The role of somatostatin and dopamine D2 receptors in endocrine tumors

Federico Gatto and Leo J Hofland

Division of Endocrinology, Department of Internal Medicine, Erasmus Medical Center, Room Ee530b, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

(Correspondence should be addressed to L J Hofland; Email: l.hofland@erasmusmc.nl)

Abstract

Somatostatin (SS) and dopamine (DA) receptors have been highlighted as two critical regulators in the negative control of hormonal secretion in a wide group of human endocrine tumors. Both families of receptors belong to the superfamily of G protein-coupled receptors and share a number of structural and functional characteristics. Because of the generally reported high expression of somatostatin receptors (SSTRs) in neuroendocrine tumors (NET), somatostatin analogs (SSA) have a pronounced role in the medical therapy for this class of tumors, especially pituitary adenomas and well-differentiated gastroenteropancreatic NET (GEP NET). Moreover, NET express not only SSTR but also frequently dopamine receptors (DRs), and DA agonists targeting the D2 receptor (D2) have been demonstrated to be effective in controlling hormone secretion and cell proliferation in in vivo and in vitro studies. The treatment with SSAs combined with DA agonists has already been demonstrated efficacious in a subgroup of patients with GH-secreting pituitary adenomas and few reported cases of carcinoids. The recent availability of new selective and universal SSA and DA agonists, as well as the chimeric SS/DA compounds, may shed new light on the potential role of SSTR and D2 as combined targets for biotherapy in NET. This review provides an overview of the latest studies evaluating the expression of SSTR and DR in NET, focusing on their co-expression and the possible clinical implications of such co-expression. Moreover, the most recent insights in SSTR and D2 pathophysiology and the future perspectives for treatment with SSA, DA agonists, and SS/DA chimeric compounds are discussed.

Endocrine-Related Cancer (2011) 18 R233–R251

Introduction

In recent years, somatostatin (SS) and dopamine (DA) receptors have been highlighted as two critical regulators involved in the negative control of hormonal secretion in a wide group of neuroendocrine tumors (NET), including pituitary adenomas (Ben-Jonathan & Hnasko 2001, Guillemin 2005, Ferone et al. 2009). Moreover, SS, DA, and their receptors represent two major systems that share a number of structural and functional characteristics (Rocheville et al. 2000a, Baragli et al. 2007, Srirajaskanthan et al. 2009). SS is a peptide present in mammals in two biologically active isoforms, consisting of 14 (SS-14) and 28 (SS-28) amino acids. Up to date, five human SS receptor subtypes (sst) have been cloned and characterized (Lamberts et al. 1996, Patel 1999, Moller et al. 2003). The transcript of the sst2 gene can be present in two splice variants that differ only for the length of the cytoplasmic portion of the receptor (sst2A and sst2B). Somatostatin receptors (SSTRs) belong to the seven-transmembrane segment receptor superfamily and functionally couple to G proteins (Lamberts et al. 1996, Hofland & Lamberts 2003, Moller et al. 2003). SS binding to SSTR subtypes activates a series of second messenger systems, resulting in the inhibition of calcium channels and adenylate cyclase activity, ultimately leading to inhibition of hormone secretion (Reisine & Bell 1995, Patel 1997, 1999). Stimulation of other second messengers, such as phosphotyrosine phosphatases (PTPs), plays a role in SS- and somatostatin analogs (SSA)-mediated control of cell growth (Schally 1988, Lamberts et al. 1991, Hofland et al. 1995, Patel 1999, Hofland & Lamberts 2003, Florio 2008, Schonbrunn 2008). Among the SSTR, sst3
appears to be the subtype mostly related to the pro-apoptotic and antiproliferative effects of SS and SSA (Ferone et al. 2002, Hofland & Lamberts 2003).

DA is the predominant catecholamine neurotransmitter in the human central nervous system but also plays multiple roles in the periphery as a modulator of cardiovascular and renal function, gastrointestinal (GI) motility, as well as the endocrine system (Missale et al. 1998). DA exerts its functions via binding to dopamine receptors (DRs; Missale et al. 1998). DR are G protein-coupled receptors (GPCRs) acting predominantly via adenylate cyclase modulation. Up to now, five different DR, divided into two subfamilies, have been characterized. Activation of the D1-like receptors (D1 and D5) results in a stimulation of adenylate cyclase activity, whereas on the contrary D2-like subfamily (D2, D3, and D4) stimulation leads to a Gi/Go protein-mediated decrease of intracellular cAMP (Missale et al. 1998). The DR subtypes have been demonstrated to exert heterogeneous roles in a number of different tissues and organs and show a tissue-specific distribution. Moreover, two different isoforms of the D2 receptor have been isolated, the short (D2 short) and long (D2 long) form, hypothesized to activate distinct intracellular pathways and to mediate differential effects following ligand activation (Missale et al. 1998).

The SSTR and DR families share a 30% sequence homology and appear to be structurally related. Behavioral and clinical evidence in brain research indicated an interaction between the somatostatinergic and dopaminergic systems (Chneiweiss et al. 1985, Martin-Iverson et al. 1986, Izquierdo-Claros et al. 1997, Marzullo et al. 1999), recently confirmed by in vitro studies (Roccheville et al. 2000a, Baragli et al. 2007, Kidd et al. 2008). It is well known that SSTR and DR are widely expressed both in normal human neuroendocrine tissues and in tumors (Reubi et al. 1987, Pivonello et al. 2007b), such as pituitary adenomas (Stefaneau et al. 2001, Moller et al. 2003, Saveanu et al. 2008, Ferone et al. 2009) and adrenal tumors (Pivonello et al. 2007a, b, de Bruin et al. 2009a). Since SSTR and D2-like receptors mainly exert inhibitory functions, medical therapies targeting these receptors with selective agonists have been developed for the treatment of a number of neuroendocrine disorders. In the last decades, the knowledge on the pathophysiology of these two families of GPCRs in NET has progressively increased due to the new insights in receptor dimerization, internalization, and trafficking (Hofland & Lamberts 2003, Ferone et al. 2009, Lesche et al. 2009, Poll et al. 2010). Moreover, the recent availability and use of novel selective and universal SSAs and DA agonists, as well as the chimeric SS/DA compounds, has shed light on the potential role of SSTR and D2 as combined targets for biotherapy in NET. Since SSTR and D2 expression, co-expression, and their role as targets for medical treatment of patients with pituitary tumors have recently been extensively reviewed (Saveanu et al. 2006, Ferone et al. 2009), we will highlight in this review mainly the large and heterogeneous family of NET (previously known with the name of ‘carcinoids’), which is emerging as a main field for SSTR and DR targeting therapy.

**SSTR expression in endocrine tumors**


As for pituitary adenomas, well-differentiated gastroenteropancreatic (GEP) NET represent a major target for SSA treatment (Oberg 2002, Oberg et al. 2004a, b, Batista et al. 2006, Falconi et al. 2006, O’Toole et al. 2006a).

Both pancreatic NET (including gastrinomas, glucagonomas, and VIPomas) and GI NET express SSTRs in 80–100% of the cases. Insulinomas have a lower incidence of SSTR expression (50–70%). The receptor density can vary among tumors, but it can be considered high in the majority of cases. Undifferentiated NET express SSTR (mainly sst2 subtype) less frequently (and in lower density) than well-differentiated ones (Reubi 2007). On the other hand, sst5 mRNA expression was demonstrated to be positively correlated with histopathological features of tumor aggressiveness in primary insulinomas (de Sa et al. 2006). There is consensus, based on various methodologies, that among the different SSTR subtypes, sst2 is usually the most prominent, followed by sst1 and sst5.
while sst_3 is less frequently expressed and sst_4 almost absent (Reubi 2003, 2004, Reubi & Waser 2003). This high and heterogeneous expression did not show any relevant correlation between the subtype(s) expressed and the primary tumor origin, or a specific hormone secretion (Reubi et al. 1998b, Papotti et al. 2002, Volante et al. 2008).

Moreover, also lung NET, both well- and poorly differentiated, have been shown to express SSTR in vivo, being subtypes 2, 3, and 5 the most represented (Berenger et al. 1996, Reubi et al. 1996, 1998b, 2000, Janson et al. 1998, Reisinger et al. 1998, Hofland et al. 1999, Papotti et al. 2000, 2001a). A recent study evaluating the tissue distribution of sst_2A and sst_3 in a large series (>200 cases) of pulmonary NET with clinically aggressive features (Righi et al. 2010) confirmed previous data from small series, showing a decrease in the expression of sst_2 and sst_3 from low-grade/intermediate-grade to high-grade tumors, and confirming a good correlation between immunohisto-chemical results and octreotide scintigraphy (Reisiger et al. 1998, Granberg et al. 2003, Reubi & Waser 2003).

The catecholamine-producing and -secreting tumor pheochromocytoma has been shown to express SS (Reubi et al. 1992c) and more than one SSTR receptor both at mRNA and protein level (Reubi et al. 1992c, Kubota et al. 1994, Mundschenk et al. 2003, Kolby et al. 2006), being the subtypes 1–3 the most represented (Hofland & Lamberts 2003, Unger et al. 2004). Similarly, in vivo and in vitro studies on medullary thyroid carcinomas detected the presence of all sst subtypes, except sst_4, and showed a clear positivity for SS, indicating that possible autocrine/paracrine circuits may be active in this tumor (Pacini et al. 1991, Kwekkeboom et al. 1993, Mato et al. 1998, Papotti et al. 2001b, Zatelli et al. 2006). As indicated above, SSTR expression is largely heterogeneous both within the same tumor type and even more between NET arising from different tissues. However, sst_2 is reported to be the mostly represented subtype, even if frequently other SSTR subtypes are co-expressed.

Furthermore, immune cells and human lymphatic tissues represent other important sites of SSTR expression in the human body. Monocyte-derived cells and mature T-lymphocytes have been clearly demonstrated to predominantly express sst_2 and sst_3 respectively (Ferone et al. 2002, Lichtenauer-Kaligis et al. 2004). Moreover, human lymphoid follicle centers (Reubi et al. 1998a), thymus, and spleen (Ferone et al. 2011) have been reported to express SSTR subtypes 2 and 3 as well. Sst_1, sst_2, and sst_3 have been demonstrated as the most represented SSTR in thymic tumors, both at the protein and at the mRNA level (Ferone et al. 2001). Moreover, a number of malignancies arising from immune cells, such as Hodgkin disease, T and B non-Hodgkin lymphomas (Reubi et al. 1992d, Lugtenburg et al. 2001a,b), myeloma, and plasmacytoma (Georgii-Hemming et al. 1999, Duet et al. 2005) heterogeneously express SSTR, although the role of SSTR targeting for diagnosis or treatment in these tumors is still debated. Finally, a clear positivity by SSTR scintigraphy has been observed in a number of chronic inflammatory diseases, such as sarcoidosis (van Hagen et al. 1994, Ameri et al. 2007) and rheumatoid arthritis (ten Bokum et al. 1999).

**DR expression in endocrine tumors**

It has been extensively demonstrated, with various techniques, that DR are expressed in the large majority of pituitary adenomas, including GH-secreting, prolactin (PRL)-secreting, ACTH-secreting, and clinically non-functioning tumors (Panetta & Patel 1995, Stefaneanu et al. 2001, Pivonello et al. 2004b, Zatelli et al. 2005, Ferone et al. 2008, Saveanu et al. 2008). Furthermore, DR expression has been well characterized in other NET, such as pheochromocytomas (Pupilli et al. 1994, Pivonello et al. 2004a, 2007b) and paragangliomas (Wu et al. 2001).

Beyond the above-mentioned NET, the presence of DR – mainly D_2 – has been demonstrated in a small group of well-differentiated endocrine tumors (including lung and thymic carcinoids), associated with ectopic ACTH secretion and Cushing’s syndrome (Pivonello et al. 2007a).

The observation of the expression of D_2 in GEP NET cell lines (Lemmer et al. 2002) has been followed by recent studies evaluating the expression of D_2 in a series of patients with NET (mainly GEP NET).

O’Toole et al. evaluated the quantitative expression of D_2 mRNA by RT-PCR in a series of 35 GEP NET. The expression of D_2 was detected in all samples, although D_2 expression level was similar to those observed in somatotroph adenomas only in the 17% of cases (O’Toole et al. 2006b).

Recently, Grossrubatscher et al. demonstrated the presence of D_2 in well-differentiated NET of different sites and in normal islet cells by IHC. They found a high expression of D_2 receptors among the tumors examined (85%), being bronchial carcinoids, islet cell tumors, and the NET of the duodenum the tumors with the highest receptor protein expression (Grossrubatscher et al. 2008). Moreover, they observed that a worse clinical outcome was more frequent among patients with less D_2 immunoreactivity,
although they could not find a significant correlation between the presence of D2 receptors and Ki-67 expression in the tumors.

It has to be emphasized that, as for SSTR, comparison between results obtained with different techniques is difficult, and in some cases, mRNA detection with PCR may overestimate quantities expressed.

SS and DA receptor co-expression in endocrine tumors

Co-expression of SSTR and D2 in pituitary adenomas has been recently reviewed extensively (Jaquet et al. 2005b, Ferone et al. 2009, Saveanu & Jaquet 2009). Here, we would like to underline few important aspects: the D2 receptor is the GPCR mostly represented in the pituitary tumors, overall it is associated with two or more SSTR subtypes (preferentially sst2 and sst5), and there is a high variability in SSTR and D2 expression due to the heterogeneity of these tumors (Zatelli et al. 2005, Ferone et al. 2008, Saveanu et al. 2008, de Bruin et al. 2009c).

Up to date, only few studies in the literature demonstrated SSTR and D2 co-expression in endocrine tumors beyond the above-mentioned pituitary adenomas (Table 1). The majority of these studies were carried out on immortalized cell lines from lung and GEP carcinoids, small cell lung cancer (SCLC), and medullary thyroid carcinoma (Ferone et al. 2005, Kidd et al. 2007b, 2008, de Bruin et al. 2009b, Arvigo et al. 2010). To our knowledge, only two studies have been published so far describing SSTR and D2 co-expression in patients with NET (not including pituitary adenomas). O’Toole et al. (2006b) evaluated quantitative co-expression of sst1, sst2, sst3, and sst5 with D2 mRNA by RT-PCR in a series of 35 GEP NET. They observed co-expression of sst2 and D2 in 100% of cases and sst3 and sst5 in 89%. Moreover, they correlated quantitative mRNA expression in tumors of this group of patients with that observed in GH-secreting adenomas and showed that sst2 levels were similar between GEP and somatotroph tumors, whereas sst5 and D2 levels were higher in the latter.

Recently, Srirajaskanthan et al., using IHC, demonstrated co-expression of sst2, sst5, and D2 in a wide variety of NET (56 cases). They observed an overall expression of D2 in 81% of the cases and sst2 and sst5 were identified in 93% of cases. Both D2 and SSTR expression was higher in low- and intermediate-grade tumors compared with high-grade tumors (Srirajaskanthan et al. 2009).

Preliminary data by Pivonello et al. (2008) described sst2, sst5, and D2 co-expression in ten different cases of pancreatic endocrine tumors, evaluated by IHC.

Table 1 Most recent studies reporting co-expression of somatostatin and D2 receptors in human tumors (excluding pituitary adenomas)

<table>
<thead>
<tr>
<th>Author (years)</th>
<th>Materials</th>
<th>Receptor evaluation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferone et al. (2005)</td>
<td>NSCLC cell line</td>
<td>mRNA/protein (W.B.)</td>
<td>sst2, sst3, sst4, and D2 co-expression</td>
</tr>
<tr>
<td>O’Toole et al. (2006b)</td>
<td>35 GEP NET patients</td>
<td>mRNA</td>
<td>100% sst1, sst2, and D2 co-expression</td>
</tr>
<tr>
<td>Kidd et al. (2008)</td>
<td>Atypical BP NET cell line</td>
<td>mRNA</td>
<td>sst2, sst5, and D2 co-expression</td>
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<tr>
<td></td>
<td>Typical BP NET cell line</td>
<td>mRNA</td>
<td>sst1, sst2, sst3, sst5, and D2 co-expression</td>
</tr>
<tr>
<td></td>
<td>GI NET cell line</td>
<td>Protein (IHC)</td>
<td>D2 absent</td>
</tr>
<tr>
<td>Pivonello et al. (2008)</td>
<td>Ten pancreatic NET patients</td>
<td>Protein (IHC)</td>
<td>100% sst2 and D2 co-expression</td>
</tr>
<tr>
<td>De Bruin et al (2009b)</td>
<td>Pancreatic carcinoid cell line</td>
<td>mRNA</td>
<td>80% sst2, sst5, and D2 co-expression</td>
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<td></td>
<td>MTC cell line</td>
<td>sst2, sst5, and D2 co-expression</td>
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<tr>
<td></td>
<td>SCLC cell line</td>
<td>sst2, sst5, and D2 co-expression</td>
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<tr>
<td></td>
<td>56 NET patients</td>
<td>Protein (IHC)</td>
<td>sst2, sst5, and D2 co-expression</td>
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<tr>
<td></td>
<td>(48 GEP NET, seven unknown primary NET</td>
<td>92.9% sst2 and sst5 co-expression</td>
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<td></td>
<td></td>
<td></td>
<td>80.3% sst2, sst5, and D2 co-expression</td>
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<tr>
<td>Srirajaskanthan et al.</td>
<td>18 ectopic ACTH-producing tumors patients (five BP NET, three SCLC, three thymic carcinoid, two pancreatic NET, two MTC, one gastrinoma, one NSCLC, one esthesioneuroblastoma)</td>
<td>mRNA/protein (W.B.)</td>
<td>sst1, sst2, sst3, sst5, and D2 co-expression</td>
</tr>
<tr>
<td>(2009)</td>
<td></td>
<td>Protein (IHC)</td>
<td>50% sst2 and D2 co-expression</td>
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<tr>
<td>Arvigo et al. (2010)</td>
<td>PCa cell line</td>
<td></td>
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<tr>
<td>Feelders et al. (2010)</td>
<td>18 ectopic ACTH-producing tumors patients (five BP NET, three SCLC, three thymic carcinoid, two pancreatic NET, two MTC, one gastrinoma, one NSCLC, one esthesioneuroblastoma)</td>
<td>mMnRNA/protein (W.B.)</td>
<td></td>
</tr>
</tbody>
</table>

NSCLC, non small cell lung cancer; W.B., western blot; GEP, gastroenteropancreatic; NET, neuroendocrine tumors; BP, bronchopulmonary; GI, gastrointestinal; MTC, medullary thyroid cancer; SCLC, small cell lung cancer; PCa, prostate cancer.
Moreover, other preliminary data by Feelders et al. showed sst2 and D2 co-expression by IHC in 18 patients with ectopic ACTH-producing tumors from different sites. Sst2- and D2-positive staining was found in 10/18 and 13/18 tumors respectively. Co-expression of both receptors was observed in 9/18 tumors (Feelders et al. 2010).

Figure 1 shows a schematic representation of human tumors already demonstrated to co-express SS and DA D2 receptors.

**SS and DA analogs treatment in endocrine tumors**

NET previously considered as ‘carcinoids’ are heterogeneous and ubiquitous neoplasias that account for about 1% of all malignancies.

Recent estimates have shown an increasing incidence (3–10% per year) over the last 30 years and a sharp increase in their prevalence, as well as survival (Gustafsson et al. 2008, Yao et al. 2008). Surgery is still the only curative therapy for NET, but unfortunately it can be really effective in a minority of patients (about 15%). This is due to the very high prevalence of metastatic disease already at the time of diagnosis (85% of diagnosed NET; Modlin et al. 2006). In this respect, the control of clinical symptoms and the stabilization of the disease are the primary goal of the currently available therapies (Modlin et al. 2006).

Most of the NET of any site have a more or less indolent clinical trend and can benefit from specific therapeutic approaches with biological agents with antiproliferative and antisecretory activity (Colao et al. 2010). The use of these agents requires knowledge of the biological mechanisms underlying neuroendocrine tumorigenesis, and in this respect up to now, medical therapy of NET is mainly based on biotherapy with SSAs (Arnold et al. 2009).

The ‘classical’ clinically available SSA octreotide and lanreotide, with a preferential binding affinity for sst2, at standard dosages have been demonstrated to improve the symptoms related to functioning NET in 64% of patients, with a biochemical response in 66% of cases (Grozinsky-Glasberg et al. 2008). Only in about 10% of cases there is a significant reduction of tumor mass, while tumor stabilization occurs in 35–50% of patients (Grozinsky-Glasberg et al. 2008, 2009).

![Figure 1](image_url)

**Figure 1** Schematic representation of human tumors already demonstrated to co-express somatostatin and D2 receptors. The presence of both SSTR and D2 receptors in pituitary adenomas is well known and has already been extensively reviewed. Recently, a significant number of studies have been published (listed in Table 1) focusing on SSTR and D2 receptor co-expression in a number of neuroendocrine neoplasms, such as GEP and lung NET, and non-endocrine tumors. These new studies arise from the availability of new universal and subtype-specific SSAs and D2-selective DA agonists and from the possibility to test these compounds as targeted combination therapy, with potentially synergistic effects. NETs, neuroendocrine tumors; NSCLC, non small cell lung cancer.
Colao \textit{et al.} 2010). A first recent double-blind, placebo-controlled randomized phase IIIb study of octreotide LAR or placebo in patients with well-differentiated metastatic midgut NET demonstrated that octreotide more than doubled the time to tumor progression compared with placebo, in both functioning and non-functioning NET (Rinke \textit{et al.} 2009). More in detail, patients with low-grade (Ki-67 <2\%) and low hepatic tumor load NET, even if non-functioning, are likely to benefit from octreotide treatment as first-line therapy (Rinke \textit{et al.} 2009).

Different from GEP NET, and due to the limited number of clinical studies investigating the effect of ‘cold’ (Kosmidis 2004, De Dosso \textit{et al.} 2007) or radiolabeled SSA in pulmonary NET (van Essen \textit{et al.} 2007), there are no standardized guidelines yet for SSA treatment in these kind of tumors, especially for the most aggressive histotypes, where the role of biotherapies is still debated and not well clarified (Righi \textit{et al.} 2010). Well-defined therapeutic approaches still reside on surgery (McMullan & Wood 2003), chemoradiotherapy, or liver embolization (Kosmidis 2004).

In addition, patients with GEP or lung NET receiving sst2-prefering analogs frequently experience a loss of response (the ‘escape from response’ phenomenon or tachyphylaxis) to treatment, either in the short or in the long term, following initiation of treatment (Wynick & Bloom 1991, Ricci \textit{et al.} 2000, Hofland & Lamberts 2003, Oberg 2010). In this context, although final evidences are lacking, receptor desensitization and degradation (leading to receptor downregulation) may be involved in an ‘early’ escape (weeks–months) to SSA treatment (Hofland & Lamberts 2003). On the other hand, cellular events such as the overgrowth of SSTR-negative clones, mutations in SSTR genes, and/or tumor dedifferentiation, frequently observed in cancer natural history, are more likely related to the ‘late’ escape to SSA (years) observed in NET (Hofland & Lamberts 2003; Tulipano & Schulz 2007).

Higher doses of the drug may reverse, even if temporarily, the ‘early’ escape but, going forward with the treatment, most patients with lung and GEP NET eventually become un-responders (Lamberts \textit{et al.} 1996, Hofland & Lamberts 2003).

Targeting D2 has been shown as an effective mechanism for suppressing secretion of hormones by NET, and especially in pituitary adenomas (Ren \textit{et al.} 2003\textit{a}, Pivonello \textit{et al.} 2004\textit{b}, Jaquet \textit{et al.} 2005\textit{a}, Ferone \textit{et al.} 2007\textit{a}). In particular, in patients with prolactin-secreting pituitary adenomas, the role of D2 agonist treatment is well established (i.e. inhibition of circulating prolactin levels and induction of tumor shrinkage). However, despite the multiple evidences for antiproliferative and antisecretive effects by DA agonists from \textit{in vitro} studies, the \textit{in vivo} efficacy of these compounds has not well been established yet in NET, particularly in lung and GEP NET (Kidd \textit{et al.} 2008).

Many years ago, for the first time, Child \textit{et al.} (1978) treated two cases of hypergastrinemia due to a gastrinoma with bromocriptine, unfortunately without any results. Ishibashi \textit{et al.} (1994) showed the ability of bromocriptine, a dopaminergic agonist, in inhibiting the growth of human SCLC cells, implanted as tumor xenograft in athymic nude mice, in a dose-dependent manner. In the human neuroendocrine pancreatic cell line BON some authors have demonstrated the expression of D2 (de Bruin \textit{et al.} 2009) and the effect of the D2 receptor agonist drug quinpirole in decreasing intracellular cAMP levels (Lemmer \textit{et al.} 2002). Recent studies also demonstrated a significant inhibitory effect of cabergoline, a D2 preferential compound, in inhibiting cell proliferation both in lung carcinoma and in prostate cancer cell lines, endogenously expressing the D2 (Ferone \textit{et al.} 2005, Arvigo \textit{et al.} 2010, Ruscica \textit{et al.} 2010).

Recently, bromocriptine and cabergoline have been shown to be effective in suppressing ACTH secretion in a small group of patients with lung carcinoid (Reith \textit{et al.} 1987, Pivonello \textit{et al.} 2007\textit{a}), as previously observed \textit{in vitro} by Farrell \textit{et al.} (1992). Moreover, preliminary data by Pivonello \textit{et al.} (2008) demonstrated an \textit{in vitro} inhibitory effect of cabergoline on cell growth in ten different cases of pancreatic endocrine tumors (seven cases defined as well-differentiated tumors and three defined as well-differentiated carcinomas). In this context, a recent report showed a sevenfold decrease in the pancreatic polypeptide levels and the reduction of liver metastases in a patient with pancreatic polypeptide secreting islet cell tumor during therapy with DA agonists (Pathak \textit{et al.} 2004).

Despite the fact that combined treatment with SSAs, DAs have already been demonstrated efficacious in a subgroup of patients with GH-secreting pituitary adenomas (Colao \textit{et al.} 2007) and in one case of an ACTH-secreting lung carcinoid (Pivonello \textit{et al.} 2005), up-to-date clinical studies reporting the effect of combined SSA/DA in NET are lacking.

\textbf{New insights in SS and DA receptor signaling, trafficking, and interaction}

In recent years, a number of studies have investigated the pathophysiological role of SSTR and D2, leading to novel insights with possible important clues for clinical management of NET (Hofland & Lamberts 2003, Tulipano & Schulz 2007).
It is well known that the five SSTR subtypes share common signaling pathways, although particular SSTR subtypes can activate distinct signaling pathways as well (Ben-Shlomo & Melmed 2010, Hofland et al. 2010).

Recently, Duran-Prado et al. (2009) reported the first evidence for the existence of two novel sst5-truncated variants (termed sst5TMD4 and sst5TMD5) in pituitary adenomas, which are absent in the normal pituitary gland. The sst5TMD4 variant was reported as particularly abundant in octreotide-resistant somatotropinomas and the authors speculated about its possible role in the attenuated response to SSA observed in some pituitary tumors (Duran-Prado et al. 2010).

The intracellular pathways activated by SSTR activation appear different in different types of tumor cells and depend on the specific SSTR distribution pattern, signaling elements, as well as to receptor desensitization, internalization, and cross talk (Lahlou et al. 2004, Schonbrunn 2008). Moreover, it has been suggested that different SSA, in the same cell type, may elicit differential effects, due to the activation of different subsets of intracellular mediators. This phenomenon, also named biased agonism, seems to depend on the typical agonist–receptor interactions (Schonbrunn 2008, Ben-Shlomo et al. 2009, Cescato et al. 2010). In two recent in vitro studies octreotide and pasireotide (SOM 230) have been demonstrated to modulate sst2A receptor phosphorylation and trafficking in a clearly distinct manner, despite their approximately similar binding affinity to this SSTR subtype (Poll et al. 2010). Pasireotide appeared to be more potent than octreotide in inducing internalization and signaling of human sst3 and sst5 receptors (Lesche et al. 2009). The observed behavior of SOM 230 as only a partial agonist of sst2 sheds light on the importance of the agonist-induced receptor conformation in affecting receptor signaling and regulation, more than binding affinity alone.

It has to be reminded that like many other GPCRs, SSTR undergo agonist-induced endocytosis following the agonist binding to the receptors. The activated receptor is then phosphorylated by the G protein-coupled receptor kinases (GRKs) and subsequently

Figure 2 Simplified representation of SSTR signaling and trafficking. SS (or SSA) binding to SSTR activates G proteins and inhibits adenylyl cyclase (AC) activity, activates K⁺ channels, and inhibits Ca²⁺ channels. An antiproliferative effect may be mediated via the stimulation of PTP and modulation of mitogen-activated protein kinases (MAPK). An increase in apoptosis via p53 has been shown as well (Ferone et al. 2009). After agonist activation, SSTR are phosphorylated (mainly involving G protein coupled receptor kinase (GRK)) and recruited by cytoplasmic proteins termed arrestins that interrupt coupling between the receptor and G proteins (desensitization process). β-Arrestins also function as the link between the receptor and the components of the endocytic machinery, such as dynamin and clathrin. The internalized receptor is then directed to early endosomes in which it is dephosphorylated and dissociated from β-arrestins. The receptor is then directed to different intracellular compartments, leading to either recycling or degradation. Finally, the recycled receptor is back to the plasma membrane as functional (resensitized) receptor (Tulipano & Schulz 2007). The rate of recycled or degraded receptors seems to be influenced mainly by β-arrestin interaction and other regulatory intracellular proteins, such as ubiquitins (Gray & Roth 2002). NSF, N-ethylmaleimide sensitive factor; GASP, GPCR-associated sorting protein; SNX-1, sortin nexin-1; pHᵢ, intracellular pH. Adapted, with permission, from Tulipano & Schultz (2007), Ferone et al. (2009).
recruited by cytoplasmic proteins, named arrestins, determining uncoupling between the receptor and its related G proteins (Oakley et al. 2000, 2001, Gurevich & Gurevich 2006). The receptor/arrestin complex is then internalized by dynamin-dependent endocytosis.

In this context, β-arrestins seems to play a pivotal role in the desensitization–internalization process of GPCRs, including SSTR (Bohm et al. 1997, Koenig & Edwardson 1997, Bloch et al. 1999, Tulipano & Schulz 2007). Different SSTR subtypes display a differential interaction with β-arrestins. sst₃ and sst₅ bind β-arrestin 2 with higher affinity than β-arrestin 1, resulting in a less stable receptor/arrestin complex and a faster recycling on cell membrane. On the contrary, sst₂ displays the same affinity for both β-arrestin 1 and 2 and is internalized into endosomes forming a tight SSTR/β-arrestin complex.

Moreover, while the recycling seems to be the most common process following the internalization of sst₂ and sst₅, degradation seems to be the most common for sst₃ (Jacobs & Schulz 2008, Reubi et al. 2010). In this respect, other regulatory factors of this already very complex system are intracellular proteins such as ubiquitins, SNX-1, GASP, and NSF that lead the early endosome to either cell membrane or the lysosomal pathway (Yu et al. 1993, Pippig et al. 1995, Gagnon et al. 1998, Tsao & von Zastrow 2000, Gray & Roth 2002).

The co-expression of different SSTR subtypes or the presence of different intracellular components involved in this trafficking could form the basis for differences in the SSTR trafficking in different kinds of tumors (Hofland & Lamberts 2003, Tulipano & Schulz 2007, Grant et al. 2008, Jacobs & Schulz 2008; Fig. 2).

These above-mentioned new insights are extremely important for the development of new therapeutic strategies targeting SSTR with SSA, especially for lung and GEP NET, that after an initial response frequently show escape from the effect of SSA. The mechanism behind such an escape from treatment has not been elucidated yet (see above) but could include receptor downregulation as result of SSA-activated receptor trafficking (Lamberts et al. 1996, Hofland & Lamberts 2003, Hofland et al. 2005b).

The regulation of D₂ seems even more complicated, with the receptor activation variably resulting in functional desensitization, sensitization, and up or downregulation (Pivonello et al. 2007b). Namkung and colleagues demonstrated that D₂ homologous and

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**Figure 3** Simplified representation of D₂ signaling and trafficking. DA (or DA agonists) binding to D₂, via interaction with G proteins, inhibits adenyly cyclase (AC) activity and phosphatidylinositol metabolism, activates voltage-activated potassium channels and decreases voltage-calcium currents, and modulates the activity of phospholipase C (PLC) and the mitogen-activated protein kinases (MAPKs). These processes result in lowering of gene expression, as well as to antisecretive effects in endocrine cells, and, via modulation of MAPK, in increased cell apoptosis and inhibition of cell growth (Pivonello et al. 2007b, Ferone et al. 2009). Like SSTR, D₂ phosphorylation after agonist activation is mediated by GRKs and heterologous receptor phosphorylation may occur via protein kinase C activation (PKC; Namkung & Sibley 2004). Moreover, subsequent recruitment of arrestins by the activated receptor may result not only in signal desensitization but β-arrestins can also act as a second messenger leading to glycogen synthase kinase 3β (GSK 3β) activation and increased gene expression (Hofmann et al. 2009). It has to be underlined that recent studies suggested that GRK and β-arrestins may exert their functions in the absence of receptor phosphorylation, and phosphorylation-independent association with β-arrestin seems to play a major role in agonist-induced D₂ desensitization (Cho et al. 2010). Adapted, with permission, from Ferone et al. (2009).
heterologous phosphorylation (mediated by GRK and protein kinase C activation respectively) differently affect receptor internalization and recycling (Namkung & Sibley 2004, Namkung et al. 2009). Moreover, in a recent study, Cho et al. (2010) observed that D2 desensitization and resensitization, different from the classical GPCR model, are mainly mediated by β-arrestin in a phosphorylation-independent manner (Fig. 3). Since SSTR and D2 are often co-expressed in endocrine tumors, it is likely to hypothesize some interaction between the receptor/β-arrestin complex of the two receptor families, affecting signaling and trafficking of the activated receptors.

In addition, it is well known that both SSTR and DR may act not only as monomers but also as homo- and heterodimers as well. Such receptor oligomerization results in modified functional and pharmacological properties of the receptor complex (Rocheville et al. 2000b, Pfeiffer et al. 2001, Patel et al. 2002, Lee et al. 2003, Grant et al. 2004, Duran-Prado et al. 2008, Grant et al. 2008). SSTR and D2 have been reported to physically interact by forming heterodimers with enhanced functional activity (Rocheville et al. 2000a) involving cellular events such as modified SSTR internalization, trafficking (Baragli et al. 2007, Grant et al. 2008), and signal transduction (Kidd et al. 2008; Fig. 4).

In the light of these insights, new SSA, which bind more than one SSTR subtype (Weckbecker et al. 2003), as well as chimeric compounds binding both SSTR and D2, have been developed for treatment of SSTR and D2 co-expressing tumors (Ferone et al. 2007b).

However, almost all studies carried out on cell lines showed receptor dimerization in transfected models highly expressing at least two receptor subtypes (Rocheville et al. 2000b, Pfeiffer et al. 2001, Ren et al. 2003b) and most of the in vitro studies evaluating the effect of these new chimeric compounds did not evaluate receptor interactions (Ferone et al. 2005, Saveanu et al. 2006, Florio et al. 2008, Kidd et al. 2008). In a very recent study, Arvigo et al. investigated the effect of this new generation of chimeric compounds in two non-neuroendocrine cell lines endogenously expressing SSTR and D2 (Ferone et al. 2005, Ruscica et al. 2010) observing for the first time a direct and significant positive correlation between the amount of ligand induced SST5/D2 dimers and the observed antiproliferative effect (Arvigo et al. 2010).

Figure 4 Schematic representation of possible interactions between SSTR and D2 receptor. Like the majority of GPCRs, SSTR and D2 can also interact at the cell membrane, when co-expressed. It is well known that these receptors can act as heterodimers after agonist binding (Rocheville et al. 2000a, Kidd et al. 2008). Kidd et al. suggested a possible explanation for the intracellular pathway following the activation of the heterodimer represented by an upregulation of p21WAF1/CIP1, via c-Jun N-terminal phosphorylation and a concomitant inhibition of Ki-67 transcription. However, they observed that a different dimer complex composition can lead to a decrease in P21 transcription and to an increase in p53 (Kidd et al. 2008). It could also be hypothesized that receptor dimerization can influence the single receptor phosphorylation and β-arrestin interaction, resulting in the modulation of receptor desensitization, recycling, and degradation. Moreover, recent evidences suggested a possible heterologous phosphorylation of both SST2 and D2 by co-expressed phospholipase C (PLC)-coupled receptors (e.g. cholecystokinin (CCK) or bombesin (BBS) receptors) that may result in the modulation of receptor desensitization and internalization (Elberg et al. 2002, Namkung & Sibley 2004). IP3, inositol phosphate 3. Adapted, with permission, from Ferone et al. (2009).
Keeping all these data together, this suggests that not only the single receptor signaling and trafficking but also the cell types, as well as the receptor dimerization, are important components determining the final effect of a given ligand.

Future perspectives of SSA and DA agonist treatment

On the basis of the reported data on NET treatment using the classical SSA and DA, the newly developed compounds and the combined targeting of SSTR and D₂ all aim as primary goal to increase the tumor response rate (especially in terms of antiproliferative effect) and to reduce the impact of the escape to treatment.

In order to perform a really tailored target therapy, several SSA with different SSTR binding affinity have been developed and used for in vitro and in vivo studies (Weckbecker et al. 2003). Among these, an interesting compound is the BIM-23244, a SSA with a comparable high binding affinity for sst₂ and sst₅. In vitro studies using this compound have demonstrated that a co-activation of sst₂ and sst₅ suppresses GH production in octreotide-resistant GH-secreting adenomas, suggesting that this generation of compounds could improve the clinical utility of SSA (Saveanu et al. 2001).

Pasireotide (SOM 230), a novel multi-receptor ligand analog with high binding affinity for four of the five SSTR subtypes (sst₁₋₃ and sst₅), exhibits an affinity binding profile for human SSTR more similar to native SS than to either octreotide or lanreotide (Schmid & Schoeffter 2004, Schmid 2008, Ben-Shlomo et al. 2009). A number of preclinical studies suggest that SOM 230 is a promising candidate for clinical applications in situations where octreotide and lanreotide were shown to be weakly active or even ineffective, such as ACTH-secreting pituitary adenomas and octreotide-resistant GH-secreting adenomas (van der Hoek et al. 2005, Hofland et al. 2005a, Batista et al. 2006, Ben-Shlomo & Melmed 2007). Moreover, pasireotide was described to significantly reduce cell proliferation in a lung NET cell line (Ono et al. 2007) and to inhibit cell growth and catecholamine secretion in cell cultures of pheochromocytoma (Pasquali et al. 2008). Therefore, this new SSA is currently under clinical investigation to treat patients with acromegaly, Cushing’s disease, and NET.

The SSA/DA chimeric molecules have been largely tested in in vitro studies. Saveanu et al. (2006) demonstrated that the chimeric compounds BIM-23A387 and BIM-23A760 were more effective in controlling hormonal hypersecretion in vitro in a subgroup of GH-secreting adenomas that were partially responders to octreotide, compared to both sst₂ and sst₅ monospecific analogs, as well as to octreotide in combination with cabergoline. The same molecules tested in a non-small lung cancer cell line (Calu-6) and in a prostate cancer cell line (LNCaP) showed a greater antiproliferative effect than subtype-specific SS and DA agonists, alone or in combination (Ferone et al. 2005, Arvigo et al. 2010). In addition, a differential cytotoxicity of chimeric compounds was recently observed in bronchopulmonary and small intestinal NET cell lines (Kidd et al. 2008). Conversely, in a gastric enterochromaffin-like tumor cell line, the dopastatin BIM-27A760 did not display any additive effect in the inhibition of cell proliferation and hormone secretion (Kidd et al. 2007a). All these data suggest that specific compounds based on the individual tumor lesion receptor profile might be needed to achieve a significant antiproliferative effect in the different NET cell types (Kidd et al. 2008). Moreover, a possible explanation for the lack of efficacy of agonist drugs in the presence of a ‘suitable’ SSTR/D₂ profile may reside in the dynamic behavior of GPCRs, leading to receptor cross talk both at cell membranes and/or intracellular level (Ferone et al. 2007b, Saveanu et al. 2008).

The in vitro studies reported above are now to be supported by clinical evidences. There are several ongoing pre-clinical and clinical trials, involving pasireotide, dopastatin, and combined treatments with SSA and everolimus (a mammalian target for rapamycin inhibitor), aimed at demonstrating the in vivo safety and efficacy of these newly developed compounds (Ferone et al. 2009, Colao et al. 2010). It should be mentioned, however, that a recent phase II trial with BIM-23A760 in acromegaly was stopped due to a lack of efficacy. Therefore, further studies investigating the clinical efficacy of dopastatins are required.

Conclusions

SSTR and D₂ are widely co-expressed in pituitary adenomas. Recent studies demonstrated their co-expression in a large variety of well-differentiated NET, originating from different tissues such as GI tract, pancreas, lung, adrenal, and thymus as well.

These findings, together with the availability of new universal and subtype-specific SSAs and D₂-selective DA agonists, initiated researchers and clinicians to test targeted combination therapy and treatment with the so-called dopastatins, compounds with affinity for both SSTR and D₂.
However, the controversial results coming out from a number of in vitro studies highlight the importance for a better understanding of the pathophysiological basis, which regulates the complex system of GPCRs. The most recent insights of SSTR and D2 pathophysiology show that not only cell and tissue specificity, receptor pattern, and single receptor binding affinity of drugs might affect receptor signaling and trafficking after ligand exposure.

Many other variables, such as the presence of truncated receptor forms with different biological effects than the wild-type receptor, receptor cross talk, receptor homo and heterodimerization, and different expression of intracellular regulatory molecules (e.g. β-arrestin and ubiquitins), can determine the success of a given receptor targeted therapy. In this context, the increasing need for a better understanding of the molecular mechanisms regulating SSTR and D2 signaling, trafficking, and cross talk is the ultimate step for developing more specific, selected, and effective medical treatment for patients with NET.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding
This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received in final form 5 October 2011
Accepted 20 October 2011
Made available online as an Accepted Preprint 20 October 2011