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► To cite this version:

Leo J. Hofland. Somatostatin and somatostatin receptors in cushing's disease. Molecular and Cellular Endocrinology, 2008, 286 (1-2), pp.199. 10.1016/j.mce.2007.10.015 . hal-00531958

HAL Id: hal-00531958

<https://hal.science/hal-00531958>

Submitted on 4 Nov 2010

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Accepted Manuscript

Title: Somatostatin and somatostatin receptors in cushing's disease

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PII: S0303-7207(07)00391-7
DOI: doi:10.1016/j.mce.2007.10.015
Reference: MCE 6730

To appear in: *Molecular and Cellular Endocrinology*

Received date: 4-7-2007
Revised date: 4-10-2007
Accepted date: 24-10-2007

Please cite this article as: Hofland, L.J., Somatostatin and somatostatin receptors in cushing's disease, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2007.10.015

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Figure 1
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Figure 2
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SOMATOSTATIN AND SOMATOSTATIN RECEPTORS IN CUSHING'S DISEASE

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Abstract

Cushing's disease is caused by an ACTH secreting pituitary adenoma. Surgery is the treatment of choice and cure rates between 60 and 90% are reported. For patients in which surgery fails, effective medical treatment options are needed. Somatostatin (SS) receptors (sst) are expressed on normal and tumoral corticotroph cells. However, the role of somatostatin and in particular the current clinically available sst₂-preferring SS analogs in the regulation of normal ACTH secretion, as well as in lowering ACTH and cortisol hypersecretion in patients with Cushing's disease, has been shown to be limited. Recent studies have provided renewed insights into the expression of sst subtypes, as well as into the functional role of SS-analogs in the regulation of ACTH secretion by corticotroph tumors. Sst₂ and sst₅ seem the predominantly expressed sst in corticotroph adenoma cells and targeting both these receptors with a new generation of multiligand SS analogs showed promising effects in terms of lowering ACTH release and urinary free cortisol (UFC) levels in patients with Cushing's disease. In this review an overview of the current insights into the role of SS and sst in the regulation of normal and pathological ACTH secretion is provided.

Introduction

Cushing's disease is associated with significant mortality and morbidity [1]. Removal of the ACTH secreting pituitary adenoma by transsphenoidal surgery results in cure rates between 60 and 90%, depending on the localization of the tumor and the experience of the neurosurgeon [1-5]. If surgery fails, radiotherapy [6], alone or in combination with adrenolytic drugs, may be used [7-10]. Nevertheless, none of the current treatment modalities ensure a full and permanent cure as, depending on the criteria of initial cure, recurrence rates vary between 5 and 24% [6,11-15]. Therefore, new medical treatment options are certainly required. One of the potential targets for such treatment include somatostatin (SS) receptors (sst) which are expressed on the majority of corticotroph adenomas [16,17]. SS was originally characterized as a hypothalamic peptide with a strong inhibitory activity on growth hormone (GH) secretion by the pituitary gland [18]. Thereafter, it was shown that the peptide has a widespread activity on various (neuro-)endocrine systems including the brain, pituitary gland, the pancreas and the gastrointestinal tract [19]. Two biologically active forms of SS consisting of 14 (SS-14) or 28 (SS-28) amino acids are present in the circulation and display a tissue specific expression. SS-14 and SS-28 act via high affinity G-protein coupled membrane sst's. Five sst subtypes have been cloned and characterized. The genes encoding the five sst's are localized on different chromosomes [19]. The five sst subtypes, codenamed sst₁-sst₅, share a coupling to several second messenger systems known to be activated upon SS binding to its receptor. These systems include inhibition of adenylyl cyclase activity and activity of calcium channels, as well as stimulation of phosphotyrosine phosphatase (PTP) or MAP kinase activity. While the inhibitory effects on adenylate cyclase activity and on the influx of Ca²⁺ are linked to inhibition of secretion processes, the activation of PTP or MAP-kinase activity may play a role in the regulatory effects that SS may exert on cell proliferation [19]. More recently, another SS-like peptide, named cortistatin (CST), has been cloned and

characterized [20,21]. Due to its high structural resemblance to SS, CST binds with high affinity to all five known sst subtypes [22]. CST has overlapping as well as differential activities compared to SS, particularly in the brain [23]. In the present review, the role of SS, CST, and related receptors in the regulation of normal and tumoral ACTH secretion by corticotroph (tumor) cells is discussed.

SS and sst in the regulation of normal ACTH secretion

In normal rat corticotrophs, co-localization of all five sst with ACTH expressing cells was shown [24]. Several studies demonstrated that both sst₂ and sst₅ receptors are expressed in rat corticotrophs [25,26]. The role of the individual sst subtypes in the regulation of normal ACTH secretion seems dependent, however, on the hormonal environment of the corticotroph cells. SS, at concentrations between 1-100 nM did not inhibit basal and corticotrophin releasing hormone (CRH)- induced ACTH secretion by cultured normal rat pituitary cells and pituitary halves [27,28]. On the other hand, SS inhibited CRH- and vasopressin induced ACTH release by cultured cells prepared from long-term adrenalectomized rats [29]. Moreover, in serum-deprived- or in rat pituitary cells pre-treated with the glucocorticoid blocking agent RU-38146, SS inhibited CRH-induced ACTH secretion already at low nanomolar concentrations, whereas in serum cultured cells SS was unable to influence ACTH secretion [30]. In humans, systemic SS infusion did not suppress basal- or angiotensin II-, ghrelin- or CRH-induced ACTH or cortisol concentrations [31-33]. Although differential activities of SS and CST on brain function have been reported, the endocrine activities of both peptides appear similar. Similar to SS, basal or ghrelin- and hexarelin- stimulated ACTH secretion was not suppressed by systemic CST infusion [31,34,35]. However, these studies only evaluated exogenous administration of SS or CST. Recent studies showed elevated pituitary proopiomelanocortin (POMC) mRNA levels in SS

knockout mice compared with wild type controls [36]. Moreover, *sst₅* knock out mice had significantly elevated ACTH and cortisol levels compared to wild type animals (Figure 1) [37]. In addition, it was recently shown in a group of ten healthy postmenopausal women that L-arginine infusion, as a putative suppressor of endogenous SS outflow, potentiates ghrelin- and CRH-induced ACTH secretion, suggesting that endogenous SS has a direct inhibitory role on ACTH secretion [38]. Finally, a recent study in rats showed that a 1h pre-treatment with 10µg/kg SOM230 (binds with high affinity to *sst₂*, *sst₃* and *sst₅*) inhibited CRH-induced ACTH and corticosterone release in vivo by 51 and 27%, respectively, whereas octreotide (binds mainly *sst₂* receptors) was significantly less potent at this dosage [39]. These latter data suggest a differential activity of *sst* subtypes in the control of ACTH release (see below). The stronger activity of SOM230 may be related to its higher binding affinity to *sst₃*, and in particular *sst₅* receptors, compared with octreotide.

Summarizing, the currently available data suggest that SS may have regulatory effects on ACTH release under physiological conditions, but also that *sst* (or specific *sst* subtypes) expression is under negative regulatory control of glucocorticoids and that endogenous glucocorticoid levels may thus modulate SS action on the pituitary corticotroph cell.

Expression of *sst* in corticotroph adenomas

Initial studies using receptor autoradiography on cryostat sections of corticotroph adenomas failed to detect *sst* binding sites using the *sst₂*-selective radioligand [¹²⁵I-Tyr³]-Octreotide [40]. In addition, in vivo *sst* scintigraphy using ¹¹¹In-diethyltriamine pentaacetic acid (DTPA) octreotide showed no increased uptake in eight ACTH secreting pituitary adenomas [41,42], whereas positive uptake was found in two invasive ACTH macroadenomas and in two cases of Nelson's

syndrome [41]. By using qualitative PCR, several studies showed the highest frequency of expression of *sst*₅ and *sst*₂, and to a lesser extent *sst*₁ in corticotroph adenomas [43-46]. The high frequency of *sst*₂ mRNA expression in corticotroph adenomas, together with observation of the absence of octreotide (*sst*₂)-binding sites in vitro and in vivo, suggest that *sst*₂ expression is present on corticotroph adenoma cells, but at generally too low level to generate significant *sst*₂ receptor protein expression. More recent studies using real-time PCR indeed showed that most corticotroph adenomas express *sst*₂, *sst*₅ and *sst*₁, and that *sst*₂ expression is in the majority of cases low compared to *sst*₅, the latter being expressed at highest level [47,48]. Moreover, also at the protein level, *sst*₅ receptors were expressed at the highest level in corticotroph adenomas [48]. These data suggest that the *sst*₅ receptor may be a novel potential target for medical therapies aiming to lower ACTH secretion by corticotroph adenomas.

Role of somatostatin and its analogs on ACTH secretion by corticotroph adenomas

The *sst*₂-selective SS-analog octreotide has no effect on basal and CRH-induced ACTH release in patients with Cushing's disease [49-51]. On the other hand, octreotide and somatostatin suppressed ACTH serum levels in patients with adrenal insufficiency (Addison's disease) [52] and in patients with Nelson's syndrome [51,53]. Since Addison's disease and Nelson's syndrome are accompanied by hypocortisolism, it has been suggested that the missing effect of octreotide in patients with Cushing's disease could be due to a down-regulation of *sst* at the hypercortisolemic state [54]. This conclusion was further underlined by the in vivo observation that octreotide had no effect on circulating basal- or CRH-induced ACTH levels, whereas in the same study octreotide inhibited basal- and CRH-induced ACTH release by corticotroph adenomas in vitro in four of six and three of three cell cultures, respectively. Moreover, hydrocortisone pre-treatment in vitro abolished the inhibitory effect of octreotide on tumoral ACTH secretion [54]. We

recently showed that the multiligand somatostatin analog SOM230 (pasireotide), as well as SS-14, were significantly more potent than octreotide in suppressing basal ACTH release by human corticotroph adenomas in vitro (figure 2) [47]. The potency of SOM230 in inhibiting ACTH secretion by corticotroph adenomas was confirmed in another study, in which ACTH secretion was inhibited by SOM230 in five of six corticotroph adenoma cultures by 23-56% [48]. The high affinity of SOM230 for the ss_{t5} receptor, and the high expression of ss_{t5} receptors in corticotroph adenomas may form the basis for this enhanced potency of SOM230 in suppressing ACTH secretion [47,48]. Indeed, studies in AtT20 cells expressing both ss_{t2} and ss_{t5} receptors, showed that SOM230 was also much more potent in suppressing basal and CRH-induced ACTH release than octreotide [47,55]. IC_{50} values of inhibition suggested a ss_{t5} -like response of ACTH-inhibition. Both ss_{t2} and ss_{t5} are involved in the inhibition of ACTH release and cAMP production in AtT20 cells [56] and a functional superiority of ss_{t5} over ss_{t2} has been suggested by others as well [57,58]. On the other hand, ss_{t2} receptors, albeit at low level, are co-expressed with ss_{t5} receptors in corticotroph adenomas as well. Since SOM230 also binds with high affinity to ss_{t2} receptors a coordinated enhanced ACTH-suppressive activity via both ss_{t2} and ss_{t5} receptors cannot be excluded. Such an enhanced activity is not readily explained by in vitro data, however. In CHO cells co-expressing ss_{t2} and ss_{t5} , as well as in AtT20 cells, SS-14 treatment (targeting both ss_{t2} and ss_{t5}) induced a loss of 60-70% of sst binding sites and a massive ss_{t2} endocytosis. On the other hand, selective stimulation of ss_{t2} receptors resulted in only a loss of 20-40% of sst binding sites and markedly reduced ss_{t2} internalization. This was accompanied by desensitization of the response to SS-14, and absence of desensitization by the ss_{t2} -specific analog. These findings suggest that ss_{t5} modulates trafficking and cell surface regulation of ss_{t2} and cellular desensitization to the effects of SS [59]. It also suggest that tumors co-expressing ss_{t2} and ss_{t5} have sustained responsiveness to ss_{t2} -selective SS-analogs and that SS-analogs targeting

both ss_{t2} and ss_{t5} would induce desensitization more rapidly. This seems not in agreement with the *in vitro* observations in corticotroph adenomas in which prolonged exposure to SOM230 and SS-14 induced a more potent suppression of basal- and CRH-induced ACTH release, compared with octreotide (see above). Therefore, likely other mechanisms are involved as well. In addition, a recent study in AtT20 cells showed that SS-28 exhibited a higher potency (femtomolar), compared with octreotide (picomolar potency) on cAMP accumulation and ACTH secretion, while it was shown that, using monoreceptor-selective peptide agonists, ss_{t5} regulates a dominant ss_{t2} action, attenuating ss_{t2} effects on intracellular calcium oscillation and internalization [60]. Besides the suppression of ACTH and cortisol secretion, a secondary beneficial activity of medical therapy would be the inhibition of tumor cell proliferation. In AtT20 cells, neither octreotide, nor SOM230 inhibited cell proliferation or induced apoptosis, however [47]. Moreover, in rats, both octreotide and SOM230 inhibited adrenalectomy-induced mitotic activity in the pituitary gland of male rats. However, both drugs did not inhibit proliferation of ACTH-immunoreactive cells, while hormonally null cells were inhibited [61]. Taken together, it seems that ss_{t2} and ss_{t5} receptors in corticotroph tumor cells do not mediate a growth inhibitory effect. On the other hand, a recent *in vitro* study in six primary corticotroph adenoma cell cultures showed that SOM230 inhibited cell number in five of six cultures, as measured by a colorimetric (MTS) assay [48]. Since pituitary adenoma cells have very low proliferative capacity, it remains to be established which is the exact mechanism of this inhibitory effect of SOM230 on MTS activity in primary corticotroph adenoma cultures.

Preliminary *in vivo* data in patients with Cushing's disease showed that SOM230 has ACTH lowering activity. In an open label, single arm, Phase II multicenter study in 27 patients with *de novo* or persistent/recurrent Cushing's disease, SOM230 treatment for 15 days (600 μ g s.c. bid)

resulted in normalization of free urinary cortisol (UFC) levels in 4 patients (19%), while in 9 patients (43%) there was a 50% or more higher decrease in UFC. Overall, UFC levels decreased in all but one patient. Significant improvement of body weight was reported in 24% of patients. Mild-to-moderate gastrointestinal events and hyperglycemia were the most frequent adverse events. One patient with pre-existing diabetes mellitus had to discontinue because of worsening of glycemic control [62,63]. A more long-term treatment study should demonstrate whether this multiligand SS-analog can indeed be a novel medical tool to control ACTH hypersecretion and related severe symptomatology in patients with Cushing's disease. Moreover, since patients with Cushing's disease are known to suffer from glucose intolerance, such studies need to address the potential adverse effects on glycemic control. Impaired GH secretion usually accompanies Cushing's syndrome, in a significant percentage of patients even after long-term remission of hypercortisolism obtained by surgery alone [64]. In this respect, the potent suppression of circulating GH and IGF-I levels by SOM230 [65] should be taken into account as well, in particular in patients who are already significantly catabolic.

Role of glucocorticoids on the regulation of sst expression of corticotroph adenomas

The absence of inhibition of ACTH secretion by SS and the SS-analog octreotide in patients with Cushing's disease and the inhibition of ACTH release by SS(-analogs) in patients with absent endogenous glucocorticoid feedback, strongly suggest that glucocorticoids have a negative regulatory effect on the expression of sst receptors, most likely sst₂ (see above). Indeed, dexamethasone treatment induced a down-regulation of sst binding sites in GH₄C₁ and AtT20 cells by 20-40% [66]. In GH₄C₁ cells, high dose dexamethasone (1 μM) treatment for 24-48 hr resulted in a decrease in sst₁ (-50%) and sst₂ (-30%) mRNA level, whereas sst₃ levels showed a biphasic time-dependent response, e.g. inhibition at early time points and a significant increase

after more long-term exposure [67]. Moreover, in adrenalectomized rats, eight days following adrenalectomy a specific upregulation of pituitary ssr_2 expression was observed [68]. Finally, high dose dexamethasone treatment induced a downregulation of ssr_{1-4} and an upregulation of ssr_5 [68]. Our in vitro studies showed that pretreatment of AtT20 cells with dexamethasone abolished the inhibitory effect of octreotide, but not that of SOM230, on ACTH release. This was accompanied with a decrease of ssr_2 mRNA expression, whereas ssr_5 expression was significantly more resistant to the inhibitory effect by dexamethasone [55].

Taken together, these data provide strong evidence for the fact that ssr_2 receptor expression is under negative regulatory control of endogenous glucocorticoids, and could thus form an explanation for the observed low levels of ssr_2 and high levels of ssr_5 expression in corticotroph adenomas of patients with Cushing's disease, as well as for the lack of efficacy of ssr_2 -specific SS-analogs and the enhanced potency of ssr_2/ssr_5 specific SS-analogs on tumoral ACTH secretion.

Summarizing conclusions

From the current knowledge it can be concluded that multiple ssr are expressed in corticotroph cells. Exogenous administration of SS or CST does not lower ACTH levels. However, suppression of endogenous hypothalamic SS outflow by L-arginine increases CRH- and ghrelin-induced ACTH secretion. A role for endogenous SS in the control of ACTH secretion seems further substantiated by the increased ACTH and cortisol levels in ssr_5 knockout mice, as well as by the increased pituitary POMC expression level in SS knock out mice. The expression of ssr_2 is under negative control of glucocorticoids, whereas ssr_5 seems more resistant to the suppressive effect of glucocorticoids. Corticotroph adenomas express predominantly ssr_1 , ssr_2 and ssr_5 . ssr_5 are expressed at highest level. Targeting multiple ssr by SS-14, SS-28 or the multiligand

pasireotide (SOM230), results in a more potent suppression of ACTH release, compared to the effect of the sst₂-selective SS-analog octreotide. These observations formed the basis for a first Phase II clinical study with pasireotide in Cushing's disease and promising preliminary data have been reported. On the basis of this current knowledge a hypothetical model of the interrelationship between cortisol levels and the expression of sst subtypes (e.g. sst₂ and sst₅) on corticotroph adenomas of patients with Cushing's disease is proposed (Figure 3).

Figure legends

Figure 1: Basal ACTH and corticosterone levels in fasted wild type (WT) and *sst₅* knock out (KO) mice. Animals were 3- to 6-month-old males fasted for 12-16h. Values are expressed as mean $\pm$ SEM. Numbers in parentheses indicate the number of animals tested. * $P < 0.05$ vs WT mice.

Adapted from [37].

Figure 2: Effect of Octreotide (OCT, 10nM), SS14 (10 nM) and SOM230 (10nM) on ACTH secretion by cultured cells from a human corticotroph adenoma (A) and by AtT20 pituitary tumor cells (B). Incubation time was 72 hr. Values are the mean $\pm$ SEM. * $p < 0.01$ vs control (CON).

Adapted from [47].

Figure 3: Hypothetical model of the regulation of *sst₂* and *sst₅* receptors on human corticotroph adenomas by cortisol. A de novo human corticotroph adenoma has predominant expression of *sst₅* and low expression of *sst₂* (left panel). The elevated ACTH and cortisol levels down-regulate *sst₂* expression on the ACTH-secreting pituitary adenoma, which may form an explanation for the lack of efficacy of the *sst₂*-preferring SS-analog octreotide on circulating ACTH and cortisol levels in patients with Cushing's disease. Lowering of ACTH and cortisol levels by for example *sst₂/sst₅* specific SS-analogs via targeting *sst₅* may lower ACTH and cortisol levels.

Subsequently, the down-regulation of *sst₂* receptors by cortisol may be abrogated (right panel).

When *sst₂* receptor expression increases, a combined *sst₂/sst₅* activation may result in enhanced ACTH and cortisol suppression. The model may also provide an explanation for the ACTH- and cortisol lowering effect of octreotide in patients with adrenal insufficiency (Addison's disease) and in patients with Nelson's syndrome, which have a hypocortisolemic state. Adapted from [69].

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