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

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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.

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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

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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 bisselective 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 sst₅/sst₂ 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 initiated a proof-of-concept clinical trial with SOM230 in 12 patients with active acromegaly (27). A single s.c. injection of 250 µg SOM230 compared with 100 µg OCT showed comparable efficacy in suppressing circulating GH concentrations in eight patients, and showed a significantly enhanced suppression in 3 of 12 acromegalic patients (27; Fig. 1). The observation that SOM230 demonstrates 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 sst₁ in the control of GH-secreting pituitary adenoma cell function has been proposed.

Table 1 Percentage of human pituitary tumors expressing SRIF receptors.

Tumor type	SRIF receptor subtype (mRNA levels)				
	sst ₁	sst ₂	sst ₃	sst ₄	sst ₅
Pituitary tumor					
Somatotrope	44	96	44	5	86
Lactotrope	84	63	35	6	71
Non-functioning	38	75	43	13	48
Corticotrope	56	67	25	0	86

The values represent the percentage of tumors expressing the sst subtype.

Figure 1 *In vivo* effects of SOM230 in acromegaly. The bars represent mean $\pm$ S.E.M. percentage GH suppression induced by 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 ($n=8$). B: Group showing higher sensitivity to SOM230 ($n=3$; $*P<0.05$). Adapted from (27).

Activation of ss_{t1} receptors by the ss_{t1} -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 ss_{t1} -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. Transsphenoidal surgery is the treatment of choice for pituitary-dependent CD. Although transsphenoidal 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 ss_{t2} -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 ss_{t2} and ss_{t5} in regulating ACTH release in mouse corticotrope AtT-20 cells, predominantly expressing ss_{t2} and ss_{t5} (41). In the absence of dexamethasone (DEX), OCT and SOM230 potentially inhibited corticotropin-releasing hormone (CRH)-induced ACTH release, while the ss_{t5} -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 IC_{50} values for OCT and BIM-23 268 after DEX treatment shift toward their ss_{t5} -binding affinity. The high potency for SOM230 was not affected by DEX. These data suggest down-regulation of ss_{t2} by DEX, while ss_{t5} receptors seem more resistant to DEX. This was indeed confirmed by the mRNA as well as by the ss_{t} membrane-binding studies. These data suggest that in untreated patients with CD the expression level of ss_{t2} is too low for OCT to lower ACTH and cortisol levels. Indeed, relatively low levels of ss_{t2} mRNA in a series of primary human corticotrope adenomas of patients with CD were found. On the other hand, a predominant expression of ss_{t5} mRNA in corticotrope adenomas was observed (42). This ss_{t} mRNA profile in human corticotrope adenomas, i.e. a low ss_{t2} and a significant ss_{t5} mRNA expression, supports the concept that glucocorticoids down-regulate ss_{t2} expression in human corticotrope adenomas, while ss_{t5} 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 ss_{t2} expression is too low and ss_{t5} -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 $sst_2 + sst_5$ preferring SRIF analogs, such as SOM230, might become of therapeutic interest in CD. The suppression of ACTH levels by activation of sst_5 in patients with CD might lower cortisol levels. Since cortisol lowers sst_2 expression, these suppressive effects might subsequently be (partially) abrogated, and enhanced ACTH inhibition via restored sst_2 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 sst_5 and sst_2 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 neuro-endocrine 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 sst_5 mRNA in the adenoma cells and the percentage inhibition of PRL secretion by SOM230. In one prolactinoma culture, which expressed high levels of sst_5 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 sst_5 mRNA levels. The lower potency of OCT in reducing PRL secretion by prolactinomas seems related to the very low sst_2 levels as demonstrated in a series of 10 prolactinomas by Jaquet *et al.* (47). These data further underline the role of sst_5 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 sst_5 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 corticotrope adenoma has predominant expression of sst_5 and low expression of sst_2 . The elevated ACTH and cortisol levels down-regulate sst_2 expression on the tumor, making it almost impossible for the current clinically available sst_2 -preferring SRIF-analogs to inhibit ACTH and cortisol secretion (left). SOM230, however, through activation of sst_5 , should be able to lower ACTH and cortisol levels. In this relative hypocortisolemic state, the down-regulation of sst_2 by cortisol might be abrogated. Subsequently, because SOM230 also binds with good affinity to sst_2 , enhanced suppression of ACTH and cortisol via restored sst_2 expression becomes suggestive (right).

Figure 3 Upper: effects of SOM230 (10 nM), OCT (10 nM) and bromocriptine (10 nM) on PRL release in a primary culture of a prolactinoma. * $P < 0.01$ vs untreated control cells. Incubation time: 72 h. Lower: sst subtype mRNA expression in the prolactinoma cells. Adapted from (18).

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 sst_2 receptors seem to be involved in the beneficial effects of the current clinically available sst_2 -preferring SRIF analogs.

Clinically non-functioning pituitary adenomas

Clinically non-functioning pituitary adenomas (NEAs) 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*. NEAs express sst subtypes, allowing researchers to attempt treatment with SRIF analogs. Despite favorable anti-proliferative effects of OCT treatment on NEA cells *in vitro*, few clinical trials have been reported in NEAs, 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 sst_3 mRNA in this group of pituitary adenomas (Hofland *et al.*, unpublished data) and the *in vitro* support that sst_3 can induce apoptosis via the induction of p53 and BAX (53), might reveal a new medical treatment option for patients with NEAs.

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 sst_2 -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 sst_2 and sst_5 or to sst_1 alone, resulting in greater efficacy to suppress GH in acromegaly; binding to sst_5 to suppress PRL in prolactinomas; binding to predominantly sst_5 and concomitantly to sst_2 resulting in suppression of ACTH release in CD; and finally, binding to sst_3 might induce pituitary adenoma cell shrinkage in NEAs. 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.

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