Role of Somatostatin Receptors in Normal and Tumoral Pituitary Corticotropic Cells

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Abstract

Normal and tumoral pituitary corticotropic cells express sst₂ and sst₅, of which sst₅ is the predominantly expressed receptor subtype. Somatostatin (SS) inhibits pituitary adrenocorticotropic hormone (ACTH) secretion in vitro, but the sensitivity to SS is strongly regulated by glucocorticoids. In pathological conditions of a low endogenous cortisol level, i.e. in patients with adrenal insufficiency and in patients with Nelson's syndrome, SS and sst₂-preferring SS analogs (SSA), such as octreotide, are able to lower circulating ACTH and cortisol levels. On the other hand, sst₂-preferring SSA seem not effective in lowering ACTH and cortisol levels in patients with untreated Cushing's disease (CD), in which circulating cortisol levels are high. This is likely due to the downregulation of sst₂ receptors by glucocorticoids. sst₅ receptor expression is more resistant to the inhibitory effect of glucocorticoids. In recent years, novel sst subtype-selective and universal SSA have been developed. In particular, SSA with a high sst₅-binding affinity are potent inhibitors of ACTH secretion by pituitary corticotropic adenoma cells. This knowledge has initiated clinical trials evaluating the efficacy of these novel SSA in patients with CD, with the aim to lower circulating ACTH and cortisol levels by targeting multiple sst₅ on the corticotropic adenoma cells. In this minireview, the effects of SS in the regulation of normal and tumoral ACTH secretion, the role of sst subtypes involved herein, as well as the potentials of novel SSA in the treatment of patients with recurrent or persisting CD are discussed.

Introduction

Somatostatin (SS) was originally characterized as a hypothalamic peptide with a direct inhibitory activity on the secretion of growth hormone (GH) by the anterior pituitary gland [1]. Since this original discovery, numerous studies have established that SS exists in two molecular forms in the circulation, i.e. a 14- and a 28-amino acid cyclic peptide, named SS-14 and SS-28, respectively. Both peptides have a widespread biological activity due to the presence of SS receptors (sst) in many organ systems, including the brain, the pituitary gland, the gastrointestinal tract, pancreas and adrenals [2, 3]. ssts are seven-transmembrane receptors that are coupled to G-proteins and of which five subtypes, named sst₁, sst₂, sst₃, sst₄ and sst₅, have been identified. In normal physiology, sst₅ is primarily expressed in the pancreatic β-cells, where it plays a role in the regulation of insulin and glucagon secretion. In contrast, sst₂ is predominantly expressed in the pituitary gland, where it regulates the secretion of various hormones, including growth hormone (GH), prolactin (PRL), and ACTH. Overexpression of sst₅ has been observed in a variety of tumors, including pancreatic islet cell tumors, thyroid cancers, and neuroendocrine tumors of the gastroenteropancreatic tract. This suggests that sst₅ has a role in tumor growth and progression. More recently, sst₅ has been shown to play a role in the development of insulin resistance and type 2 diabetes. In this minireview, we will discuss the role of sst subtypes in the regulation of ACTH secretion and the potential of novel SSA in the treatment of patients with recurrent or persisting CD.
ssts, have been identified. On the basis of structural and pharmacological characteristics, two subclasses have been identified. To one class of sst, consisting of sst2, sst3 and sst5, structural SS analogs (SSA), such as octreotide and lanreotide, bind with high affinity, whereas these SSA do not bind to the other class of sst, consisting of sst1 and sst4 (table 1) [4]. Among the multiple physiological effects of SS is its potent inhibitory effect on pituitary hormone secretion [2, 3]. SS is considered as a physiological regulator of GH secretion. In vitro, the peptide inhibits the secretion of GH, prolactin (PRL), thyroid stimulating hormone (TSH), as well as adrenocorticotropin hormone (ACTH) by rat anterior pituitary cells, although its effects are strongly influenced by the respective physiological feedback hormones [5]. In human fetal pituitary cell cultures, SS inhibits the secretion of GH, TSH and PRL, whereas the release of ACTH and luteinizing hormone (LH) is only modestly influenced [6]. The effects of SS on normal and tumoral ACTH secretion are strongly regulated by glucocorticoids, representing the physiological feedback system [5].

### Role of Somatostatin Receptors in Normal Corticotrophic Cells

In normal rat corticotropes, all five sst colocalize with ACTH-expressing cells [7]. In another study, it was demonstrated that sst3 mRNA is expressed in 38% of corticotropes, whereas the expression of sst2 mRNA is found in only 3% of the corticotrophic cell population [8]. By immunohistochemistry, <60% and 10–20% of rat corticotropic cells express detectable sst2A and sst3 at the protein level, respectively [9]. In vitro, SS does not inhibit basal and CRH-induced ACTH release by normal rat anterior pituitary cells [10, 11], whereas CRH- and vasopressin-induced ACTH release is inhibited in cultured pituitary cells from long-term adrenalectomized rats [12]. In serum-deprived or in rat pituitary cells pretreated with the glucocorticoid receptor-blocking compound RU-38486 in vitro, SS inhibits CRH-stimulated ACTH secretion, but not in serum cultured cells. Moreover, pretreatment with dexamethasone abolished the inhibitory effect of SS on ACTH release [5]. Therefore, it can be concluded that SS is able to inhibit CRH-induced ACTH secretion by rat pituitary cells in vitro, but primarily in the absence of glucocorticoids.

In humans, systemic SS infusion does not inhibit basal or stimulated ACTH secretion [13–15]. On the other hand, in patients with adrenal insufficiency, SS infusion lowers circulating ACTH and cortisol levels [16]. These latter data again suggest the importance of the endogenous cortisol level in the regulation of the inhibitory effects of SS on ACTH secretion by the anterior pituitary gland. In vivo evidence in rats shows the importance of both sst2 and sst5 receptors in the regulation of ACTH secretion. A 1-hour pretreatment of rats with 10 μg/kg pasireotide (targeting sst2, sst3 and sst5 receptors) inhibited circulating ACTH and corticosterone levels by 51 and 27%, respectively, whereas octreotide (sst2-preferring SSA) was significantly less potent (34% and no inhibition, respectively) at this dosage [17]. These data suggest that the combined activation of both sst2 and sst5 receptors in corticotropic cells results in a more potent suppression of ACTH secretion compared with the selective targeting of sst2 alone.

In conclusion, endogenous glucocorticoid levels modulate the effects of SS on ACTH secretion by normal pituitary corticotropes. SS has an inhibitory effect on pituitary ACTH secretion, particularly when cortisol levels are low.
Role of Somatostatin Receptors in Corticotropic Adenoma Cells

Already in 1981, Richardson and Schonbrunn [18] showed the presence of SS-binding sites in the ACTH-secreting AtT20/D16V mouse corticotropic tumor cell line and demonstrated inhibition of ACTH secretion by SS in vitro [18]. Since the cloning and characterization of the five sst, also the expression of these sst subtypes in human corticotropic adenomas has been evaluated. Human corticotropic adenomas show a predominant expression of sst₃ mRNA, whereas the majority of adenomas express sst₃ mRNA as well. Compared with sst₂ mRNA, sst₃ mRNA expression has been reported to be approximately 5- to 10-fold higher [19–23]. Limited studies have evaluated the expression of sst₁ and sst₇ receptors at the protein level. Batista et al. [19] showed that sst₅ had the highest immunohistochemistry score, compared with sst₁, sst₂, sst₃ and sst₄ protein expression, in 83% of a series of 13 corticotropic adenomas, Hassaneen et al. [24] showed an absence of sst₅ immunostaining. The reason for these discordant results is unclear, but may be the result of the use of different antibodies. Coexpression of sst₁ receptors has been reported in only a proportion of corticotropic adenomas [20–23]. Interestingly, in silent corticotropic adenomas a considerable higher sst₁ and sst₂, but lower sst₅ mRNA expression was found, compared with corticotropic adenomas causing Cushing’s disease (CD) [20].

In primary cultures of corticotropic adenomas the universal SSA pasireotide (high sst₂-, sst₃- and sst₅-binding affinity) was significantly more potent in inhibiting ACTH secretion, compared with the sst₂-preferring SSA octreotide [22]. Following a 72-h incubation, octreotide (10 nM) inhibited ACTH secretion by 28% in only 1 of 5 cultures, whereas pasireotide (10 nM) induced significant suppression of ACTH secretion in 3 of 5 cultures (30–40% suppression) [22]. Moreover, Batista et al. [19] demonstrated significant suppression of ACTH secretion in 5 of 6 cultures (23–56% suppression). In AtT20 corticotropic adenoma cells, both pasireotide, as well as sst₂-selective SSA were more potent inhibitors of basal and CRH-induced ACTH secretion, compared with sst₂-preferring SSA (fig. 1) [22, 25]. Figure 1a shows that AtT20/D16V cells selectively express sst₂ and sst₃ mRNA. Moreover, pasireotide inhibits ACTH secretion with an IC₅₀ of 0.2 nM, whereas octreotide induced a significant suppres-
The observation that SS and the sst2-preferring SSA octreotide do not inhibit circulating ACTH levels in patients with untreated CD [27, 28], in combination with the inhibitory effects of SS on ACTH levels in patients with adrenal insufficiency (Addison’s disease) [16] and Nelson’s syndrome [27, 29], suggests that glucocorticoids have a negative regulatory role on the expression of sst receptors, particularly sst2, and indicates that glucocorticoids are potent inhibitors of ACTH secretion in patients with elevated ACTH levels due to a lack in steroid feedback. Downregulation of SS-binding sites on AtT20 corticotropic tumor cells was previously shown by Schonbrunn [30]. More recently, we found that dexamethasone treatment of AtT20 cells induced a significant suppression of sst2 mRNA expression, whereas sst5 mRNA expression was not significantly affected [25]. Moreover, the number of binding sites for the sst2-preferring SSA octreotide was lowered by 72% by dexamethasone treatment, whereas the total number of binding sites for SS-14 was lowered only by 17%. These data suggest that the sst5 protein expression, compared with sst2, is more resistant to downregulation by glucocorticoids. The functional consequence of this effect was further underlined by the observation that the effects of octreotide on CRH-induced ACTH secretion by AtT20 cells were abolished by dexamethasone treatment, whereas pasireotide potently suppressed CRH-induced ACTH secretion, even in the presence of 10 nM dexamethasone (fig. 2) [25].

In conclusion, sst2 receptor expression on corticotropic adenoma cells is downregulated by glucocorticoids, whereas sst5 receptor expression is less sensitive to this downregulation. These data may form an explanation for the low sst2 and relatively high sst5 expression levels in corticotropic adenomas of patients with CD. Moreover, these observations may explain the lack of efficacy of sst2-preferring SSA in patients with CD and suggest an enhanced potency of sst2–sst5 targeting SSA on ACTH secretion by corticotropic adenomas.
Outlook

The observation that sst5 receptors are expressed at significant levels in human corticotropic adenomas, together with the more important role of sst5, compared with sst2, in the regulation of tumoral ACTH secretion, has initiated clinical trials testing the efficacy of the universal SSA pasireotide in patients with CD. Promising results of a first phase II clinical study with pasireotide in CD have been recently reported [31]. On the basis of the potent inhibitory effect of glucocorticoids on sst2 expression in corticotropic adenoma cells, it can be hypothesized that lowering of circulating cortisol levels in patients with CD results in an upregulation of sst2 expression on the corticotropic adenoma, thereby further contributing to an ACTH-lowering effect of sst2–sst5 targeting SSA. This lowering of circulating cortisol in patients with recurrent or persisting CD may be achieved with sst2–sst5 targeting SSA, but also with dopamine D2 agonists, such as cabergoline, or with drugs inhibiting cortisol production at the adrenal level, such as ketocnazole. Corticotropic adenomas express D2 in about 70% of the cases [32] and cabergoline induces long-term normalization in approximately 40% of patients with CD unsuccessfully treated by surgery [33]. D2 mRNA receptor expression in corticotropic adenomas is significantly higher compared to sst3 and sst2 mRNA expression [21, 23]. Interestingly, unlike sst2, but comparable to sst5, D2 receptor expression seems not under the negative regulatory control by glucocorticoids [34]. We recently found that biochemical remission can be achieved in 90% of patients with CD (n = 17) with pasireotide monotherapy (29%), with combined pasireotide-cabergoline treatment (in an additional 24%) and in another 35% with triple therapy with pasireotide, cabergoline and ketoconazole [35, and this issue]. Therefore, a future approach for medical treatment of recurrent or persistent CD may involve combination therapy with drugs that have additive or potentiating effects.

Disclosure Statement

The authors have nothing to disclose.


