Multiple intracellular effectors modulate physiological functions of the cloned somatostatin receptors

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Introduction

Somatostatin is a peptide widely distributed in both the central nervous system (CNS) and peripheral tissues. It is found as two bioactive peptides of 14 and 28 amino acids, the latter being an N-terminal-extended form (Reichlin 1983a).

The name somatostatin comes from its initial discovery as an inhibitor of growth hormone (GH) release from anterior pituitary cells (Brazeau et al. 1973). Since then, numerous other physiological activities of somatostatin have been discovered associated with differing peptide and receptor localization (Reichlin 1983a,b). Besides GH, somatostatin is also able to inhibit secretion of prolactin (PRL) and thyroid-stimulating hormone (TSH) (Reichlin 1983a). In the CNS, the highest somatostatin concentrations have been detected in the hypothalamus in the tuberoinfundibular neurons where, acting as a neurohormone, the peptide regulates the hypothalamic–hypophyseal axis (Schettini 1991). Somatostatin-containing neurons are also present in many other areas of the brain, such as the cerebral cortex, the limbic system (including the hippocampus and the amygdala) and the nigro-striatal pathway (Schettini 1991). In these areas somatostatin acts as a true neurotransmitter or as a neuromodulator of release of neurotransmitters such as acetylcholine (Gray et al. 1990) and dopamine (Thal et al. 1986). In the past few years clinical and post-mortem observations on Alzheimer’s patients, as well as experimental studies in animal models, have suggested that this peptide may be an important regulator of cognitive functions (Schettini 1991). In support of this are the observations that the concentration of somatostatin in the brain (Davies et al. 1980) and cerebrospinal fluid (Atack et al. 1988) is reduced in patients with Alzheimer’s dementia, neuritic plaques contain somatostatin (Morrison et al. 1985), neurofibrillary tangles have been identified in somatostatinergic neurons (Roberts et al. 1985) and a strict correlation has been demonstrated between somatostatin levels and cognitive performance in patients affected by Alzheimer’s disease (Tamminga et al. 1987). In animal models, depletion of rat brain somatostatin by cysteamine administration (a thiol-containing agent the action of which mimics the natural decline in somatostatin content in aged animals (Florio et al. 1991)) caused memory loss as assessed by many different behavioural paradigms, including active and passive avoidance tests (Vecsei et al. 1984, Harotunian et al. 1987, Schettini et al. 1988a, Florio et al. 1988), the Morris swim test (Fitzgerald & Dokla 1989) and the eight-arm radial maze (Sessions et al. 1989).

In the peripheral nervous system, somatostatin is produced by δ-cells of the pancreas where it plays an important role in the control of both insulin and glucagon secretion (Koerker et al. 1974). Somatostatin is also produced in the gastroenteric tract, mainly in the antral portion of the stomach where it controls the release of enteric hormones (vasoactive intestinal peptide, cholecystokinin (CCK), motilin and secretin) as well as gastric acid secretion (Reichlin 1983b). Finally, somatostatin has been recently demonstrated to play an important role as an endogenous inhibitor of cell proliferation in various normal and neoplastic tissues, and somatostatin analogues are now being used for the treatment of different human tumours (Schally 1988, Reubi & Laisse 1995).

The wide range of tissues releasing or responding to somatostatin and the variety of physiological

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activities regulated by this peptide suggest that multiple somatostatin receptors may exist that activate different intracellular transducing mechanisms through different G-proteins, leading to different cellular responses.

Since 1992, when the first three somatostatin receptors (SSTR) were cloned, a whole family of these proteins has been identified (for review see Reisine & Bell 1995). Extensive effort has been directed toward identification of the possible physiological roles and intracellular transducing mechanisms specifically coupled to each SSTR subtype. Although numerous articles have been published in this field, no definitive results have yet been obtained. Two major problems have limited these studies: (1) the presence of multiple SSTR subtypes on the tissues and cell lines used for these studies, (2) a paucity of somatostatin analogues which are truly selective for different receptors. As a consequence, all the studies have been performed by transfection of the SSTRs in heterologous cell lines. This approach, aimed at evaluating the activity of single receptor subtypes, has demonstrated that the resulting effects are dependent not only on the SSTR expressed but also on the recipient cell system. Indeed, often the cell lines selected do not provide the correct cellular environment for SSTR activity (e.g. specific G-proteins or enzymatic effectors). This has led to unexpected and misleading results, especially in the initial studies (for example, the reported lack of coupling between SSTR1 and SSTR2 to adenylate cyclase (AC) (Rens Domiano & Reisine 1992)).

The aim of this review is to summarize and organize, in a critical fashion, the extensive data produced to date. We hope to provide the reader with information that may facilitate the future development of new experimental approaches that may contribute to a better understanding of the role of somatostatin in physiology and to increase its therapeutic potential for numerous diseases.

**Cloning of the somatostatin receptors**

The existence of multiple SSTRs was hypothesized long before the molecular cloning of five different cDNAs encoding SSTRs. Binding studies using radioiodinated somatostatin showed different sensitivity in different tissues to somatostatin-14 and -28 (Srikant & Patel 1981). Moreover, somatostatin-14 and -28 exerted opposite effects on a voltage-dependent delayed rectifier K⁺ current, the former being an activator and the latter an inhibitor (Wang et al. 1989). The somatostatin analogue SMS 201-995 was found to displace all of the somatostatin binding in the pituitary, but not in the brain, suggesting the presence of at least two different binding sites for somatostatin in the brain (Tran et al. 1985). From a functional point of view it has been reported that somatostatin-14 was able to inhibit AC activity in both pituitary and striatum, but the somatostatin derivative MK678 was effective only in the pituitary, again suggesting the possibility of multiple SSTRs selectively recognized by natural and synthetic peptides (Raynor & Reisine 1992). Finally, solubilization of SSTRs from membranes revealed the presence of multiple bands with different molecular masses (for review see Rens Domiano & Reisine 1992).

In 1992 five SSTRs were cloned and named (SSTR1-5) according to their order of identification (Bruno et al. 1992, O’Carroll et al. 1992, Yamada et al. 1992, Yasuda et al. 1992). SSTR1 was isolated from a number of G-protein-coupled receptors cloned from pancreatic islet RNA by means of the reverse transcriptase (RT)-PCR technique (Yamada et al. 1992). In these experiments a generic set of degenerate primers was used, following those utilized by the group of Vassart, using the conserved amino acid sequences in the third and sixth transmembrane segments of all the G-protein-coupled receptors (Libert et al. 1989). This approach had already been used for the molecular cloning of many other genes for receptors of this family, since most of them do not have introns (Bell & Reisine 1993).

Amplification of the pancreatic islet RNA resulted in the identification of a group of new receptors that were highly similar to, but not identical with, other members of the G-protein-coupled receptor family. Although it appeared clear that these new clones belonged to the G-protein-coupled receptor family, PCR amplification did not provide any useful data on possible ligands. However, as the clones were obtained from pancreatic islets, it seemed reasonable that the ligand should be some kind of regulator of insulin or glucagon secretion. Expression of the two receptors in mammalian cells allowed screening of a number of possible ligands. One of the clones showed high-affinity binding for ³²¹Tyr-somatostatin and was named SSTR1 (Yamada et al. 1992).

Subsequently, using a radiolabelled human SSTR1 as a probe in Southern blotting experiments, a few weakly hybridizing bands were identified in addition to the SSTR1 gene. The screening of a human genomic library resulted in the identification of a new receptor that was more similar to SSTR1 than any other cloned G-protein-coupled receptor, which was named SSTR2 (Yamada et al. 1992).
The SSTR3, SSTR4 and SSTR5 sequences were then identified (Bruno et al. 1992, O’Carroll et al. 1992, Yasuda et al. 1992, Corness et al. 1993, Demchysyn et al. 1993, Panetta et al. 1994) together with a number of species-specific variants (Kluxen et al. 1992, Li et al. 1992, Meyerhof et al. 1992, Yamada et al. 1993a). All of these receptors showed seven putative transmembrane regions on hydropathy analysis of their sequences, three extracellular and three intracytoplasmic loops, the N-terminal portion of the protein oriented toward the outside of the cell membrane and the C-terminus inside. All these are characteristic features of the G-protein-coupled receptor family (Johnson & Dhanasekaran 1989). Interestingly, compared with the other seven transmembrane receptors, all the SSTRs showed a short third intracytoplasmic loop which is believed to couple the receptor to the G-proteins (Bell & Reisine 1993).

SSTRs vary in size ranging, for the human sequences, from the 418 amino acids of SSTR3 to 391 for SSTR1, 388 for SSTR4, 369 for SSTR2 and 364 for SSTR5 (Yamada et al. 1993a). The human SSTR genes have been localized on separate chromosomes, SSTR1 is on chromosome 14, SSTR2 on chromosome 17, SSTR3 on chromosome 22, SSTR4 on chromosome 20 and SSTR5 on chromosome 16 (Corness et al. 1993, Demchysyn et al. 1993, Yamada et al. 1993b, Panetta et al. 1994).

Analysis of the sequences of the SSTRs indicated that, along with a general high similarity between all these receptors (almost 50% amino acid identity), SSTR1 and SSTR4 sequences are the most similar (more than 60% amino acid identity) (Yamada et al. 1993a). This similarity is probably the basis for the pharmacological subdivision of the SSTRs into two subclasses (SSTR1/4 and SSTR2/3/5) according to their ability to bind synthetic somatostatin analogues. Indeed, while SSTR1 and SSTR4 bind with high affinity (nanomolar range) only somatostatin-14 and -28, SSTR2, SSTR3 and SSTR5 also bind restricted hexapeptide (for example MK678 and BIM 23050), linear peptide and cyclic octapeptide (SMS 201-995, RC 160) somatostatin analogues (Patel & Srikant 1994). The similarity between SSTR1 and SSTR4 was also observed at the signal-transduction level, where both receptors are able to activate phosphotyrosine phosphatase (PTP) activity as an intracellular mechanism to reduce cell proliferation (Florio et al. 1994, 1996).

Interestingly, all these receptors except SSTR5 showed similar affinities for the two natural forms of somatostatin (14 and 28), with a slightly higher affinity for the 14-amino acid peptide. Only SSTR5 displayed an almost 13-fold higher affinity for the somatostatin-28 peptide (O’Carroll et al. 1992, Patel & Srikant 1994). Recently Srikant and Patel’s group identified a novel SSTR in the AtT20 mouse pituitary cell line that showed high-affinity binding for somatostatin-28 yet hybridized weakly with an SSTR5 radiolabelled probe, and differed from the murine SSTR5 mRNA size (3·8 vs 2·4 kb) (Patel et al. 1994b), suggesting that the molecular cloning of the SSTR family is not complete.

The molecular heterogeneity among SSTRs has been further increased by the discovery of SSTR2 isoforms generated by mRNA alternative splicing that results in a long and a short form of the receptor (Vanetti et al. 1992, Patel et al. 1993). The two forms of SSTR2, named A and B, differ in the C-terminus where SSTR2A is 23 amino acids longer than SSTR2B and the last 15 amino acids of SSTR2B are specific for this ‘short form’. The biological significance of these different isoforms of the SSTRs is not completely clear, although it has been reported that SSTR2B couples more efficiently to some intracellular effectors, such as AC (Vanetti et al. 1993) and is under the transcriptional control of cAMP (Patel et al. 1993).

SSTR localization

As previously discussed, SSTRs are widely distributed in tissues and organ systems, ranging from the CNS and endocrine glands such as the pituitary and thyroid, to the pancreas, the gastroenteric tract, the kidney, the lung and other organs.

One of the first unexpected results obtained after the molecular cloning of the family of SSTRs was the absence of tissue-specificity for any SSTR subtype. Indeed, although some receptors show higher expression in particular areas, and SSTR2 shows a generally wider distribution, almost all the five SSTRs are present in all the tissues and systems studied.

The tissue distribution of SSTRs has been studied in rats by Northern blotting (Kaupmann et al. 1993, Kong et al. 1994), RNase protection (Bruno et al. 1993), in situ hybridization (Breder et al. 1992, Wulfsen et al. 1993, Kong et al. 1994) and semiquantitative PCR (Wulfsen et al. 1993). The distribution of SSTRs in the CNS is reported in Table 1. SSTR1 and SSTR2 are widely expressed, with the highest levels in the cortex, hippocampus, amygdala and hypothalamus, and to a lesser extent in the cerebellum and spinal cord (Bruno et al. 1993). SSTR1 expression in the cerebral cortex is readily detected in mouse (Breder et al. 1996).
et al. 1992) but not in human cortex (Li et al. 1992). The reason for this discrepancy is not yet clear. SSTR3 is also widely expressed in the CNS with the highest levels in the cerebellum (Bruno et al. 1993, Kaupmann et al. 1993). Notably, in the cerebellum at birth SSTR1 is the most strongly represented SSTR, but the levels of this receptor rapidly decline several-fold until day 14 when SSTR3 increases, becoming the most abundant SSTR at about the 28th postnatal day (Wulfsen et al. 1993). At birth, SSTR4 is also expressed in most brain areas, but its concentration gradually declines postnatally (Wulfsen et al. 1993). High concentrations of this receptor in the adult have been found mainly in the hippocampus and in the olfactory bulb, while it is not present in the cerebellum (Bruno et al. 1993, Bito et al. 1994). SSTR5 shows a unique pattern of expression in the rat CNS, being present in the hypothalamus and in the preoptic area but always at very low levels (Bruno et al. 1993).

All the cloned SSTRs are expressed in all pituitary cell types (Day et al. 1995, O’Carroll & Krempels 1995). However, in situ hybridization studies demonstrated that SSTR1 is expressed mainly on TSH-secreting cells, SSTR2 on both TSH- and luteinizing hormone-secreting cells, SSTR3 on prolactin (PRL)- and follicle-stimulating hormone-secreting cells and SSTR4 and SSTR5 mainly on GH-secreting cells (O’Carroll & Krempels 1995). The different ratios of expression of the single SSTRs has been suggested to represent an indication of which SSTR is involved in the control of which pituitary hormone secretion (Day et al. 1995, O’Carroll & Krempels 1995).

In the periphery, all five SSTRs have also been detected in the small intestine and spleen (Bruno et al. 1993). Other localizations were heart (SSTR1/3/4), liver (SSTR3), stomach (SSTR1-4), pancreas (SSTR2), kidney and lung (SSTR1/3/4) (Bruno et al. 1993).

Intracellular signalling of endogenous somatostatin receptors

Before the cloning of a whole family of SSTRs, the coupling of endogenous SSTRs to a wide variety of intracellular effectors had been reported. SSTR coupling to different intracellular effectors was, in almost all the cases, mediated through the activation of G-proteins belonging to the G_i or G_o family which are sensitive to the ADP-ribosylation induced by pretreatment with pertussis toxin. The only intracellular pathway activated by the binding of somatostatin to its receptors that was reported not to be mediated by G-protein activation was modulation of the Na^+/H^+ antiporter (Barber et al. 1989).

Of the intracellular effectors coupled to SSTRs, the most widely studied is the AC-cAMP-protein kinase A (PKA) system. In many different cell types, including pituitary and brain, which represent two major somatostatin targets, somatostatin is able to reduce basal and stimulated cAMP production, acting through a G_i-protein to reduce the AC activity (Dorflinger & Schonbrunn 1983, Schettini et al. 1988b, 1989).

The modulation of ion channels is also well characterized. Somatostatin has been reported to modulate both Ca^{2+} and K^+ channels. The activation of SSTRs causes a reduction in intracellular Ca^{2+} concentrations through a G-protein-dependent inhibition of L-type voltage-dependent Ca^{2+} channels (Luini et al. 1986, Wang et al. 1990). The reduction in voltage-dependent Ca^{2+} channel activity was reported to be independent of both somatostatin effects on cAMP production and K^+.
conductance (Reisine 1990). Somatostatin modulation of K+ channels is also mediated through a G-protein and results in the activation of at least two different types of outward currents: an ‘inward rectifying’ (Inoue et al. 1988) and a voltage-dependent ‘delayed rectifying’ current (Janquín et al. 1988, Wang et al. 1989). Thus, SSTRs coordinate the activity of certain Ca2+ and K+ channels causing an increase in K+ efflux, hyperpolarization and an increase in the threshold for Ca2+ action potential (Mollard et al. 1988). Moreover, the inhibition of PRL secretion, induced by somatostatin treatment of clonal pituitary cells, was completely prevented by blocking the ATP-sensitive K+ channels with glibenclamide, suggesting that the activity of this class of channels is also regulated by somatostatin (Meucci et al. 1992). More recently, somatostatin has also been reported to indirectly modulate Ca2+ currents through the activation of cGMP-dependent protein kinase (Meriney et al. 1994) or a large K+ conductance through the activation of the Ser/Thr phosphatases PP2A (White et al. 1991). SSTRs are also coupled to phospholipase A2 (PLA2) stimulation, the activity of which leads to the production of arachidonic acid (AA) (Schweitzer et al. 1990). Particular interest has been recently devoted to the regulation of the PTP activity mediated by somatostatin activation of SSTRs (Hierowski et al. 1985, Colas et al. 1992, Pan et al. 1992). It has been proposed that the activation of these enzymes is responsible for the somatostatinergic control of cell proliferation (Pan et al. 1992, Buscail et al. 1994). An SH2-domain-containing tyrosine phosphatase (SHPTP1) has been demonstrated to co-purify with an acinar pancreatic SSTR, and its activity was increased after somatostatin-28 treatment, thus suggesting that this PTP activity was modulated by somatostatin (Zeggari et al. 1994).

Less understood are the effects of somatostatin on phospholipase C (PLC), since in ‘native’ cell systems there are no changes in the production of inositol triphosphate and diacylglycerol following somatostatin treatment (Cook et al. 1988), but in COS-7 cells, transfected with each of the five SSTRs, stimulation of PLC was reported (Akbar et al. 1994). These discrepancies may be due to a certain degree of receptor-effector promiscuity present in the transfected cell system, probably because of the excessive amount of transfected receptors on the plasma membrane. Similar atypical coupling was reported also in fibroblasts transfected with the D2 dopamine receptor subtype (Vallar et al. 1990). In CHO-K1 cells transfected with SSTR4, no changes in PLC activity were detected (Bito et al. 1994), while the transfection of SSTR5 resulted in inhibition of CCK-induced PLC activation (Buscail et al. 1995).

### Specific coupling of the cloned SSTRs to intracellular effectors and their physiological role

The presence of multiple SSTRs in almost all the tissues and cell lines studied did not allow the identification of the existence of a selectivity between SSTR subtypes and specific intracellular effectors. Only after the cloning of five SSTRs and their expression in heterologous cell systems has the characterization of the second-messenger systems modulated by a single SSTR begun.

In the following, the available data on the coupling between the single SSTRs and the intracellular transducing systems are reported. The data reported are also summarized in Tables 2 and 3 and in Fig. 1.

#### SSTR1

When SSTR1 was cloned, and in few subsequent studies, it was reported not to couple to AC and G-proteins (Rens Domiano et al. 1992, Buscail et al. 1994, Castro et al. 1996). It now appears clear that the lack of effects on this transducing mechanism was mainly due to the expression system used in the

### Table 2. Specific physiological effects of SSTRs. (Only physiological activities of somatostatin reported to be mediated by specific SSTR subtypes are given)

<table>
<thead>
<tr>
<th>SSTR</th>
<th>AC</th>
<th>Ca2+ channels</th>
<th>K+ channels</th>
<th>Na+/H+ exchanger</th>
<th>PA2/MAP kinase</th>
<th>PLC</th>
<th>PTPases</th>
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<td>SSTR1</td>
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<td>SSTR4</td>
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<td>SSTR5</td>
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<td>↑†</td>
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<td>↑†/↑†</td>
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†, Inhibition; =, no effect.

### Table 3. Specific intracellular transducing mechanisms coupled to SSTRs

<table>
<thead>
<tr>
<th>SSTR</th>
<th>AC</th>
<th>Ca2+ channels</th>
<th>K+ channels</th>
<th>Na+/H+ exchanger</th>
<th>PA2/MAP kinase</th>
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<td>SSTR5</td>
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<td>↑†/↑†</td>
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</table>

†, Activation. ↓, inhibition; =, no effects.

*Indirectly through AA production.
†Only in transfected COS-7 cells.
‡Inhibition of CCK-stimulated PLC activity.
initial experiments or depended on alterations caused by the transfection process itself. In fact, many reports have since demonstrated that G-proteins mediate the effects of this receptor and that its activation results in inhibition of AC activity (Kaupmann et al. 1993, Garcia & Myers 1994, Hadcock et al. 1994, Hershberger et al. 1994, Hou et al. 1994, Patel et al. 1994). The lack of expression of specific G-proteins was probably responsible for the uncoupling of SSTR1 from AC in early reports.

Moreover, differential analysis of the G-protein content in the two subclones of the CHO cell lines transfected with SSTR1 that were used in the original cloning paper and in the subsequent studies (CHO-DG44 vs CHO-K1) have given possible identification of the G-protein mediating the effects of SSTR1 on AC activity. In the CHO-DG44 line, where somatostatin did not inhibit cAMP formation, only the G3 subtype is present (Rens Domiano et al. 1992). In the CHO-K1 line, the ability of the peptide to reduce AC activity was associated with the presence of both G3 and G2a (Hershberger et al. 1994), the latter being a likely candidate for mediation of SSTR1 activity on the AC enzyme. Subsequent studies also demonstrated an inhibitory modulation of AC by SSTR1 activation in CHO-DG44 cells (Kubota et al. 1994).

Another transduction mechanism coupled to SSTR1 is modulation of PTP activity. Similar to that reported for the activation of the D2 dopamine receptor, which causes pertussis-toxin-sensitive activation of PTPs (Florio et al. 1992), SSTR1-transfected CHO-K1 cell lines show an increase in PTP activity in a G-protein-dependent fashion (Florio et al. 1994). This stimulatory activity was not generalized but involved only a subset of PTPs (Florio et al. 1994). It has also been shown that activation of PTP by SSTR1, transfected in NIH3T3 cells, parallels inhibition of cell proliferation (Buscail et al. 1994). However, in this case the activation of PTPs was not G-protein-mediated (Buscail et al. 1994). Further studies will be necessary to clarify this discrepancy. Another G-protein-independent intracellular mechanism coupled to SSTR1 is the activation of the Na+/H+ ion exchanger (Hou et al. 1994).

SSTR2A and SSTR2B
SSTR2 is one of the most widely studied SSTRs since it is present in many of the cell lines commonly used for the study of SSTR activity. Similarly to SSTR1, discordant data on SSTR2 coupling to AC are present in the literature. This receptor, initially considered to be not coupled to AC (Rens Domiano et al. 1992), is now recognized as exerting some of its cellular effects through G-protein-dependent coupling to AC (Kaupmann et al. 1993, Strnad et al. 1993, Garcia & Myers 1994, Hershberger et al. 1994, Hou et al. 1994, Patel...
et al. 1994a). One possible reason for the different results obtained on analysing the intracellular effectors that mediate somatostatin activation of SSTR2 may be the existence of two SSTR2 isoforms, A and B, which show different sensitivity in inhibiting cAMP formation (Vanetti et al. 1992). All tissues examined to date express mRNA for both receptors (Law et al. 1995), therefore it is likely that most tissues express a mixture of both SSTR2 isoforms. Functionally these structural differences result in a twofold higher efficiency of SSTR2B inhibition of AC activity in transfected CHO-K1 cells (Vanetti et al. 1993), although SSTR2A is also able to significantly reduce cAMP formation (Vanetti et al. 1993, Hershberger et al. 1994). Another physiological difference between the two isoforms is the lack of homologue desensitization of SSTR2B, after agonist binding, whereas this does occur with the SSTR2A isoform (Vanetti et al. 1993). The molecular basis for the different behaviour between the two receptors is probably the different C-termini of the receptor sequences. In the SSTR2B C-terminus, possible phosphorylation sites (e.g. Ser\textsuperscript{333}, Thr\textsuperscript{335}, Ser\textsuperscript{341}, Ser\textsuperscript{345}) for β\textsubscript{2}-adrenergic receptor kinase (β-ARK) are lost (Vanetti et al. 1993). Although originally discovered as a down-regulator of the β\textsubscript{2}-adrenoreceptor through the phosphorylation of specific serine and threonine residues at the C-terminus of the receptor, β-ARK has recently been shown to be involved in the regulation of SSTR activity. In particular, it has been reported to be involved in the phosphorylation of SSTRs in S49 lymphoma cells which occurs upon desensitization-dependent somatostatin treatment (Mayor et al. 1987). Moreover, in a dominant negative mutant cell line for β-ARK, somatostatin-dependent SSTR2 desensitization was prevented (Hines et al. 1993).

Recently, the coupling between SSTR2 and AC was reported to be mediated by G\textsubscript{1α} (Kagimoto et al. 1994). In fact, somatostatin-14 was able to inhibit cAMP formation only when SSTR2 was co-transfected with a cDNA encoding G\textsubscript{1α} (Kagimoto et al. 1994).

Beside its AC activity, SSTR2 (but not SSTR1) was reported to inhibit high-voltage-activated Ca\textsuperscript{2+} current in the rat insulinoma cell line RINm5F (Fujii et al. 1994). Similar results were obtained in brain neurons in culture, where somatostatin inhibited voltage-dependent Ca\textsuperscript{2+} currents and activated K\textsuperscript{+} channels, modulating a receptor with pharmacological characteristics like SSTR2 (Law et al. 1995). Finally in the Atf120 pituitary cell line a somatostatin analogue that preferentially binds to SSTR2 was found to inhibit L-type Ca\textsuperscript{2+} channel activity and potentiate an inward rectifying K\textsuperscript{+} current (Reisine et al. 1994). Although SSTR2 activation was found to inhibit cell proliferation (Buscail et al. 1994, 1995), discordant results have been reported on its ability to stimulate PTP activity, being either an activator (Buscail et al. 1994, 1995) or ineffective (Florio et al. 1994). Further studies, using different cell models, will be required to better address this issue.

Aside from the possible physiological role for SSTR2 activation in the control of cell proliferation, a statistically significant correlation was reported between synthetic SSTR2 agonist binding potency and their ability to inhibit GH secretion, whereas no correlation was observed for any of the other four SSTRs (Raynor et al. 1993).

SSTR3
More limited studies have been carried out on the specific coupling between SSTR3 and second-messenger systems. The data reported show that expression of SSTR3 in COS-1 or HEK293 cell lines resulted in inhibition of AC activity (Yasuda et al. 1992), while no changes in the cAMP formation were detected following the expression in CHO-DG44 cells (Law et al. 1994). Expression of specific G-proteins was considered responsible for this discrepancy. In the COS-1 cells, all of the G-proteins believed to be responsible for mediating somatostatin effects (G\textsubscript{q} and G\textsubscript{i}α) are present, and in HEK293 cells G\textsubscript{1α} and G\textsubscript{3α} are expressed, whereas in CHO-DG44 cells only G\textsubscript{3α} and G\textsubscript{i}α have been detected. It is likely that SSTR3 requires the presence of G\textsubscript{1α} to inhibit AC activity (Law et al. 1994). This observation was further demonstrated by co-expressing SSTR3 and G\textsubscript{1α} or G\textsubscript{2α} in CHO-DG44 cells. In these experiments only the cells that were expressing G\textsubscript{1α} responded to SSTR3 activation with inhibition of cAMP formation, confirming that G\textsubscript{1α} is necessary for SSTR3 to couple to AC (Law et al. 1995). Finally, SSTR3 was reported not to be involved in the control of cell proliferation (Buscail et al. 1995).

SSTR4
SSTR4, transfected in HEK293 or CHO-K1 cell lines, was also reported to be coupled in an inhibitory way to AC (Kaumann et al. 1993, Bito et al. 1994). This effect was sensitive to pertussis toxin pretreatment, suggesting G-protein mediation (Kaumann et al. 1993, Bito et al. 1994). Conversely, in CHO-K1 cells transfected with SSTR4, as reported for SSTR activation in ‘native systems’, somatostatin did not affect PLC activity (Bito et al. 1994).

Another pertussis-toxin-sensitive mechanism modulated by somatostatin binding to SSTR4 is
the activation of PLA$_2$ (Bito et al. 1994). PLA$_2$ activation results in an increase in AA release. AA is reported to be a modulator of many K$^+$ currents such as a delayed rectifier K$^+$ current, Sk current and Im current. Since somatostatin has been reported to activate some of these currents (Inoue et al. 1988, Janquin et al. 1988), it was proposed that AA produced after somatostatin stimulation may be the direct activator of these currents. Similarly, SSTR4 activation via G-protein activated mitogen-activated protein (MAP) kinase kinase and MAP kinase (Bito et al. 1994). Both PLA$_2$ and MAP kinase activation were blocked by similar concentrations of wortmannin, a phosphatidylinositol 3-kinase (PI3-K) inhibitor, supporting the idea that PI3-K may be a somatostatin effector upstream of MAP kinase and PLA$_2$ (Sakanaka et al. 1994).

More recently, SSTR4 was demonstrated to be the only SSTR natively expressed in a clonal thyroid cell line, PC Cl3 (Florio et al. 1996). In this cell line somatostatin was able to inhibit AC activity and stimulate PTP activity, the latter effect being responsible for the antiproliferative activity of the peptide in these cells (Florio et al. 1996).

**SSTR5**

SSTR5, similarly to all the other SSTR subtypes, is able to inhibit AC activity when transfected in CHO-K1 cells (O’Carroll et al. 1992, Panetta et al. 1994). It has been reported that SSTR5 agonists, but not SSTR2- or SSTR3-stimulating ligands, are able to inhibit insulin release (Rossowsky & Coy 1993). This observation is in agreement with previous observations showing that somatostatin-28, which binds SSTR5 with higher affinity than all the other SSTRs (O’Carroll et al. 1992), is 100-fold more potent than somatostatin in reducing insulin release from pancreatic islets (Mandarino et al. 1981). Interestingly it was reported that somatostatin modulation of insulin release in the HIT cell line was mediated, in a G-protein-dependent manner, by the inhibition of voltage-dependent Ca$^{2+}$ channel activity (Hsu et al. 1991). Thus, it is possible that the modulation of Ca$^{2+}$ currents may represent another intracellular transducing mechanism regulated by the activation of SSTR5.

Finally, in CHO-K1 cells expressing SSTR5, the somatostatin analogue RC 160 was able to block serum-induced cell proliferation (Buscail et al. 1995). This effect was not dependent on inhibition of cAMP accumulation induced by the peptide since cAMP is per se an antiproliferative agent in these cells. Blockage of either PTPs or Ser/Thr phosphatases by vanadate and okadaic acid respectively had no effect on the RC 160 action. In contrast, RC 160 inhibited the proliferative activity of CCK in CHO-k1 cells, and this effect was paralleled by the reversal of IP$_3$ production and increase in intracellular Ca$^{2+}$ concentration induced by CCK (Buscail et al. 1993). This suggested that, in contrast with the effects observed in cells transfected with SSTR1 (Buscail et al. 1994, Florio et al. 1994), SSTR2 (Buscail et al. 1994) and SSTR4 (Florio et al. 1996), the antiproliferative effects mediated by SSTR5 are not dependent on the stimulation of PTPs, but by the inhibition of PLC activity (Buscail et al. 1995)

**Concluding remarks**

Although the molecular cloning of many different SSTR subtypes has greatly improved our knowledge of their physiological role, most of the unsolved questions in this field still have not been answered. This apparent failure is probably due to several different reasons: first of all each receptor subtype may activate multiple intracellular pathways and, secondly, the same intracellular pathway may be activated by different SSTR subtypes, often expressed on the same cell. Furthermore, significant selectivity for the different peptides in the somatostatin family has not been identified. Thus, the need for so many different receptors is not yet clearly understood.

However, it is important to remember that the studies carried out to date have been performed mainly on heterologous cell lines transfected with single receptor subtypes, since it is only very recently that more specific synthetic somatostatin analogues have been developed. Thus, it is not known whether the promiscuity among the different SSTRs in signal transduction is actually present in native cell lines.

It is possible to speculate that fine tuning of the selectivity of these receptors towards the intracellular effectors is regulated by the cellular environment. For example, the G-protein content of a single cell population may direct the activity of different SSTRs on different second-messenger systems. Another possibility is that two or more SSTRs present on the same cell may activate the same intracellular pathway with different efficiency or sensitivity, allowing a different intensity of the response according to the needs of the cell.

All these aspects represent significant goals for research over the next few years.

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