Somatostatin receptors in gastroenteropancreatic neuroendocrine tumours

W W de Herder, L J Hofland, A J van der Lely and S W J Lamberts

Department of Internal Medicine, Section of Endocrinology, Erasmus MC, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands

(Requests for offprints should be addressed to W W de Herder; Email: w.w.deherder@erasmusmc.nl)

Abstract

Five somatostatin receptor (sst) subtype genes, sst₁, sst₂, sst₃, sst₄, and sst₅, have been cloned and characterised. The five sst subtypes all bind natural somatostatin-14 and somatostatin-28 with high affinity. Endocrine pancreatic and endocrine digestive tract tumours also express multiple sst subtypes, but sst₂ predominance is generally found. However, there is considerable variation in sst subtype expression between the different tumour types and among tumours of the same type. The predominant expression of sst₂ receptors on pancreatic endocrine or carcinoid tumours is essential for the control of hormonal hypersecretion by the octapeptide somatostatin analogues such as octreotide and lanreotide. Somatostatin and its octapeptide analogues are also able to inhibit proliferation of normal and tumour cells. The high density of sst₂ or sst₅ on pancreatic endocrine or carcinoid tumours further allows the use of radiolabelled somatostatin analogues for in vivo visualisation.

The predominant expression of sst₂ receptors in these tumours and the efficiency of sst₂ receptors to undergo agonist-induced internalisation is also essential for the application of radiolabelled octapeptide somatostatin analogues. Currently, [¹¹¹In-DTPA]octreotide, [²⁵²Y-DOTA⁰,Tyr³]octreotide, [¹⁷⁷Lu-DOTA³Tyr³]octreotate, [¹¹¹In-DOTA⁰]lanreotide and [³⁵⁵Y-DOTA⁰]lanreotide can be used for this purpose.

Somatostatin

Somatostatin is a small cyclic peptide. It circulates in the blood in two biologically active forms: somatostatin-14, consisting of 14 amino acids and somatostatin-28, consisting of 28 amino acids (Reichlin 1983a,b). Somatostatin is formed by proteolytic processing of larger precursor molecules: preprosomatostatin and pro-somatostatin. This peptide was detected accidentally during studies of the distribution of growth hormone-releasing factor in the hypothalamus of rats (Kruilich et al. 1968, Brazeau et al. 1973). Somatostatin inhibits a variety of physiological functions in the gastrointestinal tract, such as gastrointestinal motility, gastric acid production, pancreatic enzyme secretion, bile secretion and colonic fluid secretion. It also inhibits the secretion of pancreatic and intestinal hormones such as insulin, glucagon, secretin and vasoactive intestinal polypeptide. In addition to playing an important regulatory role in neurotransmission and secretion, the peptide may control cell proliferation in normal tissues and tumours (Reichlin 1983a,b, Schally 1988, Lamberts et al. 1991). In view of the ability of somatostatin to inhibit such a variety of physiological processes, it was predicted that this peptide might be of therapeutic value in clinical conditions involving hyperfunction or hypersecretion of the organ systems mentioned above. However, the multiple simultaneous effects of pharmacological concentrations of somatostatin in different organs, the need for intravenous administration, the short duration of action (a half-life in the circulation of less than 3 min) and the post-infusion rebound hypersecretion of hormones considerably hampered the initial enthusiasm, as well as its clinical use (Lamberts et al. 1996).

Somatostatin receptor subtypes

Somatostatin-14 and somatostatin-28 act through high-affinity G protein-coupled membrane receptors. Five somatostatin receptor (sst) subtype genes have been cloned and characterised. They were code-named sst₁, sst₂, sst₃, sst₄, and sst₅ (Hoyer et al. 1994). The genes encoding the five sst subtypes are localised on different chromosomes (Patel 1997). Two forms of the sst₂ receptor (sst₂A and sst₂B) can be generated through alternative splicing (Vanetti et al. 1992, Patel et al. 1993). Upon binding of somatostatin to its receptor subtype(s) second messenger systems will become activated.
These systems include (1) inhibition of adenylate cyclase activity and (2) activity of calcium channels, as well as (3) stimulation of phosphotyrosine phosphatase or (4) MAP (mitogen-activated protein) kinase activity (Reisine & Bell 1995, Patel 1997, 1999). The inhibitory effects of somatostatin on adenylate cyclase activity and on the influx of calcium are linked to inhibition of secretion processes. The activation of phosphotyrosine phosphatase or MAP kinase activity by somatostatin may play a role in the regulation of cell proliferation (Schally 1988, Lamberts et al. 1991, Hofland et al. 1995).

Classical somatostatin-target tissues such as the central nervous system, the anterior pituitary gland and the pancreas express multiple sst subtypes. Pancreatic islet cells express all five sst subtype proteins (Reubi et al. 1998b, Kumar et al. 1999). In these cells, sst1, sst3 and sst5 receptors are the most abundantly expressed subtypes, with a high percentage of β-cells expressing sst1, and sst2, α-cells expressing sst2, and δ-cells expressing sst5 (Kumar et al. 1999).

Tumours arising from somatostatin-target tissues frequently express a high density of sst receptors (Reubi et al. 1992a,b, 1994, 1996, 2001). The sst-expressing tumours include pituitary adenomas, pancreatic endocrine tumours, carcinoids, paragangliomas, pheochromocytomas, small cell lung cancers, medullary thyroid carcinomas, breast cancers and malignant lymphomas (Reubi et al. 1992b, Vikic-Topic et al. 1995). The sst subtype expression in different tumours has been demonstrated at the mRNA level using in situ hybridisation, RNase protection assays and RT-PCR (Kubota et al. 1994, Panetta & Patel 1995). The majority of sst-positive tumours simultaneously express multiple sst subtypes, although there is a considerable variation in sst subtype expression between the different tumour types and among tumours of the same type (Reubi et al. 1998a, Schulz et al. 1998, Kimura et al. 1999, Hofland et al. 1999b). Endocrine pancreatic and endocrine digestive tract tumours can also express multiple sst subtypes, but sst5 predominance is generally found in more than 80% (Reubi et al. 1994, 2001, de Herder et al. 1996a, Papotti et al. 2002, Reubi & Waser 2003).

The five sst subtypes all bind somatostatin-14 and somatostatin-28 with high affinity. The sst2, and sst5 receptors do not bind the currently available octapeptide somatostatin analogues octreotide and lanreotide (see later), whereas sst1, sst3, and sst4 receptors display a high, low, and moderate affinity respectively towards these octapeptide somatostatin analogues (Table 1). The predominant expression of sst2 receptors on pancreatic endocrine or carcinoid tumours forms the basis for the successful clinical application of octapeptide somatostatin analogues such as octreotide and lanreotide in controlling symptoms related to hormonal hypersecretion (Lamberts et al. 1996, de Herder et al. 1996b, de Herder & Lamberts 2002). The high density of sst subtypes on these tumours further allows the use of radiolabelled somatostatin analogues to visualise sst-positive tumours in vivo (see later) (Krenning et al. 1982, 1993, 1994a,b, 1999, Kwékkeboom et al. 1993, Kwékkeboom & Krenning 1996). Therefore, knowledge of the sst subtype expression patterns in endocrine tumours may be very important for the development of the concept of sst-targeted radiotherapy or chemotherapy (see later).

Also, ssts may form homo- or heterodimers or may heterodimerise with other G protein-coupled receptors such as the dopamine D2 receptor or the µ-opioid receptor (MOR-1), resulting in a novel receptor state with properties different from the individual receptors (Rocheville et al. 2000a,b, Pfeiffer et al. 2001, 2002).

### Table 1 Binding affinities of somatostatin analogues to the five sst subtypes (Bruns et al. 2002). Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; value (nM)</th>
<th>sst&lt;sub&gt;1&lt;/sub&gt;</th>
<th>sst&lt;sub&gt;2&lt;/sub&gt;</th>
<th>sst&lt;sub&gt;3&lt;/sub&gt;</th>
<th>sst&lt;sub&gt;4&lt;/sub&gt;</th>
<th>sst&lt;sub&gt;5&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatostatin 14</td>
<td>0.93 ± 0.12</td>
<td>0.15 ± 0.02</td>
<td>0.56 ± 0.17</td>
<td>1.5 ± 0.4</td>
<td>0.29 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Lanreotide</td>
<td>180 ± 20</td>
<td>0.54 ± 0.08</td>
<td>14 ± 9</td>
<td>230 ± 40</td>
<td>17 ± 5</td>
<td></td>
</tr>
<tr>
<td>Octreotide</td>
<td>280 ± 80</td>
<td>0.38 ± 0.08</td>
<td>7.1 ± 1.4</td>
<td>&gt;1000</td>
<td>6.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Chromosomal location</td>
<td>14</td>
<td>17</td>
<td>22</td>
<td>20</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Somatostatin analogues

As mentioned above, there are several limitations to the use of native somatostatin-14 and -28 in daily practice. Therefore, attempts have been made to synthesise somatostatin analogues for clinical use. Octreotide (Sandostatin, Novartis, Basel, Switzerland) was the first octapeptide somatostatin analogue that was synthesised. Its elimination half-life after subcutaneous administration is 2 h, and rebound hypersecretion of hormones does not occur (Bauer et al. 1982). Somatostatin and its analogues exert their effects through interaction with sst subtypes 1 through 5 (sst<sub>1–5</sub>). Somatostatin binds with high affinity to all somatostatin subtypes, whereas octreotide binds only with a high affinity to sst2 and sst5 (Patel 1999). Other cyclic analogues with almost similar affinity and activity profiles, such as lanreotide (Somatuline, Ipsen Biotech, Paris, France) have been subsequently developed (Lamberts et al. 1996). Octreotide (Sandostatin) and lanreotide (Somatuline) have been registered in most countries for the control of hormonal symptoms in patients with carcinoids and endocrine pancreatic tumours and in patients with acromegaly. Octreotide and lanreotide can be administered by

---

*de Herder et al.: Somatostatin receptors and neuroendocrine tumours*
multiple subcutaneous injections or by continuous subcutaneous infusion as well as by the intravenous route, either as a single injection or as a continuous infusion over many hours or days. The slow-release depot intramuscular formulation of octreotide (Sandostatin LAR, Novartis Pharma, Basel, Switzerland) has to be administered once every 4 weeks and that of lanreotide (Somatuline-PR, Ipsen Biotech, Paris, France) once every 2 weeks. A new slow-release depot preparation of lanreotide, Lanreotide Autogel (Ipsen Biotech, Paris France), has been introduced in several European countries. This drug has to be administered deep subcutaneously once every 4 weeks.

In the majority of patients with metastatic carcinoids and pancreatic endocrine tumours, treatment with octreotide induces a rapid improvement of clinical symptomatology, such as diarrhoea, dehydration, flushing attacks, hypokalaemia, peptic ulceration, hypoglycaemic attacks and necrotic skin lesions (Kvols et al. 1986, 1987, Ruszniewski et al. 1996, Caplin et al. 1998, Kulke & Mayer 1999, Wymenga et al. 1999). On the other hand, the majority of these patients show desensitisation of the inhibition of hormone secretion by octreotide and lanreotide within weeks to months. In a series of 57 patients with the carcinoid syndrome, octreotide therapy was ended in 23 patients after periods ranging from 1 week to 12.5 months (median 4 months), whereas the other responding patients could be controlled for periods extending to 2.5 years. The estimated mean duration of response to octreotide therapy in the whole group of responding patients was approximately 1 year (Moertel 1987). The potential mechanisms responsible for this desensitisation, as well as for the considerable variability in the duration of the responses to octreotide therapy are not known at present. Potential mechanisms of tachyphylaxis and resistance to somatostatin analogue therapy in patients with sst-positive tumours are (1) receptor down-regulation; a decrease in the number and/or affinity of ssts, (2) desensitisation; a decrease in responsiveness due to receptor uncoupling from second messenger activation, (3) non-homogeneous expression of ssts in tumours, (4) outgrowth of sst-negative cell clones, (5) resistance due to the absence of sst subtypes with high affinity for octapeptide somatostatin analogues, (6) resistance due to tachyphylaxis of the inhibitory effect of somatostatin analogues on indirect tumour growth-promoting mechanisms (like growth hormone or gastrin) and (7) mutations in sst genes leading to the absence of functional receptor proteins (Lamberts et al. 1988, Hofland & Lamberts 2003).


**sst scintigraphy**

Tumours and metastases that bear sst2 or sst3 can be visualised *in vivo* after injection of radiolabelled octapeptide analogues. The technique of sst scintigraphy to visualise sst-positive tumours in man was first developed using the radiolabelled somatostatin analogue [*123I-Tyr*]octreotide (Krenning et al. 1989). Because the use of this radiopharmaceutical had a number of drawbacks (such as costs, lack of availability, short physical half-life and predominant hepatic clearance resulting in accumulation of radioactivity in liver, gall bladder, bile ducts and gastrointestinal tract), novel somatostatin analogues were developed to circumvent these disadvantages. The most widely used somatostatin analogue for sst scintigraphy is currently [*111In-DTPA*]octreotide (Krenning et al. 1993). Apart from [*111In-DTPA*], [*111In-DOTA*]lanreotide can also be used (Krenning et al. 1994b, Virgolini et al. 2001).

**sst targeted radiotherapy**


In tumour tissue obtained after the administration of [*111In-DTPA*]octreotide to patients harbouring OctreoScan-positive metastatic midgut carcinoids, the subcellular distribution of radioactivity using ultrastructural autoradiography was subsequently analysed. This radioactivity could be found at the plasma membrane, in the cytoplasmic areas among secretory granules and vesicular compartments, but also in the perinuclear area. This localisation of [*111In*] in close prox-
iminity to the cell nucleus is especially important for this short range Auger electron-emitting radioisotope to exert its cytotoxic effect in the form of DNA double-strand damage (Janson et al. 2000). The predominant expression of sst2 receptors in most sst-positive endocrine tumours and the efficiency of sst3 receptors to undergo agonist-induced internalisation is very important for the application of sst-targeted radiotherapy. However, [111In-DTPA]octreotide may not be the most suitable compound to carry out radiotherapy because the Auger electron-emitter 111In has a low tissue penetration. In addition, a stable coupling of α- or β-emitting isotopes to [DTPA]octreotide could not be achieved, which initiated the development of a novel compound, such as [DOTA,Tyr3]octreotide, allowing a stable binding with the β-emitter yttrium-90 (90Y) ([90Y-DOTA,Tyr3]octreotide (OctreoTher, Novartis Pharma, Basel, Switzerland) and lutetium 177 ([177Lu-DOTA[Tyr3]octreotate). Furthermore, [111In-DOTA]lanreotide and [90Y-DOTA]lanreotide can also be used for radiotherapy of sst2- and sst-positive advanced or metastatic endocrine tumours (Hofland et al. 1999a, Anthony et al. 2002, Kwekkeboom et al. 2002, 2003, Valkema et al. 2002a,b, Virgolini et al. 2002).

Several mechanisms may determine the amount of uptake of radiolabelled somatostatin analogues. These include: (1) the stability of the radioligand, (2) the density of sst expression on the tumour, (3) the type of ssts expressed by the tumour, (4) affinity of the radioligand for the sst, (5) the efficiency of sst-mediated internalisation and recycling, (6) the final trapping of the radioisotopes within the tumour cells, as well as (7) the mass of the injected peptide (Nouel et al. 1997, Hukovic et al. 1999, Hofland 1999a, Hofland & Lamberts 2003).

New developments

Because every sst has distinct biologic functions, the development of new classes of somatostatin subtype-selective analogues may provide valuable information for tumour diagnosis, prognosis and prediction of somatostatin analogue efficacy, not only in tumours that are sensitive to the currently available octapeptide analogues, but also in tumours that express sst other than sst1 and sst2. A new so-called ‘universal’ somatostatin analogue, named SOM230, with high affinity for sst1, sst2, sst3 and sst5 receptors is currently under evaluation in phase I–III trials (Bruns et al. 2002, Lamberts et al. 2002, Weckbecker et al. 2002). New drugs interacting with multi-receptor family cross-talk are being developed. These sst subtype homo- or heterodimers may have properties which are distinct from the individual receptors in terms of internalisation, agonist-induced desensitisation and functional activity (Rocheville et al. 2000a,b, Pfeffer et al. 2001, 2002). The hybrid somatostatin–dopamine molecule, BIM-23A387, has high-affinity binding to both sst2 and dopamine D2 receptors and has an enhanced potency on growth hormone and prolactin release by primary cultures of pituitary adenoma cells, compared with sst2- and D2-specific analogues alone or in combination. This significant enhanced potency, however, cannot be explained on the basis of the binding affinity of the compounds for sst2 and dopamine D2 receptors (Saveanu et al. 2002).

Like peptide receptor-targeted radiotherapy, targeted chemotherapy to deliver the chemotherapeutic compounds selectively to tumour cells might be a promising approach as well (Plonowski et al. 2000, 2001, 2002, Kiaris et al. 2001). Although still at a very early stage, gene therapy may represent an exciting new treatment alternative for patients with advanced tumours. Transfer of genes that encode for the expression of sst to sst-negative cancers may render these tumours responsive to the currently available (radiolabelled or cytotoxic) octapeptide somatostatin analogues (Benali et al. 2000, Vernejoul et al. 2002, Guillermet et al. 2003).

References

Alderton F, Fan TP, Schindler M & Humphrey PY 1998 Rat somatostatin sst2(a) and sst2(b) receptor isoforms mediate opposite effects on cell proliferation. British Journal of Pharmacology 125 1630–1633.


Buscail L, Esteve JP, Saint-Laurent N, Bertrand V, Reisine T, O’Carroll AM, Bell GI, Schally AV, Vaysses N & Susini C 1995 Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms. *PNAS* 92 1580–1584.


Krenning EP, Kwekkeboom DJ, Oei HY, de Jong RJ, Dop FJ, Reubi JC & Lamberts SW 1994b Somatostatin-receptor...
de Herder et al.: Somatostatin receptors and neuroendocrine tumours


Krulich L, Dharwal AP & McCann SM 1968 Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in *vitro*. *Endocrinology* 83 783–790.


de Herder et al.: Somatostatin receptors and neuroendocrine tumours

