Somatostatin Receptors in the Diagnosis and Therapy of Neuroendocrine Tumors


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Abstract

The expression of somatostatin receptors in neuroendocrine tumors has facilitated the diagnosis and surgical treatment of patients with these tumors. After injection of a radiolabeled long-acting somatostatin analog, 111In-octreotide, scintigraphic tumor imaging can be performed as well as intraoperative tumor localization. During localization studies very high 111In concentration values were found in tumor tissues versus normal tissues, especially in carcinoid tumors and endocrine pancreatic tumors. Studies on such tumors in cell culture further indicated internalization of 111In into tumor cells, which is a prerequisite for a radiobiological effect from short range Auger and conversion electrons. Attempts to systemic radionuclide therapy via somatostatin receptors in patients with neuroendocrine tumors have been initiated.

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Introduction

The expression of somatostatin receptors (SSTRs) on neuroendocrine tumor cells opens new possibilities to diagnose and treat patients with these tumors. Long-acting somatostatin analogs are today clinically used to reduce excessive hormone production and accompanying symptoms. Receptor subtype-specific somatostatin analogs with antiproliferative effects may prove to be a new basis for individualized therapy. Radiolabeled somatostatin analogs can be used to localize neuroendocrine tumors scintigraphically with high specificity and sensitivity and can also be used for intraoperative tumor localization. Detailed studies using 111In-octreotide in patients have shown high uptake of the radionuclide by the tumors. Studies on cultured tumor cells have demonstrated internalization and long retention of 111In. These findings are prerequisites for adequate radiation therapy with 111In via SSTRs after careful characterization of the individual tumor. It is possible that these diagnostic and therapeutic principles can be applied for other types of tumors expressing SSTRs, e.g., breast cancer and prostatic carcinoma. Long-acting ligands to SSTRs have rapidly been developed, and in the future similar principles may also be employed for other types of peptide receptors expressed by tumor cells, e.g., ligands to growth factor receptors.

Somatostatin and Its Receptors

In 1968, Kruhlich and collaborators identified a hypothalamic substance which suppressed the secretion of growth hormone [1]. The following year, Hellman and Lernmark found a factor in pancreatic islets which also reduced the secretion of insulin [2]. The suppression of both growth hormone and insulin secretion was later shown to be mediated by a peptide hormone with general inhibitory effects on the secretion of hormones. As the inhibitory effect on the secretion of growth hormone was first described, the peptide was named somatostatin (SS) [3] or somatotropin release-inhibiting factor (SRIF).

Somatostatin is present in several organ systems and has an important function in the regulation of both endocrine and exocrine secretion [4-6]. In the gastrointestinal tract, somatostatin is localized to endocrine D cells and certain neurons. In man, the gene is located on chromosome 3, coding for preprosomatostatin [6]. Preprosomatostatin is converted to the precursor prosomatostatin, which includes both SS 14 (14 amino acids) and the N-terminally extended form of somatostatin, SS 28. Both SS 14 and SS 28 have biological activity, and they have a tissue-specific distribution with a relative dominance of SS 14 in the pancreas and stomach and of SS 28 in the intestine [6]. The therapeutic value of somatostatin treatment was limited due to the short biological half-life of the peptide (<3 min) [7]. This fact stimulated the
development of more stable analogs. The clinically most-used analog is octreotide, an octapeptide (8 amino acids) with a biological half-life of about two hours [8].

The effects of somatostatin are mediated via specific cell membrane-bound high-affinity receptors located on target cells. Five subtypes of SSTRs, SSTRs 1-5, have been cloned, and they belong to a distinct group within the superfamily of G-protein-coupled receptors with seven transmembrane regions. The genes for SSTRs 1-5 are located on different chromosomes and are lacking introns in their coding regions [9]. For SSTR 2, alternative splicing will generate two isoforms of the receptor. About 50% of the amino acids are identical for the different receptor subtypes, and the homology is most pronounced in the transmembrane regions. Of practical importance is the division of the five receptor subtypes into two groups, where SSTRs 2, 3, and 5 differ from SSTRs 1 and 4 regarding amino acid homology and pharmacological profile. Short somatostatin analogs bind to the first group of receptor subtypes. Octreotide has its highest affinity to SSTR 2 and lower affinity to SSTRs 3 and 5, while SS 14 and SS 28 bind to all subtypes with high affinity [9].

**Somatostatin Analogs in the Treatment of Neuroendocrine Tumors**

Almost 10 years ago, octreotide was introduced as medical therapy for hormone-producing neuroendocrine tumors [10]. Somatostatin analogs reduce excessive secretion of hormones from carcinoid tumors and certain types of endocrine pancreatic tumors (glucagonoma, VIPoma and gastrinoma), which leads to marked symptom palliation. They also have a major role in the prophylaxis against life-threatening hormonal crises, e.g., the carcinoid crisis. Antiproliferative effects have been reported in individual patients. It is likely that the antiserotory and antiproliferative profiles vary among somatostatin analogs due to their different affinity for SSTR subtypes. In studies of human carcinoid tumors in primary culture, octreotide was shown to have a direct effect on the tumor cells, causing rapid suppression of the secretion of both amines (serotonin) and peptides (tachykinins). A gradual inhibition of the synthesis of serotonin could also be demonstrated. In such short-term studies, no antiproliferative effect of octreotide could be demonstrated, even though this has been demonstrated for various endocrine tumor cell lines in vitro [11]. In studies of tumor cells transfected with SSTR 2, the antiproliferative effect seemed to be independent of adenyl cyclase, but dependent on tyrosine phosphatase [12].

**Somatostatin Receptors in the Diagnosis of Neuroendocrine Tumors**

Autoradiographic studies of tumor biopsies and binding studies of tumor cell membranes have verified the presence of SSTRs in several types of neuroendocrine tumors [13]. These findings have been employed clinically for scintigraphic tumor localization after injection of radiolabeled octreotide. The first radiopharmaceutical used was an iodinated modified octreotide molecule (substitution with tyrosine versus phenylalanine in position 3, i.e., $^{123}$I-Tyr$^3$-octreotide) [14]. This construction had limited clinical use for several reasons; e.g., the binding of the radionuclide modifies the receptor-binding part of the molecule and the rapid biliary excretion necessitates abdominal imaging shortly after injection. Today, $^{111}$In-labeled octreotide is clinically used where $^{111}$In is indirectly bound via DTPA (diethylentriamine-penta-acetic acid) to the N-terminally located phenylalanine of the octreotide molecule ($^{111}$In-DTPA-D-Phe$^1$-octreotide) [15]. Scintigraphy with $^{111}$In-DTPA-D-Phe$^1$-octreotide was first evaluated in pioneering work by Krenning and Lamberts in Rotterdam, and later documented in a European multicenter study. The scintigraphic technique has high sensitivity (80%-95%) for carcinoid tumors and endocrine pancreatic tumors, except for insulinomas, which are only detected in about half of the patients [16].

In our own investigation of a well-characterized group of 27 patients with disseminated midgut carcinoid tumors, octreotide scintigraphy, including tomography (SPECT), had higher sensitivity and specificity than the combination of ultrasound and CT [17]. The strength of the method was that it could reveal tumors in patients who were considered to be in complete remission after surgery (normal tumor markers and normal radiological findings). In a few patients with hepatic metastases, clinically silent metastases in other regions were demonstrated, predominantly in the neck and mediastinum. The clinical management was influenced by the scintigraphic findings in one-third of the patients. These patients underwent further surgical treatment to reach a stage of tumor remission or to remove metastases with unfavorable localization (infiltration of the recurrent vagal nerve or large vessels). By using a single injection of the radiopharmaceutical prior to surgery, it was possible to perform preoperative and postoperative scintigraphic imaging, thereby documenting the completeness of the surgical intervention [17].

In several clinical series, about half of the patients with medullary thyroid carcinoma (MTC) had scintigraphically visualized tumors [16, 18, 19]. A positive scintigraphic finding was earlier considered to correlate with favorable prognosis. Dedifferentiation of the tumor was supposed to reduce the expression of SSTRs by the tumor [20]. We have evaluated 22 patients with MTC with biochemical signs of recurrent disease (elevated serum calcitonin levels) after total thyroidectomy [18]. Eleven patients with scintigraphically positive metastases had considerably higher calcitonin concentrations (indicating larger tumor burden) than those with negative scintigraphy. It is of clinical interest to note that the patients...
Somatostatin Receptors and Neuroendocrine Tumors

with receptor-positive tumors had a significantly higher annual increment of their calcitonin levels. These tumors also had higher proliferation indices (studied histopathologically with nuclear proliferation antigens). Therefore, these findings indicate that a positive octreotide scintigraphy is correlated with aggressive tumor growth and worse prognosis. For these patients, surgical treatment has been the only treatment alternative. However, these data may indicate that aggressive tumors which are difficult to cure by surgery may be suited for SSTR-mediated radionuclide therapy following the surgical treatment (completion radiotherapy).

**INTRAOPERATIVE LOCALIZATION OF NEUROENDOCRINE TUMORS WITH A SCINTILLATION DETECTOR**

Realizing the high diagnostic sensitivity and specificity of octreotide scintigraphy, it was logical to bring this technique to the surgical theater in order to localize neuroendocrine tumors intraoperatively. This has been done according to a standardized protocol after injection of $^{111}$In-octreotide in 23 patients with carcinoid tumors, endocrine pancreatic tumors, or MTC [21]. The uptake of radionuclide in suspect tumor lesions was investigated by a hand-held scintillation detector (probe) both in the surgical field in situ and after excision ex vivo. The ratio ($R_{\text{in situ}}$ and $R_{\text{ex vivo}}$) between the signal from the tumor-suspect lesion and the signal from adjacent normal tissue was calculated and evaluated statistically (Fig. 1). Before histopathological analysis, the $^{111}$In concentration in all removed tissue was measured in a gamma-counter and related to the corresponding levels in blood sampled during surgery for calculation of a tissue-to-blood $^{111}$In concentration ratio (Ti/B). The evaluation of the results showed that the probe measurements gave erroneous results (both false positive and false negative results) in 12% of in situ measurements versus 7% of ex vivo measurements. It was not possible in any case to detect microscopic tumor growth in lymph node metastases or in microadenomas in the pancreas with the present technique. The most reliable measurements were seen in regions with low $^{111}$In background (i.e., pelvis, mediastinum, and neck). The accuracy decreased close to parenchymatous organs with high background $^{111}$In concentration (liver, spleen, and kidneys). The tumor/blood ratios (T/B) were very high for some endocrine pancreatic tumors (910-1,500), high for carcinoids (27-650) and lower for MTC tumors (3-86) (Table 1). For all different tumor types, T/B values seemed to be independent of the time interval between injection and measurement [22]. The high T/B values contrasted with the low ratios, which were seen intraoperatively using the probe (Fig. 1). To improve the results of intraoperative examination, the sensitivity of the detector system should be increased. A radionuclide with lower photon energy than $^{111}$In, for instance $^{125}$I or $^{161}$Tb, would give a lower signal from the background. Moreover, the accuracy would be improved so that smaller tumors might be detected. After modification, this technique could be developed to a valuable adjunct in the surgery of small endocrine pancreatic tumors, recurrent MTC (surrounded by scar tissue), and for evaluation of the completeness of tumor removal, e.g., carcinoid lymph node metastases in the mesenteric root.

**DETERMINATION OF RECEPTOR SUBTYPES IN NEUROENDOCRINE TUMORS**

The binding of somatostatin analogs to tumor cells is dependent on the expression of specific receptor subtypes by the individual tumor as well as on the affinity of...
Table 1. Tumor-to-blood $^{111}$In concentration ratios (T/B) for 28 patients after various time intervals after i.v. injection of $^{111}$In-octreotide. The carcinoid tumors were of midgut type, except (*) indicating a foregut type.

<table>
<thead>
<tr>
<th>Time after injection (d)</th>
<th>Carcinoid tumor</th>
<th>Medullary thyroid cancer (MTC)</th>
<th>Other neuroendocrine tumors</th>
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<tr>
<td>1 d</td>
<td>100-170</td>
<td>2-39</td>
<td>110-120</td>
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<tr>
<td></td>
<td>($n = 6$)</td>
<td>($n = 9$)</td>
<td>($n = 2$)</td>
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<tr>
<td>10$^2$-200</td>
<td>32-210</td>
<td>2$^2$-41</td>
<td>1,500</td>
</tr>
<tr>
<td>($n = 5$)</td>
<td>($n = 8$)$^a$</td>
<td>($n = 10$)</td>
<td>($n = 1$)$^b$</td>
</tr>
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<td>2 d</td>
<td>51-220</td>
<td>4-33</td>
<td>6</td>
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<tr>
<td></td>
<td>($n = 3$)</td>
<td>($n = 6$)</td>
<td>($n = 1$)$^b$</td>
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<td></td>
<td>17-47</td>
<td>($n = 5$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>650-910</td>
<td>($n = 2$)$^b$</td>
</tr>
<tr>
<td>3 d</td>
<td>100</td>
<td>9</td>
<td>4-13</td>
</tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>3$^2$-240</td>
<td>($n = 9$)</td>
</tr>
<tr>
<td>5 d</td>
<td>46-270</td>
<td>3-36</td>
<td>6-14</td>
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<td></td>
<td>($n = 4$)</td>
<td>($n = 5$)</td>
<td>($n = 7$)</td>
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<td></td>
<td></td>
<td>27-34</td>
<td>($n = 2$)</td>
</tr>
<tr>
<td>6 d</td>
<td>150-650</td>
<td>3$^2$-360</td>
<td>21-32</td>
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<td></td>
<td>($n = 4$)</td>
<td>($n = 5$)</td>
<td>($n = 16$)</td>
</tr>
<tr>
<td>7 d</td>
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<td>6-14</td>
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<td>($n = 7$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27-34</td>
<td>($n = 2$)</td>
</tr>
</tbody>
</table>

$^1$micrometric tumor growth
$^2$neuroendocrine carcinoma
$^3$endocrine pancreatic tumor
$n$ = number of examined tumor biopsies

the analog to the respective receptor subtype. Northern analysis and reverse transcriptase polymerase chain reaction (RT-PCR) can be used to determine the expression of receptor subtypes in fresh tumor biopsies. By Northern analysis, we could demonstrate that all five SSTRs were frequently expressed in midgut carcinoid tumors [23] (Fig. 2). However, in similar studies on MTC tumors, it was evident that scintigraphic visualization of tumors was possible even in the absence of SSTR 2. Also, other types of thyroid tumors (papillary cancer and Hurthle cell adenoma) were shown to express multiple SSTR-subtypes [24]. In one study of non-medullary thyroid cancers, octreotide scintigraphy visualized specific uptakes in all patients with primary tumors and in 75% of the metastatic lesions [25]. The examination was complementary to radioiodine scintigraphy in the detection of distant metastases. After withdrawal of thyroxine in preparation for radioiodine scintigraphy, the uptake of $^{111}$In-octreotide appeared to be increased, indicating upregulation of SSTRs by thyrotropin. Intense interest to develop receptor subtype-specific ligands is shown by the pharmaceutical industry. With detailed knowledge about the receptor profile of a given tumor, there will be future opportunities to use tailor-made analogs for therapeutic purposes.

**Somatostatin Receptor Manipulation**

Most of the information on the expression of SSTRs under various hormonal influences has been obtained from rat pituitary cell lines. Incubation of such cell lines with somatostatinins (SS14 and SS28) or analogs showed that the SSTR expression could be manipulated with a cell-type-specific responsiveness [26]. Addition of glucocorticoids decreased the SSTR expression, while estrogens caused an increase. Short-term treatment with thyrotropin increased SSTR expression in contrast to chronic treatment, which decreased the receptor expression. Rat prolactinoma cells do not express SSTR in vivo, a finding similar to the human prolactinoma. However, if rat prolactinoma cells were incubated with estrogen, expression of SSTRs 2 and 3 was induced. Also, administration of estrogen to tumor-transplanted rats induced expression of SSTR 2 in tumor transplants [26]. One plausible explanation is that the high levels of prolactin in tumor-bearing rats suppress estrogen production in the ovaries and thus also the SSTR expression in the tumor. First after addition of estrogen, these tumors would be targeted for imaging studies.

**Prerequisites for Somatostatin Receptor-Mediated Radiation Therapy**

Radiopharmaceuticals are used for treatment of a few malignant diseases, e.g., $^{131}$I for thyroid carcinoma. A prerequisite for therapy is that the absorbed dose to tumor tissue is considerably higher than the absorbed dose to normal tissue, either due to high uptake or to long biological half-life in the tumor tissue. During decay, a radionuclide emits different types of radiation, i.e., electromagnetic radiation (photons) and particles (e.g., electrons). Photons with appropriate energy (70-400 keV) can be used for scintigraphy, as photons can pass through the body and be detected by a gamma camera. Electrons can be used for therapy and are completely stopped in tissue within their ranges (about 5 nm-1 cm), and during retardation the electron transfers its energy to the tissue. Absorbed dose means absorbed energy per mass unit. The risk, or, in case of therapy, the possibility, for tissue damage is proportional to the absorbed dose. During certain types of radioactive decay, Auger and conversion electrons are emitted. These electrons may have very low energy, but since the range is short, their energy is absorbed within a small volume. The absorbed dose can, therefore, be locally very high. After uptake into the tumor cells (internalization), a decay at a position close to the DNA may have potent cytotoxic effects. The therapeutic potential for a radionuclide is determined by: A) the fraction of energy emitted as electrons and other charged particles; B) the range of the electrons versus the subcellular distribution of the radionuclide in both tumor and normal tissue, and C) the half-life of the radionuclide versus pharmacokinetic properties in tumor and normal tissue.
The biokinetics of $^{111}$In have been followed in 13 patients during the first 72 h after i.v. injection of $^{111}$In-octreotide. Whole-body scintigraphy visualized the liver, spleen, kidneys, and the urinary bladder. Absorbed doses to various organs and tissues were estimated. The $^{111}$In content in the visualized organs varied by a factor of 5-6 among the patients. Similar interindividual variation was seen for the absorbed dose to the organs, which underlines the importance of individual dose-planning (Table 2) [27]. Radiation from $^{111}$In consists mainly of photons, and Auger and conversion electrons with very short ranges. For therapy via these electrons, a specific receptor binding of octreotide is required as well as internalization of $^{111}$In into tumor cells. The cellular handling of the SSTR-ligand complex is not well-characterized, but for other G-protein-coupled receptors, a receptor-mediated endocytosis has been demonstrated. Internalization of somatostatin has been described for mammalian endocrine cell lines [28]. We have studied human neuroendocrine cells in vitro to try to corroborate and visualize the internalization of $^{111}$In [29]. Figure 3 shows an experiment on midgut carcinoid tumor cells in primary culture from a patient with high T/B values (150-650). During continuous incubation with $^{111}$In-octreotide, there was a steady increase of specific binding and uptake of $^{111}$In over time. After pulse incubation for 1 h with $^{111}$In-octreotide, followed by incubation with standard medium, there was a redistribution of the cell-bound $^{111}$In. The amount of internalized $^{111}$In was diminished over the subsequent 6 h to about 50%, and the amount of $^{111}$In released to the medium increased correspondingly. The incubation was continued over 40 h with no further release of $^{111}$In. The subcellular distribution of $^{111}$In in tumor cells was analyzed by electronmicroscopic autoradiography. Part of the $^{111}$In was distributed over the nucleus, but the majority over the cytoplasm (Fig. 3).

**Radiation Therapy with $^{111}$In-Octreotide**

Krenning and collaborators have reported SSTR-mediated radionuclide therapy in six patients with advanced neuroendocrine tumors and disseminated disease [30]. The detailed knowledge obtained during perioperative localization and scintigraphic studies gave us a good background to consider SSTR-mediated radiotherapy in individual cases. We have treated one patient with an advanced midgut carcinoid syndrome with very high levels of 5-HIAA in urine (680 µmol/24 h) and chromogranin A in plasma (1500 IU/l) [31]. Tumor dissemination to the mesentery, retroperitoneum, liver, mediastinum, and skeleton made surgical therapy impossible. Individual dose-planning prior to therapy and subsequent estimation of absorbed doses was performed for

<table>
<thead>
<tr>
<th>Organ</th>
<th>Absorbed dose (mGy/MBq)</th>
<th>mean</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenals</td>
<td>0.047</td>
<td>(0.030-0.062)</td>
<td></td>
</tr>
<tr>
<td>Gallbladder wall</td>
<td>0.047</td>
<td>(0.029-0.067)</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.23</td>
<td>(0.13-0.45)</td>
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</tr>
<tr>
<td>Liver</td>
<td>0.094</td>
<td>(0.051-0.17)</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>0.020</td>
<td>(0.016-0.036)</td>
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<tr>
<td>Pancreas</td>
<td>0.054</td>
<td>(0.040-0.070)</td>
<td></td>
</tr>
<tr>
<td>Red marrow</td>
<td>0.030</td>
<td>(0.015-0.032)</td>
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<tr>
<td>Spleen</td>
<td>0.032</td>
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<tr>
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<td>(0.011-0.024)</td>
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<tr>
<td>Urinary bladder wall</td>
<td>0.082</td>
<td>(0.042-0.22)</td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>0.024</td>
<td>(0.016-0.035)</td>
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</tbody>
</table>
each therapy. The pharmacokinetics of $^{111}$In were studied by whole-body scintigraphy, allowing an estimation of absorbed dose to critical organs. Four therapies with $^{111}$In-octreotide (12.4 GBq total), corresponding to 50-100 times the diagnostic amounts, were given with one- to five-month intervals. The patient had marked symptom palliation, and the levels of the tumor markers were reduced to less than half. Repeat CT examinations one year following the first therapy showed stationary tumor status [31]. We noticed that the $^{111}$In uptake both in tumor and normal tissues was reduced after single injections with therapeutic amounts of $^{111}$In-octreotide. After fractionated administration (1.3 + 1.4 GBq), the kinetics were similar to that seen after diagnostic amounts [32].

**Future Development of Radionuclide Therapy**

$^{111}$In is not optimal for radiation therapy due to the high amount of energy emitted as photons, so other radionuclides have been discussed but not yet used in clinical trials. $^{131}$I has better radiation properties for therapy than $^{111}$In but is usually bound to the receptor-binding site of the octreotide molecule, which may cause reduced affinity to SSTRs. The production of $^{131}$I-Tyr$^3$-octreotide with the high specific activity needed for therapy is still a problem [33]. Several radioactive metal ions have also been suggested, e.g., $^{67}$Ga, $^{90}$Y, $^{153}$Sm, $^{161}$Tb, $^{177}$Lu, and $^{186}$Re, and methods for labeling octreotide with such radionuclides have been proposed [33-38]. Considerable work is still needed to accomplish a radiolabeled octreotide molecule for radiation therapy with suitable properties, in vivo stability, and pharmacokinetics.

**Antiproliferative Effects of Somatostatin Receptor Activation**

Over the last years, interest has been focused on the antiproliferative effects of somatostatin and its analogs on tumor cells and the possible use of these analogs in cancer treatment. Experimental studies on SSTR-expressing cell lines from

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**Figure 3.**

A) Human midgut carcinoid tumor cells were incubated for 1 h at 37°C with $^{111}$In-octreotide (10nM). The cells were washed and further incubated at 37°C in cell medium without octreotide. The $^{111}$In contents of the media were measured over 48 h. B) Tumor cells from the above-described experiment were studied by electron microscope autoradiography. Two silver grains (arrows) were located over the cytoplasm of the tumor cell. The cellular uptake was low, but highly specific; cells incubated with excess of unlabeled octreotide showed no cellular uptake of $^{111}$In. n = nucleus, bar = 1 µm. (Used with permission [29]). C) A putative model for internalization of peptide hormones. After binding to cell surface receptors, ligand-receptor complexes aggregate in coated pits, which are internalized as coated vesicles and transported via endosomes to lysosomes. The complex can be dissociated and the receptors recycled to the plasma membrane, while the ligand is degraded (route 1). Degradation without recycling of receptors may also occur (route 2).
rodent and man have demonstrated a direct inhibitory effect of somatostatin analogs on tumor cell growth. The rat pancreatic cell line (AR42J), the mouse pituitary cell line (AtT-20), and the rat pituitary cell line (7315b) were all shown to be inhibited by somatostatin analogs, as were certain human breast cancer cell lines [39-43]. The effect on human thyroid cell lines was variable with inhibitory effects observed only in differentiated cell lines [44]. In cell lines derived from human acute lymphoblastic and myeloid leukemias, somatostatin analogs were shown to have an antiproliferative effect in about one-third of the cases [45]. There are multiple mechanisms by which somatostatin and its analogs may exert their antiproliferative actions. A direct effect on SSTR-expressing cells has been demonstrated, e.g., in HeLa cells and gerbil fibroma cells where somatostatin was shown to inhibit the mitogenic effect of epidermal growth factors on tumor cells [46]. However, in mouse pituitary cell line (AtT-20), octreotide was shown to induce apoptosis [42]. Another direct effect of somatostatin analogs has been demonstrated in rat pancreatic cells (AR42J), in which octreotide was shown to potentiate the antiproliferative effects of mitomycin C, doxorubicin, taxol, and 5-fluorouracil [43]. It has not yet been determined by which SSTR subtypes the antiproliferative effects are mediated. Suggested candidates are SSTR 1, SSTR 2, and SSTR 5, which are thought to activate tyrosine phosphatases [47, 48]. An indirect effect of somatostatin and its analogs on tumor cell growth has also been demonstrated. This effect is not dependent on SSTR expression by tumor cells but is the result of an inhibition of circulating growth factors, which in turn stimulate tumor cell growth or neovascularization. Inhibition of circulating IGF-1 has thus been suggested to be one mechanism by which octreotide may inhibit growth of breast cancer cells and chondrosarcomas [49, 50]. In the hypergastrinemia-induced gastric carcinoid model, it has been suggested that octreotide inhibits the development of carcinoid tumors by lowering circulating gastrin levels [51]. While there is substantial experimental evidence that somatostatin and its analogs may inhibit tumor growth, there is yet limited clinical evidence for an antitumor effect of these compounds. A majority of gastrointestinal endocrine tumors express a high number of SSTRs. Patients with these tumors have been treated with somatostatin analogs for long periods, and retrospective studies have indicated an antiproliferative effect in individual cases [10]. In one prospective study on metastasized endocrine gastrointestinal tumors, an antiproliferative effect was demonstrated in a minority of patients [52].

**Other Tumor Types**

Somatostatin receptors are expressed in two-thirds of breast cancers, which suggests that somatostatin analogs may be of value in the treatment of these tumors. Experimental studies on mammary tumor cell lines have shown that octreotide alone inhibits tumor cell growth and that octreotide potentiates the effect of anti-estrogen treatment (tamoxifen) [50]. Ongoing studies on octreotide in combination with tamoxifen to breast cancer patients will determine the role of somatostatin analogs as adjuvant treatment of breast cancer.

In a series of breast cancer biopsies, SSTR expression was detected by autoradiography in two-thirds of cases [53]. In 41 tumors, the expression was homogeneous, and in 17 it was heterogeneous. In a minority of tumors with SSTR expression, two or more cytotoxic markers of neuroendocrine differentiation were demonstrated. No correlation was seen between the expression of SSTR and alterations in the retinoblastoma gene or specific oncogene amplification (neu, c-myc, L-myc and int-2). When 111In-octreotide was used, 39 out of 52 primary breast cancers were visualized scintigraphically in one clinical study [54]. Significantly more invasive ductal carcinomas were visualized than invasive lobular carcinomas, and more T2 tumors were visualized than T1 tumors. Special images of the axillae could reveal nonpalpable lymph node metastases in one-third of the patients.

To distinguish between exocrine and endocrine pancreatic tumors can be difficult but of crucial importance for proper diagnosis and adequate treatment. The high expression of SSTRs on neuroendocrine cells may be helpful to increase the diagnostic accuracy by the use of octreotide scintigraphy and in vitro analyses of SSTR expression. In a study of 62 patients with an initial diagnosis of pancreatic ductal cancer, 12 patients were alive three years after subtotal or total pancreatectomy [55]. Five of these patients had metastatic disease. Octreotide scintigraphy was positive in these five patients at follow-up. Re-evaluation of the tumor biopsies with specific neuroendocrine markers revealed that all patients had nonfunctioning neuroendocrine islet cell tumors.

In prostatic cancer, a significant degree of neuroendocrine differentiation is often observed [56]. In experimental prostatic cancer, somatostatin analogs have a therapeutic value as adjuncts to LH-releasing hormone agonists [57]. In a study of patients with skeletal metastases of hormone-refractory prostatic carcinoma, 30/31 patients had at least one metastasis demonstrated by octreotide scintigraphy [58]. Of all the lesions detected at bone scintigraphy, 37% showed uptake of 111In-octreotide. This indicates a heterogeneity among the metastatic lesions of prostatic cancer, but still somatostatin analogs may be an option in the treatment of hormone-refractory tumors.

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