Preclinical and clinical experiences with the role of somatostatin receptors in the treatment of pituitary adenomas

Joost van der Hoek, Steven W J Lamberts and Leo J Hofland
Department of Internal Medicine, Section Endocrinology, Room Ee30b, Erasmus MC, Dr Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands
(Correspondence should be addressed to L J Hofland; Email: l.hofland@erasmusmc.nl)

Abstract
The patho-physiological role of somatostatin receptor subtypes (sst) in neuro endocrine diseases has gained enhanced scientific interest in the past few years. The development of novel somatotropin-release inhibiting factor analogs, both sst-specific and universal ligands, seem promising as a tool to further increase fundamental insights in sst function. Eventually, this research should result in novel medical therapeutic opportunities in patients suffering from neuro-endocrine diseases. In the present review, the functional role of sst in all types of pituitary adenomas, based on recent preclinical and clinical studies, is being discussed.

Introduction
Somatostatin, also known as somatotropin-release inhibiting factor (SRIF), is a cyclopeptide that has broad inhibitory effects on the secretion of hormones such as growth hormone, insulin and glucagon. These effects have formed the basis for the clinical use of SRIF-analogs in the treatment of acromegaly and endocrine tumors. The discovery of the five SRIF receptor subtypes (sst) in the 1990s further enhanced the understanding of the biological roles of SRIF and SRIF analogs, and paved the way for new therapeutic opportunities. In this review, recent preclinical and clinical advances in sst-targeted treatment of human pituitary adenomas are discussed.

GH-secreting pituitary adenomas
Acromegaly is predominantly caused by a growth hormone (GH) secreting pituitary adenoma, resulting in high circulating GH and insulin-like growth factor I (IGF-I) hormone concentrations. Over the years, a triad of therapeutical options has been formed: surgery, irradiation and medical treatment (1). These treatment modules had the aim of inducing tumor shrinkage and normalizing GH and IGF-I levels, thereby reducing the risk of long-term complications including the development of malignant neoplasms, cardio- and cerebrovascular disease, respiratory and metabolic dysfunction (2). The clinical introduction of the long-acting SRIF analogs in the early 1980s added a new dimension to the treatment of acromegaly (3–5). The short half-life of SRIF and the subsequent need for i.v. administration however, as well as the post-infusion hypersecretion of GH, insulin and glucagon, has rendered the native peptide impractical for therapeutic use (6). The short stable synthetic octapeptide SRIF analogs, octreotide (OCT) and lanreotide, appeared not to have these disadvantages and were administered in acromegalic patients to assess their role as a novel treatment option in acromegaly. Indeed, after the first reports that demonstrated the long-acting inhibitory effect of OCT on plasma GH levels, as well as a rapid amelioration of the clinical signs and symptoms, 20 years of endocrine practice and science has turned OCT and lanreotide into the widely accepted first medical treatment option for acromegaly (7).

The current clinically available octapeptide SRIF analogs have been consistently shown to be able to reduce hormonal hypersecretion and to normalize IGF-I levels in a significant proportion of treated patients. Recently, Freda, summarizing the literature data on this topic (8), showed the achievement of safe GH levels in 56% of patients treated with sandostatin-LAR (OCT incorporated into microspheres of a biodegradable polymer that results in therapeutical blood concentrations of the peptide for 24–42 days) and in 49% of those treated with lanreotide 30 mg. The respective figures for IGF-I normalization were 66 and 48%. However, a large majority of the patients enrolled in these studies were preselected for SRIF analog responsiveness (8). Therefore, several studies were
initiated in which newly diagnosed patients were treated with somatostatin analogs. To date, efficacy numbers have been reported for normalizing GH (43–79%) and IGF-I (53–68%), which are comparable with efficacy numbers for adjuvant SRIF analog therapy (9–15). Human GH-secreting pituitary adenomas express multiple sst subtypes. sst2 and sst5 receptors are the predominantly expressed sst, both at the mRNA (Table 1; (16–19)) and the protein level (20). Several studies reported a variable sst2 mRNA expression and a relatively high expression of sst5 (16, 18, 21). The sst2 seems a predominant receptor in determining the inhibitory effect of OCT or lanreotide on circulating GH release in acromegalic patients. sst2 mRNA expression in GH-secreting pituitary adenomas shows a positive correlation with the in vivo GH suppression induced by an acute test using a single injection of 200 μg OCT (21), as well as with the in vitro and in vivo responsiveness to OCT in another series of patients (18). Moreover, it has recently become clear that apart from sst2, sst5 receptors play an important role in regulating GH secretion by human GH-secreting pituitary adenoma cells as well. In this respect, the regulation of fetal human GH secretion (22) is similar to that in human GH-secreting pituitary adenomas. In primary cultures of human GH-secreting pituitary adenomas, new SRIF-analogs with enhanced sst2-binding affinities inhibit GH secretion more potently compared with the clinically used octapeptide SRIF analogs, OCT and lanreotide. In addition, some adenomas show a better response to sst2-specific analogs, whereas in others sst5-specific analogs are more potent in suppressing GH release (23). Moreover, the combined activation of sst2 and sst5 results in additive inhibitory effects on GH secretion. Interestingly, the sst5 preferential analog BIM-23 268 inhibited GH release in only 7 of 15 cases, whereas, in agreement with the results of Shimon and coworkers (23), partial additive effects in suppressing GH release were found in OCT-partially responding cultures when the sst2- and sst5-specific compounds were used in combination. Taking these data together, it can be concluded that sst2 is the predominant receptor in regulating GH release by GH-secreting pituitary adenoma cells, whereas sst5 receptors may mediate an inhibitory effect on GH secretion as well. The additive inhibitory effects on GH release following activation of both sst2 and sst5 are probably mediated via a functional association of both sst subtypes. Ren and coworkers (24) demonstrated in human fetal pituitary cell cultures that an sst2 selective antagonist was capable of reversing the GH suppressive effects of sst2/sst5 biselective agonists or that of sst2 and sst5 agonists in combination, suggesting a functional interaction between both sst subtypes. In adenomas co-secreting GH and prolactin (PRL), PRL secretion is preferentially inhibited by sst5-specific SRIF analogs (16, 18, 23). The observed additive GH-suppressive effect of activating both sst2 and sst5 also initiated the development of analogs with selectivity to multiple sst subtypes. One of these compounds, the sst2- and sst5-bispecific compound BIM-23 244, indeed inhibits GH release in a subgroup of partially OCT-sensitive adenomas more potently when compared with OCT. In this subgroup of adenomas sst2 mRNA expression was ninefold lower, and sst5 mRNA expression approximately sevenfold higher than in the OCT-sensitive adenomas (21). These studies suggest that in tumors expressing a low sst2 level and a high sst2/sst5 ratio, sst5 is of increasing importance in regulating GH release (21). Another recently developed compound has a more universal sst-binding profile. This compound, named SOM230, has a 25-, 5- and 40 times higher binding affinity to sst1, sst3, and sst5 receptors respectively, and 2.5 times lower affinity to sst2, when compared with OCT (25). The SOM-230 inhibits GH release in a higher number of GH-secreting pituitary adenomas, both in vitro (18) and in vivo (see below). SOM230 suppressed GH secretion in primary cultures from human fetal pituitary cells (26). The preclinical reports of enhanced GH suppression via the combined activation of sst2 and sst5 receptors in cultures of GH-secreting pituitary adenomas, both in vitro and in vivo, demonstrated potent inhibitory effects upon GH release in patients with GH-secreting pituitary adenomas expressing a low amount of sst2 and a high amount of sst5 suggests that the sst5 subtype may indeed play an essential role in mediating in vivo GH suppressive actions in OCT-partial responders. Therefore, the enhanced efficacy of SOM230 can be explained by high sst5 expression in this type of adenomas and its 40-fold higher binding affinity for sst5 as compared with OCT. Since SOM230 is able to lower GH levels in both OCT responders and partial responders, this novel SRIF analog has a clear advantage over OCT and might increase the number of patients that can be biochemically controlled during long-term medical treatment. Finally, a role for sst5 in the control of GH-secreting pituitary adenoma cell function has been proposed.

Table 1 Percentage of human pituitary tumors expressing SRIF receptors.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>sst1</th>
<th>sst2</th>
<th>sst3</th>
<th>sst4</th>
<th>sst5</th>
</tr>
</thead>
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<tr>
<td>Pituitary tumor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatotrope</td>
<td>44</td>
<td>96</td>
<td>44</td>
<td>5</td>
<td>86</td>
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<tr>
<td>Lactotrope</td>
<td>84</td>
<td>63</td>
<td>35</td>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>Non-functioning</td>
<td>38</td>
<td>75</td>
<td>43</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>Corticotrope</td>
<td>56</td>
<td>67</td>
<td>25</td>
<td>0</td>
<td>86</td>
</tr>
</tbody>
</table>

The values represent the percentage of tumors expressing the sst subtype.
Group showing higher sensitivity to SOM230 (showing equal response to octreotide and SOM230 2–8 h after s.c. injection compared with the control day. A: Group octreotide 100 μg (black bars) and SOM230 250 μg (white bars) 2–8 h after s.c. injection compared with the control day. A: Group showing equal response to octreotide and SOM230 (n=6). B: Group showing higher sensitivity to SOM230 (n=3; *P<0.05). Adapted from [27].

Activation of sst₁ receptors by the sst₁-selective agonist BIM-23 296 results in a dose-dependent inhibitory effect in the nanomolar range on GH and PRL secretion by GH-secreting pituitary adenomas. In addition to lowering GH and PRL secretion this SRIF analog induced a decrease in cell viability as well (28). Moreover, BIM-23 745, another sst₁-selective agonist, was further demonstrated to significantly suppress GH secretion in vitro in a series of GH-secreting pituitary adenomas from patients resistant or partially responsive to OCT or lanreotide in vivo (29).

**ACTH-secreting pituitary adenomas**

The endogenous Cushing’s syndrome is predominantly caused by excess adrenocorticotropin (ACTH) release from an ACTH-secreting pituitary adenoma, also known as Cushing’s disease (CD; [30]). Since significant mortality and morbidity accompany this condition, proper medical intervention is necessary. Transphenoidal surgery is the treatment of choice for pituitary-dependent CD. Although transphenoidal surgery allows cure of CD, the reported success rates vary between 50 and 90% (31–35). If surgery fails, radiotherapy, either alone or in combination with adrenolytic agents, may be used. Unfortunately, none of the current treatment modalities ensure a full and permanent cure, as the rate of recurrence of the disease, depending on the criteria of initial cure, varies between 5 and 24% in the literature (36, 37). Therefore, physicians have explored new medical strategies, preferably based on fundamental and (patho-)physiological pathways, with the hope to increase the chances of cure in this group of patients. Neuromodulatory agents, such as dopamine (DA) and SRIF, have been proposed to be of therapeutic interest in the medical treatment of CD. The conclusion of various case reports, however, is that the sst₂-preferring analog OCT is ineffective in treating CD (38–40). Recently, it was demonstrated that glucocorticoid treatment induces remarkable differences with respect to the role of sst₂ and sst₅ in regulating ACTH release in mouse corticotrope AtT-20 cells, predominantly expressing sst₂ and sst₅ (41). In the absence of dexamethasone (DEX), OCT and SOM230 potently inhibited corticotropin-releasing hormone (CRH)-induced ACTH release, while the sst₅-specific analog BIM-23 268 appeared to be the least potent. In the presence of DEX, a physiological nanomolar concentration of SOM230 and BIM-23 268 still inhibited CRH-induced ACTH release, whereas the suppressive effects of OCT were almost completely blocked. In addition, the IC₅₀ values for OCT and BIM-23 268 after DEX treatment shift toward their sst₅-binding affinity. The high potency for SOM230 was not affected by DEX. These data suggest down-regulation of sst₂ by DEX, while sst₅ receptors seem more resistant to DEX. This was indeed confirmed by the mRNA as well as by the sst membrane-binding studies. These data suggest that in untreated patients with CD the expression level of sst₂ is too low for OCT to lower ACTH and cortisol levels. Indeed, relatively low levels of sst₂ mRNA in a series of primary human corticotrope adenomas of patients with CD were found. On the other hand, a predominant expression of sst₅ mRNA in corticotrope adenomas was observed (42). This sst₅ mRNA profile in human corticotrope adenomas, i.e. a low sst₂ and a significant sst₅ mRNA expression, supports the concept that glucocorticoids down-regulate sst₂ expression in human corticotrope adenomas, while sst₅ receptors are more resistant to glucocorticoid pressure. These observations also support the lack of efficacy of OCT in lowering circulating ACTH and cortisol levels in these patients, because sst₂ expression is too low and sst₅ membrane binding affinity of OCT is not high enough to make OCT therapeutically active in patients with CD. Support is formed by SOM230-induced inhibition of basal and CRH-stimulated ACTH release by the corticotrope adenomas, which appeared comparable with the observations in AtT20-cells, i.e. significant inhibition by SOM230 when compared with OCT, even in the presence...
of DEX. SOM230 did not inhibit AtT20 cell-proliferation or pro-opiomelanocortin synthesis during 72 h incubation in vitro. Therefore, increased ACTH breakdown may form an additional explanation for the inhibitory effect of SOM230 (and SRIF) on basal ACTH secretion. It is suggested, therefore, that sst₂ + sst₅ preferring SRIF analogs, such as SOM230, might become of therapeutic interest in CD. The suppression of ACTH levels by activation of sst₅ in patients with CD might lower cortisol levels. Since cortisol lowers sst₂ expression, these suppressive effects might subsequently be (partially) abrogated, and enhanced ACTH inhibition via restored sst₂ expression becomes suggestive. Therefore, prolonged treatment with SOM230 may be able to lower ACTH levels in CD even more, because it could now function via both sst₅ and sst₂ receptor subtypes (Fig. 2). An open label phase II trial in nine patients with untreated or recurrent CD has demonstrated promising results with SOM230 (43). After 2 weeks of SOM230 treatment, 600 μg s.c. twice daily, free urinary cortisol levels normalized in three patients, while in the remaining six patients urinary cortisol levels were suppressed by 17–60%. A prolonged treatment shall reveal whether this multiligand can indeed be of clinical potential for this serious neuroendocrine condition. On the other hand, glucose intolerance in all patients studied was documented during this short treatment period, and one patient even withdrew from the trial due to overt diabetes mellitus. In addition, a transient increase in glucose levels after SOM230 was already observed in acromegalic patients (44), whereby SOM230 was well tolerated and, in contrast to OCT, did not suppress insulin concentrations. Even though patients with CD and acromegaly are already known to suffer from glucose intolerance, it seems crucial for the future clinical development of SOM230 to retrieve answers with respect to this serious side effect.

Prolactinomas

Dopamine agonists have been shown to be highly effective in the medical treatment of PRL secreting pituitary adenomas (45, 46). Still, several patients seem intolerant for these drugs and some of them are resistant or partially responsive to DA agonist treatment. Therefore, can SRIF analogs become of interest to treat this small group of prolactinoma patients? In three DA agonist sensitive prolactinomas, SOM230 was significantly more potent than OCT in lowering PRL secretion in vitro (18). In two of the prolactinomas, there was a clear relationship between the expression of sst₅ mRNA in the adenoma cells and the percentage inhibition of PRL secretion by SOM230. In one prolactinoma culture, which expressed high levels of sst₅ mRNA and no other sst mRNAs, PRL secretion was reduced to the same extent as that induced by bromocriptine (Fig. 3). One other prolactinoma, which showed a significantly lower responsiveness to SOM230, had very low sst₅ mRNA levels. The lower potency of OCT in reducing PRL secretion by prolactinomas seems related to the very low sst₂ levels as demonstrated in a series of 10 prolactinomas by Jaquet et al. (47). These data further underline the role of sst₅ in mediating its suppressive effect on PRL secretion. However, as already mentioned, the potential clinical importance of these findings should be considered in view of the very high proportion of patients with prolactinomas responding to DA agonist treatment with a normalization of PRL levels and tumor shrinkage (45, 46). In addition, Jaquet et al. (47) showed previously that the effects of sst₅ selective compounds on prolactinoma cells are superimposable, at higher concentrations to those of the DA agonists, but not additive, particularly in adenomas resistant to dopaminergic suppression of PRL release.

Figure 2 SOM230 in untreated pituitary-dependent Cushing’s disease. A de novo human corticotroph adenoma has predominant expression of sst₂ and low expression of sst₅. The elevated ACTH and cortisol levels down-regulate sst₂ expression on the tumor, making it almost impossible for the current clinically available sst₂-preferring SRIF-analogs to inhibit ACTH and cortisol secretion (left). SOM230, however, through activation of sst₅, should be able to lower ACTH and cortisol levels. In this relative hypocortisolemic state, the down-regulation of sst₂ by cortisol might be abrogated. Subsequently, because SOM230 also binds with good affinity to sst₂, enhanced suppression of ACTH and cortisol via restored sst₂ expression becomes suggestive (right).
**TSH-secreting pituitary adenomas**

TSH-secreting pituitary adenomas are rare and transsphenoidal surgery is considered the first treatment approach for these tumors, leading to normalization of thyroid hormone levels and the disappearance of pituitary tumors in approximately 44% of patients (48, 49). The current clinically available SRIF analogs OCT and lanreotide induce normalization of thyroid hormone levels in the large majority of patients with TSH-secreting adenomas, although limited data are available. In approximately 80% of patients normalization of TSH levels has been reported, while significant tumor shrinkage is observed in 50% of cases (49–51). These data suggest that sst2 receptors seem to be involved in the beneficial effects of the current clinically available sst2-preferring SRIF analogs.

**Clinically non-functioning pituitary adenomas**

Clinically non-functioning pituitary adenomas (NFAs) represent a very heterogeneous group of tumors because a consistent proportion of them (up to 90%) are shown to secrete low amounts of intact follicle-stimulating hormone and luteinizing hormone and/or their α- and β-subunits either *in vitro* or *in vivo*. NFAs express sst subtypes, allowing researchers to attempt treatment with SRIF analogs. Despite favorable antiproliferative effects of OCT treatment on NFA cells *in vitro*, few clinical trials have been reported in NFAs, but tumor reduction was observed only in 11–13% of cases, indicating a weak correlation between sst expression and treatment efficacy with OCT in these patients (52). More recently, the suggested predominant expression of sst3 mRNA in this group of pituitary adenomas (Hofland et al., unpublished data) and the *in vitro* support that sst3 can induce apoptosis via the induction of p53 and BAX (53), might reveal a new medical treatment option for patients with NFAs.

**Conclusion**

In the last two decades, SRIF analogs were used for the treatment of hormone-secreting pituitary adenomas. However, a significant proportion of these patients were found to be (partially) resistant to the suppressive effects of current clinically available sst2-preferring SRIF analogs. With the improved understanding of SRIF and sst physiology and interactions, and with the increasing involvement of the industry in novel SRIF formulation production, well-characterized sst selective and universal SRIF analogs were developed. Using these novel SRIF analogs in *in vitro* pituitary cell cultures resulted in new concepts and potential mechanisms for the treatment of pituitary hormone hypersecretion. These concepts include ligand binding to both sst3 and sst5 or to sst1 alone, resulting in greater efficacy to suppress GH in acromegaly; binding to sst5 to suppress PRL in prolactinomas; binding to predominantly sst5 and concomitantly to sst2 resulting in suppression of ACTH release in CD; and finally, binding to sst3 might induce pituitary adenoma cell shrinkage in NFAs. In addition, interactions among sst subtypes and receptors of other G-protein coupled receptors, such as the dopamine D2 receptor, may form another therapeutic tool in the medical treatment of pituitary adenomas (54–59). This interactive multireceptor system of ligand activation and cross-talk at the membrane and intracellular levels may improve manipulation of pituitary hormone regulation, and enhance efficacy of medical therapy for pituitary hormone hypersecretion.

**References**


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