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Illuminating somatostatin analog action at neuroendocrine tumor receptors

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Abstract

Somatostatin analogs for the diagnosis and therapy of neuroendocrine tumors (NETs) have been used in clinical applications for more than two decades. Five somatostatin receptor subtypes have been identified and molecular mechanisms of somatostatin receptor signaling and regulation have been elucidated. These advances increased understanding of the biological role of each somatostatin receptor subtype, their distribution in NETs as well as agonist-specific regulation of receptor signaling, internalization and phosphorylation, particularly for the sst₂ receptor subtype which is the primary target of current somatostatin analog therapy for NETs. Various hypotheses exist to explain differences in patient responsiveness to somatostatin analog inhibition of tumor secretion and growth as well as differences in the development of tumor resistance to therapy. In addition, we now have a better understanding of the action of both first generation (octreotide, lanreotide, Octreoscan) and second generation (pasireotide) FDA-approved somatostatin analogs, including the biased agonistic character of some agonists. The increased understanding of somatostatin receptor pharmacology provides new opportunities to design more sophisticated assays to aid the future development of somatostatin analogs with increased efficacy.

Keywords

sst receptors; octreotide; lanreotide; pasireotide

Somatostatin

Somatostatin was originally isolated from hypothalamic extracts based on its ability to inhibit the secretion of growth hormone from pituitary cells^{1,2} but is now known to regulate many specific targets in the endocrine, gastrointestinal and nervous systems³⁻⁶.

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Conflict of interest

The authors declare no conflict of interest.

Physiologically active somatostatin is secreted in two forms, 14 and 28 amino acids in length, generated by alternative proteolytic processing from a single precursor. Although the ratio of the two peptides varies among tissues, both SS14 and SS28 activate all somatostatin receptor subtypes at nanomolar concentrations because they share the peptide region critical for receptor interaction. Interestingly, two additional endogenous neuropeptides bind to somatostatin receptors with high affinity. The cortistatins, although encoded by a different gene than somatostatin, share sufficient sequence identity that they potently activate all somatostatin receptor subtypes^{7,8}. However cortistatins, which also exhibit biological actions distinct from somatostatins, bind to the Mas-related GPCR MrgX2 albeit with a lower affinity than to sst receptors⁹.

Somatostatin's well-established physiological actions include inhibition of hormone secretion by the pituitary gland (predominantly growth hormone (GH) but also adrenocorticotrophic hormone (ACTH), prolactin and thyroid stimulating hormone (TSH)), inhibition of insulin and glucagon secretion by the endocrine pancreas, inhibition of motility and exocrine secretion in the gastrointestinal tract, regulation of motor, cognitive and sensory functions in the central nervous system (CNS), and pain transmission in the peripheral nervous system (PNS)³⁻⁶. In addition, somatostatin inhibits both secretion by and the growth of many different types of neuroendocrine tumors (NETs). As a consequence, metabolically stable somatostatin analogs have been widely used both for the diagnosis and therapy of such tumors. However, the clinical utility of somatostatin analogs in the treatment of NETs is limited because (1) a subgroup of tumors either do not respond to somatostatin analog therapy or respond poorly, and (2) tumors may become resistant to somatostatin analog treatment after several months of therapy¹⁰⁻¹². During the search for more effective therapeutic agents some unexpected aspects of somatostatin analog action have been uncovered. This review will describe recent insights into somatostatin receptor signaling and regulation and discuss how new understanding can help guide the use of somatostatin analogs in the treatment and diagnosis of neuroendocrine tumors.

Somatostatin receptor subtypes

Somatostatin receptors were first identified by radioligand binding studies in a pituitary tumor cell line in which somatostatin potently inhibited the secretion of prolactin and growth hormone¹³. Subsequently, radioligand binding assays showed that somatostatin receptors were widely distributed in many different tissues¹⁴⁻¹⁶. More than a decade later, five distinct somatostatin receptor genes, named *sst*₁ through *sst*₅, were cloned and shown to belong to the Rhodopsin-like Family of G protein coupled receptors (GPCRs)¹⁷. The *sst*₂ gene can be alternatively spliced to produce two receptor proteins, *sst*_{2A} and *sst*_{2B}, that differ in the length and sequence of their carboxy termini¹⁸. Human tissues contain the unspliced *sst*_{2A} variant almost exclusively, whereas both spliced forms have been identified in rodents. Although the other *sst* genes do not contain introns, Castaño's group has recently identified novel variants of *sst*₅ mRNA formed by splicing of noncanonical donor and acceptor splice sites¹⁹. These *sst*₅ variants encode truncated receptors missing one or more of the seven transmembrane domains that provide the characteristic architecture of GPCRs. The human variant mRNAs contain the 5' coding region of the *hssst*₅ gene linked to alternative 3' open reading frames, and encode proteins containing five (*sst*5TMD5) or four (*sst*5TMD4) of the amino-terminal trans-membrane domains of full length *sst*₅ and distinct C-termini. Recently the *sst*5TMD4 protein was detected by immunohistochemical staining in pituitary tumors²⁰. Although the abundance, distribution, and physiological functions of truncated *sst*₅ receptor variants are not yet known they have been hypothesized to contribute to tumor resistance to somatostatin analog treatment by inhibiting the signaling of full length *sst*₂ and *sst*₅ receptors¹⁹.

The five sst subtypes show a characteristic and distinct cell and tissue distribution but interestingly, both normal and tumor target cells frequently appear to express multiple somatostatin receptor subtypes. Although the co-expression of sst subtypes may be overestimated by nonspecific antibody reactivity (see below)^{21,22}, functional overlap of sst subtypes has been confirmed in a number of instances using somatostatin analogs. Moreover, experiments in cell lines expressing multiple transfected sst subtypes have demonstrated that some sst receptors are able to heterodimerize with other members of the sst receptor family as well as with unrelated GPCRs, including D2 dopamine receptors, $\beta 1$ and $\beta 2$ adrenergic receptors and ghrelin receptors^{23,24}. Co-expression of such interacting receptors in a cell can affect sst receptor trafficking, signaling or biological activity. Thus, the role that such heterodimers play under physiological or pathological conditions is an interesting area for future research.

The complexity resulting from the expression of five sst receptor subtypes, two of which can produce alternatively spliced variants, and a number of which can heterodimerize provides a substantial challenge for the therapeutic targeting of this receptor family.

Targeting of sst receptors in neuroendocrine tumors

Distribution and importance of ssts in NETs

NETs consist of a heterogeneous group of relatively rare tumors that originate either from endocrine organs such as pituitary, pancreas, thyroid and adrenals, or from neuroendocrine cells in the disseminated endocrine system present in organs such as gut, lung or thymus. Found originally in growth-hormone-producing pituitary tumors²⁵, somatostatin receptor overexpression was subsequently shown to occur in a large variety of other tumors²⁶. The incidence and density of sst receptors are particularly high in NETs and brain tumors²⁶, with often large individual variation in receptor density. Several different *in vitro* techniques have been used to detect these receptors, including measurement of receptor mRNA, protein and binding activity, each with specific advantages and limitations. Although RT-PCR or quantitative real time PCR are widely used to detect sst subtype mRNA in tumor samples, these methods suffer from the absence of morphological information. Thus, small amounts of normal and non-tumoral tissues located adjacent to a receptor-negative tumor sample may erroneously suggest tumor positivity. Such proximal somatostatin receptor positive tissue may include blood vessels, nerves, lymphocytes as well as non-tumoral endocrine cells^{27–30}. Because tumor samples are usually highly heterogeneous, it is preferable to use a morphological method of receptor analysis. Measurement of somatostatin receptor mRNAs by *in situ* hybridization does provide a morphological correlate, however, this method still suffers from the disadvantage that receptor mRNA levels may not directly reflect levels of functional receptor protein. Again, it is preferable to detect the somatostatin receptor protein itself, and if possible, active somatostatin-binding sites, because these represent the molecular targets for the clinical application of radiolabeled or non-radiolabeled somatostatin analogs²⁶. The gold standard in this respect is *in vitro* quantitative somatostatin receptor autoradiography on fresh-frozen tissue sections that combines morphology, highly specific binding site detection and receptor quantification. Because of its limited cellular resolution, however, somatostatin receptor autoradiography is optimal for the detection of receptors in cell groups (especially tumors) rather than in single cells. An attractive morphological alternative is immunohistochemical analysis of the receptors on readily-available formalin-fixed tissues^{22,31,32}, with the limitations that (i) highly specific antibodies of sufficient sensitivity were initially lacking; (ii) quantification is not possible; and (iii) an epitope distinct from the binding site is usually identified.

The existence of five somatostatin receptor subtypes in human tissues has made the evaluation of the somatostatin receptor profile more complex: in principle, all three methods

(receptor mRNA measurement; ligand autoradiography; receptor immunohistochemistry) are capable of detecting each of the somatostatin receptor subtypes. Unfortunately, not all antibodies raised against the five receptors are sufficiently sensitive and specific for immunohistochemical detection. They need therefore to be carefully validated, for instance by correlating the immunohistochemical results with an established morphological parameter such as binding with receptor autoradiography^{22,33}. The availability of adequate antibodies against sst₂, the most predominantly expressed somatostatin receptor subtype, [i.e. the polyclonal R2-88 more than a decade ago or, more recently, the commercially available monoclonal UMB-1 (Epitomics)] has meant considerable progress for pathologists^{22,32–34}. The same may apply to a novel sst₅ antibody³⁵. However, it should be emphasized that, in general, high affinity antibodies against GPCRs are extremely difficult to develop and that a majority of commercial receptor antibodies lack the sensitivity and the specificity required to detect receptors in native tissues, even though they may detect the high receptor levels seen in transfected cell lines^{36,37}. Although the current belief is that the final proof for GPCR antibody specificity comes from studies done in tissues from receptor KO animals, these necessary control experiments are not sufficient as they do not exclude the possibility that an antibody may show different immunohistochemical staining in human and mouse tissue samples, especially if the sequence of the target receptor differs in the two species.

Receptor expression in tumors

Although somatostatin receptor incidence and density reported in various tumors depends on the methodology used, there is, however, general agreement that the majority of NETs express somatostatin receptors in high density (Table 1). These tumors include pituitary adenomas (in particular GH- and TSH-producing adenomas), gastroenteropancreatic and lung neuroendocrine tumors, pheochromocytomas, and paragangliomas²⁶. Tumors of the nervous system, such as medulloblastomas, meningiomas and neuroblastomas, also express somatostatin receptors at high density²⁶. Furthermore, non-neural and non-neuroendocrine tumors also can express somatostatin receptors, although clearly with a lower incidence and/or density than NETs. Such tumors include breast cancer, small cell lung cancer, lymphoma, hepatocellular carcinoma, renal cell carcinoma, and gastric carcinoma²⁶.

Among all somatostatin receptor expressing tumors, pituitary and gastroenteropancreatic (GEP) NETs express the highest density of the sst₂ receptor subtype, which can be targeted by currently available analogs *in vivo*. The great majority of pituitary NETs, including GH and TSH-producing tumors, express large amounts of sst₂³⁸. In addition pancreatic NETs (including gastrinomas, glucagomas, vipomas) and gut NETs (foregut, midgut and hind gut tumors) express sst₂ in 80–100% of cases³⁹ whereas insulinomas have a lower incidence of sst₂ expression (50–70%). In general, somatostatin receptors are expressed homogeneously in GEP-NET⁴⁰, a characteristic that represents an advantage for optimal *in vivo* targeting of as many tumor cells as possible during peptide receptor radiotherapy. Of note, well differentiated GEP-NET usually express a greater density and frequency of somatostatin receptors than do undifferentiated GEP-NETs⁴⁰, an observation which is relevant for therapeutic options.

Whereas an early study showed evidence for a relationship between protein sst₂ expression levels and the magnitude of the secretory response to somatostatin analogs⁴¹, lack of such correlation by measuring sst₂ mRNA expression were published subsequently⁴². Such variability is likely to be due to differences in the methods used to measure the expression of sst₂ receptor mRNA or protein, as discussed above. Indeed, recent studies have reported that, as expected, there is a direct correlation between sst₂ receptor protein expression and tumor responsiveness to a high affinity sst₂ agonist (octreotide)^{33,43–45}. Moreover,

transfection of human adenoma cells in culture with sst₂ receptor expressing adenovirus vectors improves octreotide sensitivity⁴⁶.

Table 1 also lists tumors which may, in addition to or instead of sst₂, express different or additional somatostatin receptor subtypes, such as sst₁, sst₃, or sst₅. Among pituitary adenomas a fraction of GH-adenomas preferentially express sst₅, most ACTH-adenomas have sst₅⁴⁷; and many inactive pituitary adenomas have sst₃³⁸. GEP-NET often express more than one somatostatin receptor subtype, with sst₂ usually the most prominent, followed by sst₁ and sst₅, whereas sst₃ is less frequent and sst₄ almost absent³⁹. In contrast, insulinomas often express sst₁ or, alternatively, sst₅ together or instead of sst₂^{39,48}. In general, very few human tumors express sst₄^{26,39}. Other than for the sst₂ receptor subtype, no data exists correlating the level of sst subtype expression and responsiveness to targeting somatostatin analogs.

Rationale for the use of somatostatin analogs in NET

There are three rationales for the use of somatostatin analogs in the diagnosis and treatment of NETs.

First, many NETs secrete specific hormones, often in amounts that if untreated are clinically debilitating (i.e. vasoactive intestinal polypeptide (VIP)-induced diarrhea) or life-threatening (i.e. insulin-induced hypoglycemia). Somatostatin markedly inhibits via its receptors this pathophysiological hormonal secretion and thus alleviates the symptoms associated with tumoral hypersecretion^{49–51}. These actions require potent somatostatin agonists sufficiently metabolically stable to induce a strong and persistent hormone inhibition during long-term treatment.

Second, the massive overexpression of somatostatin receptors in specific NETs is the molecular basis for *in vivo* targeting of these receptors for diagnostic purposes with radiolabeled somatostatin analogs containing a gamma-emitting isotope⁵². The accumulated radioactivity permits visualization of the tumor. The same principle, (i.e. binding to the tumoral receptors of a radioligand linked to an α - or β -emitting isotope) permits selective radiotherapy of the tumor *in vivo*⁵².

Third, somatostatin has been shown in animal tumor models *in vivo* to inhibit tumor growth both directly and indirectly⁵³. *Indirect inhibition* occurs through suppression of the synthesis and secretion of growth factors such as growth hormone and IGF1 or through inhibition of angiogenesis⁵⁴. *Direct inhibition* is accomplished through tumoral somatostatin receptors which can inhibit cell growth or promote apoptosis through several signal transduction pathways^{3,53,55}. Therefore, potent and metabolically stable somatostatin analogs given systematically in a long-term regimen can reduce tumor size or slow tumor growth⁵⁶.

Mechanisms of somatostatin receptor signaling

The most widespread and best characterized action of somatostatin is its strong inhibitory effect on secretion. Both in endocrine cells and in neurons this inhibition is mediated by coupling of ssts to Gi/Go proteins, members of the heterotrimeric guanine-nucleotide binding protein family characterized by their sensitivity to pertussis toxin. G-protein activation by ssts subsequently leads to a reduction in two critical second messengers: cyclic AMP and cytosolic calcium. The reduction in cAMP results from somatostatin receptor inhibition of adenylyl cyclase via Gi/Go proteins^{3,57–60}. In addition, ssts act via Gi/Go to inhibit calcium channels both directly and indirectly, the latter by opening potassium channels to produce hyperpolarization and, as a consequence, inhibition of calcium influx through voltage-dependent calcium channels^{61–65}. Reduction of either cyclic AMP or

calcium will lead to inhibition of secretion but the simultaneous reduction of both second messengers by somatostatin results in a synergistic inhibitory effect on hormone release^{61,65}. In addition to these second messenger-mediated actions, early studies showed that sst activation of Gi/Go proteins inhibits the secretory process directly^{66,67}. The second messenger-independent inhibition of secretion is now known to involve direct regulation of the vesicle exocytotic machinery by Gi/Go subunits⁶⁸.

The signaling events responsible for sst inhibition of cell proliferation and stimulation of apoptosis in NETs are understood less well than those that inhibit hormone secretion, but multiple pathways have also been implicated. Two important factors need to be considered when identifying the key signaling pathways producing growth inhibition (1) Because NETs arise from various cell types often containing different growth promoting mutations^{69–71}, the resulting tumors represent diseases with distinct properties and genetic abnormalities. Thus the *key* sst receptor signaling pathway that inhibits the growth of these different tumors is also likely to vary and may well differ from those that occur in the undifferentiated model cell lines commonly used to study sst signaling (2) The action of somatostatin to inhibit secretion in NETs can occur independently of the inhibition of tumor growth suggesting that at least some different intracellular signaling events are involved in producing these biological effects^{72,73}.

Two general signaling pathways are likely to be important for somatostatin analog inhibition of NET cell proliferation. One pathway involves sst activation of protein tyrosine phosphatases, such as the Src homology phosphatases, SHP-1 and SHP-2^{53,74–77}. The ensuing dephosphorylation of specific substrates is proposed to counteract growth factor stimulated tyrosine kinase activity and thus to inhibit multiple mitogenic signaling pathways.

The second implicated signaling pathway involves sst inhibition of adenylyl cyclase, leading to a reduction in cyclic AMP levels and, as a consequence, to changes in CREB (cAMP response element-binding protein) and ERK (extracellular receptor kinase) signaling. Reduction of cyclic AMP production by somatostatin inhibits protein kinase A (PKA)-catalyzed phosphorylation and activation of the transcription factor CREB⁷⁸. Although the role of this pathway in NET cell proliferation is not fully understood, new studies show that the cAMP/CREB signaling cascade is highly upregulated in NETs⁷⁹. Further, recent studies have identified a new PKA-independent mechanism by which cAMP activates ERK phosphorylation in neuroendocrine cells⁸⁰. This mechanism involves cAMP binding to the guanine nucleotide exchange factor Rapgef2 resulting in its activation of Rap1 and subsequent downstream activation of ERK via the B-Raf-MEK-ERK protein kinase cascade⁸⁰. Chronic stimulation of cyclic AMP production both by activating mutations in the GNAS1 gene encoding Gs and by hyperactivation of GPCRs linked to Gs are well known to stimulate NET cell proliferation^{81–83}. Thus, inhibition of adenylyl cyclase and the subsequent cAMP-dependent signaling cascades is likely to contribute to somatostatin analog reduction of NET cell growth as well as to its inhibition of secretion.

Numerous additional sst-regulated signaling events have been identified and proposed to play a role in somatostatin inhibition of cell growth. However, their importance for the inhibition of NET cell proliferation specifically is mostly unknown although it is clear from studies in model cell lines that the ability of sst receptors to regulate a particular signaling pathway depends both on the receptor subtype involved and the nature of the signaling machinery expressed in each target cell. For example, in contrast to the inhibition of cytosolic calcium usually observed in pituitary cells, somatostatin elevates intracellular calcium in smooth muscle cells as well as in pancreatic tumor cells^{84–86}. Similarly, sst receptors inhibit phosphorylation of ERKs in pituitary tumor and medullary thyroid

carcinoma cells^{87–89} but stimulate ERK phosphorylation in some NET cell lines^{86,90}. In addition, the signal transduction mechanisms activated by endogenous ssts in NET cells often differ from those observed when ssts are transfected into heterologous host cells^{91,92}. Thus, although somatostatin regulation of proliferative signaling pathways has been intensely studied in a variety of model systems, the mechanisms responsible for somatostatin receptor inhibition of cell proliferation in different NET types remain to be defined. Most importantly, we do not yet understand why somatostatin analogs inhibit cell proliferation in some NETs but not others of the same type, despite the expression of the same sst receptor subtypes.

A detailed review of the numerous intracellular signaling events triggered by different sst receptors in various model systems is beyond the scope of this article but many of these have been summarized in recent reviews^{3,21,53,76,77,91–93}. Overall, it is clear that somatostatin receptor signaling depends on three critical factors: (i) the sst subtype involved; (ii) the signaling machinery present in the target cell in which the sst receptor is expressed; and (iii) the particular agonist activating the receptor. Deciphering the signaling mechanisms that are activated by somatostatin analogs acting at individual sst receptor subtypes in each NET type will aid the therapeutic use of somatostatin agonists.

Somatostatin receptor regulation

At the time that somatostatin triggers sst signaling it also initiates counter-regulatory processes that prevent receptor overstimulation and can affect tumor responsiveness to somatostatin analog treatment. Numerous studies have demonstrated that sst subtypes show distinct patterns of receptor desensitization and internalization in response to agonist stimulation^{94–97}. Regulation of the sst_{2A} subtype, which is most widely expressed in human NETs, is the best understood and the only one which has been examined in patients.

Somatostatin treatment causes desensitization and internalization of sst_{2A} within minutes in both pituitary and pancreatic tumor cells^{98,99}. The desensitization can produce a marked shift in the potency of somatostatin stimulation, as found in pancreatic tumor cells, or can lead to a decrease in the maximal inhibition achieved, as observed in pituitary tumor cells. However, desensitization usually does not lead to a complete absence of responsiveness^{98,99}. Somatostatin stimulated receptor internalization reduces the steady state level of cell-surface receptors by 50 to 80% but residual receptors remain at the plasma membrane^{98,99}. Also *in vivo* in rats, a potent and very rapid sst₂ receptor internalization (within 2.5 min) is observed in transplanted AR42J pancreatic tumors¹⁰⁰. A similar massive sst_{2A} receptor internalization occurs in NET tumors *in situ* in patients treated acutely with the somatostatin analog octreotide, whereas sst_{2A} receptors are localized only at the cell surface in tumors from control patients (Figure 1)¹⁰¹. These data demonstrate that the sst_{2A} internalization found in cell culture also occurs rapidly *in vivo* in NET tumor patients.

Desensitization and internalization are both triggered by receptor phosphorylation. Early studies showed that somatostatin treatment of pituitary and pancreatic tumor cells causes more than a 10-fold increase in ³²PO₄ incorporation into sst_{2A} within 2 min^{98,99}. Peptide mapping of the radiolabeled receptor demonstrated that phosphorylation occurs in two receptor regions, the third intracellular loop (IC3) and the C-terminal tail (CT)^{99,102}. The other intracellular domains of sst_{2A} were not detectably phosphorylated^{99,102}. Five phosphorylated residues were identified in the C-terminus of sst_{2A} following hormone stimulation using a combination of biochemical analysis, site-directed mutagenesis, and reactivity with phospho-site specific antibodies: Ser341, Ser343, Ser348, Thr353, Thr354 (Figure 1)¹⁰³. Subsequently, two additional C-terminal phosphorylation sites, Thr356, and Thr359, were recognized using another phospho-site specific antibody¹⁰⁴. However, the

IC3 loop residues that are phosphorylated upon somatostatin treatment remain to be identified. Since a high level of sequence identity occurs in these regions of the sst_{2A} receptor in humans and rodents (Figure 1) it is not surprising that identical sites are phosphorylated in both the rat and human sst_{2A} receptor^{88,91,94}.

Phosphorylation of the sst_{2A} receptor occurs in a sequential manner within seconds following somatostatin stimulation: phosphorylation of Ser341/Ser343 precedes phosphorylation of Thr353/Thr354¹⁰⁵. Agonist stimulated sst_{2A} phosphorylation is independent of receptor signaling and is catalyzed, at least at some phospho-sites, by two G protein coupled receptor kinase subtypes, GRK2 and/or GRK3^{98,103,104}. Thus the rate and extent of agonist-stimulated receptor phosphorylation depends on the expression level of these kinases in target cells. Indeed overexpression of GRK2 has been shown to stimulate agonist induced sst_{2A} phosphorylation in some cells.

Phosphorylation of different sst_{2A} residues has distinct functional consequences¹⁰⁶. Phosphorylation of carboxy-terminal Thr residues is absolutely required for the stable binding of arrestins¹⁰⁶, receptor-interacting proteins that desensitize GPCRs by blocking receptor-G protein coupling, that link the receptor to the endocytic machinery and that can produce G-protein independent signaling¹⁰⁷. Mutation of all C-terminal Thr sites in the sst_{2A} receptor abrogates both efficient receptor internalization and arrestin recruitment. However, this Thr-negative mutant is still phosphorylated on Ser residues¹⁰⁵ and desensitizes within 5 min of somatostatin pretreatment even in the absence of receptor endocytosis. Thus, the C-terminal Thr residues, although required for internalization, are not needed for uncoupling sst_{2A} from signaling¹⁰⁶. In contrast, phosphorylation of Ser residues is not required for sst_{2A} internalization: hormone stimulated phosphorylation of Thr residues and endocytosis are unaffected when all Ser residues in the IC3 and CT regions of sst_{2A} are mutated to Ala and this Ser-negative receptor internalizes normally^{105,106}. However, Ser phosphorylation is critical for sst_{2A} desensitization/uncoupling¹⁰⁶. Finally, when all Ser and Thr residues in the IC3 and CT regions of sst_{2A} are mutated, somatostatin does not cause any sst_{2A} phosphorylation or desensitization, and its effect on internalization is severely compromised¹⁰⁶. These studies show striking differences in the functional consequences of different phosphorylation events and indicate that understanding the mechanisms by which individual phosphorylated residues alter sst_{2A} receptor function will provide important insights into its regulation and signaling.

Both sst_{2A} phosphorylation and receptor internalization are rapidly reversed upon hormone washout. Receptor dephosphorylation occurs within minutes of somatostatin removal, and recycling of internalized receptors then follows dephosphorylation more slowly^{105,108}. Because recycled receptors are dephosphorylated prior to reinsertion into the plasma membrane they are resensitized and ready to respond to hormone activation. However this is not the situation for receptors that are not internalized and recycled. Although Thr 353/354 can be dephosphorylated both at the cell surface and in endocytic vesicles, Ser341/343 cannot be dephosphorylated unless the receptor is internalized¹⁰⁵. These unexpected results indicate that following agonist stimulation, full dephosphorylation and recovery of active sst_{2A} depends on receptor internalization, a conclusion of great significance for the use of biased somatostatin analogs that stimulate sst_{2A} phosphorylation but do not lead to receptor endocytosis^{105,108}.

At least two phosphatases act on sst_{2A} to specifically dephosphorylate different receptor residues: PP2A dephosphorylates Thr353/354, whereas a different phosphatase dephosphorylates Ser341/343¹⁰⁵. Interestingly, a subsequent study found that PP1 rather than PP2A dephosphorylated Thr353/354 in HEK293 cells¹⁰⁹. The finding that different phosphatases act on sst_{2A} in different cell lines suggests that the phosphatases that catalyze

sst_{2A} dephosphorylation may be cell specific. However, the enzymes regulating sst_{2A} phosphorylation in NETs have not been characterized.

Unraveling the mechanisms that regulate sst_{2A} receptor function in human tumors *in situ* is far more challenging than in cultured cells. However, phosphorylation of sst_{2A} has been demonstrated in human tumors stimulated *in vivo*, and the phosphorylation state of the receptor appears to determine its subcellular localization, paralleling the results observed in model cell lines. Thus, in pheochromocytomas and somatostatinomas, which secrete somatostatin and also express sst_{2A}, the receptor is highly phosphorylated and is localized intracellularly, consistent with the effect of agonist stimulation observed in culture ¹¹⁰. Moreover, analysis of NETs obtained from patients acutely treated with octreotide just prior to surgery using phospho-site specific antibodies showed that the sst_{2A} was both phosphorylated and internalized in excised tumors (Figure 1) ¹¹¹. In contrast, in untreated patients, the receptor was localized at the cell surface and was not detectably phosphorylated ¹¹¹. These results demonstrate that sst_{2A} phosphorylation and internalization occur in NETs during acute somatostatin analog treatment of patients and show that sst_{2A} regulation is similar in model cell lines in culture and in human tumors *in vivo*. This observation contrasts with the widely held belief that sst_{2A} receptors do not desensitize in NETs until many months of treatment, if at all. Rather it suggests that the steady state response to somatostatin analog therapy even after short treatment times represents a “desensitized” response, according to standard pharmacological definitions. Indeed, it suggests that the magnitude of a patient’s response to somatostatin analog therapy will depend on the extent that phosphorylation-dependent sst_{2A} desensitization and internalization occur *in vivo* and, thus, on the steady state level of cell surface, active receptors present in each individual tumor during therapy.

The sst_{2A} receptor is also subject to heterologous receptor regulation. Stimulation of protein kinase C (PKC), either directly with phorbol esters or indirectly by activation of Gq-linked GPCRs, rapidly increases sst_{2A} phosphorylation ^{98,99,102}. In contrast, PKA activation has no effect ⁹⁸. PKC increases sst_{2A} phosphorylation in both the IC3 and CT regions ^{99,102} and two C-terminal PKC phospho-sites have been identified, namely Ser343 and Ser348 ¹⁰³ (Figure 1). Use of a panel of phospho-site specific antibodies further showed that a different pattern of sst_{2A} phosphorylation is produced following PKC activation than after somatostatin stimulation ¹⁰³. Interestingly, whereas PKC phosphorylation stimulates sst_{2A} receptor internalization, it has a smaller desensitizing effect on sst_{2A} signaling than somatostatin pretreatment ^{99,102}. Because the activation state of PKC in NETs is likely to be variable, heterologous regulation may well produce variability in the responsiveness of individual NETs to somatostatin analogs.

Regulation of the sst₅ receptor in model systems differs from that of sst_{2A}. Although agonist stimulation also produces rapid receptor internalization, sst₅ appears to recycle more rapidly than sst_{2A}, and less intracellular accumulation of this receptor is observed at steady state ⁹⁷. There is disagreement as to the region of the sst₅ receptor that is critical for arrestin binding and agonist stimulated receptor internalization. Phosphorylation of Ser242 in the third intracellular loop was implicated in a study with pituitary tumor cells ¹¹², whereas phosphorylation of Thr333 in the C-terminal region was implicated when the receptor was examined in HEK cells ¹¹³. Clearly additional studies will be required to understand the role of site-specific phosphorylation in sst₅ regulation and signaling, particularly in NETs.

The sst₃ receptor is also rapidly phosphorylated, desensitized and internalized ¹¹⁴. Phosphorylation of four residues in the C-terminus appears to be critical for sst₃ regulation. Although the sst₁ receptor also undergoes rapid agonist stimulated phosphorylation and desensitization, it internalizes to a lesser extent than sst₂, sst₃ or sst₅ and does not

accumulate intracellularly with prolonged treatment^{115–117}. The sst₄ receptor subtype resembles sst₁ in that it also internalizes poorly. Sst₄ desensitization has been reported in some systems but not others^{118,119}. Although sst₄ receptors contain multiple phosphorylation sites in both the IC3 and CT regions of the receptor, agonist stimulation did not cause detectable receptor phosphorylation¹¹⁹.

In summary, sst receptor subtypes exhibit complex and distinct patterns of homologous and heterologous regulation in model cell lines. However few studies have examined the mechanisms that modulate sst receptor function in NETs. Thus the extent to which sst regulation varies in different types of tumors or among individual tumors of the same type remains to be established and is an important area for future research, given the recognized impact of target cell environment on GPCR regulation^{120,121}. However, because somatostatin receptor phosphorylation and internalization clearly occur *in vivo* in human NETs even after short stimulation times (Figure 1), such acute regulation must affect the magnitude of tumor response to somatostatin analog therapy. In fact, the steady state response measured in patients even after hours or days of analog treatment must represent a “desensitized” receptor response. In contrast, the development of resistance to somatostatin analog treatment over many months or years is unlikely to be due to the rapid phosphorylation, desensitization (uncoupling) and internalization of the receptor and is more likely to depend on changes in the expression or activation state of effectors of somatostatin signaling and/or in the proteins that regulate receptor function, perhaps resulting from a selection process during tumor growth and development.

Development of unlabeled compounds for symptomatic treatment

Established sst_{2A} selective compounds

Two FDA-approved synthetic somatostatin analogs, octreotide and lanreotide, constitute the standard of care for the medical treatment of NETs. They have a highly improved stability compared to natural somatostatin, allowing for long-term treatment. They are, however, not true somatostatin mimics, as they have a restricted receptor affinity profile, with high sst₂ affinity, lower sst₃ and sst₅ affinity and no sst₁ or sst₄ affinity (Table 2)¹²². Both these peptides were developed before knowledge of sst subtypes and were selected for their high potency to inhibit growth hormone secretion^{123,124}. Only much later, in 1992, were sst subtypes identified. It was actually octreotide itself that gave the first clue that more than one sst subtype existed¹²⁵ and that octreotide was not a true somatostatin mimic, since it was unable to bind to all sst receptors^{126,127}. The exact receptor subtype profile of octreotide was established later. Luckily, NETs express predominantly sst₂, as summarized above.

These drugs produce a strong inhibition of tumoral hormone secretion in sst₂ expressing NET tumors, thus reducing or abolishing the symptoms due to hormone overproduction in patients^{11,128}. Also a growth inhibition has been identified in many tumors, as shown by the phase III, randomized, placebo controlled PROMID study in GEP tumors¹²⁹, as well as in acromegalics^{11,130}. In other types of tumors, non-randomized studies have failed to detect much tumor inhibition^{131,132}. Unfortunately, not all NET tumors are responsive to these somatostatin analogs. Moreover, some responsive tumors escape after prolonged somatostatin analog treatment and become resistant to treatment. This has been observed with gastrointestinal NETs and with GH-producing pituitary adenomas^{10,12}.

New compounds: multireceptor ligands

To optimize and expand the clinical applications of somatostatin analogs, multi-somatostatin analogs have been developed. These analogs bind to several somatostatin receptor subtypes

and include SOM230 (pasireotide), with high affinity for sst₂, sst₃ and sst₅, (but lower affinity for sst₁), and KE108, with high affinity for all five somatostatin receptors (Table 2)^{122,133}. Interestingly, although multi-somatostatin analogs were originally designed to mimic natural somatostatin actions, it turned out that both pasireotide and KE108 exhibit signaling properties distinct from somatostatin⁸⁶ and are biased agonists rather than true somatostatin mimics (Table 2). Pasireotide was recently approved for the treatment of Cushing's disease and is presently being evaluated in clinical trials for the treatment of acromegaly and octreotide-resistant carcinoids^{122,134}. Ideally, target tumors should express sst₅, the receptor subtype with highest affinity for pasireotide. Pasireotide has been reported to reduce and sometimes normalize urinary free cortisol in a subgroup of patients with Cushing's disease¹³⁵ and to control diarrhea and flushing in about 30% of patients with advanced NETs whose carcinoid syndrome symptoms were resistant to octreotide¹³⁴.

The peptide analog somatoprim (DG3173 or PTR-3173), with high affinity for sst₂, sst₄, and sst₅ (Table 2),¹³⁶ has shown some promising results in pituitary tumors. It is reported to inhibit GH-secretion from human pituitary adenomas that are responsive to octreotide and, in some cases, even from those that are unresponsive to octreotide¹³⁷.

Dopastatin (BIM 23A760) is a chimeric compound that covalently links two pharmacologically active moieties: a peptide somatostatin analog and a dopamine agonist¹³⁸. This compound was designed to produce synergistic effects by simultaneously activating two inhibitory GPCRs that can hetero-dimerize, namely the sst₂ and D2 receptors. In vitro, BIM23A760 binds to sst₂ with at least a 100-fold greater affinity than either to other sst subtypes or to D2 dopamine receptors. Although initially promising, the compound unexpectedly lacked efficacy at somatostatin receptors during chronic administration to acromegalic patients and this Phase IIb study was therefore discontinued early^{139,140}. However, development of new compounds combining somatostatin and dopamine activity into a single molecule is continuing.

Mode of action

Agonists that activate GPCRs are broadly classified into three groups (i) **full agonists** that can effectively trigger the same signal transduction machinery and regulatory pathways as the native ligand; (ii) **partial agonists** that are less effective than full agonists at producing downstream signaling and regulatory actions by a receptor; and (iii) **biased agonists** that effectively elicit only a subset of a receptor's multiple actions, differentially activating signaling cascades or regulatory events¹⁴¹. A systematic analysis of somatostatin analogs first identified biased agonism at the sst_{2A} receptor with the finding that a nonpeptide analog (L779,976) had full activity for inhibiting adenylyl cyclase but was unable to promote stable association of sst_{2A} with arrestin or to maximally induce receptor internalization^{96,142}. In contrast octreotide was a full agonist at sst_{2A}¹⁴². Subsequent studies showed that pasireotide and KE108 are similar to L779,976 and behave as biased agonists at sst_{2A}. Both peptides fully inhibit adenylyl cyclase but are less effective than the native ligand at stimulating receptor internalization and arrestin recruitment^{86,108,143}. In addition pasireotide and KE108 induce a different pattern of sst_{2A} phosphorylation than does octreotide or somatostatin (Figure 1)^{104,108}. Strikingly, pasireotide and KE108 antagonize somatostatin induced calcium mobilization and ERK phosphorylation via the sst_{2A} receptor⁸⁶ demonstrating that the intracellular signaling pathways activated by these ligands differ from those regulated by the native hormone.

These observations raise some interesting dilemmas. Whereas the effectiveness of pasireotide and KE108 could be increased compared to octreotide when treating some NETs because of their ability to activate multiple sst receptor subtypes and reduced stimulation of sst_{2A} receptor internalization, their effectiveness may be compromised in other instances

because of their inability to fully activate all signaling pathways via sst_{2A}, the major receptor subtype expressed in these tumors. Further, their receptor-selective or biased agonist activity may influence specific physiological pathways in humans differently from octreotide. For example, the more frequent occurrence of hyperglycemia during treatment with pasireotide than with octreotide^{144,145} may result from the different affinities of these drugs for sst receptor subtypes or from the biased agonist property of pasireotide at sst_{2A} which is expressed in human islets^{146,147}. Because biased agonism is now known to occur at several sst receptor subtypes (Table 2)⁹⁶ it must be considered in the development of more effective drugs for the treatment of NETs⁹⁶.

Development of radiolabeled compounds

Established compounds

For somatostatin receptor tumor targeting, radioactive ligands based on the octreotide or lanreotide backbone are now available in the clinic. Details on general nuclear medicine principles and strategies needed for the development of such radioligands can be found in a recent review⁵². The first commercially available agent was ¹¹¹In-diethylenetriaminepentaacetic acid (DTPA)⁰-octreotide (Octreoscan) (Table 1), originally designed for scintigraphy. This remains the only FDA-approved somatostatin imaging agent. New developments have been the DOTA-coupled radio-peptides [DOTA⁰,Tyr³]-octreotide (DOTATOC) and [DOTA⁰,Tyr³,Thr⁸]-octreotide (DOTATATE) both with a high sst₂ affinity. These two sst₂-preferring analogs are successfully and widely used in nuclear medicine¹⁴⁸ even though they are not yet FDA-approved. More recently, development of somatostatin-based radioligands with a broader receptor subtype affinity profile has been initiated⁵². Such compounds may not only extend the range of targeted cancer candidates but also increase net tumor uptake when several receptor subtypes are present in a tumor^{26,149}. Several compounds have therefore been developed that show high affinity to sst₂, sst₃, and sst₅, the best of them containing the unnatural amino acids 1-naphtyl-alanine (1-Nal) such as DOTA-NOC^{150,151}. The clinical characteristics of these compounds are a more precise localization of somatostatin receptor expressing tumors and a higher tumor uptake for more efficient subsequent radiotherapy. DOTA-TOC and DOTA-TATE are better targeting agents than Octreoscan due to their higher sst₂ affinity. DOTA-NOC is better than DOTA-TATE to localize NET due to its wider receptor profile¹⁵².

New compounds: multi-somatostatin DOTA-KE108 and DOTA-SOM230

Attempts have also been made to develop multi-somatostatin radioligands using the backbone of pasireotide or KE108. KE108 modified by coupling DOTA to the N-terminus and labeled with ¹¹¹Indium (In-KE-88)¹⁵³ is still able to bind with high affinity to all five somatostatin receptor subtypes, but its expected imaging capabilities are not fulfilled, because ¹¹¹In-KE-88 has a disappointingly low *in vitro* internalization and *in vivo* uptake in sst₂ tumors¹⁵³. Similarly, the radioactive pasireotide derivative DOTA-SOM230¹⁵⁴ was designed to perform scans that would identify NET patients suitable for pasireotide treatment. No human data are available yet. Interestingly, pan-somatostatin radioligands based on native somatostatin have recently been developed and show better sst_{2A} internalization capabilities than synthetic pan-somatostatins such as KE108 or SOM230^{155,156}.

New compounds: sst antagonists

Although agonists that readily internalize represent excellent targeting agents *in vivo*¹⁵⁷, it has recently been shown that potent somatostatin receptor antagonists, known to poorly internalize into tumor cells, can visualize tumors *in vivo* as well or even better than agonists¹⁵⁸. This observation was found in animal experiments both for sst₂- and for sst₃-

selective somatostatin analogs¹⁵⁸. In both sst₂- and sst₃-expressing tumors the antagonists bound to many more receptor sites than the agonists¹⁵⁸. This is because agonists preferably recognize the active state of receptors, whereas antagonists bind with high affinity to both active and inactive receptor states. Since receptors are primarily in the inactive state in cells, agonist binding will represent a fraction of what can be labeled with an antagonist. A pilot clinical trial with radiolabeled DOTA-linked sst₂ antagonist recently confirmed the animal data¹⁵⁹. More effective radiolabeled sst₂ antagonists for tumor imaging are in development¹⁶⁰.

Mode of action

Historically, the use of radioligands for tumor imaging was based on the ability of these peptide agonists to undergo extensive receptor-mediated internalization and prolonged intracellular retention. The recent observation that radiolabeled derivatives of biased agonists such as KE108 and pasireotide show reduced sensitivity for NET imaging is consistent with this hypothesis, because they are less effective at stimulating sst_{2A} internalization than full agonists such as radioactive DOTA-TATE or DTPA-octreotide^{108,143}. The observation that some radiolabeled somatostatin antagonists provide greater sensitivity than agonists for NET imaging clearly demonstrates that receptor-mediated internalization of radioligand does not represent the unique principle for tumor imaging. Further studies are needed to define the optimal combination of binding kinetics, receptor affinity, and receptor-mediated internalization for a compound to provide sensitive and specific *in vivo* tumor visualization.

Concluding remarks

The results summarized here show that our understanding of the complexity of somatostatin analog action has exploded in the last few years with the elucidation of many of the molecular details involved in somatostatin receptor signaling and regulation as well as by the characterization of biased somatostatin agonists. Development of the currently used FDA-registered somatostatin analogs was focused on only a few parameters of receptor activity at a time when far less was known about the somatostatin receptor family. Despite the tremendous value of the established drugs for many patients with NETs a great need exists for compounds to treat those tumors that are unresponsive to the current drugs or that become insensitive during prolonged therapy. Thus, although important aspects of somatostatin receptor function remain to be elucidated, opportunities now exist to exploit our increased understanding of somatostatin receptor signaling and regulation. For example it will be interesting to determine whether biased agonists that produce little or no sst_{2A} receptor internalization exhibit enhanced efficacy at this receptor compared to unbiased agonists, especially in tumors with low receptor levels, and whether they induce the counter-regulatory mechanisms that cause tumors to escape from long term therapy. Even though at present we do not know which signaling pathways are the most important for analog inhibition of tumor growth, the agonist bias exhibited by several somatostatin analogs indicate that multiple post-receptor signaling pathways must be investigated with new compounds in order to develop more effective somatostatin receptor directed agents that control tumoral growth and hypersecretion as well as for use in tumor imaging and radiotherapy.

Glossary

Neuroendocrine tumors (NETs)

Tumors that originate from endocrine cells in endocrine organs (pituitary, thyroid, pancreas) or from neuroendocrine cells in disseminated endocrine tissues such as lung or gut. They often

	secrete high levels of biologically active hormones or neurotransmitters that produce characteristic symptoms and disease
Acromegaly	Endocrine disturbance caused by a growth hormone producing somatotroph pituitary adenoma
Cushing's disease	Endocrine disturbance caused by an ACTH-producing corticotroph pituitary adenoma
Octreotide lanreotide	First generation FDA-approved somatostatin analogs
Pasireotide (=SOM230)	Second generation somatostatin analog with multiple sst receptor affinity
Octreoscan =¹¹¹In-DTPA-octreotide	First generation FDA-approved somatostatin analog for tumor imaging
Agonists	are compounds that activate receptors to produce a characteristic set of biological effects
Full agonists	are compounds that act at receptors to effectively trigger the same signal transduction machinery and regulatory pathways as the native ligand
Partial agonists	are compounds that are less effective than full agonists at producing the downstream signaling and regulatory actions by a receptor.
Biased agonists	are compounds that effectively elicit only a subset of a receptor's multiple actions for signaling or for receptor regulation.
Antagonists	bind to receptors but do not activate them and hence are characterized by their ability to block agonist binding and action at a receptor. One needs to distinguish between
neutral antagonists	which have no activity on their own but will block the effects of an agonist as opposed to
inverse agonists	which will inhibit receptor activity in the absence of agonist, if the receptor exhibits constitutive activity. A neutral antagonist does not distinguish between the active or inactive state of the receptor, whereas an inverse agonist prefers the inactive state. While somatostatin receptor agonists, biased agonists and neutral antagonists are known, no somatostatin receptor inverse agonists have been described

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Highlights

Overview of somatostatin receptor signaling

Role of sst receptor phosphorylation in the mechanisms of sst receptor regulation

Discussion of somatostatin analogs used for cancer therapy and diagnosis

Biased agonism at sst receptors: surprises and opportunities

Figure 1. Phosphorylation of the ss_{2A} receptor

Panel A. Diagram of the potential and demonstrated phosphorylation sites in the ss_{2A} receptor. The structure depicts the carboxy-terminal portion of the rat ss_{2A} receptor. Identified phosphorylation sites are colored orange or torquoise whereas potential, but not yet identified, phosphorylation sites are shown in grey. The rat and human ss_{2A} receptors differ only by the two residues shown in purple (AE in $rs_{ss_{2A}}$ ->TD in $hs_{ss_{2A}}$). Somatostatin stimulates the GRK-catalyzed phosphorylation of all orange and torquoise Ser and Thr residues whereas protein kinase C activation only stimulates the phosphorylation of the sites shown in orange. (Modified from ¹⁰³).

Panel B Octreotide treatment of patients stimulates ss_{2A} phosphorylation in NETs. Immunohistochemistry was carried out with either an ss_{2A} antibody that is insensitive to receptor phosphorylation (UMB-1) (Left panels) or an antibody specific for pSer341/343 (Ra-1124) (Right Panels). The top panels show immunohistochemistry of a NET from an octreotide-treated patient and identifies phosphorylated, internalized receptors. The lower panels show immunohistochemistry of a NET from an untreated patient and identifies unphosphorylated, cell surface receptors. (Bars = 0.1 mm) ¹¹¹.

Panel C Agonist specific phosphorylation of the sst_{2A} receptor. CHO cells expressing the rat sst_{2A} receptor were incubated at 37°C for 30 min with varying concentrations of either somatostatin (left panel) or KE108 (right panel). Receptor phosphorylation was measured at steady state using phospho-site-specific monoclonal antibodies with an ELISA. Somatostatin stimulates the phosphorylation at both sets of phosphosites with similar potency (Left panel). KE108 is both less efficacious at stimulating receptor phosphorylation than somatostatin and, in addition, stimulates phosphorylation at pSer341-343 more potently than at pT353-354. (Adapted from¹⁰⁸¹⁰⁸).

Table 1

NET Tumors expressing somatostatin receptors

Tumors with predominance of sst₂	Tumors with predominance of other ssts (with or without sst₂)
Pituitary adenomas (GH, TSH)	Selected GH pituitary adenomas (sst ₅ ; sst ₂ + sst ₅) ACTH pituitary adenomas (sst ₅) Inactive pituitary adenomas (sst ₃)
Gastroenteropancreatic neuroendocrine tumors	Selected gastroenteropancreatic neuroendocrine tumors (insulinomas: sst ₁ , sst ₅ , with or without sst ₂)
Lung neuroendocrine tumors	Selected lung neuroendocrine tumors
Pheochromocytomas	Medullary thyroid carcinomas
Paragangliomas	

Table 2

Affinity and agonist bias of somatostatin analogs.

	Binding Affinity for Somatostatin Receptor Subtype				
	sst ₁	sst ₂	sst ₃	sst ₄	sst ₅
Somatostatin Analog					
Peptide Agonist					
Somatostatin 14	++	++	++	++	++
Somatostatin 28	++	++	++	++	++
Octreotide	–	++	+	–	+
Lanreotide	–	++	–	–	+
Pasireotide	–	+ b)	+	–	++
KE108	++	++ b)	++	++	++
Somatoprim	–	++	–	++	++
Nonpeptide Agonist	++	++	++	++	++
L779,976	–	++ b)	–	–	–
L817,818	–	–	–	–	++

The table shows the relative affinities of peptide and nonpeptide agonists for each somatostatin subtype. Data were obtained from membrane binding studies using cells transfected with individual receptor subtypes. Since the absolute affinities obtained in different reports depend on the conditions of the assay used, we have expressed data from the literature as the *relative affinities* of each analog for different sst subtypes.

++ indicates the highest affinity of an analog for sst receptors.

+ indicates affinity of an analog that is between 10 – 50 fold lower than its highest affinity for an sst receptor subtype.

– indicates affinity of an analog that is at least 50-fold lower than its highest affinity for an sst receptor subtype.

Compounds acting on the sst₂ receptor behave either as full agonists or as biased agonists (shown by b) (see⁹⁶ for review). Except for KE108 and L817,818, which behave as biased agonists at sst₅ based on their inability to induce receptor internalization, it is not known whether other analogs act as full or biased agonists at the different sst subtypes to which they bind.