Neuropeptide Receptors in Health and Disease: The Molecular Basis for In Vivo Imaging

Jean Claude Reubi

Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Berne, Switzerland

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Neuropeptides have been the focus of an increasing interest by nuclear physicians within the last few years (*1*). This has mainly been due to the successful development of one of these neuropeptides, somatostatin, as a tool to visualize various pathologic conditions known to express a high number of somatostatin receptors (*2*). Somatostatin receptor imaging, which is based on the specific binding of radiolabeled somatostatin analogs to somatostatin receptors expressed by specific tissues, is a powerful technique (*3,4*). To understand and interpret the imaging data, basic knowledge about somatostatin receptors is necessary as well as information about the conditions under which somatostatin receptors are expressed. Most of these data, which are the result of a close cooperation between clinicians, nuclear physicians, pathologists and cell biologists, have been and are currently obtained mainly by in vitro investigations. This review therefore looks beyond the applications in nuclear medicine and especially at the molecular basis for somatostatin receptor imaging as it is understood from basic in vitro studies. This article summarizes what is known about the biology of somatostatin receptors in normal tissue and what is known about somatostatin receptors in pathologic conditions. It compares the in vitro results with in vivo data and tries to evaluate critically the pitfalls and limitations of each technique.

Although the main part of this review focuses on somatostatin receptors, a number of emerging new neuropeptides are also discussed and compared with somatostatin to evaluate their respective effects on nuclear medicine.

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For correspondence or reprints contact: J.C. Reubi, MD, Professor of Pathology, Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Murtenstrasse 31, CH-3010 Berne, Switzerland.

NEUROPEPTIDES AND NEUROPEPTIDE RECEPTORS IN GENERAL

Neuropeptides represent a family of substances discovered during the last 25 yr that consist of only a few amino acids (e.g., thyrotropin-releasing hormone) or several dozen amino acids (e.g., insulin). These highly potent molecules act in nanomolar to femtomolar concentrations and are primarily synthesized in the brain, especially in neurons, which led to the term neuropeptide (*5*). There is, however, extensive evidence that shows that most, if not all, of these neuropeptides are also synthesized and act in non-neuronal tissues. The gut, with the brain, is the organ with the highest concentration and incidence of neuropeptides; there, they are generally called gut peptides, although most often they are identical to those found in the brain (*6*). The endocrine system (e.g., pituitary and pancreas) and the lymphatic tissue are also important production sites and targets for neuropeptides. The multiple production sites of these peptides reflect the multiple actions they have in the human body, which is primarily to regulate essential biologic processes. A nonexhaustive list of the major neuropeptides is shown in Table 1.

The action of neuropeptides is mediated by their binding to specific, membrane-associated neuropeptide receptors. Most of these receptors belong to the family of G protein-coupled receptors (*7*). These are membrane-bound molecules consisting of a single polypeptide chain with seven transmembrane domains, an extracellular domain with the ligand binding site and an intracellular domain with sites linked to the activation of second messengers. As other receptors, the neuropeptide receptors, once activated by ligand binding, can be internalized as a receptor-ligand complex into the cell where they will be either degraded by lysosomes or recycled and reintegrated into the cell membrane.

TECHNIQUES TO IDENTIFY NEUROPEPTIDE RECEPTORS IN VITRO

Because the basic knowledge on neuropeptide receptors is based on in vitro data, a brief introduction to the available in vitro techniques to identify such receptors is appropriate. Basically, biochemical, molecular biologic and immunologic techniques are available to identify the receptor protein or the receptor messenger RNA (mRNA), either in tissue homogenates or tissue sections (Table 2). Whereas the methods that deal with tissue homogenates are usually relatively simple, the more difficult handling of tissue sections has the great advantage of a high anatomic resolution. In the past, biochemical techniques have provided evidence for the existence of receptors for the different neuropeptide families, including somatostatin (*8,9*). The use of the so-called high-affinity radioligand binding techniques allowed the study of binding to

TABLE 1
Major Neuropeptides (Including Gut and Pituitary Peptides and Hypothalamic Releasing Hormones)

Opioid peptides	Bradykinin
Substance P	Bombesin/Gastrin-releasing peptide
Gastrin	Neuropeptide Y
Cholecystokinin	Galanin
Vasoactive intestinal peptide	Atrial natriuretic factor
Pituitary adenylate cyclase activating peptide	Neurotensin
Alpha-melanocyte-stimulating hormone	Secretin
Arginine-vasopressin	Melatonin
Oxytocin	Somatostatin
Angiotensin	Thyrothropin-releasing hormone
Insulin	Luteinizing hormone-releasing hormone
Calcitonin	Corticotropin-releasing factor
Endothelin	Growth hormone-releasing factor

membrane preparations of radiolabeled molecules with high specific activity. These techniques permitted the characterization of sites with high affinity (in the low nanomolar range) and low capacity (femtomoles per milligram of protein) for a given peptide and its analogs. In recent years, this relatively simple approach allowed the detailed study of the structure-activity relationship and the biochemical mechanisms involved in neuropeptide action. The main limitation of this technique was, however, the lack of sufficient anatomic resolution. The availability of quantitative receptor autoradiography, which measures radioligand binding on tissue sections, represented a major progress to localize binding sites at the microscopic level and to identify specific cell populations in a given tissue or organ enriched in a particular neuropeptide receptor (*10,11*). Such morphologic evaluations are particularly relevant in tissues with complex pathologic alterations.

The recent cloning and sequencing of several neuropeptide receptors has provided the opportunity to use potent molecular biologic techniques to identify receptors or their mRNA. Northern blotting of isolated mRNA from tissue homogenates is one of the currently used methods. In addition, mRNA amplification by reverse transcription (RT) and polymerase chain reaction (PCR) provides an extremely sensitive method that can detect a few gene transcripts in a tissue segment. However, RT-PCR does not provide for precise anatomic localization of gene expression. The same limitation applies to another highly sensitive molecular biologic technique, the ribonuclease protection assay. Conversely, the identification of the receptor mRNA by in situ hybridization on tissue sections with isotopic or nonisotopic labels allows a precise anatomic localization of the investigated receptor mRNA (*12*). If specific antibodies against neuropeptide receptors are available, immunologic techniques (immunoblotting and immunoprecipitation) on homogenates can be used. The use of receptor antibodies in morphologic studies allows immunohistochemical detection of receptors at the cellular level. An advantage of molecular biologic techniques over biochemical techniques is to identify precisely the various neuropeptide receptor subtypes. Table 2 gives an overview of the available techniques and shows, as an example, the present state of methodologic development for the in vitro detection of one of these neuropeptide receptors, the somatostatin receptor.

Because the visualization of neuropeptide receptors in nuclear medicine is based on the binding of a radiolabeled ligand to the receptor, it is evident that, among all the in vitro data available, the ligand binding techniques are most appropriate to mimic and therefore understand better what happens in vivo. Clearly, one of the most adequate techniques is quantitative receptor autoradiography, which indeed has been used over the last several years to identify neuropeptide receptors in pathologic tissues (*13–17*). Receptor autoradiography has the following advantages over homogenate binding assays for the evaluation of the receptor status of surgical human samples. First, the type of tissue that contains the receptor can be clearly identified. Second, the homogeneity of the receptor distribution in the tissue sample can be evaluated. Third, different histopathologic techniques (e.g., immunohistochemistry) can be combined on adjacent sections with that of receptor autoradiography for more extensive characterization of the type of tissue involved. Fourth, very small tissue samples that weigh only a few milligrams may be sufficient for receptor identification with receptor autoradiography.

TABLE 2
Available Techniques to Identify Human Somatostatin Receptors In Vitro

Technique	Tissue	Detected material	References
Biochemical			
Radioligand binding	Homogenate	Receptor protein	27, 60
Quantitative receptor autoradiography	Sections	Receptor protein	13, 14
Molecular biologic			
Northern blotting	Homogenate	Receptor mRNA	24
Reverse transcription/polymerase chain reaction	Homogenate	Receptor mRNA	29
Ribonuclease protection assay	Homogenate	Receptor mRNA	29
In situ hybridization	Sections	Receptor mRNA	32
Immunologic			
Immunoblotting	Homogenate	Receptor protein	61 (rat only)
Immunoprecipitation	Homogenate	Receptor protein	61 (rat only)
Immunohistochemistry	Sections	Receptor protein	NR

NR = not yet reported for somatostatin receptors; mRNA = messenger RNA.

SOMATOSTATIN RECEPTORS

This section reviews specifically present knowledge based on in vitro studies about the biology and pathology of somatostatin and somatostatin receptors in humans, including a critical evaluation of the limitations or advantages of in vitro methods.

In Vitro Detection of Somatostatin Receptors

Somatostatin Receptors in Normal Tissues. Somatostatin is a cyclic peptide that consists of 14 amino acids (somatostatin-14), but there is also a biologically active, longer form with 28 amino acids (somatostatin-28) (*18*). These molecules play, as do other neuropeptides, a regulatory role in several different organ systems. For instance, they modulate neurotransmission in the central nervous system (as a neurotransmitter) and regulate pituitary growth hormone (GH) and thyroid-stimulating hormone release (as a neurohormone). They also act in the gastrointestinal tract (inhibition of transit time, fluid resorption, gastrointestinal hormone release and acid production) and in the exocrine and endocrine pancreas (inhibition of exocrine secretions and insulin and glucagon release). Somatostatin, which is synthesized and released by endocrine and/or nerve cells in these organs, locally inhibits the physiologic actions mentioned above in an autocrine and/or paracrine regulatory manner. Somatostatin also inhibits the proliferation and immunoglobulin production of activated lymphocytes. It is furthermore considered to be a strong vasoconstrictory agent in the gut. Other somatostatin actions, i.e., at the kidney level, remain unclear.

These different actions of somatostatin are mediated via specific membrane receptors on the target cells. The presence of somatostatin receptors has been demonstrated in various regions of the normal brain, the leptomeninx, the anterior pituitary, the endocrine and exocrine pancreas, the mucosa of the gastrointestinal tract and the normal human kidney; parts of the peripheral nervous system (gastrointestinal plexus); and cells of the immune and vascular systems (Fig. 1 A, C, E, and G) (*19–21*). Specifically, the following cell types express somatostatin receptors: neuronal and astroglial cell populations in the brain (cortex, hippocampus and so forth) and in the vegetative nervous system (plexus myentericus and submucosus); endocrine cells in several organs, including the pituitary, pancreas and gastrointestinal tract; lymphoid cells localized in germinal centers of lymphatic follicles; smooth muscle cells in the veins of various tissues, including the kidney and gut; parietal cells in the gastric mucosa; and proximal tubular epithelia of the kidneys.

Autoradiographic studies of somatostatin receptor distribution in some organs pointed to a differential affinity for somatostatin-14, somatostatin-28 and small synthetic analogs (*22*), which suggests the presence of somatostatin receptor subtypes. The recent cloning of several somatostatin receptor genes has increased understanding of receptor structure and function. To date, the five human somatostatin receptor subtypes SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 have been cloned and partially characterized (*23–25*). These subtypes belong to a superfamily of receptors that have seven membrane-spanning domains. They have distinct, often overlapping, patterns of expression in human organs such as the brain, pituitary, gastrointestinal tract, pancreas and kidneys. All five receptor subtypes can functionally couple to the inhibition of adenylyl cyclase. Pharmacologic studies showed that all five human subtypes bind somatostatin-14 and somatostatin-28 with a high affinity. The SSTR2 subtype is also able to bind small synthetic somatostatin analogs such as octreotide, BIM-23014 (lanreotide) or MK 678 (seglitide) with very high affinity [IC_{50}, peptide dose required for 50% binding inhibition: 0.5 μM]. SSTR5 and SSTR3 are also able to bind these small analogs, albeit with less affinity (IC_{50} of 7 and 30 nM, respectively). SSTR1 and SSTR4 do not bind these octreotide-like molecules. The precise role of these five somatostatin receptor subtypes is not yet fully elucidated (*26*).

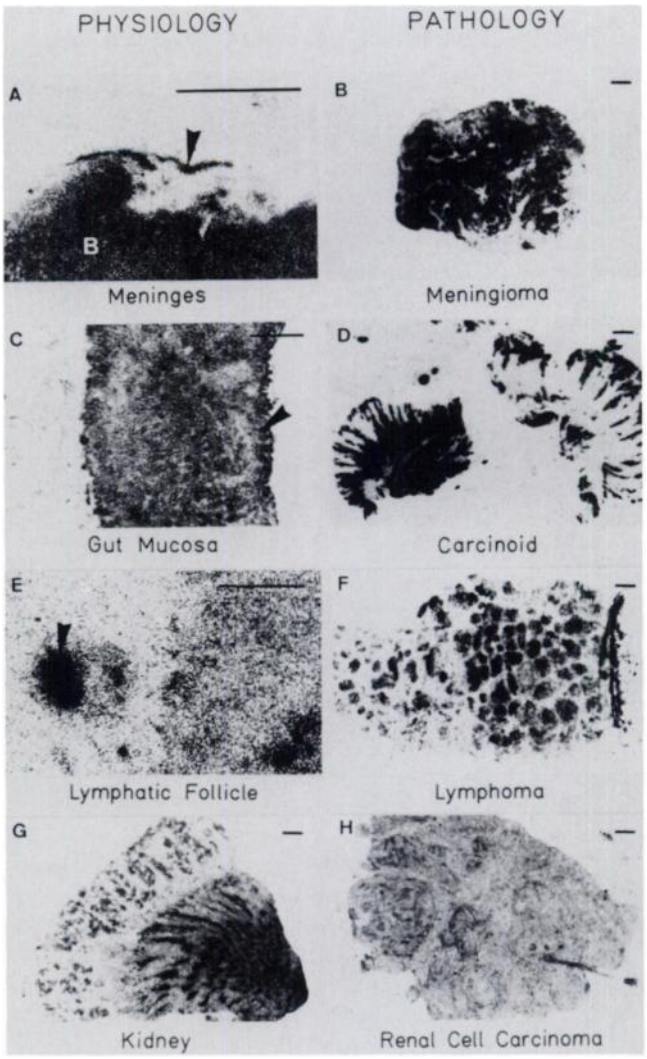

FIGURE 1. Somatostatin receptors measured with in vitro receptor autoradiography in various normal human somatostatin target tissues (A, C, E and G) and in human tumors derived from these targets (B, D, F and H). The panels represent autoradiograms showing total binding of ^{125}I-[Tyr^3]-octreotide. Black areas represent somatostatin receptor-positive tissues (bars = 1 mm). (A,B) Meninges (arrowhead) are strongly receptor positive as is meningioma. Underlying brain (B) is also receptor positive. (C,D) Gut mucosa (arrowhead) is receptor positive as is corresponding gut carcinoid tumor. (E,F) Germinal center of a lymphatic follicle (arrowhead) is receptor positive as is malignant follicular lymphoma. (G,H) Kidney (proximal tubules in cortex, vasa recta in medulla) is receptor positive as is renal cell carcinoma.

Somatostatin Receptors in Human Tumors. In vitro receptor autoradiographic studies have clearly shown that most human tumors that originate from somatostatin target tissues have conserved somatostatin receptors, which are often expressed at a high density (Fig. 1 B, D, F and H). This was first demonstrated by Reubi and Landolt (*27*) for human GH-producing pituitary

TABLE 3
Human Tumors that Express Somatostatin Receptors*

Neuroendocrine tumors	High incidence
Pituitary adenomas, islet cell tumors, carcinoids, paraganglioma(s), medullary thyroid carcinomas, pheochromocytomas, small cell lung cancers	
Tumors of the nervous system	High incidence
Astrocytomas, neuroblastomas, medulloblastomas, meningiomas	
Renal cell carcinomas	High incidence
Malignant lymphomas	High incidence
Breast cancers	About 50%
Ovarian and colonic cancers	Low incidence

*Data were obtained with in vitro ligand binding and autoradiographic studies with radiolabeled Tyr^3-octreotide.

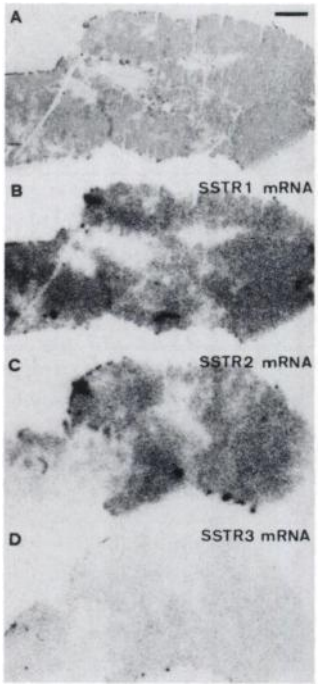

FIGURE 2. SSTR1, SSTR2 and SSTR3 receptor subtypes in GH-producing pituitary adenoma by the in situ hybridization technique. (A) Hematoxylin-eosin-stained section (bar = 1 mm). (B) Autoradiogram showing SSTR1 mRNA. (C) Autoradiogram showing SSTR2 mRNA. (D) Autoradiogram showing SSTR3 mRNA. SSTR1 and SSTR2 mRNAs are particularly abundant.

tumors, in which a high number of somatostatin receptors were found in most cases. These receptors are the pathophysiologic basis for the successful control of GH secretion in most acromegalic patients during treatment with octreotide; the acute suppressive response to octreotide on GH secretion in an acromegalic patient indeed depends in most cases on the number of octreotide receptors on the pituitary tumor. Islet cell tumors and carcinoids also resemble the cells from which they originate in that they express somatostatin (analog) receptors in more than 80% of cases (Fig. 1 F and Table 3). Most islet cell tumors and carcinoids are slow growing but malignant tumors, which produce clinical symptoms related to hypersecretion of hormones that incapacitate the patients. Treatment with the stable somatostatin analog octreotide is helpful in controlling clinical symptoms through inhibition of the hormone hypersecretion in most patients with metastatic carcinoids, vipomas, gastrinomas, insulinomas and glucagonomas. As in GH-producing pituitary adenomas, the number of octreotide receptors on carcinoid tumors closely predicts the suppressive effect of chronic therapy with the drug on hormonal hypersecretion (*17*). There is evidence that octreotide controls tumor growth in part of these patients; in about 20% of the cases, shrinkage of enlarged lymph nodes and liver metastases has been demonstrated.

Apart from pituitary adenomas, islet cell tumors and carcinoids, high numbers of somatostatin receptors are expressed by other endocrine tumors, like paraganglioma(s), pheochromocytomas, medullary thyroid cancers (MTC) and small cell lung cancers (SCLC) (Table 3) (*14*). A number of adenocarcinomas that originate from the breast, kidney, colon, ovary and other organs express somatostatin receptors to a varying degree. In some of these tumors, other histopathologic neuroendocrine characteristics such as positive reactivity to chromogranins, synaptophysin and/or neuron-specific enolase were also demonstrated. Tumors that originate from other tissues that normally express somatostatin receptors such as the leptomeninx (meningiomas) and glia (well-differentiated astrocytomas) (*14*) and from lymphocytes (malignant lymphomas) (*28*) express high numbers of somatostatin and/or octreotide receptors (Fig. 1 A, B, E and F and Table 3).

Human tumors may express different somatostatin receptor subtypes. As shown with RT-PCR and ribonuclease protection assay, pituitary adenomas appear to express all five SSTR subtypes, with particularly high levels of SSTR2 and SSTR5 (*29–31*). Reubi et al. (*32*) have preliminary evidence, based on in situ hybridization for SSTR subtype mRNAs, that other human tumor types can express one or several of the somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3. The most frequently expressed subtype is SSTR2, which has the highest affinity for octreotide. Figure 2 shows a case of a somatostatin receptor-positive GH-producing pituitary adenoma that expresses simultaneously SSTR1, SSTR2 and SSTR3 mRNA. Receptor binding studies in this tumor showed high affinity binding for octreotide and for somatostatin-28.

The fact that many tumors express the SSTR2 subtype is extremely important for somatostatin receptor imaging with octreotide-like ligands because human SSTR2 represents the subtype with the highest affinity for octreotide. Conversely, it should be emphasized that tumors that do not express octreotide-preferring somatostatin receptors, such as ovarian and prostate carcinomas, and several insulinomas and MTC, will not be visualized by ^{111}In-diethylenetriaminepenta-acetic acid (DTPA)-octreotide imaging. A different type of radioligand, structurally more closely related to somatostatin-14 or somatostatin-28 than to octreotide, would be required for the visualization of such tumors.

What is known about the role of somatostatin receptors expressed by human tumors? Basically, somatostatin analogs are used therapeutically to inhibit excessive hormone secretion in patients with somatostatin receptor-positive GH-producing adenomas, metastatic carcinoids or islet cell carcinomas (*33*). Little is known, however, concerning the potential role and value of octreotide therapy on tumor growth in patients with these and other somatostatin receptor-positive neuroendocrine tumors, breast cancers, malignant lymphomas, renal cell carcinomas or meningiomas (*26*). In cultured human neuroendocrine tumors, octreotide can inhibit both hormone release and cell growth in vitro. In cultured human meningiomas, however, octreotide inhibits adenylyl cyclase activity but stimulates the growth of tumor cells, which suggests that octreotide should not be used in the treatment of patients with inoperable meningiomas. Furthermore, several clinical trials of somatostatin analogs to treat diverse tumor types,

including tumors of the breast, lung, pancreas or carcinoids, have been generally disappointing, showing only little, if any, antiproliferative effects of somatostatin (*26,33*).

These human data are in contrast with data obtained in various animal models in which somatostatin analogs were clearly shown to inhibit the growth of a variety of tumors, including chondro- and osteosarcomas, pancreatic, mammary and prostatic adenocarcinomas. As summarized recently (*26*), combinations of the following direct and indirect mechanisms of somatostatin actions in tumors might apply: (1) via the inhibition of the secretion of GH, insulin and other gastrointestinal hormones; (2) via the direct or indirect (via GH) inhibition of the production and biologic activity of insulin-like growth factor type 1 or other tumor growth factors; (3) via a direct inhibitory effect on angiogenesis; and (4) via direct antiproliferative effects on tumor cells by specific tumoral somatostatin receptors. Recent studies indicate that at least SSTR1 and SSTR2 can exert direct antiproliferative effects through stimulation of a tyrosine phosphatase. Somatostatin thus may reverse the growth-promoting activity of the tyrosine kinase group of oncogenes.

Somatostatin Receptors in Nontumoral Diseases. High expression of somatostatin receptors is not restricted to tumors but can also be found in selected chronic inflammatory lesions and diseases, namely in granulomas, Crohn's disease, colitis ulcerosa and rheumatoid arthritis. Receptor autoradiographic studies show that, in active granulomatous diseases, including sarcoidosis and tuberculosis, the epitheloid cells within the active granulomas express somatostatin receptors (*16*). Inactive, i.e., successfully treated, granulomas lose their somatostatin receptors. An example of active tuberculous granulomas that express somatostatin receptors is shown in Figure 3. The presence of somatostatin and somatostatin receptors within granuloma cells might indicate that somatostatin participates in the regulation of the intensity of the granulomatous response to specific microorganisms (tuberculosis) or to unknown stimuli (sarcoidosis). Because somatostatin inhibits proliferative and other functions of activated lymphoid cells, it is conceivable that similar effects may take place in the cells from active granulomas. In accordance with this hypothesis, Weinstock (*34*) recently showed that octreotide therapy decreases the intensity of the granuloma response by approximately 45% in murine schistosomiasis.

In another study, Reubi et al. (*15*) identified in vitro somatostatin receptors in intestinal samples of patients with inflammatory bowel diseases (IBD) such as Crohn's disease or ulcerative colitis. The receptors were present in high density in most intramural veins, but not in the arteries, of the intestines in florid IBD. They were, however, not detected in the veins of noninflamed control intestine. No significant changes in the somatostatin receptor content of the intestinal mucosa were observed between IBD samples and control samples. This suggests a primarily vascular site of the somatostatin receptor alterations. The presence of somatostatin receptors on veins and venules in IBD may be of functional interest, inasmuch as small, dilated blood vessels are the site of extravasation of plasma and of leukocytes associated with inflammation. The vasoconstrictory action of somatostatin may therefore regulate and influence inflammatory and reparatory processes in the gut. Furthermore, it is conceivable that interactions between various peptide systems take place, that is, between substance P, somatostatin and vasoconstrictory intestinal peptides, as this has been extensively described for the immune system or for the central nervous system. For instance, somatostatin may counterregulate the vasodilatory effects of substance P or the influence of substance P on the extravasation of plasma and white blood cells and therefore reverse the inflammatory process triggered by substance P.

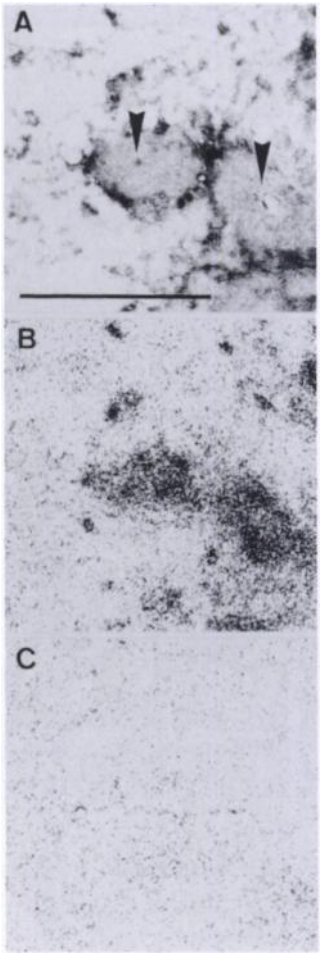

FIGURE 3. Somatostatin receptors in tuberculosis. (A) Hematoxylin-eosin-stained section showing two tuberculous granulomas (arrowheads) (bar = 1 mm). (B) Autoradiogram showing total binding of ^{125}I-[Tyr^3]-octreotide. Both granulomas are labeled. (C) Autoradiogram showing nonspecific binding of ^{125}I-[Tyr^3]-octreotide (in presence of 10^{-6}*M* octreotide).

A high expression of somatostatin receptors in blood vessels seems not to be restricted to gastrointestinal inflammatory diseases. Preliminary observations indicate that other tissues that display marked inflammatory changes, such as lymph nodes with reactive changes, may also express somatostatin receptor-positive veins, similar to those seen in IBD (Reubi JC, unpublished data).

In a recent in vitro study with tissues from patients with rheumatoid arthritis, somatostatin receptors were detected in the synovium with signs of active disease (*35*). The somatostatin receptors were preferentially located in the extremely abundant blood vessels, with specific labeling of the veins but not of the arteries, as seen in an overview picture in Figure 4. The whole vessel wall was homogeneously labeled, including the smooth muscle cells and probably the endothelium. Somatostatin may act through these venous receptors to influence the inflammatory process by induction of vasoconstriction, inhibition of plasma extravasation and cell migration or inhibition of neovascularization.

Somatostatin Receptors in the Peritumoral Vascular System. Recently, the peritumoral vascular system of the host has emerged as an additional possible site of somatostatin action in tumor development (*36*). A vascular action of somatostatin on neoplasms may turn out to be widespread and very important indeed because the presence of strongly somatostatin receptor-

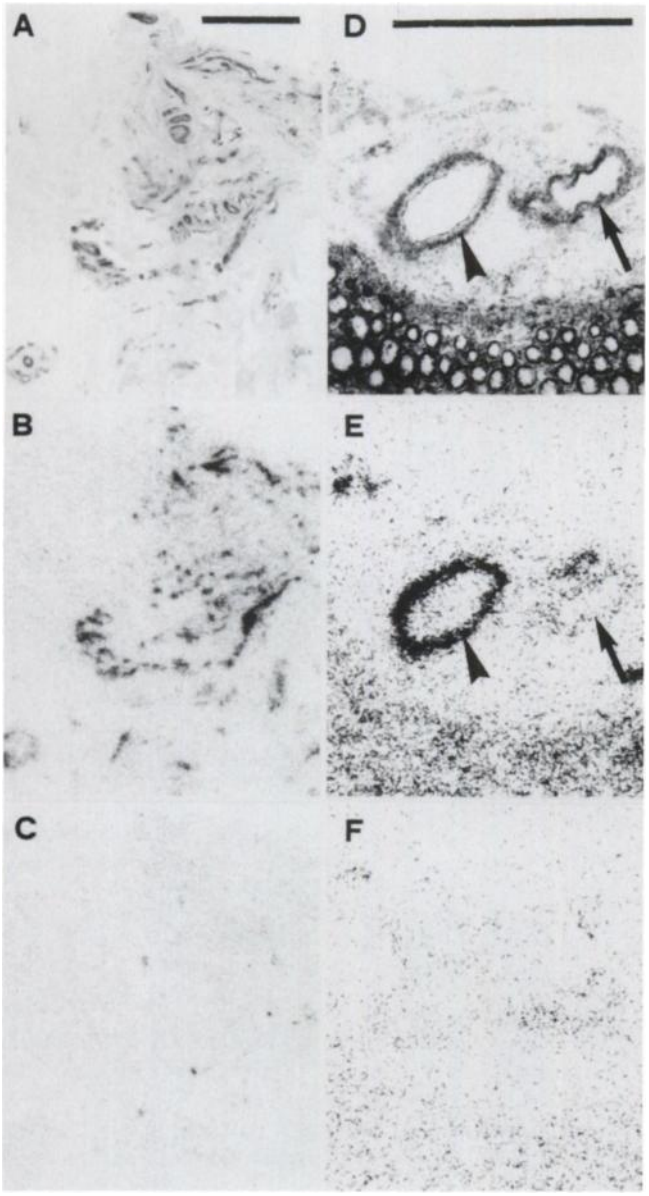

FIGURE 4. Vascular somatostatin receptors in synovium from patient with rheumatoid arthritis (A–C) and in submucosal, peritumoral tissue adjacent to colonic carcinoma (D–F). (A) Section stained for factor VIII-like immunoreactivity shows high density of vessels in the synovium (bar = 1 mm). (B) Autoradiogram shows total binding of ^{125}I-[Tyr^3]-octreotide. Veins are strongly labeled. (C) Autoradiogram shows nonspecific binding. (D) Hematoxylin-eosin-stained section shows vein (arrowhead) and artery (arrow) in colonic submucosa. Mucosa is seen on bottom. Colonic carcinoma (not depicted) is localized less than 5 mm away (bar = 1 mm). (E) Autoradiogram shows total binding of ^{125}I-[Tyr^3]-octreotide. Vein, not artery, is strongly labeled. (F) Nonspecific binding.

positive veins has been identified in the peritumoral zone of several types of malignant neoplasms, including colon carcinoma, nonsmall cell carcinoma of the lung (nSCLC), breast cancer, renal cell carcinoma and malignant lymphoma. Figure 4 shows an example of a submucosal vein located in the vicinity of a colon carcinoma that is expressing a high density of somatostatin receptors. In a large series of human colonic carcinomas, a high density of vascular somatostatin receptors has been observed in the immediate vicinity of all the tumors; as the distance from the carcinomas increases, the density of somatostatin receptors in the colon decreases considerably, which suggests a local phenomenon related to the presence of the tumor. The presence of vascular somatostatin receptors seems to be independent of the presence or absence of somatostatin receptors in the tumor itself. Because a vasoconstrictive effect of somatostatin, in particular in the gut, is well established, an increased somatostatin receptor density in veins may allow a strong and rapid vasoconstriction, which may result in local hypoxia and necrosis of the tumor or in prolonged vasoconstriction directed against metastatic tumor dissemination. This mechanism may explain the occasional clinical observation of a marked decrease in tumor size during octreotide therapy in some patients. The high expression of somatostatin receptors in peritumoral veins may be seen as a defense of the host against tumor angiogenesis, as the latter can be inhibited by somatostatin analogs in the chick chorioallantoic membrane system, possibly through somatostatin receptors similar to those identified in human peritumoral vessels.

Peptides and peptide receptors may therefore represent a novel group of substances that influence the pre-existing peritumoral vasculature of the host, and perhaps tumor angiogenesis, in addition to the large number of already defined humoral factors (*37*).

Correlations Between In Vitro and In Vivo Somatostatin Receptor Measurements in Tumors

What is the practical value of the above-mentioned in vitro data for in vivo somatostatin receptor imaging? How do in vitro data correlate with in vivo data and to which extent can in vitro data be used to explain and interpret in vivo somatostatin receptor scans? The answers that have been provided over the last few years to these questions can be summarized as follows. Studies that directly compare the somatostatin receptor imaging in vivo with the somatostatin receptor status in vitro on the resected tumor from the same individual have been performed in more than 150 patients with cancer (*4,38–40*). They generally support the concept that the pathologic tissues visualized in vivo with ^{125}I-[Tyr^3]-octreotide or ^{111}In-[DTPA, DPhe^1]-octreotide scanning represent somatostatin receptor-positive tissues. An excellent correlation between in vitro and in vivo data has been found, in particular, in tumors with a high density and a homogeneous distribution of somatostatin receptors, such as gastroenteropancreatic (GEP) tumors, meningiomas, paraganglioma(s) or pheochromocytomas. Other tumors with a homogeneous somatostatin receptor distribution but lower receptor density, such as lymphomas, neuroblastomas or renal cell carcinomas, showed, in general, a good correlation, too (Bihl H, Dörr U, Reubi JC, unpublished) (*4*). Even when the overall results with the two techniques are compared in different patient groups, a good correlation is found in most cases. Nevertheless, there are significant discrepancies in selected tumor types. In breast tumors, SCLC and MTC, a larger number of tumors was found to be somatostatin receptor positive in vivo than it was in vitro (*4*). In nSCLC or glioblastomas, no tumoral somatostatin receptors were identified in vitro, but all tumors were identified by somatostatin receptor scanning in vivo (*39,41*). The reasons for such discrepancies and the general precautions necessary for interpretation of some in vitro and in vivo data are discussed below and summarized in Table 4.

False-Negative In Vitro Status. A tumor may have several particularities that may lead to misinterpretations, i.e., as a tumor with false-negative receptor status. First, a low tumor cellularity combined with a low receptor density may not give sufficient

TABLE 4
List of Potential Reasons for False Somatostatin Receptor Tumor Status In Vitro or In Vivo

False-negative in vitro status	False-negative in vivo status
Tumors with low cellularity and low SS-R density	Tumors with low cellularity and low SS-R density
Tumors with high endogeneous SS production (SSoma, MTC, pheo)	Tumors with high endogeneous SS production (SSoma, MTC, pheo)
Tumors with SS-R subtypes, not recognized by certain ligands (i.e., octreotide)	Tumors with SS-R subtypes, not recognized by certain ligands (i.e., octreotide)
Tumors with nonhomogeneous SS-R distribution (breast tumors)	Tumors located in region with high background
Technical	Tumors located inside intact blood-brain barrier (poor ligand permeability)
Loss of SS-R	
Long delay between removal and freezing of sample	
Uncontrolled thawing of frozen sample	
Tumors with high nonspecific binding	
Poorly representative sample	
Low tumor-to-stroma ratio	
Metastasis rather than PT	
Patient undergoing drug therapy (i.e., corticosteroid)	
False-positive in vitro status	**False-positive in vivo status**
SS-R analysis with tissue homogenates (uncontrolled contamination with nontumoral SS-R-positive tissues)	Tumors located inside disturbed blood-brain barrier (nonspecific trapping of ligand)
	Identification of nontumoral SS-R-positive tissues (activated lymphocytes, vessels and granulomas)
	Binding to antibodies against chronically injected octreotide (seldom)

SS-R = somatostatin receptor; SSoma = somatostatinoma; MTC = medullary thyroid cancers; pheo = pheochromocytomas; PT = primary tumor.

specific binding to conform to the strict definition of "twice the background radioactivity" for positive tumors, which is used routinely in in vitro autoradiography experiments. It may be argued that the amount of radioactivity needed to consider a tumor to be positive is set too high and that it artificially gives rises to a number of false-negative results. However, in a large comparative study, a highly significant correlation was shown between the somatostatin receptor status quantitatively measured in vitro and the antisecretory properties of somatostatin in the respective tumors in vivo, which therefore validated the threshold of positivity set in these and further studies (*42*). Second, tumors with high somatostatin content may be found to be receptor negative because of occupancy of the available somatostatin receptor by endogeneous somatostatin (*43,44*). Although the excess of somatostatin in the sections can be washed out by adequate preincubations, a lack of receptors may still occur as a result of downregulation of somatostatin receptors after chronic exposure to excess endogenous somatostatin (i.e., somatostatinomas). Third, some somatostatin receptors may not be recognized because they belong to subtypes (e.g., SSTR1) that are not recognized by certain types of ligands (e.g., octreotide). In in vitro experiments, universal ligands that measure all subtypes such as somatostatin-14 or somatostatin-28 analogs can be used to complement octreotide compounds to overcome this problem. This is, however, a significant problem with in vivo imaging in which only octreotide-like compounds can presently be used. Fourth, a further problem specific for in vitro investigations may be the nonhomogeneous somatostatin receptor distribution, which is particularly evident in breast cancers; receptor-positive cases may be missed when small samples that contain only the receptor-negative part, are evaluated (*45*). Finally, there are several technical problems that may lead to false-negative in vitro data; the most important is the loss of somatostatin receptors as a result of an excessively long delay between surgical sample removal and freezing or of uncontrolled thawing of an already frozen sample (in general, receptors do not survive thawing and refreezing). Another one is the occasional high nonspecific binding of the ligand to a variety of tumors such as MTC, which contain macromolecules (e.g., amyloid), that can bind the ligand unspecifically. This high nonspecific binding can mask the presence of specific binding in the same area. Furthermore, it may be extremely difficult to obtain a representative biopsy sample of certain tumors, for instance, in SCLC tumors. Sometimes only one metastasis is available for the in vitro investigation; its somatostatin receptor status may not be representative for the patient's primary tumor or other metastases.

In general, it should be emphasized that the somatostatin receptor status for a given patient is performed in vitro on a single tumor or metastasis sample, whereas in vivo it is based on a whole body scan (primary tumor plus all metastases). This difference may explain discrepancies between in vitro and in vivo receptor evaluation; the in vivo evaluation has more chances to identify a receptor-positive pathologic state, particularly in patients with nonhomogeneous tumoral receptor distribution. Finally, the application of selected drugs may alter the somatostatin receptor expression of a tumor; corticosteroids, for instance, are known to downregulate somatostatin receptors. It is therefore not excluded that, in tumor samples obtained during brain surgery (often performed under corticosteroid treatment), a drug-dependent reduction or a lack of tumoral somatostatin receptor expression is observed.

False-Negative In Vivo Status. The low cellularity and low receptor density of a tumor, the high endogenous somatostatin content, the receptor subtype involved and the drug treatment are

reasons that may lead to a false-negative in vivo status, as previously discussed for the in vitro situation. In addition, there are false-negative in vivo results caused by specific in vivo conditions, e.g., inadequate location of the tumors. This can be a region of high background activity (kidney, spleen or gut) where the receptor-positive tumor will be masked (*4*); it can also be a site located inside the intact blood brain barrier, which is impermeable to peptides, including octreotide analogs (*39*). Imaging-specific technical reasons have been reviewed elsewhere (*4*).

False-Positive Status. Although false-positive results appear less frequent than false-negative ones, they may also represent a cause for discrepancies between in vitro and in vivo studies and for misinterpretations.

The main reason for false-positive findings in vitro lies in the use of binding assays with tissue homogenates, which are morphologically poorly characterized. Indeed, tumor samples may often be contaminated with somatostatin receptor-positive tissue of nontumoral origin such as peritumoral vessels, activated lymphocytes or a normal somatostatin target tissue. Special caution should be taken when amplification methods such as RT-PCR are used on homogenates to identify tumoral receptor mRNA because this method also amplifies the signal that originates from contaminating nontumoral tissues.

Tumors located inside the disturbed (i.e., permeable) blood-brain barrier, such as glioblastomas, are usually identified in vivo with somatostatin receptor scanning, even if they do not express somatostatin receptors (*39*). This may possibly be due to the trapping of the ligand within this particular compartment. Another reason for false in vivo positivity may be the identification of nontumoral somatostatin receptor-positive elements (vessels or lymphocytes) in or around a receptor-negative tumor. Such a situation may occur with the nSCLCs identified with somatostatin receptor scanning (*41*); although they lack specific tumoral somatostatin receptors, these tumors are often surrounded by somatostatin receptor-positive lymphocytes and vessels, which could theoretically account for a positive signal in vivo. Moreover, it remains to be established to which extent an increased vascular perfusion and/or permeability, as seen in inflammation and neoplasms [newly formed vessels are hyperpermeable to plasma proteins, possibly because of gaps in the endothelial lining (*46*)], can produce a significantly higher ligand accumulation in vivo than would be expected from the in vitro detected density of vascular or tumoral somatostatin receptors. Finally, a very rare situation of a false-positive determination is the binding of labeled octreotide to antibodies against octreotide developed in the course of chronic octreotide therapy (*47*).

Correlation In Vitro/In Vivo in Nontumoral Diseased Tissues

The correlation between in vitro and in vivo findings, and the unequivocal identification of the cellular elements responsible for hot spots with somatostatin receptor imaging, is much more difficult to establish in nontumoral tissues than in tumors because nontumoral tissues are rarely removed surgically and therefore are often unavailable for in vitro testing.

In the granulomas of sarcoidosis, which are rarely surgically removed, the correlation between in vitro and in vivo data is limited to the observation that somatostatin receptors were found in vitro in epitheloid cells in active lesions but not in inactive lesions and that hot spots were identified also in vivo in active lesions only. In vitro and in vivo studies, however, compared different patient groups (*16*).

The same applies to lymph nodes with reactive changes, e.g., those characterized in vitro by a high number of lymphoid follicles with numerous somatostatin receptor-positive germinal centers (*48*). In one in vivo scintigraphy study on lymphomas, reactive lymph nodes were reported to be visualized (*49*), whereas normal lymph nodes were not. Again, in vitro and in vivo studies compared different patient groups.

In two patients with rheumatoid arthritis, in vitro and in vivo studies identified positive lesions in the same patients (*50*). Whereas early speculations pointed toward activated lymphoid tissue as the source of the hot spots, recent experimental evidence in vitro clearly showed that the major source of somatostatin receptors was the veins (*35*). Because rheumatoid arthritis is characterized by a high proliferation of vessels, it is likely that the high density of vessels, which are somatostatin receptor positive at the same time, is responsible for the hot spots. It will be important to know whether a minimal density of such vessels is required to visualize a lesion (*50*). This question is equally important in the case of peritumoral vessels, which were shown to be highly somatostatin receptor positive in all samples of colonic carcinomas tested (*36*). Since only 20% to 30% of this tumor type can be visualized in vivo (*4*), the density of these somatostatin receptor-positive vessels may not be sufficient to produce hot spots, partly because of the high gastrointestinal background.

Correlation In Vitro/In Vivo in Normal Tissues

Various normal tissues in the human body express somatostatin receptors, such as the brain, pituitary, gut mucosa, thymus, kidneys, spleen and germinal centers in lymphoid follicles. Whereas the brain is not visualized in vivo because of the blood-brain barrier, the normal pituitary and the spleen are usually well identified (*4*). The somatostatin receptors in the kidneys are masked in vivo by the high quantity of ligand excreted through this organ. The intestinal mucosa, however, cannot be unequivocally identified in vivo; moreover, neither the child or adult thymus nor the normal lymph nodes are clearly visualized (*48*). The lack of in vivo visualization of these somatostatin receptor-positive normal tissues is intriguing. Whereas, in healthy lymph nodes, the small percentage of the total cell population with somatostatin receptors (e.g., germinal centers) might explain a lack of visualization, this argument does not account for the gut mucosa or the thymus, where the percentage of somatostatin receptor-positive cells should be large enough to be seen. Conversely, it is not understood why the normal thyroid gland can be identified on somatostatin receptor scans (*4*), although no somatostatin receptors can be detected in vitro. A receptor-independent mechanism of uptake of the radioligand by thyroid tissue in vivo is conceivable; however, it cannot be excluded that a subpopulation of thyroid cells (C-cells) express somatostatin receptors in low amounts that are not readily detectable in vitro.

One should be aware that in vitro somatostatin receptor identification is static, whereas in vivo scanning also measures dynamic processes. A major dynamic process is receptor turnover and receptor internalization. Is the generally better visualization of tumors compared with normal tissues related to a higher somatostatin receptor turnover and internalization rate in tumors? What is the exact role of the vascular perfusion, drainage and permeability of a tissue in respect to ligand binding to its target? Definitive answers to these two questions are of great importance.

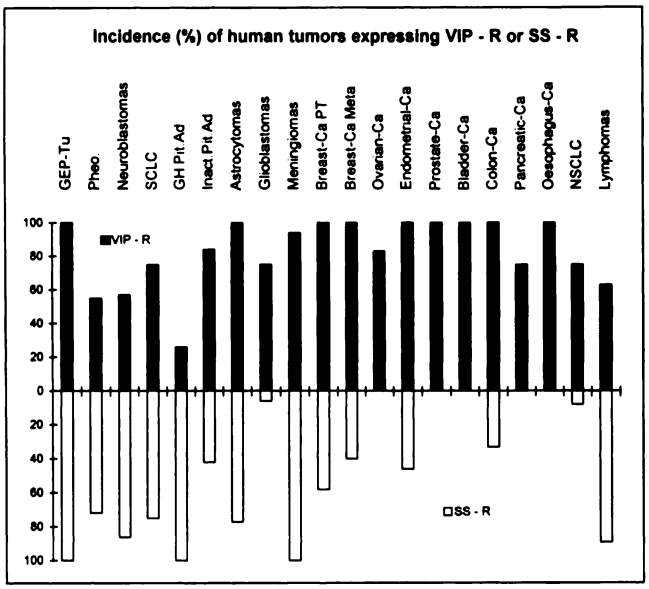

FIGURE 5. Incidence (%) of human tumors expressing VIP receptors (in comparison with somatostatin receptors (SS-R) identified in the same tumors). Adapted from Reubi (*53*). Pheo = pheochromocytomas; Pit.Ad = pituitary adenomas; PT = primary tumor; Meta = metastases.

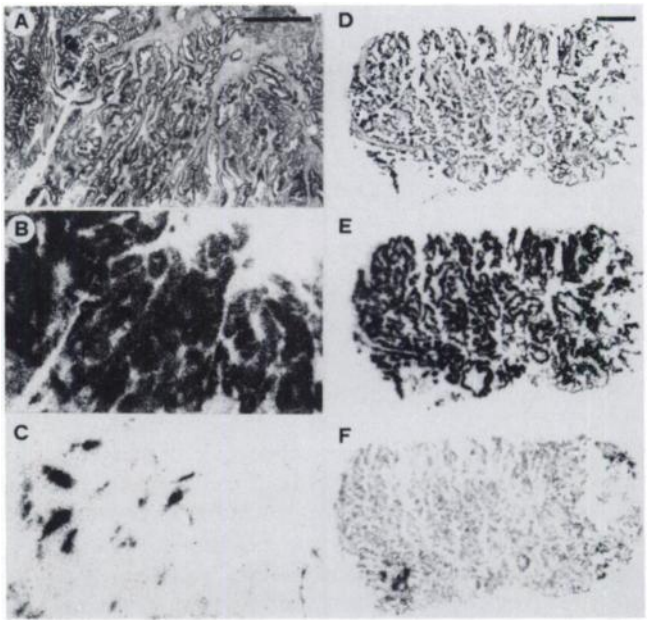

FIGURE 6. VIP receptors in colonic (A–C) and ovarian (D–F) carcinomas. (A,D) Hematoxylin-eosin-stained sections (bars = 1 mm). (B,E) Autoradiograms showing total binding of ^{125}I-VIP. Notice strong labeling of tumor tissue in both tumors. (C,F) Autoradiograms showing nonspecific binding of ^{125}I-VIP (in presence of 10^{-6}M VIP).

OTHER EMERGING NEUROPEPTIDES OF INTEREST FOR NUCLEAR MEDICINE

A major drawback of somatostatin receptor imaging is the fact that several categories of tumors, including frequently occurring and devastating tumors such as pancreatic carcinomas, are not visualized because they do not express somatostatin receptors (Fig. 5). Therefore, it has been of interest to search for alternative neuropeptide receptors expressed particularly in tumors that lack somatostatin receptors. Ideally, primary human tumors, rather than tumor cell lines, which often show altered biologic properties, therefore have to be screened for their receptor expression. With such strategies, a few neuropeptide receptors are emerging as potential tools for in vivo imaging.

Vasoactive Intestinal Peptide Receptors

Recent in vitro and in vivo data have suggested that the 28-amino acid long neuropeptide named vasoactive intestinal peptide (VIP) could represent a promising tumor imaging agent. First, with ^{123}I-VIP as the ligand, Virgolini et al. (*51*) were able to localize in vivo gastrointestinal neuroendocrine tumors and pancreatic and colonic adenocarcinomas. Second, a great number of human tumors have been shown in vitro to express VIP receptors. They were identified in homogenates of neuroendocrine tumors, breast cancer and intestinal adenocarcinomas (*52*). Furthermore, a recent in vitro receptor autoradiography study (*53*) evaluated the incidence of VIP receptor-positive tumors in a wide range of cancer types. With mono-^{125}I-[Tyr^{10}]-VIP as the radioligand, VIP receptors were characterized and localized on tissue sections in the neoplastic cells of most adenocarcinomas and squamous cell carcinomas. As seen in Figure 5, all breast carcinomas and their metastases, all prostate, bladder, endometrial, colonic and esophageal carcinomas, and astrocytomas expressed VIP receptors. Moreover, most ovarian, pancreatic, nSCLC and glioblastomas were VIP receptor positive. In all these cases, VIP receptors were found much more frequently than somatostatin receptors, which were measured simultaneously in adjacent sections of the same tumors (*53*). Among neuroendocrine tumors, the GEP tumors, pheochromocytomas, SCLC, neuroblastomas and inactive pituitary adenomas were often found to express VIP receptors. They were in general characterized by an extremely homogeneous distribution in the tumors. Figure 6 shows an example of a VIP receptor-positive colonic carcinoma and of an ovarian carcinoma. In all the tumors tested, the VIP receptors were of high affinity and specific for VIP. These in vitro results offer strong arguments in favor of a great potential for VIP receptor imaging in the diagnosis of tumors and metastases and represent a solid molecular basis for an extensive in vivo evaluation. However, it will be important to assess whether the normal, VIP receptor-expressing VIP targets (lung, lymph nodes and gut mucosa) will interfere in vivo with the signal given by the tumors. Previous experience with somatostatin receptor imaging is promising in this regard because it has revealed that the imaging signal from somatostatin receptor-positive normal tissues is, in general, weak compared with the signal from the tumors.

Substance P Receptors

Primary human neoplasms were also recently examined for the presence of substance P receptors by receptor autoradiography with [^{125}I]-labeled Bolton-Hunter substance P (*54*). Substance P receptors that belong to the neurokinin 1 (NK1) subtype were localized and characterized in the neoplastic cells of most tumors such as astrocytomas, glioblastomas, MTCs, breast carcinomas and ganglioneuroblastomas. Conversely, substance P receptors were not or only rarely identified on nSCLC of the lung, neuroblastomas, adenocarcinomas of the colon or the pancreas or malignant lymphomas. Interestingly, in most tumors investigated, substance P receptors were found on intra- and peritumoral blood vessels, even when the tumor itself did not express substance P

receptors. The substance P receptors detected in vessels had the pharmacologic characteristics of the NK1 receptor subtype.

The presence of tumoral and vascular substance P receptors as measured in vitro in tumors may have diagnostic implications. The use of radiolabeled substance P for in vivo scintigraphy of substance P receptor-positive tumors, e.g., glial tumors or MTC, may supplement the current set of diagnostic tools. Moreover, it will be of eminent interest to know, in analogy to the visualization of vascular somatostatin receptor in rheumatoid arthritis, whether a high density of substance P receptor-positive vessels, as found, for instance, in lymphomas, will also permit the visualization of these tumors, even if the tumor tissue itself does not express substance P receptors. Furthermore, it will be important to know if nontumoral diseases, characterized by a proliferation of substance P receptor-positive vessels, such as rheumatoid arthritis (*55*), will be visualized by in vivo substance P receptor scanning.

Alpha-Melanocyte-Stimulating Hormone Receptors

Recently, it has been found that melanomas express alpha-melanocyte-stimulating hormone (α-MSH) receptors (*56*). This has led to the design of chelating derivatives of α-MSH suitable for in vivo imaging (*57*). Successful visualization of tumors known to express α-MSH receptors have indicated the potential use of such compounds for the diagnosis of human tumors. However, the incidence and density of α-MSH receptors in human melanomas are not yet fully evaluated. Moreover, additional in vitro studies should investigate which other human tumors, and perhaps which nontumoral tissues, also express α-MSH receptors.

FUTURE APPLICATIONS

This review shows that the in vitro identification of neuropeptide receptors in various diseases, both historically and conceptually, plays a pivotal role for the development of suitable neuropeptide analogs as imaging agents and for the evaluation of the main indications in which these agents should be used. Further progress in the neuropeptide receptor imaging field will depend on three factors: (1) a precise description of the human pathologic states in which such neuropeptide receptors are expressed by the use of in vitro technologies, (2) the development of stable analogs of these neuropeptides with an adequate clearance from the circulation and (3) well-designed studies that directly compare in vivo with in vitro data to evaluate the sensitivity and specificity of the imaging method in the various pathologic conditions.

In the somatostatin receptor field, stable somatostatin analogs and extensive knowledge about the modalities of somatostatin receptor expression in vitro in a wide range of pathologic conditions were available at an early stage. They were the basis to test the in vivo feasibility rapidly and to develop the concept of in vivo somatostatin receptor imaging toward a standard method in nuclear medicine.

For the less advanced VIP receptor field, the general procedure to develop a simple and reliable in vivo VIP receptor imaging method may be copied from the somatostatin receptor example. A major advantage is the large number of tumors that express VIP receptors, which also appear to be adequately visualized in vivo. A search for the chemically optimal radioligand may be necessary because only an iodinated natural VIP has been used as an imaging agent; a natural peptide ligand is likely to be degraded more rapidly than stable synthetic analogs such as octreotide and may produce a high background and perhaps artifacts. More stable VIP analogs suitable for imaging, i.e., which retain high affinity for the VIP receptor, such as those recently designed and developed for the treatment of asthma (*58*), may therefore be required.

Along these lines, it can be foreseen that further neuropeptide receptors taken from the list in Table 1 may become target candidates for in vivo imaging in the near future. They may, ultimately, allow practitioners to reach what has recently been called one of the major goals of nuclear medicine, i.e., in vivo tissue characterization (*59*).

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