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Somatostatin and dopamine receptor regulation of pituitary somatotroph adenomas

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Abstract Somatostatin and dopamine receptors are expressed in normal and tumoral somatotroph cells. Upon receptor stimulation, somatostatin and the somatostatin receptor ligands octreotide, lanreotide, and pasireotide, and to a lesser extent, dopamine and the dopamine analogs bromocriptine and cabergoline, suppress growth hormone (GH) secretion from a GH-secreting pituitary somatotroph adenoma. Somatostatin and dopamine receptors are $G_{\alpha i}$-protein coupled that inhibit adenylate cyclase activity and cAMP production and reduce intracellular calcium concentration and calcium flux oscillations. Although their main action on somatotroph cells is acute inhibition of GH secretion, they also may inhibit GH production and possibly somatotroph proliferation. These receptors have been reported to create complexes that exhibit functions distinct from that of receptor monomers. Somatostatin suppression of GH is mediated mainly by somatostatin receptor subtype 2 and to a lesser extent by SST5. Human somatostatin receptor subtype 5 has also been shown to harbor mutations associated with GH levels, somatotroph tumor behavior, and somatostatin receptor ligand (SRL) responsiveness. Reviewing current knowledge of somatostatin and dopamine receptor expression and signaling in normal and tumoral somatotroph cells offers insights into mechanisms underlying SRL and dopamine agonist effectiveness in patients with acromegaly.

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Introduction

Hypothalamic somatostatin and dopamine regulate growth hormone (GH) secretion and production from pituitary somatotrophs. Somatostatin, a polypeptide, and dopamine, a biogenic amine biosynthesized from the amino acid tyrosine, both signal via G-protein coupled receptors expressed in normal and tumoral somatotroph cells. Understanding somatostatin and dopamine receptor expression and signaling pathways enables insights into mechanisms underlying somatostatin receptor ligands (SRL) and dopamine agonists regulate GH secretion in patients with somatotroph adenomas.

Somatostatin receptors in normal and tumoral somatotroph cells

Activity of endogenous somatostatin was originally thought to be limited to inhibition of GH secretion from pituitary somatotroph cells. Although later studies showed it also inhibits TSH, ACTH, and multiple central and peripheral hormones, somatostatin name remains intimately connected to GH.

Somatostatin 14, the main regulatory form of the polypeptide, is produced predominantly in the anterior periventricular nucleus of the hypothalamus, as well as in the paraventricular, arcuate, and ventromedial nuclei. These neuronal axons project into the median eminence and terminate in the hypophyseal-portal circulation,

releasing somatostatin into the blood supplying the anterior pituitary cells [1].

Somatostatin binds with high affinity to, and signals through five somatostatin receptor subtypes, labeled SST_{1-5}. Both normal and tumoral somatotroph cells express SST_5 and SST_2, and many express SST_3 and SST_1 (Table 1) [2]. In human somatotroph cells SST_5 mRNA transcripts are expressed most abundantly, followed by SST_2, SST_3 and SST_1. SST_4 mRNA is usually not detected [3]. SST_2 mRNA levels are higher in densely granulated adenomas than in sparsely granulated adenomas [4]. Delineating receptor subtype-specific signaling networks is challenging, as receptor subtypes share signaling pathways, 4 of the 5 receptor subtypes can be co-expressed in somatotroph adenomas, and SRL can regulate more than one receptor.

SSTs are encoded by different chromosomes [5], and regulate multiple downstream signaling pathways in different cells and tissues [6]. This review focuses on signaling pathways regulating pituitary somatotroph function [6, 7] in particular effects on calcium and potassium channels, adenylate cyclase-cAMP, MAPK, Na^+/H^+ exchanger, and protein tyrosine phosphatases.

Calcium

Somatostatin inhibits intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in rat GH_3 tumoral somatotroph cells [8] as well as calcium transients in GC cells, a subclone of GH_3 cells [9]. Somatostatin also dose-dependently decreases intrinsic and GHRH-dependent Ca^{2+} oscillations, Ca^{2+} spike duration and frequency in patched clamped primary rat pituitary somatotroph cultures [10]. Somatostatin signaling occurs via SST_2 [11] and $G_{\alpha o}$ protein subunit [12] in GH_3 cells, and SST_2 and $G_{\alpha i/o}$ proteins in GC cells [9, 13]. Although somatostatin reduced $[Ca^{2+}]i$ in the majority of cells derived from human pituitary somatotroph adenomas, in a small subpopulation of cells somatostatin paradoxically increased or did not affect $[Ca^{2+}]i$ [14]. A similar observation was made in porcine somatotrophs, where somatostatin could increase or decrease $[Ca^{2+}]i$ in different somatotroph subpopulations, low- and high-density. Low-density somatotrophs exhibit smaller size, fewer secretory granules and more rough endoplasmic reticulum by electron-microscopy as compared to high-density somatotroph cells [15]. Both high and low doses of somatostatin increased $[Ca^{2+}]i$ in high-density somatotroph cells, yet only high-dose somatostatin reduced $[Ca^{2+}]i$ low-density somatotrophs [16].

Adenylate cyclase-cAMP

Somatostatin reduces adenylate cyclase-cAMP production in somatotroph cells and antagonizes GHRH action [6, 7]. Somatostatin inhibition of cAMP in SST_2 overexpressing rat GH_4 tumoral somatotroph cells inhibited PKA activation and CREB phosphorylation, an effect reversed by pertussis toxin (PTX) treatment [17]. Over-expression of SST_2 in GC cells demonstrated constitutive SST_2 activity resulting in suppression of cAMP accumulation and rat GH promoter activity via deacetylation, culminating in suppression of GH synthesis [18]. Over-expression of SST_3 in these cells also constitutively suppressed cAMP levels, PKA activation and GH synthesis, albeit to a lesser extent compared with SST_2 and with partial contribution from the glycogen synthase kinase 3β (GSK3β) pathway [19]. Of note, in primary human adenomatous somatotroph co-transfected with $G_{\alpha o1}$ resistant to PTX and SST_5, the SST_5 selective SRL BIM23206 no longer reduced cAMP production [20].

Although somatostatin effect on adenylate cyclase in somatotroph cells is mainly inhibitory, both high and low dose somatostatin stimulated adenylate cyclase and cAMP accumulation and GH release in a subpopulation of pig high density somatotrophs *in vitro* but not in low density

Table 1 Somatostatin receptor subtype expression in somatotroph cells and adenomas Adapted from [2]

	Somatostatin receptor subtype expression					Detection method	References
	SST1	SST2	SST3	SST4	SST5		
Rat normal somatotroph cells[1]	5*	60	18*	24*	72	C, F	[57–59]
Rat pituitary GH-secreting cell line (GH_3)	+	+	+	+/−	+	A, C	[23, 60]
Human somatotroph adenoma[2]	61/109 (56)	159/173 (92)	80/120 (67)	2/32 (6)	150/169 (89)	A,B,C,D,E,F	[4, 31, 61–65]

[1] Percent of cells expressing the SST

[2] Number of tumors expressing the SST out of all tumors examined (percent tumors in parenthesis)

* Based on [58]; +, present; −, absent; A, solution hybridization; B, ribonuclease protection assay; C, reverse transcription PCR; D, quantitative reverse transcription PCR; E, in situ hybridization; F: immunohistochemistry

pig somatotrophs [16]. This increase in GH secretion correlated with cAMP increase, not with intracellular $[Ca^{2+}]i$ level [16].

Potassium channels

Increased intracellular K^+ levels induce cell membrane hyperpolarization and the closure of calcium channels, leading to reduced $[Ca^{2+}]i$ and inhibition of exocytosis. Somatostatin induced activation of inwardly rectifying K^+ channels in primary human somatotroph tumor cultures, as measured by whole-cell voltage clamp [21]. In primary rat pituitary cell cultures, somatostatin activated an inwardly rectifying K^+ conductance, causing hyperpolarization and inhibition of spontaneous action potential activity [22]. Finally, in rat GH_3 cells, SST_2 mediated somatostatin activation of the K^+ current [23] while somatostatin was shown to activate K^+ channels through $G_{\alpha i/o}$ proteins in GH_4C_1 cells [24].

Other pathways

SSTs regulate several other pathways in somatotroph cells. For example, both SST_5-selective SRL that signals via $G_{\alpha o}$ protein as well as pasireotide, SRL with very high binding affinity to SST_5 but also bind SST_2, SST_3 and SST_1, inhibited ERK1/2 phosphorylation in primary human pituitary somatotroph culture [20, 25] and in GH_3 cells [25]. SST_1 and SST_2 in GC cells are negatively coupled to phospholipase A2 and arachidonic acid [26]. Protein tyrosine phosphatases (PTPs) are activated by SRL in primary human pituitary tumor cultures [27] and serine/threonine phosphatases are also activated by somatostatin in GH_4C_1 cells [24].

Octreotide, SRL with high binding affinity to SST_2, and less to SST_5 and SST_3, treatment of GH_3 cells decreased PI3K regulatory subunit p85 tyrosine phosphorylation levels, leading to dephosphorylation of phosphoinositide-dependent kinase 1 (PDK1), Akt, activation of GSK3β and increased expression of ZAC1, a tumor suppressor protein that induces cell cycle arrest and apoptosis [28]. In support of cell cycle arrest mechanisms of somatostatin, pasireotide treatment of primary human somatotroph tumor cultures enhanced expression of the cyclin-dependent protein kinase (cdk) inhibitor p27kip1, while p27 immunostaining was stronger in tumor samples from patients with longer (>6 months) preoperative octreotide treatment compared to those from patients with shorter treatment periods [25]. Indeed, both SST_2 and SST_5 enhanced p27kip1 expression in primary human somatotroph tumor cultures [29].

Despite the fact that MAPK, PTP, PI3K pathways, ZAC1 and p27kip1 have all been implicated in somatotroph cell growth inhibition, it remains unclear whether the observed somatotroph tumor shrinkage in patients treated with SRL therapy is due to cell growth arrest or to increased cell death. Alternatively, this observation may result from atrophy of the cellular GH production system in somatotroph cells, resulting in reduced cell volume.

Although SRL are the preferred pharmacotherapy for patients with somatotroph tumors, many patients respond only partially or not at all to these agents [30]. One possible explanation is the SST profile of the tumor, for example, tumors expressing high levels of SST_2 are more likely to respond to octreotide. Both SST_2 protein expression and mRNA transcript number positively correlated with GH levels after acute administration of octreotide [31] and also correlated with percent decrease in GH and IGF-1 levels after treatment with octreotide long-acting-release (LAR) [3, 32]. It was further suggested that higher SST_2/SST_5 ratio is a predictor of disease control with octreotide LAR [3], and that somatotroph tumors expressing high levels of SST_5 may respond more favorably to pasireotide than to octreotide [33, 34].

Many somatotroph tumor cells express 4 receptor subtypes, all inhibit cAMP production, intracellular calcium levels and, ultimately, GH secretion. It may therefore be possible to overcome the limited clinical response in acromegaly observed for octreotide, which binds mostly SST_2, with pasireotide, which has high binding affinity for SST_5 [33], or even for the SST_1-selective SRL BIM23926 [35] or BIM23745 [36]. Clinically, pasireotide LAR has been shown to inhibit GH secretion in acromegaly patients who failed or only partially responded to octreotide LAR [33, 34].

Genetic variants of SST_5 in pituitary disease

Several functional genetic aberrations associated with GH/IGF1 changes have been described for $hSST_5$. Octreotide-resistant acromegaly was described in a patient with a single germline mutation at Arg240Trp that attenuated somatostatin 28-inhibition of cAMP accumulation and MAPK pathway activation, as well as increased cell proliferation [37].

$hSST_5$ SNPs at C1004T and T461C were associated with GH and IGF-I levels in patients with acromegaly [38]. Specifically, patients with acromegaly harboring the C1004 allele (P335) had lower IGF-I levels than did patients homozygous for 1004T (335L). $hSST_5$ SNP at the 663T allele was associated with a younger age of acromegaly at diagnosis, increased body mass index, more frequent adenoma resection, and a lack of tumor shrinkage after SRL therapy [39].

$hSST_5$ mRNA splice variant isoforms, namely sst5TMD4, are truncated at the 4th transmembrane domain,

in a variety of pituitary tumors, but mostly in somatotroph adenomas (85% of tumors). This isoform attenuates somatostatin-inhibitory effects on cAMP but not on intracellular Ca^{2+} concentration. sst5TMD4 expression negatively correlated with ability of octreotide or SST_5-selective SRL therapy to reduce GH levels, suggesting sst5TMD4 is an indicator of SRL resistance [40, 41]. sst5TMD4 mRNA and protein levels positively correlated with pituitary tumor invasiveness, and inversely with age or octreotide inhibition of GH and IGF-I. Somatotroph adenomas that also expressed the GNAS mutation had lower sst5TMD4 levels [42]. Genetic SST_5 aberration in somatotroph adenomas may thus contribute to tumor responsiveness to SRL therapy and tumor growth, but the extent of this effect remains unclear.

Dopamine receptors in normal and tumoral somatotrophs

Five dopamine receptors (DRs) subtypes are encoded by five separate genes, D_1R to D_5R. D_1R and D_5R, which share similar transmembrane domain and ligand-binding characteristics, activate adenylate cyclase. D_2R, D_3R, and D_4R are highly conserved in transmembrane sequences; these receptors inhibit adenylate cyclase [43].

Radioligand binding assay demonstrated that D_2R was present in both anterior and intermediate lobes of the pituitary, and mediated dopamine hypothalamic tonic inhibition on prolactin (PRL) and α-MSH production [44, 45]. Using quantitative real-time RT-PCR of 39 GH-secreting adenomas, D_2R was the predominant DR subtype detected, followed by D_4R, D_5R, and D_1R [46]. No D_3R expression was detected. This expression pattern is similar in GH-secreting adenomas and nonfunctioning pituitary adenomas (NFPAs), except that D_5R expression is much higher in somatotroph adenomas than in NFPAs. Of note, although D_2R has the highest level of expression among the DR subtypes in somatotroph adenomas, expression levels are significantly lower than in normal pituitary cells, as lactotrophs have higher levels of D_2R expression [46].

The human D_4R has at least three polymorphic variations in the coding sequence, with a 48-bp sequence in the putative third cytoplasmic loop as a single repeat (D4.2), 4-fold repeat (D4.4) or 7-fold repeat (D4.7). D4.4 is expressed in the pituitary but lineage specificity and pathophysiological significance are unclear [47].

Alternative splicing of D_2R generates two variants: D_2R long (D_2RL), and D_2R short (D_2RS). The coding sequences of D_2RS and D_2RL are similar, but the former lacks exon 5 [43]. Expression patterns for D_2R variants in somatotroph adenomas are heterogeneous. Some studies showed that the majority of these tumors express more D_2RS than D_2RL, while others show one-to-one expression of D_2RL and D_2RS [47]. Higher D_2RS expression has been observed in somatotroph adenomas that also stained positive for PRL; However, DR expression does not correlate with sex or age [46]. In one study of 24 somatotroph adenomas, immunohistochemistry for D_2R protein expression showed that 13 tumors were >50% positive cells for D_2R, 9 tumors were 10–50% positive, and 2 tumors were immune-negative [43].

Dopamine receptor signaling and effect on GH secretion and cell proliferation

D_2R activates multiple pituitary signaling pathways, regulating cAMP, inositol phosphate production, and voltage-activated potassium and calcium channel activity [43]. These effects, mediated by G proteins, inhibit cAMP accumulation, hormone secretion, and cell proliferation, have mostly been studied in pituitary lactotrophs [48]. In somatotroph cells, growth hormone expression is activated by Pit-1, a pituitary specific POU domain transcription factor [7]. Pit-1 positively autoregulates the Pit-1 promoter as a result of binding to the two Pit-1-binding sites, such that loss of the 5' Pit-1-binding sites eliminate positive autoregulation [49]. A 260 bp Pit-1 promoter containing the autoregulatory Pit-1-binding sites and cAMP response elements exhibits 60–65% decreased transcriptional activity following dopamine treatment [50]. It is yet to be established whether Pit-1 plays a direct role in dopamine signaling via somatotroph DNA binding or trans-activating function.

Less is known about pituitary signaling mechanisms for D_1R, D_4R, and D_5R. In non-pituitary cells, D_4R is coupled with $G_{\alpha i}/G_{\alpha o}$ to downregulate cAMP, whereas D_1R and D_5R interact with $G_{\alpha s}$ to upregulate cAMP [43]. In the rat GH/PRL pituitary cell line GH_4C_1, overexpression of human D_1R led to cAMP activation and potentiation of Ca^{2+} channel opening [51]. It is unknown whether somatotroph adenomas expressing higher levels of D_1R and D_5R are resistant to dopamine-agonist-mediated reduction in hormone release and/or cell proliferation.

Somatostatin and dopamine receptor subtype interactions

Somatostatin and dopamine receptors have been proposed to interact via hetero- or oligomerization to inhibit GH secretion. Studies in non-pituitary cells revealed complexes of SST_2-SST_2, SST_1-SST_5, SST_2-SST_3, D_2R-SST_5, D_1R-D_2R, and D_2R-D_3R, suggested to affect receptor activity and downstream signaling patterns in ways that differ from

that of the monoreceptor [52]. These receptor interactions could occur in somatotrophs as well, but this has not yet been demonstrated. These studies employed systems in which receptors are artificially overexpressed in the cell membrane and/or cytoplasm. This biological phenomenon that may not normally occur as endogenous SST expression in the cell is normally low. Technologies with better resolution and reliability than currently available are required to study whether or not this type of receptor interaction occurs in pituitary somatotrophs.

Treating patients with a somatostatin agonist together with a dopamine agonist is intuitive, especially when the adenoma expresses both ligand receptors or secretes both GH and PRL. It has long been recognized that dopamine agonists are additive to SRL efficacy when given in combination to acromegaly patients [53, 54]. In an *in vitro* analysis of somatotroph adenomas, SST_2 and D_2R mRNA expression levels correlated with octreotide and cabergoline suppression of GH levels, respectively. However, both the multi-ligand targeting D_2R-$SST_{2/5}$ chimeric BIM23A760 and $SST_{5,2,3,1}$-targeting pasireotide were more potent and efficacious in inhibiting GH [55], suggesting that effects are more likely to be additive than synergistic.

Conclusions

SST_5, SST_2, SST_3, and SST_1, as well as D_2R are expressed in somatotroph adenoma cells in different combinations, signaling as monomers, and probably also as homo- or heterdimers or oligomers. These receptors act to inhibit adenylate cyclase and cAMP accumulation and suppression of intracellular calcium concentration and oscillation, culminating in the reduction of GH secretion. Mutations in human SST5 gene were shown to be associated with GH/IGF-1 levels and tumor behavior in patients harboring somatotroph adenomas. Understanding somatostatin and dopamine receptor expression profiles and signaling enables design of new [56] and more effective SRL and dopamine agonists to treat acromegaly.

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