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Somatostatin receptors in gastroenteropancreatic neuroendocrine tumours

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Abstract

Five somatostatin receptor (sst) subtype genes, sst_1, sst_2, sst_3, sst_4 and sst_5, 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 sst_2 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 sst_2 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 sst_2 or sst_5 on pancreatic endocrine or carcinoid tumours further allows the use of radiolabelled somatostatin analogues for *in vivo* visualisation. The predominant expression of sst_2 receptors in these tumours and the efficiency of sst_2 receptors to undergo agonist-induced internalisation is also essential for the application of radiolabelled octapeptide somatostatin analogues. Currently, [^{111}In-DTPA^0]octreotide, [^{90}Y-DOTA^0,Tyr^3]octreotide, [^{177}Lu-DOTA^{0}Tyr^3]octreotate, [^{111}In-DOTA^0]lanreotide and [^{90}Y-DOTA^0]lanreotide can be used for this purpose.

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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 1983*a*,*b*). Somatostatin is formed by proteolytic processing of larger precursor molecules: prepro-somatostatin and pro-somatostatin. This peptide was detected accidentally during studies of the distribution of growth hormone-releasing factor in the hypothalamus of rats (Krulich *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 1983*a*,*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 sst_1, sst_2, sst_3, sst_4 and sst_5 (Hoyer *et al*. 1994). The genes encoding the five sst subtypes are localised on different chromosomes (Patel 1997). Two forms of the sst_2 receptor (sst_{2A} and sst_{2B}) 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.

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

Compound	IC_{50} value (nM)				
	sst_1	sst_2	sst_3	sst_4	sst_5
Somatostatin 14	0.93 ± 0.12	0.15 ± 0.02	0.56 ± 0.17	1.5 ± 0.4	0.29 ± 0.04
Lanreotide	180 ± 20	0.54 ± 0.08	14 ± 9	230 ± 40	17 ± 5
Octreotide	280 ± 80	0.38 ± 0.08	7.1 ± 1.4	>1000	6.3 ± 1.0
Chromosomal location	14	17	22	20	16

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.* 1998*b*, Kumar *et al.* 1999). In these cells, sst_1, sst_2 and sst_5 receptors are the most abundantly expressed subtypes, with a high percentage of β-cells expressing sst_1 and sst_5, α-cells expressing sst_2 and δ-cells expressing sst_5 (Kumar *et al.* 1999).

Tumours arising from somatostatin-target tissues frequently express a high density of ssts (Reubi *et al.* 1992*a*,*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.* 1992*b*, 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.* 1998*a*, Schulz *et al.* 1998, Kimura *et al.* 1999, Hofland *et al.* 1999*b*). Endocrine pancreatic and endocrine digestive tract tumours can also express multiple sst subtypes, but sst_2 predominance is generally found in more than 80% (Reubi *et al.* 1994, 2001, de Herder *et al.* 1996*a*, Papotti *et al.* 2002, Reubi & Waser 2003).

The five sst subtypes all bind somatostatin-14 and somatostatin-28 with high affinity. The sst_1 and sst_4 receptors do not bind the currently available octapeptide somatostatin analogues octreotide and lanreotide (see later), whereas sst_{2A}, sst_3 and sst_5 receptors display a high, low, and moderate affinity respectively towards these octapeptide somatostatin analogues (Table 1). The predominant expression of sst_2 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.* 1996*b*, 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.* 1992, 1993, 1994*a*,*b*, 1999, Kwekkeboom *et al.* 1993, Kwekkeboom & 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 D_2 receptor or the μ-opioid receptor (MOR-1), resulting in a novel receptor state with properties different from the individual receptors (Rocheville *et al.* 2000*a*,*b*, Pfeiffer *et al.* 2001, 2002).

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 ($sst_{1–5}$). Somatostatin binds with high affinity to all somatostatin subtypes, whereas octreotide binds only with a high affinity to sst_2 and sst_5 (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

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).

Expression of ssts by endocrine tumours is essential for the control of hormonal hypersecretion by the octapeptide somatostatin analogues. Somatostatin is also able to inhibit proliferation of normal and tumour cells. Induction of G1 cell arrest and induction of apoptosis have been demonstrated in a number of tumour cell models and several sst subtypes seem to be involved (Buscail *et al*. 1994, 1995, Cordelier *et al*. 1997, Alderton *et al*. 1998, Bousquet *et al*. 1998, 2001, Sharma & Srikant 1998, Sharma *et al*. 1999, Pages *et al*. 1999, Rochaix *et al*. 1999, Benali *et al*. 2000, Vernejoul *et al*. 2002*b*). Various reports demonstrating tumour regression or stabilisation in patients with metastatic carcinoids and endocrine tumours of the gastrointestinal tract with octapeptide somatostatin analogues are consistent with these experimental data although other mechanisms may also play a role, such as inhibition of angiogenesis and inhibition of growth factors by these drugs (Kraenzlin *et al*. 1983, Clements & Elias 1985, Wiedenmann *et al*. 1988, Woltering *et al*. 1997, Filosso *et al*. 2000, Imtiaz *et al*. 2000, Delle Fave & Corleto 2001, Garcia de la Torre *et al*. 2002, Shojamanesh *et al*. 2002, Florio *et al*. 2003).

sst scintigraphy

Tumours and metastases that bear sst_2 or sst_5 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 [^{123}I-Tyr^3]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 ^{111}In-pentetreotide ([^{111}In-DTPA^0]octreotide, OctreoScan, Tyco Healthcare, Mallickrodt, St Louis, USA) (Krenning *et al*. 1993). Apart from ^{111}In-pentetreotide, [^{111}In-DOTA^0]lanreotide can also be used (Krenning *et al*. 1994*b*, Virgolini *et al*. 2001).

sst targeted radiotherapy

In general, sst–agonist complexes follow the mechanism and route of internalisation as described for many other G protein-coupled receptor complexes (Hausdorff *et al*. 1990, Yu *et al*. 1993, Roettger *et al*. 1995, Ferguson *et al*. 1996, Koenig & Edwardson 1997, Hofland & Lamberts 2003). The sst subtypes differentially internalise somatostatin and somatostatin analogues. The sst_1 receptors show low agonist-induced internalisation, whereas sst_2, sst_3, sst_4 and sst_5 are more efficient in this respect (Patel 1999, Hofland & Lamberts 2003).

In tumour tissue obtained after the administration of [^{111}In-DTPA^0]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 ^{111}In in close prox-

imity 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 sst_2 receptors in most sst-positive endocrine tumours and the efficiency of sst_2 receptors to undergo agonist-induced internalisation is very important for the application of sst-targeted radiotherapy. However, [^{111}In-DTPA^0]octreotide may not be the most suitable compound to carry out radiotherapy because the Auger electron-emitter ^{111}In has a low tissue penetration. In addition, a stable coupling of α- or β-emitting isotopes to [DTPA^0]octreotide could not be achieved, which initiated the development of a novel compound, such as [DOTA^0,Tyr^3]octreotide, allowing a stable binding with the β-emitter yttrium-90 (^{90}Y) [^{90}Y-DOTA^0,Tyr^3]octreotide (OctreoTher, Novartis Pharma, Basel, Switzerland) and lutetium 177 ([^{177}Lu-DOTA^{0}Tyr^3]octreotate). Furthermore, [^{111}In-DOTA^0]lanreotide and [^{90}Y-DOTA^0]lanreotide can also be used for radiotherapy of sst_2- and sst_5-positive advanced or metastatic endocrine tumours (Hofland *et al*. 1999*a*, Anthony *et al*. 2002, Kwekkeboom *et al*. 2002, 2003, Valkema *et al*. 2002*a*,*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 1999*a*, 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 ssts other than sst_2 and sst_5. A new so-called 'universal' somatostatin analogue, named SOM230, with high affinity for sst_1, sst_2, sst_3 and sst_5 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*. 2000*a*,*b*, Pfieffer *et al*. 2001, 2002). The hybrid somatostatin–dopamine molecule, BIM-23A387, has high-affinity binding to both sst_2 and dopamine D_2 receptors and has an enhanced potency on growth hormone and prolactin release by primary cultures of pituitary adenoma cells, compared with sst_2- and D_2-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 sst_2 and dopamine D_2 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_2 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).

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