GLP-1 receptor, situated on the surface of pancreatic β-cells in the islets of Langerhans (331). Insulinomas derived from pancreatic β-cells express receptors for GLP-1, as shown in the rat (332) and in a recent study in humans (156).

8. Oxytocin. Oxytocin is a nine-amino-acid-long peptide with both central and peripheral actions, the latter predominantly at the mammary gland and uterus level. Recently, immunohistochemical studies have revealed specific oxytocin receptors in glial tumors, neuroblastomas, and breast and endometrial cancers (333–335).

9. Endothelin. Endothelin is a 21-amino-acid peptide with potent vasoactive properties, mediated by the two receptors ET_A and ET_B. There is increasing evidence from *in vitro* and *in vivo* studies that endothelin is mitogenic to tumor tissue. Expression of the endothelin receptors has been found in cancer of the breast (336), ovary (337), and lung (338), and in gliomas and meningiomas (339). Endothelin may initiate or support the growth and progression of these tumors.

VI. Clinical Applications

A. General considerations

The clinical implications based on the presence of peptide receptors in human tumors are threefold: 1) tumor diagnosis with radioactive analogs, 2) tumor therapy with radioactive or cytotoxic analogs, and 3) long-term therapy with nonradioactive, noncytotoxic analogs. This section discusses current and potential clinical applications for the various peptides described above.

The principle of targeting tumoral peptide receptors with radiolabeled peptide analogs for diagnostic oncology is simple (Fig. 1): iv injection of the radiopeptide (linked to a γ-emitter such as ^{111}In, ^{99m}Tc, or ^{177}Lu) followed by γ-camera scintigraphy for 24–48 h. This procedure permits the identification of tumoral lesions as radioactive hot spots in the whole body; the whole body scan identifies receptor-positive tumors not only at presumed sites but also at unforeseen sites. It has a high sensitivity, because lesions as small as 5–10 mm can be detected. However, to achieve this, it requires a sufficiently high density of tumoral receptors and a high tumor to background ratio. The receptor has to bind the radioligand with high affinity and eventually to be internalized. One of the advantages and attractions of peptide receptor scintigraphy is the fact that, in addition to the simple tumor localization, it also corresponds to a biological parameter; it tells whether or not the tumor expresses a peptide receptor that may be instrumental for a successful long-term therapy with a nonradioactive peptide or, if the density is sufficiently high, for a peptide radiotherapy program. A particular diagnostic use of radiolabeled peptides that is also worth mentioning is the tumor detection *in situ* during a surgical tumor resection with a small detector of radioactivity, which may help to localize in the operation field areas of tracer accumulation corresponding to tumor nests that can then be removed with great precision (340).

A logical consequence of peptide receptor scintigraphy is peptide receptor radiotherapy, based on the same principle. The number of receptors in the treated tumors needs, however, to be high; instead of (or in addition to) γ-emitters, radioisotopes with a short (β-emitters such as ^{90}Y, ^{188}Re, or ^{177}Lu) or very short range (Auger electrons of ^{111}In) should be used. An important key to the success of *in vivo* receptor radiotherapy is the degree of receptor-radioligand internalization. Most G protein-coupled receptors, including regulatory peptide receptors, can internalize (20–25), although considerable differences in the efficiency of internalization can exist between peptide receptors and even between peptide receptor subtypes (20, 56). Because experimental measurements of internalization rates have only been possible

with cells in culture, the characterization of receptor internalization *in vivo* in tumor tissue and, for comparison, in normal tissue has not been achieved yet. One of the critical limitations of receptor-mediated radiotherapy is the radiation-induced destruction of surrounding and/or distant receptor-positive normal target tissues, in particular radiosensitive tissues such as those of the immune system. Other critical organs that may be destroyed include kidney and liver, not only because they may express peptide receptors but mainly because they excrete and eliminate from the body large amounts of peptide radiotracers not bound to tumor. A well-controlled limitation of the radiation dose given to these vital organs is necessary to reduce potential side effects. A potential alternative to radiotherapy is a tumor therapy with peptides linked to cytotoxic drugs such as doxorubicin, a strategy extensively developed by the group of Schally (28).

The third important clinical application is the targeting of peptide receptors with nonradioactive, noncytotoxic peptides with the aim of inducing a major functional response. There are two aspects. The first is to elicit a favorable biological response that is not directly related to an effect on tumor growth. The inhibition of hormone secretion in neuroendocrine tumors by somatostatin is an example of such a therapeutic application. The second is to elicit a direct effect on tumor growth. This is a mechanism found to be valid for numerous regulatory peptides in experimental tumor models (30, 32), but it is still not completely clear to which extent it can be successfully applied to humans. It should be stressed that long-term treatment with nonradioactive peptides requires much higher peptide doses than needed for radioactive applications; this may trigger side effects in target tissues. Many peptides, for instance, are vasoactive and may thus have significant vasomotor side effects (160, 174, 298).

B. Diagnostic and therapeutic targeting with radiolabeled or cytotoxic peptides

1. Somatostatin receptors

a. Targeting agents. Among radiolabeled somatostatin analogs, the stable octapeptide analog octreotide was first used as iodinated (^{125}I- or ^{123}I-[Tyr^3]octreotide) compound (6, 341). Linking chelators (DTPA, DOTA) to this analog improved the biodistribution profile very much, with a shift from a gastrointestinal excretion pathway to a predominant renal excretion. These developments finally permitted commercialization (7). ^{111}In-DTPA-[D-Phe^1]octreotide (Octreoscan) (Fig. 2), in a dose of approximately 200 MBq, has become the most widely used tracer for somatostatin receptor scintigraphy. It emits γ-rays and Auger electrons; the γ-rays are required for scintigraphy, whereas the Auger electrons may be used for radiotherapy. The tracer differs slightly from ^{111}In-DOTA-lanreotide or from another somatostatin analog, ^{99m}Tc-P829, in its sst affinity profile (342), which is reflected in part by distinct *in vivo* scintigraphic results in selected tumors (343, 344). For radiotherapy, the most frequently used analog has been ^{90}Y-DOTA-Tyr^3-octreotide, abbreviated as ^{90}Y-DOTATOC (Fig. 2) with ^{90}Y as β-emitter. Recently, the search for improved radiolabeled somatostatin analogs has been intensified. Because sst_2 appears to be the main somatostatin receptor subtype in many human tumors (50, 156), improvement of sst_2 affinity has been one goal of recent research; it turns out that minimal changes, such as the replacement in DOTATOC of one metal (In or Y) by another (Ga) (Fig. 2) markedly improved sst_2 binding affinity (342) and *in vivo* tumor imaging (345). Also octreotate, which is an octreotide derivative lacking the alcohol moiety at threonine (Fig. 2), shows much improvement of the sst_2 affinity (342), the biodistribution profile (346), and the quality of tumor scintigraphy (347). Furthermore, somatostatin analogs with specific somatostatin receptor subtype affinity profiles [*i.e.* DOTA-[1-Nal^3]-octreotide (DOTANOC) with high affinity for sst_2, sst_3, and sst_5] have been developed recently and show an improved *in vivo* sensitivity (348) compared with Octreoscan. Octreotide analogs linked to sugar moieties have recently been reported as promising candidates for *in vivo* sst_2 imaging as well (349). The search for a universal pan-somatostatin that can be radiolabeled easily has been intensified, with the aim to detect more sst-positive tumors. However, not all somatostatin receptor subtypes can internalize to a similarly great degree ($sst_3 > sst_2 > sst_1$) (56). This fact has to be considered, too, in the development of such compounds. Among the nonradioactive compounds, Nagy and Schally (350) developed cytotoxic somatostatin drugs based on RC-160 and RC-121 somatostatin analogs coupled with the toxin doxorubicin or its superactive derivative 2-pyrrolinodoxorubicin.

b. Scintigraphy. Diagnostic somatostatin receptor scintigraphy can, in principle, be done in all somatostatin receptor-expressing tumors. The best and most consistent results are found in tumors expressing a high density of somatostatin receptors, namely the majority of neuroendocrine tumors, but also meningiomas or medulloblastomas (7, 351, 352). Successful scintigraphy has also been reported for other tumor types, with lower or nonhomogeneous somatostatin receptor density, such as breast cancer, lymphomas, or renal cell carcinomas (7, 353–357). Table 5 lists tumors frequently selected for somatostatin receptor targeting that will be discussed below in terms of clinical impact.

i. Gastrointestinal, pancreatic, and bronchial neuroendocrine tumors (carcinoids, islet cell tumors, small cell lung carcinomas). These are frequently characterized by a high to very high incidence and density of somatostatin receptors of the sst_{2A} type. The very high sensitivity of Octreoscan permits detection of not only very small primary tumors but also small metastases. As a consequence, patient classification and therapeutic strategy have often to be modified after Octreoscan (8). The impact of Octreoscan on patient management is fourfold: 1) it may detect resectable tumors that would be unrecognized with conventional imaging techniques; 2) it may prevent surgery in patients whose tumors have metastasized to a greater extent than can be detected with conventional imaging; 3) it may direct the choice of therapy in patients with inoperable tumors; and 4) it may be used to select patients for peptide receptor radionuclide therapy. Figure 5A is an example of a positive Octreoscan showing liver metastases of a neuroendocrine tumor. The 100% inci-

TABLE 5. Peptide receptors, their tumor expression and clinical applications

Peptide	Receptor subtype	Main tumor targets[a]	Clinical applications			Tools
			Scintigr.	Radiother.	Others	
Somatostatin	sst_2	Islet cell Ca/carcinoids	+	+		Octreoscan/^{90}Y-DOTATOC[b]
					LTT	Octreotide/lanreotide/vapreotide
	sst_2	Small cell lung cancer	+	+		Octreoscan/^{90}Y-DOTATOC[b]
	sst_2/sst_5	GH-expressing pituitary adenomas			LTT	Octreotide/lanreotide/vapreotide
	sst_2	Paragangliomas/pheochromocytomas	+	+		Octreoscan/^{90}Y-DOTATOC[b]
	sst_2	Neuroblastomas	+		Progn.	Octreoscan
	sst_2	Meningiomas	+	+	DD	Octreoscan/^{90}Y-DOTATOC[b]
	sst_2	Medulloblastomas	+			Octreoscan
	sst_2	Breast Ca	+			Octreoscan
	sst_2	Astrocytomas		+		^{90}Y-DOTATOC[b]
VIP	$VPAC_1$	Gastrointestinal cancers (*e.g.* colorectal Ca)	+			^{123}I-VIP/^{99m}Tc-TP3654
		Other epithelial tumors	+			^{123}I-VIP/^{99m}Tc-TP3654
CCK/gastrin	CCK_2	Medullary thyroid Ca	+	+		^{111}In-DTPA-minigastrin ^{111}In-DTPA-[Nle^{28,31}]-CCK(26–33)
		Small cell lung cancer/insulinomas	+			^{111}In-DTPA-minigastrin ^{111}In-DTPA-[Nle^{28,31}]-CCK(26–33)
Bombesin/GRP	GRP-R	Prostate Ca/breast Ca	+			^{99m}Tc-bombesin(7–14)
Neurotensin	NTR1	Pancreatic Ca/Ewing Sa	In evaluation			^{99m}Tc-neurotensin(8–13)
Substance P	NK_1	Glial tumors		+		^{90}Y-DOTA-substance P

Scintigr., Scintigraphic diagnosis; Radiother., receptor-targeted radiotherapy; DD, differential diagnosis; Progn., prognostic factor; LTT, long-term treatment; Ca, carcinoma; Sa, sarcoma.

[a] Most listed tumors have a high density and/or a high incidence of the respective receptor subtype (see text for details).

[b] Alternative tracers are ^{90}Y-DOTA-LAN (DOTA-lanreotide) and ^{90}Y- or ^{177}Lu-DOTATATE.

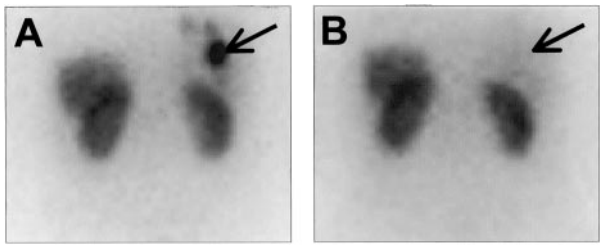

FIG. 5. A, Somatostatin receptor scintigraphy (Octreoscan) of a patient with a neuroendocrine tumor metastatic to the liver. Octreoscan shows hot spots in the liver identifying metastases (*arrow*). B, Effect of one cycle of ^{90}Y-DOTATOC radiotherapy on tumor growth in the same patient 2 months later. The Octreoscan documents the disappearance of the neoplastic liver lesion (*arrow*). In both cases, posterior views are shown. The larger spots correspond to normal renal and splenic uptake. (Photographs were generously provided by Dr. J. Müller-Brand, University of Basel, Basel, Switzerland).

dence and the high density of sst_{2A} measured *in vitro* in gastrinomas is likely to be the main reason for the high rate of their detection by scintigraphy (8). Termanini *et al.* (358) reported that Octreoscan results in gastrinomas caused physicians to alter patient management in 47% of 122 patients. Conversely, the lower rate of detection *in vivo* of insulinomas (7) can be explained by the lower incidence of somatostatin receptors in this tumor in general and of the sst_2 type in particular (125, 127, 149, 156). The 80–90% incidence of sst_{2A} measured *in vitro* in carcinoids is also reflected by the successful *in vivo* scintigraphic data (7). It should, however, be stressed here that not all somatostatin radioligands appear to have the high sensitivity of Octreoscan, as reported recently in a comparative study with the somatostatin analog ^{99m}Tc-P829 (344). Furthermore, it is worth mentioning here that the combined use of a small detector of radioactivity (359–361) and Octreoscan has recently permitted the detection and removal with great precision of small gut neuroendocrine tumors in the operation field (340). Small cell lung cancers have a somewhat lower somatostatin receptor incidence and density; accordingly, primary tumors can often be detected *in vivo*, but metastases are sometimes missed (362).

ii. Pituitary adenomas. GH-producing pituitary adenomas are characterized by a very high sst_{2A} expression, and virtually all of them can therefore be detected with Octreoscan *in vivo* (7, 363). This diagnostic method remains, however, of limited value, because there are adequate alternative radiological ways to diagnose this tumor type; neither is ^{90}Y-DOTA-[Tyr^3]octreotide radiotherapy used to treat these tumors, because there are other established therapeutic methods, surgical or medical. Among those, the long-term use of nonradioactive octreotide is well established for treating acromegalics that cannot be cured surgically (29). There is, however, a controversy as to whether Octreoscan can help physicians decide to treat an acromegalic patient with octreotide. Indeed, most investigators found only weak correlations between scanning results and GH suppressibility by Sandostatin (363, 364). Not only GH-producing but also clinically nonfunctioning and TSH-secreting pituitary tumors can be visualized using octreotide scintigraphy. Although ACTH-producing pituitary tumors are usually not detected with Octreoscan, it is worth mentioning that, on the contrary, ectopic ACTH-producing tumors frequently express somatostatin receptors that can be targeted *in vivo*. Octreoscan may often be the only method to localize this tumor (365).

iii. Pheochromocytomas, paragangliomas, and neuroblastomas. These tumors, characterized by a high sst_{2A} expression, can be detected in approximately 90% of the cases *in vivo*. Although metaiodobenzyl guanidine scintigraphy may be preferable to Octreoscan for the diagnosis of adrenal pheochromocytomas, because of the high kidney uptake of Octreoscan, paragangliomas represent an important indication for a whole body scintigraphy with Octreoscan; unexpected paraganglioma sites, not detected by conventional imaging, may often be identified by this method (49). Neuroblastomas are frequently detected *in vivo* as well (366, 367). Remarkably, patients bearing neuroblastomas that express somatostatin receptors have a longer survival than those with tumors lacking these receptors (95, 368, 369). In neuroblastomas, the presence of somatostatin receptors is also inversely correlated with the presence of N-*myc*, a marker of poor prognosis (95). *In vivo* scintigraphy of neuroblastomas may therefore also be helpful for assessing the prognosis (366).

iv. Meningiomas. Because of the very high incidence of sst_2 in meningiomas, almost all cases can be visualized *in vivo* with Octreoscan. Although meningiomas can also be adequately localized by brain computer tomography, Octreoscan is useful in patients in whom a differential diagnosis with neurinomas is required; the latter lacks somatostatin receptors and will not be detected by Octreoscan, in contrast to meningiomas (33, 351, 370).

v. Medulloblastomas. Medulloblastoma belongs to the set of tumors with highest sst_{2A} expression (100, 137, 140). Although pilot studies have shown that medulloblastomas can be identified by Octreoscan *in vivo* (352), no further large-scale studies have followed yet, despite a very high probability of successful detection. In particular, no radiotherapeutic trials have yet been performed, although adequate radiopeptides are being developed for that purpose (371).

vi. Breast cancers. Breast cancers can express somatostatin receptors. They are found *in vitro* in 50–70% of the tumors (101, 317, 353), with sst_{2A} as the predominant receptor subtype (50, 143). A marked receptor heterogeneity is noted in 50% of the tumor samples (101). Moreover, many breast cancers have a low or moderate somatostatin receptor density (101, 143). Successful scintigraphic detection of breast cancer, primary and metastatic, has been reported for Octreoscan; the percentage of positive cases varies, however, between 50 and 94%, depending on the study (353, 354, 372–374). A well-controlled study by van Eijck *et al*.(353) showed 70% somatostatin receptor positivity in breast cancers diagnosed at mammography, whereas another, also well-controlled, study by Albérini (354) found a 50% positivity. Other scintigraphic studies, without concomitant *in vitro* confirmation of receptor expression in the tumors, have reported up to 94% incidence of positive cases (373, 374); those may overestimate the tumor positivity because nontumoral breast tissue had been shown to be positive in 15% of the cases on scintigraphy (49). Up to now, *in vivo* somatostatin receptor scintigraphy has, however, not reached the status of a recognized tool as diagnostic or radiotherapeutic somatostatin receptor targeting of breast cancers. This is likely due, in a large part, to the insufficient amount and/or heterogeneous distribution of somatostatin receptor expressed by some of these tumors. These characteristics may prevent many of the breast cancer patients from being included in radiotherapy trials with ^{90}Y-DOTATOC.

vii. Other tumors. Lymphomas have a high incidence of somatostatin receptors, but of low density in most cases. Thus, although successful scintigraphic detection of these tumors has been reported, it is often difficult to detect all tumor sites in a lymphoma patient (355, 375), an information required to determine the optimal therapeutic strategies in individual patients. For the same reason, no radiotherapeutic trials have been initiated with somatostatin analogs in lymphomas despite their high radiosensitivity. Renal cell carcinomas often express somatostatin receptors, but in moderate density (104). A successful *in vivo* localization (in particular of metastases, because primaries may be masked by the high renal uptake) has been documented recently (356, 357); however, neither Octreoscan nor ^{90}Y-DOTATOC radiotherapy is clinically established yet. Sarcomas, rare connective tissue tumors of mesodermal origin, can also express somatostatin receptors (105) and be imaged *in vivo* with Octreoscan (376). In non-small cell lung carcinomas, there is a discrepancy between *in vitro* and *in vivo* somatostatin receptor data. Although no somatostatin receptor binding is identified *in vitro* in tumor cells (362), surprisingly, non-small cell lung carcinomas can be localized *in vivo* with Octreoscan, ^{111}In-DOTA-lanreotide, or $^{99\,m}$Tc-depreotide (P829) in almost all cases (362, 377, 378). Because vessels and immune cells located in the vicinity of non-small cell lung carcinomas can express somatostatin receptors, they may thus yield positive scans (362, 377). It is controversial whether targeted radiotherapy with somatostatin analogs should be initiated in such tumors. Colonic and pancreatic carcinomas are inadequate for somatostatin targeting with the current octreotide type of analogs, due to the absence of somatostatin receptors of the sst_2 type; up to now, most scintigraphic Octreoscan studies have missed pancreatic and colonic cancers; a study using ^{111}In-DOTA-lanreotide, a radiotracer with sst_2 and sst_5 affinity (342), identified few colorectal cancers, possibly due to their sst_5 expression (343, 379, 380). Hepatocellular carcinomas that have somatostatin receptors in 50% of the cases (113) also appear to be candidates for scintigraphy (381), although their moderate density of somatostatin receptors may not always be sufficient to give a detectable signal over the high liver background.

Next to *in vivo* scintigraphy, the *in vitro* somatostatin receptor detection in tumors remains as an important additional current diagnostic option. It is very useful when a tumor is removed without preoperative Octreoscan and when this tumor turns out to have neuroendocrine characteristics at the histopathological evaluation; clinicians may then want to know whether the resected tumor has somatostatin receptors to evaluate the possibility of localizing distant metastases or detecting recurrences by subsequent Octreoscan. The somatostatin receptor status can be established *in vitro*, either immunohistochemically in the formalin-fixed resected tumor (145) or, if frozen tumor

tissue samples have been secured, by somatostatin receptor autoradiography (92).

As mentioned earlier, somatostatin receptors may occur in nontumoral elements such as lymphocytes, vessels, or epithelioid cells and yield false-positive scans, *i.e.*, scans with abnormal hot spots that are not related to cancer. Pathological accumulation of lymphocytes or of vessels, or the presence of granulomas may therefore provide such false-positive scans. Also, the presence of an ectopic spleen (spleens express somatostatin receptors) has been reported to result in a false-positive scan (382). One should, however, notice that false-positive is a misnomer. It does not refer to a scanning error, because the scan is able to correctly detect such a spleen as a somatostatin receptor-positive organ; it refers to the fact that the identified structure does not correspond to the neoplasm under investigation.

c. Radiotherapy. Targeted radiotherapy of tumors expressing somatostatin receptors in high amounts was shown recently to be a most promising technique. In animal studies, a complete tumor destruction was achieved, especially of smaller tumors, by using the sst_2-preferring ^{90}Y-DOTATOC as radiotracer (383, 384). Furthermore, several pilot studies in humans have shown encouraging results of radiotherapy with ^{111}In-DTPA-octreotide or ^{90}Y-labeled DOTATOC (11, 12, 385, 386). Main indications are metastatic neuroendocrine tumors, in particular endocrine pancreatic tumors and carcinoids. However, many other tumors can also be targeted for radiotherapy, through high-affinity receptor binding and internalization of ^{90}Y-DOTATOC, if they express a sufficiently high density of somatostatin receptors. It is currently unanswered whether particularly radiosensitive tumors, such as lymphomas or small cell lung cancers, despite their low to moderate amount of somatostatin receptors, would be adequate candidates for somatostatin receptor radiotherapy.

Otte *et al.* (9, 385) described 29 patients who received injections of ^{90}Y-DOTATOC for an intrapatient dose-escalation study. Twenty of the 29 patients showed disease stabilization, two had a partial remission, four a reduction of tumor mass of less than 50%, and three only a progression of tumor growth. Paganelli *et al.* (12) treated 30 patients with injections of ^{90}Y-DOTATOC. Complete and partial tumor mass reduction was measured in 23% of the patients, with 64% showing stable disease and 13% progressive disease. Valkema *et al.* (387) in a phase 1 study with ^{90}Y-DOTATOC in 22 patients with progressive neuroendocrine tumors and a median follow-up time of 14 months, observed two partial and three minor tumor responses: 10 patients had stable disease, and 12 patients had symptomatic improvement. In a recent study by Waldherr *et al.* (11) in patients with progressive endocrine pancreatic tumors, treatment with ^{90}Y-DOTATOC resulted in tumor reduction in 24% of the patients. Tumor stabilization was achieved in 61% of the patients, and tumor progression occurred in 15%. The 2-yr overall survival of 76% compared favorably to the reports in the literature for patients with advanced tumors treated with chemotherapy or interferon (388, 389). A significant effect of ^{90}Y-DOTATOC in palliation of both malignant carcinoid syndrome and tumor-associated pain was also noticed. As an example, Fig. 5B shows the disappearance of liver metastases of a neuroendocrine tumor in a patient treated with ^{90}Y-DOTATOC. All four above-mentioned clinical studies suggest that ^{90}Y-DOTATOC is probably an effective therapeutic alternative to the chemo- and biotherapies used to date for neuroendocrine tumors. ^{90}Y-DOTATOC treatment was well tolerated, and toxicity was generally mild. Particular attention must be given to renal toxicity, which had been a problem in some previous trials (385, 390). A strict control of the total accumulated dose is essential: the cumulative renal absorbed dose was limited to 27 Gy in the study by Valkema *et al.* (387). Moreover, the use of an infusion containing amino acids to reduce kidney uptake of the radiopeptide (391, 392) further decreases the risk of renal toxicity (387).

Another strategy of ^{90}Y-DOTATOC radiotherapy has been tried recently in astrocytomas: it was hypothesized that a direct application of ^{90}Y-DOTATOC at the tumor site would not only bypass the blood-brain barrier but also largely prevent its renal excretion, therefore lowering the toxicity (393). Indeed, such local injections of ^{90}Y-DOTATOC in the tumor site had a beneficial action in terms of tumor mass reduction in several glioma patients (393, 394). This strategy allows the application of a high radioactivity dose to the tumor and, simultaneously, confers a considerable advantage in terms of low whole-body accumulation of radioactivity and limitation of side effects.

d. Cytotoxic therapy. An alternative approach to targeted somatostatin radiotherapy for tumor destruction could be the use of unlabeled somatostatin coupled to cytotoxic agents (350). One of the most efficient compounds is AN-238, a potent cytotoxic radical 2-pyrrolinodoxorubicin linked to the somatostatin octapeptide RC-121 (350); the ability of the carrier peptide portion to bind specifically to receptors on target tissues is preserved, and the cytotoxicity of the anticancer agent is retained. AN-238 was given, as a single dose, to animals bearing various types of somatostatin receptor-expressing cancers, including androgen-independent prostate cancers, renal cell cancer, ovarian and lung cancers; in most cancers, AN-238 induced a greater than 80% decrease in tumor weight and/or volume. It is presently not clear whether and to which extent the toxic radical is released before entering the cell. Nevertheless, it could be demonstrated that the cytotoxic somatostatin analog AN-238 is more effective and less toxic than its corresponding cytotoxic radical, even in experimental tumors expressing a low density of somatostatin receptors (350). In addition, AN-238 appears to be able to target somatostatin receptor-positive tumor vasculature in a model in which the tumor cells themselves are somatostatin receptor-negative (395). These cytotoxic somatostatin analogs have, however, not yet been tested in clinical trials.

2. VIP receptors

a. Targeting agents. Virgolini *et al.* (396) have used a ^{123}I-VIP for all their *in vivo* studies. This natural VIP is difficult to radiolabel and is probably sensitive to degradation. More recently, the Thakur's group (397) has developed the $^{99\,m}$Tc-labeled VIP analog TP3654 for imaging VIP receptor-expressing tissues. This compound is easily labeled; in preliminary

experiments, it disclosed VIP receptor-expressing mice tumors and selected human tumors, albeit with a significant background (398, 399). Moody *et al.* (400) have described an ^{18}F-labeled-Arg^{15}-Arg^{21}-VIP for *in vivo* imaging purposes and have shown that this compound labeled VIP receptor-positive breast cancers in animal models. Other potential VIP candidates for clinical use may be $VPAC_1$- and $VPAC_2$-selective analogs, such as KRL-VIP/GRF ($VPAC_2$-selective) or RO 25-1553 ($VPAC_2$-selective), developed by Robberecht and colleagues (175, 176), or the simplified and metabolically stable $VPAC_1$ analog developed recently by the group of Jensen (44). Those compounds would need to be labeled adequately for human use. It has been claimed by Virgolini *et al.* (401) that there is a cross-competition in the nanomolar range between somatostatin and VIP at the receptor level, implying that VIP ligands may identify somatostatin receptor-expressing tumors and vice versa. In addition, Virgolini's group has suggested that the sst_3 somatostatin receptor subtype is a high-affinity acceptor of VIP (402). However, a recent multicenter study (403) has not been able to confirm any cross-competition between somatostatin and VIP in a large series of somatostatin- and VIP receptor-expressing human tumors and in normal human VIP and somatostatin target tissues. Neither did the study show any VIP binding in cells transfected with the various human somatostatin receptor subtypes.

The identification and development of stable and easy-to-label VIP/PACAP analogs with a high affinity for VIP receptors is a current challenge of potential clinical interest and a prerequisite for a successful scintigraphic and radiotherapeutic VIP application in humans.

b. Scintigraphy. Although most tumors express a VIP/PACAP receptor density sufficient for their visualization, the optimal tumor to background ratio is of particular concern, because VIP/PACAP receptors are expressed by so many normal tissues (52). Extrapolation from *in vitro* data suggests that a successful VIP/PACAP receptor scintigraphy will be limited to those tumors located in sites in which a high tumor to tissue ratio of receptor density can be expected. $VPAC_1$-expressing colorectal cancers, for instance, are likely to be such candidates because the normal colon has a relatively moderate density of $VPAC_1$ receptors located in a very discrete area of the mucosa (182). It has been reported (396) that human colorectal cancers can indeed be localized by *in vivo* ^{123}I-VIP receptor scintigraphy. Conversely, lung cancers are poor candidates for scintigraphy because of the high lung uptake of ^{123}I-VIP, resulting from the high density of $VPAC_1$ receptors in lung acini. However, a ^{99m}Tc-labeled VIP analog (TP3654) was recently reported to have a much lower uptake by normal lung tissues (399). In the same way, $VPAC_1$-expressing prostate cancers may be inadequate candidates for VIP receptor scintigraphy due to the high $VPAC_1$ receptor expression in normal prostatic glands and in the adjacent bladder tissue (52). Likewise, $VPAC_1$-expressing primary neoplasms or metastases located in the liver may be difficult to identify with VIP receptor scintigraphy because of the high density of $VPAC_1$ receptors in the normal liver. We have shown *in vitro* that hepatocellular carcinomas have approximately one fourth the density of the VIP receptors expressed by the surrounding liver (113). A similar ratio is found between pancreatic or colorectal carcinomas and the normal liver (404). This could indicate that liver metastases of pancreatic or colorectal primaries, as well as hepatocellular carcinomas, would rarely be identified as positive hot spots with VIP receptor scintigraphy, but rather as cold spots. A recent *in vivo* study by Hessenius *et al.* (404) confirmed the poor visualization of pancreatic carcinomas and their liver metastases with ^{123}I-VIP scintigraphy. Finally, we may anticipate that lymph node metastases will be difficult to assess with VIP receptor scintigraphy because of the high VIP receptor content of normal lymphoid tissue (169, 183). One has, however, to consider that these density ratios of tumor *vs.* normal tissue are based on *in vitro* data obtained by measuring a nondynamic receptor condition in sections of normal and tumoral tissues. It cannot be excluded that, *in vivo*, VIP receptors expressed in tumoral tissues will have characteristics distinct from those expressed in normal tissues, *e.g.*, because of different internalization rates, different ligand dissociation rates, or different receptor turnover; this could lead to an accumulation of radioligand in both tissues at a rate different from that predicted by the *in vitro* measurement of receptor density. It would, of course, be particularly useful for imaging purposes if different *in vivo* receptor kinetics between tumor and normal tissue would lead to a higher accumulation in the neoplastic tissue than in the normal tissue. Experimental evidence for such mechanisms is presently lacking; it is much needed, but difficult to obtain. The fact that only very modest advances have been reported in the VIP receptor scintigraphy of tumors since the original study published in 1994 (396) is possibly a sign of the difficulties inherent to the targeting of this receptor *in vivo*.

A potential novel approach to increasing the targeting selectivity to tumors in cases of disturbingly high levels of the respective receptors in adjacent normal tissues may be achieved by using hapten-bearing peptides binding to peptide receptors and to tumor-associated antigens, mediated by bispecific antibodies. Such receptor/antigen dual targeting has been proposed recently for the neurotensin receptor (405), using a bispecific antibody to carcinoembryonic antigen and ^{111}In-DTPA-hapten (^{111}In-DTPA-neurotensin). It may be particularly attractive to develop the corresponding strategy to improve the selectivity of VIP receptor tumor targeting.

c. Radiotherapy. Because VIP/PACAP receptor-positive tumors can be targeted with radiolabeled VIP/PACAP analogs (396, 399), it appears theoretically possible to treat such tumors selectively with high doses of adequately radiolabeled VIP analogs. There are currently no reports on VIP receptor radiotherapy of human tumors. One reason may be the lack of an adequate radioligand. Another reason is certainly the inadequate tumor to background ratio mentioned above; VIP receptor radiotherapy could be highly radiotoxic to surrounding and distant VIP/PACAP receptor-positive normal target tissues, in particular to radiosensitive tissues such as the immune system, lung, kidney, or liver.

3. CCK receptors

a. Targeting agents. Different research groups have tried recently to develop peptide-based CCK_2-selective radiopharmaceuticals suitable for *in vivo* CCK_2 receptor scintigraphy and radiotherapy. One group of compounds was based on chelator (*i.e.*, DTPA or DOTA) -linked unsulfated CCK octapeptide analogs labeled with ^{111}In, such as ^{111}In-DTPA-[D-Asp^{26}, Nle^{28,31}]CCK(26–33) (406). Another group of compounds was based on ^{131}I-labeled or ^{111}In-DTPA-labeled minigastrins (407). All compounds were able to label specifically CCK_2 receptors with high affinity and to target CCK_2 receptors *in vivo* in animals, in particular in the stomach and in CCK_2-expressing TT cancer cell xenografted in nude mice (406, 407). Many potent nonpeptidic CCK_1 and CCK_2 receptor antagonists have been developed in the past two decades, primarily for gastrointestinal disturbances (214, 408). Although numerous studies in animals and with tumor cells have shown consistent antigrowth effects with these analogs, there is no clinical study that unequivocally establishes a role for CCK_1- or CCK_2-mediated growth control of tumors in man.

b. Scintigraphy and radiotherapy. In vitro receptor binding studies had shown high CCK_2 receptor incidence in medullary thyroid carcinomas (51). Therefore, these tumors were chosen for pilot clinical investigations (407, 409). Most of the tumor sites were visualized *in vivo* by CCK_2 receptor scintigraphy with ^{111}In-DTPA-minigastrin. Another study performed by Kwekkeboom *et al.* (410) in patients with advanced metastatic medullary thyroid carcinomas also visualized tumor sites *in vivo* with ^{111}In-DTPA-[D-Asp^{26}, Nle^{28,31}]CCK(26–33). However, in this study, not all carcinomas were detected. This observation suggests that some of the undifferentiated cancers may have lost CCK_2 receptors or that the radioligand used was not sensitive enough (410). An impressive example of a CCK_2 receptor scintigraphy in a patient with metastatic medullary thyroid carcinoma is shown in Fig. 6. Also encouraging are preliminary studies showing that radiotherapy with a radiolabeled minigastrin may reduce the tumor burden in medullary thyroid carcinoma patients (411). Renal toxicity, however, may be a problem (411). For that reason, new CCK analogs with reduced kidney uptake have been developed recently (412). All medullary thyroid cancer studies (407, 409–411) took advantage of an internal positive control of the scintigraphic quality in each patient, namely a nondiseased tissue, the gastric mucosa, that could always be visualized. After the brain, the gastric mucosa is the human tissue with the highest CCK_2 receptor expression.

Medullary thyroid carcinoma is the first human tumor shown to express a density of CCK_2 receptors sufficiently high to establish the proof of principle for *in vivo* CCK_2 receptor scintigraphy and radiotherapy. Targeting medullary thyroid carcinomas with CCK_2 receptors may turn out to be more valuable than targeting them with somatostatin receptors; the incidence of CCK_2 receptors is higher than that of sst_2 receptors. The differential diagnostic aspect, *i.e.*, the distinction between a CCK_2 receptor-positive medullary thyroid carcinoma and a CCK_2 receptor-negative differentiated thyroid carcinoma, may be valuable as well. The future will tell us soon whether the same targeting method can be applied to other CCK_2-expressing tumors, *i.e.*, small cell lung cancers, astrocytomas, ovarian sex-cord stromal cell tumors, or some neuroendocrine gastrointestinal tumors such as insulinomas (156). Might colorectal and pancreatic cancers, two tumor types for which *in vitro* data on CCK_2 receptor protein expression have been controversial (198, 203), also be visualized *in vivo* with the above mentioned techniques?

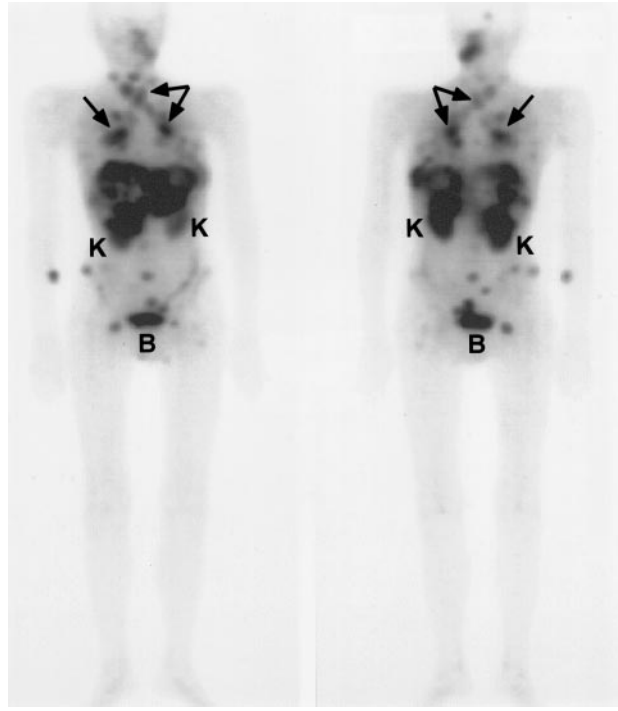

FIG. 6. Whole-body CCK_2 receptor scintigraphy of a patient with metastatic medullary thyroid cancer. CCK_2 receptor-positive metastases are particularly abundant in the thorax, abdomen, and head. *Left*, anterior view; *right*, posterior view; in both views, nonspecific labeling is seen in the kidneys (K) and urinary bladder (B). *Arrows* identify selected thoracic and intestinal metastases. (Photographs were generously provided by Dr. T. Behr, University of Marburg, Marburg, Germany).

The other major subtype of CCK receptors, the CCK_1 receptor, appears to be preferentially expressed by few human tumor types, such as gastrointestinal neuroendocrine tumors, meningiomas, and neuroblastomas. CCK_1-selective peptide radiopharmaceuticals are presently not available for the targeting of these tumors *in vivo*. Because the abovementioned tumors expressing CCK_1 receptors usually also express abundant somatostatin receptors and because the latter can be targeted successfully with Octreoscan, the priority for the need to develop CCK_1-selective radiopharmaceuticals is low.

4. GRP receptors

a. Targeting agents. Recently, several laboratories have made important progress toward developing radiolabeled bombesin analogs as potential radiopharmaceuticals (413–417). Useful guidance for designing radiolabeled bombesin derivatives that maintain high *in vitro* and *in vivo* binding

affinities for GRP receptors was provided through insights gained from earlier studies on developing bombesin antagonists for antiproliferative therapy. A recent report by Baidoo *et al*. (413) demonstrated that conjugation of ^{99m}Tc-diamine dithiol chelates to the ϵ-NH_2 group of Lys^3-bombesin produces compounds that maintain high binding affinities for GRP receptors. Breeman *et al.* (414) formulated a DTPA-conjugate of bombesin labeled with ^{111}In. Hoffman and coworkers (418, 419) demonstrated the feasibility of formulating radiolabeled truncated bombesin (7–14) analogs that retained high binding affinities for GRP receptors and that were internalized into GRP-expressing cells. Nock *et al.* (417) described a ^{99m}Tc-labeled GRP receptor antagonist. Some of these radiopharmaceuticals have been recently used in clinical trials (36, 420).

b. Scintigraphy and radiotherapy. GRP may probably be clinically applied as a radiolabeled molecule for *in vivo* diagnosis and radiotherapy of breast and prostate carcinomas. On the basis of the high density of GRP receptors in these tumors, Van de Wiele *et al.* (420) reported the results of early clinical trials with a bombesin(7–14) conjugate labeled with ^{99m}Tc in breast and prostate cancer patients. Scintigraphic images with the ^{99m}Tc-tracer demonstrated selective uptake in cancers that had spread to lymph nodes and distant sites, as well as in the primary sites (420). These preliminary studies in humans represent a proof of concept and provide first evidence that the use of radiolabeled bombesin derivatives is a promising approach for *in vivo* targeting of GRP receptor-expressing cancers. Because the number of GRP receptors in prostate and breast carcinomas is considerably higher than the density of somatostatin receptors in these tumors (421), we can foresee a more important clinical impact of *in vivo* GRP receptor scintigraphy, if optimal radiopharmaceuticals are developed. The use of radiolabeled GRP analogs to treat these tumors is of major interest. However, there are no such ongoing clinical studies.

An alternative option for tumor destruction is the use of bombesin analogs linked to cytotoxic doxorubicin, such as the conjugate consisting of the potent 2-pyrrolinodoxorubicin linked to bombesin(7–14). This compound inhibits the growth of H-69 small cell lung cancer xenografts (28, 32, 422). It has not yet entered clinical trials.

5. Neurotensin receptors

a. Targeting agents. The increasing interest in developing tools that would permit visualization of neurotensin receptor-positive tumors in patients has recently been documented by the synthesis of short and stable neurotensin analogs suitable for *in vivo* scintigraphy (39, 423–425). Adequate analogs were developed with changes in the basic structure of the minimal fragment of neurotensin, neurotensin(8–13), needed for high affinity binding to improve the metabolic stability. ^{131}I- and ^{123}I-labeled, ^{99m}Tc-, ^{188}Re-, ^{111}In-, or ^{18}F-labeled analogs with high affinity binding, strong internalization properties, improved *in vitro* stability, and adequate *in vivo* biodistribution in animals have been reported recently (39, 423–425).

b. Scintigraphy. At present, a successful clinical application of neurotensin and neurotensin receptors in oncology has not been reported. A definitive proof that NTR1-expressing tumors can be adequately visualized and, as a consequence, be subjected to peptide radiotherapy, is still missing; however, a preliminary *in vivo* study in 4 pancreatic cancer patients gave encouraging results (425a). In analogy to somatostatin, VIP, or CCK receptor scintigraphies, it will be important to see to what extent the neurotensin receptors expressed in normal tissues, such as the smooth muscle of the gut (45, 182, 285), are labeled after injection of radiolabeled neurotensin, thus providing a positive control of quality and specificity. As therapeutic options, targeted radiotherapy with neurotensin analogs should be initiated if scintigraphy is successful. Controlled clinical studies that test the long-term effect of NTR1 antagonists such as SR48692 as tumor-growth inhibiting agents could also be useful (30).

6. Substance P receptors. The visualization of the thymus in autoimmune diseases using a DTPA derivative of substance P labeled with ^{111}In, ^{111}In-DTPA-substance P, is the first report suggesting the feasibility of *in vivo* targeting of substance P receptor-positive tissues (426). However, an analogous study identifying tumors in humans by receptor scintigraphy has not been reported. Because glioblastomas often express a high density of substance P receptors (294), a recent pilot study aims to treat advanced glioblastomas with local injections of ^{90}Y-DOTA-substance P, as performed previously in astrocytomas with ^{90}Y-DOTATOC (393). The high accumulation and long residence time of the tracer restricted to the tumor site has been highly encouraging and may be the main explanation for the promising preliminary results achieved with this technique (427).

7. Other receptors. For the receptors listed below, only limited evidence, usually based on animal experimentation, suggests an interest for future clinical applications in humans.

a. α-MSH receptors. Approximately 10 yr ago, linear α-MSH analogs were labeled with ^{111}In and examined for their biodistribution and malignant melanoma-targeting properties *in vivo*; high kidney and liver uptake observed with ^{111}In-[DTPA]α-MSH compromised their imaging potential and prevented therapeutic applications (428). Recently, a novel class of cyclized α-MSH analogs that coordinate ^{99m}Tc and ^{188}Re into their three dimensional structures were developed with the potential for melanoma imaging and therapeutic applications (429, 430). There is presently no evidence for successful *in vivo* visualization of cancers expressing α-MSH receptors in humans.

b. LHRH receptors. Radiolabeled LHRH analogs for the *in vivo* visualization and possible therapy of LHRH-expressing tumors are not available for the clinic. There are, however, extensive data by Schally's group (28, 431) on LHRH analogs linked to cytotoxic drugs that have been synthesized and shown to successfully inhibit tumor growth in experimental animals. The side effects of targeted cytotoxic LHRH analogs are expected to be minor, because the receptors for LHRH are not widely distributed in normal tissues and because the DNA-intercalating cytotoxic radical 2-pyrrolinodoxorubicin is maximally cytotoxic to cells undergoing mitotic division (28). Moreover, in recent studies in which the cytotoxic an-

alog of LHRH-containing doxorubicin was linked to a two-photon fluorophore, a direct interaction of the LHRH analog with LHRH receptor-positive MCF-7 breast cancer cells was observed; its receptor-mediated entry into the cell cytoplasm and subsequently into the nucleus could be demonstrated (432). No clinical studies using this interesting cytotoxic targeting approach have yet been reported.

c. Calcitonin receptors. Several years ago, a ^{123}I-labeled calcitonin radioligand was developed for *in vivo* targeting in humans (433). This approach was not followed up, possibly due to the absence of adequate indications. In particular, the lack of a systematic *in vitro* evaluation of the calcitonin receptor content of tumors is perhaps responsible for the lack of interest for tumor targeting by calcitonin analogs.

d. ANP receptors. ^{123}I-ANP was used several years ago for *in vivo* receptor imaging of the kidneys in animals, suggesting that ANP scintigraphy could be used to diagnose diabetic nephropathy by a noninvasive method (434). Scintigraphic studies in cancer patients have not been performed.

e. GLP-1 receptors. Using the radiolabeled GLP-1 analog exendin-4, Gotthardt *et al.* (435) were recently able to visualize insulinomas by external scintigraphy in an animal model. These encouraging basic studies in animals will certainly trigger *in vivo* studies investigating GLP-1 receptors in primary human tumors, in particular in insulinomas, known to express a very high GLP-1 receptor density (156).

f. Oxytocin receptors. A new radioligand specific for oxytocin receptors has been described (436). It targets oxytocin receptor-expressing mammary mouse tumors. These promising results await, however, confirmation in humans.

g. Endothelin receptors. It was shown recently that a mixed ET_A and ET_B receptor antagonist labeled with ^{11}C, [^{11}C]L-753,037, binds to endothelin receptors *in vivo* in mice and dogs. Thus, the compound could become a candidate for *in vivo* investigations of these receptors in humans (437).

C. Long-term cancer treatment with nonradioactive, noncytotoxic peptides

1. Somatostatin receptors. Stable somatostatin analogs such as octreotide, lanreotide, or vapreotide, three octapeptides with a half-life of degradation sufficiently long to allow long-term therapy, have been at the origin of the success of long-term somatostatin therapy. The main application has been the treatment of symptoms caused by the oversecretion of hormones from neuroendocrine tumors. Thereby, the quality of life in patients with hormone-producing pituitary adenomas and gastrointestinal neuroendocrine tumors such as metastatic islet cell tumors and carcinoids was improved. I refer to the extensive literature dealing with the subject of the symptomatic therapy of tumors with octreotide and lanreotide, as well as to detailed information on the pharmacology and pharmacokinetics of these compounds (17, 29, 34, 438). Later on, long-acting compounds were introduced for the same indications and have progressively replaced the daily injectable formulations (29, 439, 440). These are the slow-release form of the octapeptide BIM 23014, named SR Lanreotide, and the slow-release form of octreotide (Sandostatin LAR) (29). Pharmacologically, these somatostatin analogs are selective, but with a receptor affinity profile different from that of natural somatostatin, because they bind primarily to $sst_2 > sst_5 > sst_3$. Recently, somatostatin analogs with a sst_1-sst_5 pansomatostatin profile resembling that of natural somatostatin have been developed (441), but they have not yet been tested for their biological behavior. Novartis Inc. (Basel, Switzerland) recently reported on a new somatostatin analog, SOM230, with a sst affinity profile close to a pansomatostatin (442) and a half-life of nearly 24 h (442), whereas Biomeasure, Inc. (Milford, MA), has developed an sst_2/sst_5 bispecific selective analog, BIM 23244 (443). The compounds of both Novartis and Biomeasure may turn out to optimally treat those acromegalics known to incompletely react to octreotide treatment and bearing tumors with a sst_5/sst_2 pattern (157, 443). Indeed, optimal GH suppression requires coactivation of sst_2 and sst_5 and can be achieved with analogs bispecific for sst_2/sst_5 (443, 444). There has also been an intensive search for analogs selective for one single subtype, both agonists and antagonists, that has led to the discovery of new compounds for potential clinical development; although subtype-selective nonpeptidic analogs have been discovered for each of the somatostatin receptor subtypes (151, 445), potent subtype-selective peptidic analogs, either agonists or antagonists, have been more difficult to identify for each of the receptors (83, 152, 153, 446–448). Specific clinical indications have still to be defined for all these subtype-selective compounds.

Long-term application of somatostatin or somatostatin analogs inhibits the proliferation of normal and neoplastic cells (31, 449). Somatostatin analogs inhibit tumor growth in a wide variety of experimental models in several species: transplantable osteo- and chondrosarcomas, transplantable acinar and ductal pancreatic carcinomas, as well as different types of rat and mouse mammary and prostatic carcinomas. Tumors developing from a number of human pancreatic, colonic, gastric, and small cell lung cancer lines xenografted in nude mice are inhibited in their growth during long-term therapy with somatostatin analogs (31). Many of these experimental tumors and cell lines are inhibited by somatostatin in a direct way, through somatostatin receptors densely and homogenously expressed on tumor cells (450). Although several experimental tumors do not express somatostatin receptors, their growth can often be inhibited by somatostatin analog administration, probably via indirect mechanisms, involving inhibitory effects on growth factors and/or angiogenesis (162, 450). Thus, there was a great hope that somatostatin analogs would act as efficient antineoplastic agents in human tumors.

Unfortunately, the antiproliferative effect of somatostatin analogs in human tumors is limited. There is evidence for control of tumor growth and for prolongation of survival in acromegalics and in some patients with gastrointestinal neuroendocrine tumors by octreotide (29, 161, 451–453). For instance, Shojamanesh *et al.* (161) showed convincingly that long-term octreotide therapy in progressive malignant gastrinomas, *i.e.*, in tumors with a very high sst_2 density, can induce long-lasting tumor stabilization in 50% of the patients. For them, octreotide treatment may be preferable to

chemotherapy. At present there is, however, no study that unequivocally established that long-term somatostatin analog therapy in patients with other somatostatin receptor-positive tumors such as cancers of the breast, lung, or prostate induces a tumor regression or at least controls tumor growth (Refs. 438 and 454–457; for review, see Ref. 458). A report suggesting a favorable effect of octreotide on tumor progression in hepatocellular carcinomas (112) could not be confirmed in a recent multicenter retrospective study (459). We can list a number of possible reasons for the generally poor efficacy of octreotide in inhibiting tumor proliferation: 1) patients selected for tumor-growth inhibitory treatment with octreotide are often in late-stage disease; 2) octreotide, which is usually the chosen drug for this treatment, does not have a high affinity for all of the five somatostatin receptor subtypes and, as such, may not be the optimal drug to inhibit proliferation; 3) the optimal octreotide dose and scheme of application has not been found for this indication; 4) somatostatin receptors are not always expressed in a high density and/or in a homogeneous way in human tumors (*e.g.*, breast carcinomas), whereas the animal tumor models chosen for *in vivo* experimentation usually have a high density and a homogeneous distribution of receptors; and 5) targeting of somatostatin receptors with octreotide does not take into account the massive overexpression of other peptide and growth factor receptors in the same tumor (Fig. 7), which may counterbalance the inhibitory action of somatostatin. It is tempting to speculate that a cocktail of adequately chosen peptides known to have their respective receptors overexpressed in a given tumor (VIP receptor antagonists, CCK_2 receptor antagonists, GRP receptor antagonists, *etc*.) should be used simultaneously with the somatostatin analog for a concerted growth-inhibitory action (460) instead of using a somatostatin analog alone. This could block counterregulatory mechanisms mediated by the various peptide and growth factor receptors.

2. VIP receptors. VIP and PACAP can affect the growth of normal and neoplastic tissues in experimental models. The great majority of the studies has shown tumor growth-promoting activities of VIP and PACAP and growth inhibitory properties of VIP and PACAP antagonists in various tumor models (30, 47, 461–464). Based on the presence of VIP/PACAP receptors in the majority of the most common human tumors, the postulate to use high doses of a growth-inhibiting VIP/PACAP antagonist may therefore be attractive, assuming that VIP/PACAP will demonstrate growth-inhibiting effects in human tumors comparable to those seen in animal tumor models. However, clinical indications in which high doses of nonradioactive VIP/PACAP analogs are necessary for long-term peptide treatment should be considered critically, because of the possible side effects related to the numerous VIP/PACAP target tissues in the human body. VIP, for instance, is a strong vasoactive peptide. Pharmacological doses may have significant vasomotor side effects (465). Other potential side effects, for instance on the immune system (171), may occur as well. Conversely, VIP receptor antagonists appear to have less effects on normal tissue, where VIP has paracrine effects, than on tumor tissue, in which VIP acts as an autocrine growth factor (30). However, complete evaluation and prediction of side effects requires a better understanding of the VIP/PACAP actions in the human body.

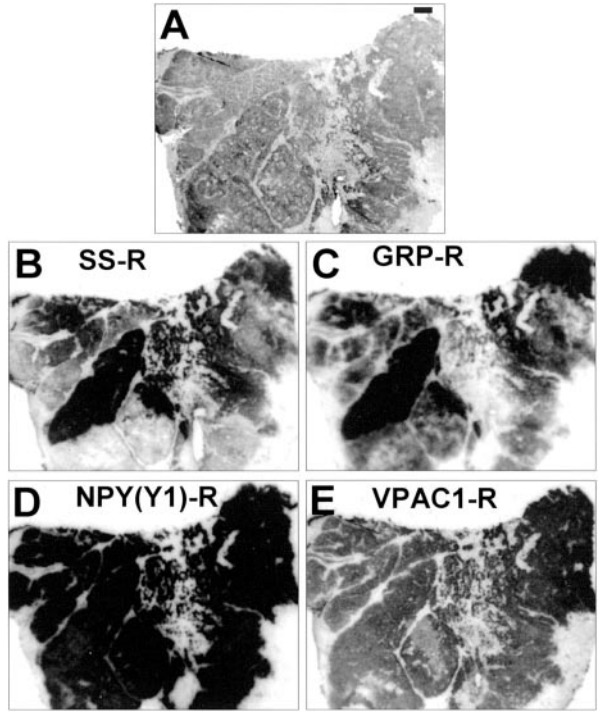

FIG. 7. Four peptide receptors expressed simultaneously in the same breast cancer tumor sample. A, Hematoxylin-eosin-stained section. *Scale bar*, 1 mm. B, Autoradiogram showing total binding of ^{125}I-[Tyr^3]-octreotide (SS-R). C, Autoradiogram showing total binding of ^{125}I-[Tyr^4]-bombesin (GRP-R). D, Autoradiogram showing total binding of ^{125}I-[Leu^{31}, Pro^{34}]-PYY [NPY(Y_1)-R]. E, Autoradiogram showing total binding of ^{125}I-VIP ($VPAC_1$-R). The four receptors are expressed in this tumor. Highest density is seen for NPY (Y_1) receptors (D); heterogeneous distribution is seen for somatostatin receptors (B) and GRP receptors (C). In all four cases, nonspecific binding was negligible.

3. CCK receptors. The growth-promoting effects of gastrin, CCK, glycine-extended gastrin, and more recently of progastrin have been documented in several instances (196, 198, 203, 466). It is, however, not clear through which CCK/gastrin receptors (CCK_1, CCK_2, CCK-C, or other types) these peptides would preferentially act (Refs. 198 and 203; for review, see Ref. 214). Despite the large number of highly potent and selective CCK_1 and CCK_2 analogs (408), there are no successful clinical studies with such nonradioactive analogs as growth inhibitors for long-term therapy of cancer (214). Conversely, the newly developed ^{111}In- and ^{90}Y-labeled CCK/gastrin radiopharmaceuticals appear to be more suitable compounds to diagnose and possibly treat CCK/gastrin receptor-expressing tumors.

4. GRP receptors. GRP receptors are present in high density in several cancers. Numerous studies in animal models show that GRP can stimulate tumor growth, whereas GRP receptor antagonists inhibit growth (30). These observations have led to the suggestion to use unlabeled GRP receptor antagonists for long-term cancer treatment (32). However, no comparable human studies are available yet. Moreover, the conten-

tion that GRP acts as a mitogen and is important for tumor cell growth has recently been challenged (261) by observations from several groups suggesting that the actions of GRP through GRP receptors may be subtler than increasing the proliferation of various tumors. According to Jensen *et al.* (261), GRP is only a modest mitogen in malignancy, with its proliferative effects subordinate to its morphogenic functions. The weak effect of GRP on tumor cell growth is supported by recent clinical studies showing that GRP/GRP receptor coexpression does not adversely affect the outcome of patients with cancers of the colon (260) or lung (467). In these studies, patients bearing small cell lung carcinoma tumors with GRP and GRP receptors actually survived longer than patients with GRP- and GRP receptor-negative tumors (467).

5. Substance P receptors. The presence of NK_1 receptors in human glial tumors (294), the role of tachykinin via NK_1 receptors in the progression of human gliomas (468), and the antitumor effect of NK_1 receptor antagonists on human glioma V373 MG xenografts (297) have led to the proposal to treat malignant gliomas with NK_1 receptor antagonists as long-term therapy in humans.

VII. Outlook

The field of peptide targeting of tumors is moving fast at the moment. There is increasing evidence that the most promising developments are linked to the targeting with radiolabeled (or possibly cytotoxic) peptides as *in vivo* scintigraphy, radiotherapy, or cytotoxic therapy. Conversely, the targeting of tumors with nonradiolabeled (and noncytotoxic) peptides with the aim of a long-term antiproliferative effect has made slower progress in the last decade. Although, for instance, the targeting of somatostatin receptors with octreotide for symptomatic treatment of neuroendocrine tumors has been continuously successful, the use of somatostatin analogs as antineoplastic agents for most other types of cancer has been disappointing (458). Cancer research on peptides is presently dominated by two active fields: one is the search for new peptide receptors overexpressed in specific tumors, *i.e.*, suitable peptide targets. The second field consists of the search and discovery of new radiopeptides and cytotoxic peptides, their development for potential clinical use in the previously defined targets, and the resulting clinical efforts to optimize peptide receptor targeting. Rapid progress is being made in the search for the optimal conditions for a successful radiotherapy of somatostatin receptor-positive neuroendocrine tumors with ^{90}Y-DOTATOC or ^{177}Lu-DOTATATE (469) (Fig. 2). Valuable new information is being extrapolated to other peptide receptor-expressing tumors. Results from studies showing the potential benefits from ^{90}Y-DOTA-substance P radiotherapy in glioblastomas should also become available soon (427). New targets, such as GRP receptors in prostate and breast cancers or NPY receptors in breast cancers are going to be included in *in vivo* targeting strategies and thoroughly investigated, a promising approach. There is also hope that cytotoxic peptides will soon enter clinical trials for cancer therapy. However, in parallel to these clinical activities, more basic information has to be gathered on peptide receptor biology and pathobiology in cancer, allowing a better understanding of the molecular mechanisms underlying the *in vivo* peptide receptor targeting. Some of the questions that need to be answered are:

1) What are the mechanisms triggering the expression of peptide receptors in cancer tissue? Is the presence of peptide receptors in the tissue of origin a prerequisite for the expression of tumoral receptors? What is the importance of the mutated peptide receptors detected occasionally in tumors? We have mentioned that receptors can be expressed in tumors originating from either peptide receptor-expressing or peptide receptor-lacking tissues. Neoplastic transformation can result in a marked increase in the number of peptide receptors that occur physiologically in a tissue, as has been shown before for somatostatin receptors in acromegalics. Conversely, high amounts of GRP receptors are expressed in prostate cancers (257), a tumor originating from the GRP receptor-negative prostate (257). There are also conditions in which the receptor expression, for instance $VPAC_1$ receptors, is not much different in the tissue of origin and the neoplasm. Conditions in which a receptor switch occurs, for instance the switch of Y_2 to Y_1 during neoplastic transformation of breast tissue (53), are also worth mentioning. Finally, cancer may induce a loss of peptide receptors, such as that of sst_2 receptors in pancreatic cancers (380). Therefore, a general rule predicting the peptide receptor expression in tumors on the basis of the receptor expression in the tissue of origin is not available. It is not known whether oncogene activation or epigenetic regulatory and compensatory mechanisms can affect peptide receptor expression. The impact of the recently discovered mutated receptors (220, 221) is not established either. Thus, a deeper insight into the receptor pathogenesis of these tumors would be welcome. If we knew exactly what controls and regulates the expression of peptide receptors, new strategic opportunities to influence the peptide receptor expression process might emerge.

2) Can we actively manipulate, in particular up-regulate, the peptide receptor expression in tumors? There is good evidence that various hormones alter the receptor density in animal experiments. For instance, corticosteroids may down-regulate (470, 471), and estrogens may up-regulate somatostatin receptors (472, 473). Although the corresponding observations are missing in humans, we may assume that certain hormone therapies are responsible for a change in receptor density. However, the effect of hormones on receptor expression is modest in comparison to the 100- to 1000-fold individual variability in receptor density observed in human cancers. This observation suggests that such pharmacological manipulation of peptide receptor expression may not be sufficiently powerful to improve significantly *in vivo* targeting, unless new classes of substances that up-regulate receptor expression more effectively than hormones would be identified.

An alternative approach to manipulate peptide receptors has been proposed by Buchsbaum *et al.* (474); they were able to introduce the peptide receptor genes in animal tumors to such an extent that the tumors expressed enough receptor proteins for detection with receptor scintigraphy. Such promising studies at the gene level are worth pursuing. But because we cannot expect its immediate clinical applicability,

we have, for the time being, to rely on nature to provide us with tumors with a sufficiently high peptide receptor density that can be targeted without complex genetic manipulations.

3) Are the receptor dynamics identical in tumors and in normal tissues? It has been striking to observe the low incidence of side effects in physiological somatostatin targets during long-term octreotide therapy of neuroendocrine tumors, despite the expression of somatostatin receptors in various normal tissues. It has also been striking to see that not all normal somatostatin receptor-positive tissues can be labeled with Octreoscan. Normal lymph nodes and thymus are virtually not detected *in vivo*, whereas they express significant amounts of somatostatin receptors *in vitro*. On the other hand, lymphomas with very low density of somatostatin receptors can often be detected with *in vivo* scintigraphy. It is therefore worthwhile questioning whether this apparent discrepancy between *in vitro* and *in vivo* data is due to different receptor dynamics or different receptor trafficking in normal tissues *vs.* tumors. Although extremely important, such questions have not been answered, due to the difficult experimental approach.

4) Is the receptor expression comparable in primary tumors and metastases? This question cannot be satisfactorily answered on the basis of the available *in vitro* data, partly because of a logistical problem: metastatic tissues are rarely resected. When they are, the corresponding primary tumor tissue is often missing. Moreover, primary tumor and metastasis are often difficult to compare, because they may reflect different stages of a disease and/or different cell populations (*e.g.*, hormone-responsive primary *vs.* hormone-resistant metastatic prostate cancer). Thus, the available information is limited. Nevertheless, most primary and metastatic gut neuroendocrine tumors appear to express similar amounts and similar subtypes of somatostatin receptors *in vitro*. There are, conversely, considerable differences in quantity and subtype of somatostatin receptors in hormone-responsive prostate cancer primaries compared with hormone-resistant prostate cancer metastases (106, 475). In breast cancers, metastases removed surgically at the same time as the primaries have frequently, but not always, a similar *in vitro* receptor pattern (421).

5) What is known about the *in situ* function of tumoral peptide receptors? Although there are no simple answers, three examples taken from the somatostatin receptor field may indicate what might be expected. First, in exocrine pancreatic carcinoma, the lack of sst_2 is considered to be a strong factor of poor prognosis and high aggressivity; accordingly, the introduction of sst_2 in sst_2-negative exocrine pancreatic tumor cells induces a significant inhibition of growth (476). Second, in neuroblastomas, the presence of sst_2 indicates a significantly better prognosis than the absence of sst_2 (95, 368, 369). Third, sst_2 is more often expressed in differentiated than in undifferentiated neuroendocrine tumors (7, 92, 96). These examples strongly suggest a functional role of sst_2 *in situ*, because its presence relates to a higher degree of differentiation and a lower state of aggressivity and growth progression. sst_2 may even be indispensable to maintain such tumor characteristics (476).

6) What is the function of the receptors (somatostatin receptors, substance P receptors, VIP receptors, GRP receptors) overexpressed in peritumoral vessels? They may mediate the vasoactive properties of these peptides (477). Can they also be targeted *in vivo*? Probably yes, as shown in a study using the cytotoxic somatostatin analog AN-238 (395). Can they be visualized with *in vivo* scintigraphy? If they can, they may give a scintigraphic signal even if the tumor is peptide receptor-negative. The visualization of non-small cell lung carcinomas by Octreoscan or DOTA-lanreotide may suggest the presence of such a mechanism (362, 377). Moreover, high doses of radiopeptides or cytotoxic peptides may be able to destroy selectively these receptor-positive peritumoral vessels and thus disturb the tumoral blood supply and induce tumor necrosis (395).

7) Do cancers express significant concentrations of endogenous peptides? Do these peptides interfere with tumoral peptide receptors? Do they affect tumor binding? Numerous tumors can express both the peptide and its receptor in large amounts: GRP and GRP receptors in small cell lung cancers; somatostatin and somatostatin receptors in pheochromocytomas; neurotensin and neurotensin receptors in Ewing sarcomas; and VIP and VIP receptors in neuroblastomas (140, 228, 282, 478). The combination of a peptide and its receptor may regulate tumor growth via autocrine feedback mechanisms (30). Moreover, it may be worthwhile knowing whether an excess of endogenous peptides would prevent an adequate targeting of these tumors, either due to dilution of the exogenous radiopeptide at the tumor site or because most of the peptide receptors have been internalized in tumor cells after binding of the corresponding endogenous peptide. An answer to these questions is crucial for the planning of diagnostic and therapeutic procedures.

8) What is the significance of the recent discovery of homo- and heterodimerization of peptide receptors in primary human tumors (64–66)? What will be the impact on receptor binding, on receptor internalization, on the development of new analogs, and, more generally, on receptor targeting strategies?

9) Which kind of radioisotopes is the best choice for optimal peptide receptor radiotherapy? Is it the proposed β-emitter particle such as ^{90}Y with a maximum energy of the electrons of 2.3 MeV and a mean range of penetration in tissue of 10 millimeters? Or is it a radioisotope with a much shorter range in tissue, such as ^{111}In, which produces, in addition to γ-rays, Auger electrons with a tissue penetration up to 10 μm (479)? Is it better to compromise with ^{177}Lu, which has a maximum tissue penetration range of 2 mm (347, 469)? Will a future combination of short- and middle-range isotopes be preferable for tumor radiotherapy (480)? Will the isotope choice depend on the tumor size? On the homogeneity of the receptor distribution? On the microvascular density in a given tumor? Will the radiosensitivity of a tumor (*e.g.*, radiosensitive lymphomas with low density of somatostatin receptors *vs.* less sensitive gut neuroendocrine tumors with high receptor density) play a role in such a decision?

10) Can we take advantage of multiple concomitant receptor expression in tumors? Is the use of a cocktail of several radiopeptides an improvement over the use of single radiopeptides? The simultaneous expression of several peptide receptors in a given tumor type, as shown *in vitro* (Fig. 7) in

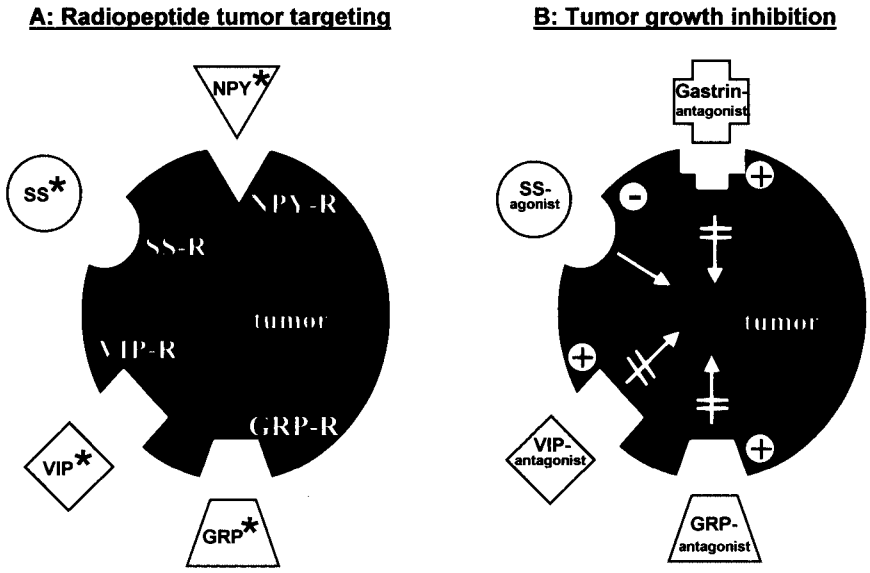

FIG. 8. Multiple peptide receptors in tumor cells. Implications for diagnosis and therapy. A, Tumor diagnosis using a cocktail of radiolabeled (*) peptides [somatostatin (SS), GRP, VIP, and NPY] to obtain an improved *in vivo* scintigraphy signal of the tumor. B, Tumor growth inhibition based on the concomitant long-term therapeutic use of multiple peptide analogs, either agonists of growth-inhibiting peptides (SS) or antagonists of growth-stimulating peptides (VIP, GRP, gastrin).

breast cancers or neuroendocrine tumors (156, 421), may lead in the near future to novel diagnostic and therapeutic strategies (Fig. 8); the use of a cocktail of peptide radioligands recognizing their respective receptors may massively increase the scintigraphic signal of the scanned tumors; the accumulated tumor dose of radioactivity may reach therapeutic levels through binding to the various tumor cell populations in polyclonal tumors. A mixture that may be of particular interest is that of radiolabeled GRP and Y_1 analogs for the diagnosis and radiotherapy of breast cancer and their metastases, because GRP and/or Y_1 receptors were found in highest density in virtually all of these tumors (421). The simultaneous use of several unlabeled peptide analogs (agonists or antagonists) for long-term therapy acting synergistically could also perhaps improve the efficacy of a single peptide (Fig. 8).

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