Somatostatin receptor biology in neuroendocrine and pituitary tumours: part 1 – molecular pathways

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Abstract

Neuroendocrine tumours (NETs) may occur at many sites in the body although the majority occur within the gastroenteropancreatic axis. Non-gastroenteropancreatic NETs encompass phaeochromocytomas and paragangliomas, medullary thyroid carcinoma, anterior pituitary tumour, broncho-pulmonary NETs and parathyroid tumours. Like most endocrine tumours, NETs also express somatostatin (SST) receptors (subtypes 1–5) whose ligand SST is known to inhibit endocrine and exocrine secretions and have anti-tumour effects. In the light of this knowledge, the idea of using SST analogues in the treatment of NETs has become increasingly popular and new studies have centred upon the development of new SST analogues. We attempt to review SST receptor (SSTR) biology primarily in neuroendocrine tissues, focusing on pituitary tumours. A full data search was performed through PubMed over the years 2000–2009 with keywords ‘somatostatin, molecular biology, somatostatin receptors, somatostatin signalling, NET, pituitary’ and all relevant publications have been included, together with selected publications prior to that date. SSTR signalling in non-neuroendocrine solid tumours is beyond the scope of this review. SST is a potent anti-proliferative and anti-secretory agent for some NETs. The successful therapeutic use of SST analogues in the treatment of these tumours depends on a thorough understanding of the diverse effects of SSTR subtypes in different tissues and cell types. Further studies will focus on critical points of SSTR biology such as homo- and heterodimerization of SSTRs and the differences between post-receptor signalling pathways of SSTR subtypes.

Keywords: somatostatin • somatostatin receptor • molecular biology • receptor signalling

Introduction

Somatostatin (SST) is a cyclic peptide and a notable physiological regulator of neuroendocrine function across multiple organ systems [1]. It is produced by the hypothalamus, throughout the central nervous system (CNS), and in different peripheral organs including gastrointestinal tract (GIT) and pancreas [1–3]. The SST gene is located in chromosome 3q28 and encodes a 116 amino acid preprohormone (preprosomatostatin) which contains the 92 amino acid SST prohormone (prosomatostatin) [4]. Prosomatostatin is the precursor peptide of the two biologically active SST forms: 14 amino acid long SST-14 and amino-terminus extended SST-28 [5]. The biological roles of the two SST isoforms strongly overlap and the relative proportions of SST-14 to SST-28 change between different tissues [6, 7]. SST-14 is the predominant form in the brain (including the hypothalamus), whereas SST-28 is the major form in the GIT, especially the duodenum and jejunum [8]. SST has a broad range of biological actions including inhibition of exocrine secretions (gastric acid production, pancreatic

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The somatostatin receptor as a GPCR

GPCRs are characterized by a core of seven transmembrane α-helices connected by three intra- and three extracellular loops [4]. The effector proteins of GPCRs are heterotrimeric G proteins, which are composed of α, β and δ subunits. SSTRs belong to the GPCR superfamily and rhodopsin-like GPCR subclass [41]. SSTRs first activate a G protein which then modulates several downstream second messenger systems after binding [42]. Activated G protein depends on the SSTR subtype, and three isoforms of the inhibitory G proteins (Goα1,-3) are all coupled to the different receptors from diverse species, and hence these data need careful interpretation [12, 25]. Third, it is now evident that GPCR function is highly dependent upon the cellular environment in which GPCRs are expressed, resulting in tissue-specific responses, even those originating from the same GPCR subtype [27]. In recent years, researchers have been obliged to find correlations between recombinant and native receptors and to ascribe specific functions to individual receptor subtypes in their own environment [28]. Fourth, although receptor-specific SST analogues have been generated, the discovery of receptor homo- and heterodimerization has added another level of complexity to the understanding of post-receptor events. SSTRs can form heterodimers with dopamine receptors [29], other SSTR subtypes [30], opioid receptors [31] or epidermal growth factor (EGF) receptors [32], which generate receptor oligomers with unique pharmacological profiles. However, it still has not been elucidated whether all these potential interactions do actually occur in the native cell, and, if they occur, what their precise functional relevance and importance are [33].

Fifth, it is known that, SSTR coupling to a given pathway can be strongly influenced by the ligand used [34–36], which is due to distinct conformations of the receptor/ligand complexes [37] induced by different SSTR analogues. Sixth and lastly, internalization, desensitization and/or receptor crosstalk [38, 39] are all known to occur in SSTRs, which may well have an impact on post-receptor signalling events. In addition, at the cellular level, crosstalk between signalling pathways may occur. All these factors might also explain the different downstream effects observed in SSTR signalling in different experimental settings.

Neuroendocrine tumours (NETs) are derived from a wide spectrum of different cell population and include, e.g. carcinoids, pituitary tumours, phaeochromocytomas, paragangliomas, medullary thyroid carcinomas and gastroenteropancreatic tumours such as insulinomas, gastrinomas and VIPomas [40]. This review will focus on the anti-tumour actions of SST, SSTR signalling pathways and the significance of our increasing knowledge on SST tissue receptor biology in terms of imaging and treatment of NETs. Cortistatin, a natural SST ligand which has both similar and different actions compared to SST, will not be included in this review (for detailed information regarding roles of cortistatin and SST, see the review by Gahete et al. recommended).
SSTRs [43]. However, using an mRNA anti-sense strategy SSTR2 was reported to couple to the $G_{o_{16}}/G_{o_{14}}$ complex to control Ca$^{2+}$ channel activity in pituitary cells [44, 45], and SSTR3 was also identified to couple to $G_{o_{16}}$ and $G_{o_{14}}$ [46]. Recent evidence indicates that, within the GPCR superfamily, there are mechanisms to increase receptor variability involving generation of splicing variants with less than seven transmembrane domains (TMDs) [47, 48]. Such truncated receptors, which may possess their own function or regulate the function of their respective long, canonical receptor isoforms, are frequently associated with tumour pathology [47, 48]. The family of SSTRs is known to uniquely comprise intronless genes except SSTR2. The only SST subtype variants known so far are the long (SSTR2A) and short (SSTR2B) isoforms of the SSTR2 gene, generated due to the presence of a cryptic splice site [49, 50]. Recently, Durán-Prado and colleagues have reported the first evidence for the existence of two functional human SSTR5 truncated isoforms of five and four TMDs, termed sst5TMD5 and sst5TMD4. These isoforms showed a unique expression pattern in normal tissues as well as in different pituitary tumour types, and display distinct functional responses to SST [51].

Binding of SST to its corresponding GPCR (SSTRs) triggers a cyclical activation and inactivation process in the G protein whereas the signal is transduced intracellularly. GPCRs are components of multiprotein networks, called ‘receptosomes’, which are organized around scaffolding proteins [28]. In this regard, GPCR-interacting proteins (GIPs), regulators of G-protein signalling (RGS) and GPCR kinases (GRKs) are the main proteins that are known to effect GPCR signalling after ligand binding. GIPs are transmembrane or cytosolic proteins which may alter either binding or functional responses of GPCR resulting in an abundance of potential receptor–protein connections [52]. RGS proteins have been found to regulate GPCR responses by binding to and stimulating the GTPase activity of the receptor-activated GTP-bound $G_{o}$ [53]. Although GIPs and RGS have been found to interact with distinct recombinant SSTRs [54, 55], the real functional role of these complexes in native systems with respect to signal transduction is still not very well documented. SST binding is known to be rapidly followed by phosphorylation of SSTR1, SSTR2A and SSTR3 by GRKs [56–58]. The GRK family consists of six serine–threonine kinases that specifically bind to and phosphorylate agonist-activated GPCRs [59, 60]. GRKs play key roles in the fundamental pathways leading to phosphorylation-dependent GPCR desensitization, endocytosis, intracellular trafficking and resensitization, as well as in the modulation of important intracellular signalling cascades by GPCR [61]. Receptor phosphorylation by GRKs results in the recruitment of cytoplasmic proteins called arrestins in a receptor subtype-specific manner [62–64]. Arrestins are proteins involved in intracellular vesicle trafficking. In general, binding of arrestins to an activated, phosphorylated GPCR blocks further interaction between the receptors and G proteins, and thus results in the desensitization of G-protein mediated signalling. In addition, arrestins play a key role in directing GPCRs to clathrin-coated vesicles and thus preparing them for endocytosis [65]. The consequent receptor internalization removes GPCRs from the cell surface so that they are no longer available for agonist stimulation. This is a second mechanism for GPCR desensitization, and the concentration of different arrestins as well as GRK subtypes influences the extent of receptor internalization [66]. However, not all SSTRs internalize equally after agonist binding [67, 68], SSTR2, SSTR3 and SSTR5 are internalized to a much higher extent than SSTR1 or SSTR4 after stimulation [25]. However, SSTR2 is rapidly recycled to the plasma membrane and does not enter any degradative pathway [25]. Desensitization and internalization are not induced by SSTR2 antagonists [69], but there is a growing body of evidence on agonist-induced desensitization and/or internalization of the SSTR2 subtypes, which have been assessed in several cell lines and tissues [21, 67, 70]. After activation and internalization through a clathrin-dependent pathway, SSTR3 and SSTR5 rapidly dissociate from arrestin and undergo ubiquitin-dependent lysosomal degradation, preventing plasma membrane recycling [64]. Arrestins have also been shown to play an important role in G-protein independent GPCR signalling by recruiting cytosolic molecules to the receptor–arrestin complex [71]. The precise role of receptor phosphorylation and arrestin binding in the desensitization, trafficking and signalling of different SSTR subtypes has not been fully elucidated as yet, but they probably play a critical role in SST function [65]. As desensitization is known to occur a few minutes after ligand binding, it is expected to have a strong impact in experimental settings in which second messenger systems are being evaluated [56, 72, 73].

Not only the nature of the SSTR subtypes present in a particular cellular environment but also the nature of the agonist is a critical determinant of the tissue response to SST [65]. In GPCRs, including SST signalling, a classical two-state model was accepted for a long time. This model proposed that the binding of an agonist shifted an equilibrium from ‘the inactive’, basal conformation of a receptor to ‘the active’ conformation by stabilizing the latter [74, 75]. According to this model the relative potencies of agonists for inducing any two biological effects from a single receptor would be the same [74, 75]. In studies evaluating receptor dynamics, analogue activities were generally retrieved from only one or two measurements; such as inhibition of hormone secretion or modulation of second messenger production [65]. Then these measurements were used to indicate the overall potency and efficacy of the analogues under investigation [65]. However, with this model it was not possible to explain the effect of many agonists which might induce only some of the possible responses following receptor activation, or to activate two effectors with different relative potencies [65]. New terms including ‘functionally selective agonism’ have been used to describe the idea that agonists can selectively activate different signalling pathways and responses via a single GPCR [65]. Described in detail in an elegant review by Schonbrunn, such selective signalling is explained by a model in which agonists not only exhibit different affinities for a receptor, but they also stabilize different active receptor conformations [65]. These differentially activated receptor structures determine the interaction of the receptor with the
In the absence of ligands, D2R forms dimers and SSTR5 remains monomeric. Treatment with ligands for any agonist-dependent dissociation of self-associated human SSTR2 stably expressed in CHO-K1 and HEK-293 cells SSTR2A and SSTR3 exist as homodimers at the plasma membrane in HEK-293 cells. Heterodimerization of SSTR2A and SSTR3 results in a new receptor with a pharmacological and functional profile resembling that of the SSTR2A. Porcine SSTR2 is a potent inhibitory receptor displaying unique features of agonist-dependent dimerization, dissociation, internalization and re-association. Cotransfection of CHO-K1 cells results in an increased ligand affinity for SST and heterodimerization between human SSTR1 and SSTR5. Direct evidence for heterodimerization of SSTR2A and MOR1 in HEK-293 cells, interaction does not alter signalling properties, induces a cross-modulation of the desensitization and internalization of both receptors. SSR2A and SSTR3 exist as homodimers at the plasma membrane in HEK-293 cells. Heterodimerization of SSTR2A and SSTR3 results in a new receptor with a pharmacological and functional profile resembling that of the SSTR2A. Porcine SSTR2 is a potent inhibitory receptor displaying unique features of agonist-dependent dimerization, dissociation, internalization and re-association. Agonist-dependent dissociation of self-associated human SSTR2 stably expressed in CHO-K1 and HEK-293 cells occurring in a concentration-dependent manner. Human SSTR5 could both homodimerize and heterodimerize with human SSTR1 in the presence of SST, activation of human SSTR5 but not human SSTR1 is necessary for heterodimeric assembly in live cells. Human SSTR1 remained monomeric when expressed alone regardless of agonist exposure in live cells. Heterodimerization between human SSTR4/human SSTR5, but not between human SSTR4/human SSTR1.

Cytoplasmic proteins, including G proteins, kinases and phosphatases, and additionally its regulation by GRKs or arrestins [65]. Thus, this model predicts that in any given cell type, the relative potencies or efficacies of agonists for various biological effects may differ even though their actions are triggered by a single receptor subtype [65]. Meanwhile, it is clear that signalling by, and regulation of, SSTR2, SSTR4 and SSTR5, are sensitive to the nature of the specific activating agonist [65]. Thus, a basic and simple assumption that all agonists are functionally equivalent and produce the same spectrum of effects at individual SSTRs cannot be accepted any longer [65].

Receptor homodimerization and oligomerization of SSTRs are other critical issues, as these processes have been shown to modify some properties of the receptors, such as ligand binding affinity, signal transduction, desensitization and up-regulation [30, 76–78]. It has been demonstrated that (excepting SSTR1) SSTRs transfected in different cell lines form homodimers and some form heterodimers with other SSTR subtypes and with different families of GPCR [29, 30, 79] (Table 1). Furthermore, GPCR dimerization is also regulated by ligand binding, yet not all receptors function similarly [80, 81]. Even in the same family, the behaviour of receptors can be opposite, as reported for SSTR2 and SSTR5, whose dimerization decreases or increases, respectively, after ligand binding [82, 83]. Interestingly, interactions among SSTRs, in other words heterodimerization between each other, seem to be highly selective, as not all the potential combinations occur. Nevertheless, not all the possibilities of SSTR heterodimers have been explored, because the interactions characterized so far are between murine SSTR2 and SSTR3 [76], human SSTR1 and SSTR5 [30], human SSTR4 and SSTR5 [84] and human SSTR2 and SSTR5, some being demonstrated under certain conditions [85]. Heterodimerization among these SSTR subtypes causes both distinct effects on SSTR functioning and modifies the endocytic process of these receptors [33]. An example for this may be the interaction between SSTR2 and SSTR3, which results in both inactivation and impaired internalization of SSTR3 [76]. Cotransfection of human SSTR1 and SSTR5 in Chinese hamster ovary (CHO)-K1 cells results in an increased ligand affinity for SST [30]. On the other hand, internalization of this heterodimer is better in response to a specific SSTR1 ligand and smaller in response to a specific SSTR5 ligand compared to monomer forms of these receptors [30]. Stabilization of human

### Table 1 Summary of the studies on receptor oligomerization

| Study            | Findings                                                                                                                                                                                                 |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Rocheville et al. [29] | In the absence of ligands, D2R forms dimers and SSTR5 remains monomeric. Treatment with ligands for any of the receptors induces heterodimerization of D2R and SSTR5.                                                   |
| Rocheville et al. [30] | Cotransfection of CHO-K1 cells results in an increased ligand affinity for SST and heterodimerization between human SSTR1 and SSTR5.                                                                       |
| Pfeiffer et al. [31] | Direct evidence for heterodimerization of SSTR2A and MOR1 in HEK-293 cells, interaction does not alter signalling properties, induces a cross-modulation of the desensitization and internalization of both receptors.               |
| Pfeiffer et al. [76] | SSTR2A and SSTR3 exist as homodimers at the plasma membrane in HEK-293 cells. Heterodimerization of SSTR2A and SSTR3 results in a new receptor with a pharmacological and functional profile resembling that of the SSTR2A. |
| Duran-Prado et al. [78] | Porcine SSTR2 is a potent inhibitory receptor displaying unique features of agonist-dependent dimerization, dissociation, internalization and re-association.                                               |
| Grant et al. [82] | Agonist-dependent dissociation of self-associated human SSTR2 stably expressed in CHO-K1 and HEK-293 cells occurring in a concentration-dependent manner.                                                   |
| Grant et al. [83] | Human SSTR5 could both homodimerize and heterodimerize with human SSTR1 in the presence of SST, activation of human SSTR5 but not human SSTR1 is necessary for heterodimeric assembly in live cells. Human SSTR1 remained monomeric when expressed alone regardless of agonist exposure in live cells. |
| Sormvanshi et al. [84] | Heterodimerization between human SSTR4/human SSTR5, but not between human SSTR4/human SSTR1.                                                                                                             |
| Grant et al. [85] | SSTR2 and SSTR5 heterodimerization. Stabilization of human SSTR2 and SSTR5 heterodimers was shown to occur following selective activation of SSTR2 but not human SSTR5 or their concurrent stimulation. Heterodimerization increases the recycling rate of internalized SSTR2 by destabilizing its interaction with -arrestin. |
| Sharif et al. [86] | Increased recycling rate and a greater propensity of SSTR2 to signal and induce growth inhibition following its heterodimerization with SSTR5.                                                                 |
| Baragli et al. [87] | Interaction between the D2R and the human SSTR2 does not seem to occur under basal conditions, but is induced by ligand binding. SSTR2 pharmacology and signalling are not altered by heterodimerization with the D2R, but its endocytic rate is increased as a consequence of this interaction. Ligand interaction results in a heterodimer with an increased affinity for dopamine and increased signalling via the D2R. |

*Abbreviations: CHO-K1, Chinese hamster ovary cells; D2R, dopamin receptor 2; (HEK)-293, human embryonic kidney cells; MOR1, μ-opioid receptor.*
SSTR2 and SSTR5 heterodimers was shown to occur following selective activation of SSTR2 but not human SSTR5 or their concurrent stimulation [85]. Moreover, an increased recycling rate and a greater propensity of SSTR2 to signal and induce growth inhibition following its heterodimerization with SSTR5 were observed [86]. Heterodimerization of SSTRs is not limited to occur between SSTR subtypes. Murine SSTR2 has been shown to heterodimerize with the µ-opioid receptor, MOR1, in cotransfected human embryonic kidney (HEK)-293 cells. Although this interaction does not alter their signalling properties it induces a cross-modulation of the desensitization and internalization of both receptors [31]. Another example is heterodimerization of the dopamine receptor 2 (D2R) and SSTR5. In the basal state, in the absence of ligands, these receptors do not interact: D2R forms dimers and SSTR5 remains monomeric [29]. However, treatment with ligands for any of the receptors dramatically induces heterodimerization and enhanced inhibition of cAMP formation occurs [29]. Binding of quinpirole, a D2R agonist, increases binding affinity of SST by 3000%, whereas a D2R antagonist, sulpiride, decreases binding affinity for the peptide by 80% [29]. An interaction between the D2R and the human SSTR2 has been also reported, which does not seem to occur under basal conditions, but is induced by ligand binding [87]. Their interaction results in a heterodimer with an increased affinity for dopamine and increased signalling via the D2R receptor [87]. However, SSTR2 pharmacology and signalling are not altered by heterodimerization with the D2R, but its endocytic rate is increased as a consequence of this interaction [87]. Additionally, heterodimerization of SSTRs appears to confer the properties of one receptor subtype onto the dimer; SSTR1 is internalized and up-regulated by octreotide when heterodimerized with SSTR5 [30], and the SSTR3/SSTR2A heterodimer has the pharmacological effects of the SSTR2A receptor [76]. However, because of the fact that the dimerization of SSTRs has been observed in recombinant systems and experimental settings, it is not clear as to what degree it can be extended to native systems [33].

Anti-tumour effects of somatostatin

There are a number of mechanisms responsible for the anti-tumour actions of SST [88]. These are the direct blockade of cell cycle progression through the activation of phosphatidylinositol phosphatases (PTPs), the indirect influence on tumour growth mediated by the inhibition of the production of growth factors that sustain tumour development, and an anti-angiogenic effect that involves the regulation of the activity of both endothelial cells and monocytes. Some of the key points in direct and indirect anti-tumour actions of SST will be summarized in this section.

In general, SSTR1, SSTR2, SSTR4 and SSTR5 produce cytostatic effects through similar downstream effector pathways, whereas SSTR2 and SSTR3 induce pro-apoptotic (cytotoxic) signals when activated [79, 89, 90] (Table 2) (for detailed information see next section). SST suppresses insulin-like growth factor (IGF)-I serum levels through a direct inhibition of its gene expression or through the inhibition of GH secretion from pituitary and the consequent reduction of the GH-stimulated IGF-1 production in liver. Moreover, SST analogues inhibit the secretion of autocrine/paracrine effectors of tumour cell survival such as the IGF-1 and -2, EGF, interleukin-6 and the transforming growth factor family. The attenuation of secretion of such survival factors in the tumour microenvironment accordingly establishes an autocrine/paracrine anti-proliferative effect [4].

Stable transfection of pancreatic cancer cell lines with human SSTR2A was reported to induce an overexpression of the connexins 26 and 43, which resulted in formation of functional intercellular gap junctions and hence restored contact inhibition of cell proliferation [91]. During the initial stages of metastatic development, malignant cells must enter the lymphatic and systemic circulation by detaching from adjacent cells and then attaching to and disrupting the endothelial basement membrane [92, 93]. SST has been shown to reduce the adhesion of carcino-sarcoma cells to blood vessels and thus attenuate the metastatic potential of these tumours [94]. For the anti-angiogenic activity of SSTRs, three signal pathways were identified: inhibition of endothelial cell activity (proliferation, migration and invasion), inhibition of the synthesis and secretion of pro-angiogenic factors such as vascular endothelial growth factor and basic fibroblast growth factor, and inhibition of monocyte activation. Very recently, thrombospondin-1 was defined as a critical effector of the inhibitory role of SSTR2 on the neoangiogenesis and oncogenesis induced by pancreatic cancer cells [95].

Post-receptor signalling pathways

Studies in the signal transduction field have demonstrated that native SST inhibits the secretion and proliferation of both normal and neoplastic pituitary cells by inducing several intracellular pathways, depending on receptor subtype and target tissue [96].
The mechanisms whereby SSTRs transduce agonist induced messages into intracellular responses under different conditions and in different cells are complex [10]. The signalling pathways used by each receptor have not been fully elucidated yet, as different tissues and cells express different subtypes of receptors and most cells have more than one subtype of receptor. Besides, the modulated intracellular cascades may vary depending on the SST analogue, the SSTR subtype and – most importantly – according to the cell type used in that experiment [6, 68, 79, 97]. Although there are a number of specific receptor agonists, some agonists used in experimental settings can activate two receptors at the same time with different levels of affinities, which makes it difficult to clarify receptor-specific effects.

When SSTRs are activated by SST, the receptor interacts with heterotrimeric G protein which consists of α, β and γ subunits [41]. When an agonist activates GPCR, the GDP-bound Gαβγ heterotrimer interacts with the receptor and the α subunit decreases the affinity for GDP [41]. As the GTP concentration is higher in the cytoplasm, this results in substitution of GDP with GTP. Thereafter, Gα protein dissociates both from the receptor and βγ subunits and both modulate the activity of several intracellular pathways [41]. Among these there are several key enzymes, including PTPs, adenylyl cyclase (AC) and pathways including mitogen-activated protein kinase (MAPK) and phosphoinositol-3-kinase (PI3K)/Akt, which are modulated along with reduction of the Ca^{2+} influx through voltage sensitive channels and the activation of K^+ channels [98].

**Phosphotyrosine phosphatases and their action on MAPK and PI3K/Akt pathways**

In the human genome around 107 PTPs have been identified, which includes 38 so-called ‘classical PTPs’ with an elevated specificity for phosphotyrosines [99]. In studies using cells transfected with individual SSTR subtypes, all the five members of this receptor subfamily have been shown to couple to a number of PTPs, including the SH2 domain-containing cytosolic tyrosine phosphatases (SHP-1 and SHP-2), and the density-enhanced protein-tyrosine phosphatase-1 (DEP-1/PTP1B) [110]. Also, PTP2B (calcineurin). The anti-proliferative action of SST-activated PTPs depends on altered growth factor signalling through the selective dephosphorylation and inactivation of their receptors have been reported for SHP-1 with the insulin receptor, for SHP-2 with the insulin, EGF and platelet-derived growth factor (PDGF) receptor [104–107], for DEP-1 with PDGF and the vascular endothelial growth factor receptor [108, 109] and for PTP1B with the EGF receptor [110].

SSTR activation of SHP-1 was reported to induce arrest of cell proliferation in human pituitary adenomas [111], GH3 rat pituitary tumour cells [112], in different tumour cell lines derived from pancreatic cancers (MIA-PaCa-2, Panc-1, PC-1) and from thyroid medullary carcinoma, among others [79, 101, 113–115]. SHP-1 activation is the critical step for SSTR2-mediated anti-proliferative signalling [101, 116]. Tyrosine phosphorylated SSTR2 interacts with and activates SHP-2 and Src, a cytosolic tyrosine kinase, inducing consequent SHP-1 recruitment and activation [117] (Fig. 1). In CHO cells, SSTR-activated SHP-1 rapidly associates to the insulin receptor causing a tyrosine dephosphorylation of both the receptor itself and its substrates (i.e. IRS-1, Shc) leading to a negative modulation of insulin mitogenic signalling [106]. Additionally, SHP-1 inhibits the ERK 1/2 pathway by directly or indirectly dephosphorylating ERK 1/2 [4], causes overexpression of the cyclin-dependent kinase inhibitor p27Kip1 and an increase in hypophosphorylated retinoblastoma gene product (Rb) which, taken together, leads to inhibition of the entry in the S phase of the cell cycle and accumulation of the cells in G1 [118]. In pancreatic and pituitary tumour cells SSTR2 activation of SHP-1 was found to be involved in the p53-independent induction of apoptosis [119–121]. Additionally, a completely novel mechanism was identified in NIH3T3 (NIH Swiss mouse embryonic fibroblast cell line) cells in which the activation of SHP-1 by SSTR2 activates the transcription factor nuclear factor-κB causing an inhibition of the anti-apoptotic effects of the MAP kinase, Jun N-terminal kinase and, in turn, hyperactivation of caspase 8 and apoptosis [122]. Of interest in this regard is the finding that SSTR2 also affects the apoptosis induced by death receptors, sensitizing the cells to tumour necrosis factor (TNF)-α and TRAIL-mediated responses through the up-regulation of their receptors: death receptor 4 and TNFR1 [119]. In essence, SHP-1 activity was also involved in SSTR3-dependent apoptosis in transfected CHO cells, but this time by involving the induction of p53 and the subsequent activation of the pro-apoptotic protein Bax [123]. In GH3 cells, SSTR2-induced activation of SHP-1 causes the dephosphorylation of p85 and hence the inhibition of PI3K activity [101]. As would be expected, inhibition of PI3K activity causes inhibition of PDK1 and Akt activities, which in turn results in the activation of the glycogen synthase kinase-3β (GSK-3β) (Fig. 2). Theodoropoulou and colleagues have reported that the enhanced GSK-3β activity up-regulated the expression of Zac1 gene, which ultimately induced growth arrest [101]. Zac1 is highly expressed in normal pituitary, mammary and ovarian glands but is down-regulated in pituitary, breast and ovarian tumours, suggesting that it might act as a tumour suppressor gene [101]. Their further study investigating the immunoreactivity of Zac1 protein in tumour tissues of patients treated with SST analogues prior to surgery has also revealed a significantly positive correlation between strong Zac1
immunoreactivity and IGF-I normalization and the presence of tumour shrinkage after SST analogue treatment [124].

The other PTP, SHP-2, was involved in the anti-proliferative activity of SST following SSTR1 [102, 125], SSTR2, SSTR3 and SSTR4 activation [126]. Besides dephosphorylating and inactivating the tyrosine kinase receptors for insulin and EGF [104, 107], SHP-2 is involved in cell growth arrest [125] (Fig. 1). It is known that the effects of ERK 1/2 on cell proliferation are also related to the duration and intensity of ERK 1/2 activation, and hyperactivation of the ERK 1/2 pathway can also cause cell cycle arrest [127]. In light of this knowledge, it is now known that the activation of both SSTR1 and SSTR2 induces cell cycle arrest via the hyperactivation of ERK 1/2 and the up-regulation of p21<sup>WAF1/CIP1</sup> and p27<sup>KIP1</sup>, respectively [102, 103]. In particular, the activation of SSTR1-induced ERK 1/2 pathway involved Src/SHP-2/Pi3K/ras/Raf-1/MEK (mitogen-activated protein) [102], the

![Diagram](image_url)
SSTR2-regulated pathway involved SHP-1/SHP-2/PI3K/rap1 and ras/B-Raf/MEK [103] while, on the contrary, SSTR3 activation caused Raf-1 inactivation and blockade of the ERK 1/2 cascade [128, 129].

In addition, besides SHP-1 and SHP-2 activity, another delayed and long-lasting PTP activity was also induced following SST treatment [125]. As opposed to SHP-1 and SHP-2 which are cytosolic, non-membrane PTPs, one of these PTPs was identified in a receptor like PTP named DEP-1 in human beings (PTP/H9257 in rats), whose tumor suppressor role had already been established [130]. It was suggested that, in endothelial cells, DEP-1/PTPγ represents a possible effector of SSTR to inhibit tumoral angiogenesis and the activity of DEP-1 seems fundamental in blocking endothelial cell migration and proliferation [109, 131, 132]; the in vivo and in vitro anti-angiogenic activity of SST was dependent on the activation of PTPs [133, 134]. SSTR1-activated DEP-1 was shown to inhibit ERK 1/2 in PC13 thyroid cells and SSTR1-, SSTR2- and SSTR5-activated DEP-1 was shown to inhibit ERK 1/2 in glioma cells [135]. In CHO-K1 cells expressing SSTR1, a large multimeric protein aggregation composed of the G protein, Jak2,
SSTR1 inhibits Ca\(^{2+}\) channels in certain cells, like GH12C1, RINm5F (rat insulinoma cell line), AT-20 (a pituitary cell line) and GH3 cells [149–152] and again SSTR5 was shown to be coupled negatively to a Ca\(^{2+}\) current in AT-20 [152].

**Other post-receptor pathways**

Following the evidence showing SST-induced increase of inositol 1,4,5-trisphosphate (IP3) in bovine adrenal medullary cells [153], it is now widely accepted that SSTRs may affect the activity of phospholipase C (PLC) in native systems and, hence, modulate intracellular levels of IP3 and/or the activity of PLC-dependent protein kinase (PKC). SSTR1 was shown to mediate phospholipase PLC activation and IP3 production in CHO cells [154, 155]. SSTR2 mediates the activation of PLC in GH4C1 (rat pituitary tumour cell line) and F4C1 (rat pituitary cell line) cells [150, 154, 156]. Activation of PLC and IP3 production by SSTR4 was also observed in transfected COS (monkey kidney cell line) cells [157].

Phospholipase A2 (PLA2) is a cytoplasmic enzyme that hydrolyses triglycerol to form arachidonic acid, the metabolites of which have been shown to regulate different physiological and pathological functions [158, 159]. SST coupling negatively to arachidonate release has been shown in the rat anterior pituitary gland [160]. In contrast, SST has been suggested to generate arachidonic acid metabolites in rat GH4C1 cells, through pertussis toxin-sensitive G proteins [161]. Nevertheless, in a few cases SST was reported to induce cell proliferation through SSTR4 activation, which was observed to increase ERK 1/2 activity and, in turn, regulate PLA2 activation [162].

Moreover, also other MAPKs, more frequently associated to the induction of cell growth arrest, are regulated by SST: p38 is activated by SSTR2 and SSTR4 in CHO-K1 cells [98] and Jun N-terminal kinase is activated by SSTR5 [163].

SST was reported to regulate nitric oxide generation through the activation of both the endothelial and neuronal nitric oxide synthases (eNOS and nNOS, respectively). However, SST has been shown to have a dual effect on the release of nitric oxide. In different paradigms it has been shown to increase or inhibit nitric oxide levels via the involvement of different receptor subtypes and intracellular pathways [164]. SSTR2 caused a SHP-1-dependent dephosphorylation and activation of nNOS in mouse pancreas acini cells, which leads to increased nitric oxide production [165]. Nitric oxide is a major regulator of soluble guanylyl cyclase [28]. Guanylyl cyclase converts GTP to cyclic nucleotide guanosine monophosphate (cGMP), which is a regulatory mediator of cell proliferation [166]. The increased nitric oxide production in these cells results in activation of cGMP levels which is believed to be instrumental in growth arrest [164, 165]. cGMP exerts its effects via modulation of cGMP-dependent protein kinase (PKG), cGMP-gated ionic channels and cyclic nucleotide phosphodiesterases [167, 168]. Conversely, in a study by Cordelier and colleagues, inhibition of nNOS activity and a reduction in intracellular cGMP...
formation which resulted in cell growth arrest was observed in CHO cells expressing SSTR5 [169]. It was noted in this study that SSTR5 inhibits cell proliferation through SSTR5-mediated Src activation and subsequent nNOS tyrosine phosphorylation and inactivation, leading to a decrease of cGMP production and subsequent MAPK inhibition [166, 169]. SST-induced negative regulation of nitric oxide was also shown to be implicated in the inhibition of tumour angiogenesis and growth via the SSTR3-mediated negative regulation of eNOS [170]. The inhibition of Na\(^{+}/\)H\(^{−}\) (NHE1) exchanger activity is responsible for anti-proliferative effects of SST in enteric endocrine cells and hepatic cells [79]. These effects are mediated by SSTR1, SSTR3 and SSTR4. Interestingly, the inhibition of NHE1 is the only intracellular signalling regulated by SSTRs that was reported to be pertussis toxin insensitive [171].

Involvement of serine–threonine phosphatase in SST action was also implied in a study by Grozinsky-Glasberg and colleagues. In this study in INS-1 rat insulinoma cell line, both octreotide and the mTOR inhibitor everolimus (RAD001, Novartis, Basel, Switzerland) were shown inhibit cell proliferation and dephosphorylate tuberous sclerosis 2 (TSC2), which could imply activation of a serine–threonine phosphatase by these agents [172]. We have also shown that in the INS-1 cell line, the presence of a serine–threonine phosphatase inhibitor (okadaic acid) significantly reversed the anti-proliferative effect of octreotide (M. Cakir and A. Grossman, unpublished observations). This implies that blockade of activation of a serine–threonine phosphatase is a necessary component to the anti-proliferative activity of octreotide in this system.

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**Conflict of interest**

The authors confirm that there are no conflicts of interest.

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