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Somatostatin Receptors in Pituitary and Development of Somatostatin Receptor Subtype–Selective Analogs

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Somatostatin receptor (SSTR) subtypes 1, 2, and 5 are expressed in the normal human pituitary. SSTR2 and SSTR5 are expressed in almost all growth hormone (GH) cell adenomas, and prolactin (PRL)-secreting tumors express SSTR5 more than SSTR2. SSTR4 is not detected in all pituitary adenoma subtypes, and SSTR1 and SSTR3 are expressed in about 50% of tumors. Human GH is regulated through ligand binding to both SSTR2 and SSTR5, but octreotide and lanreotide, the two clinically available somatostatin analogs, bind to human SSTR2 much better than to SSTR5. Novel SSTR2- and SSTR5-selective analogs with improved binding affinity for these receptor subtypes are highly potent in suppressing GH release from cultures of human fetal pituitaries or GH-cell adenomas. Only SSTR5-selective analogs suppress in vitro PRL secretion from cultured prolactinomas. A new SSTR2+5 bispecific analog with high affinity and selectivity for both SSTR2 and SSTR5, and a somatostatin analog with a unique broad receptor (SSTR1, 2, 3, and 5) binding profile, are both able to inhibit in vitro GH release in GH cell adenomas partially sensitive to octreotide. Recently, a somatostatin-dopamine hybrid molecule was introduced with potentially functional synergy on GH and PRL release. Using the expanding knowledge on SSTRs and their ligand activation, the development of novel pharmacologic concepts may open new opportunities for effective manipulation of this complex intracellular signaling system. These concepts may achieve better control of pituitary hormone hypersecretion, pituitary size, as well as antitumor effects in patients with SSTR-expressing tumors.

Key Words: Acromegaly; growth hormone; prolactin; somatostatin; somatostatin receptor.

Introduction

Somatostatin (somatotropin release–inhibiting factor [SRIF]), a cyclic tetradecapeptide hormone secreted from the hypothalamus and peripheral tissues *(1)*, is a potent inhibitor of endocrine and exocrine tissue secretion, an important regulator of cell differentiation and proliferation, and functions as a neuromodulator in the central nervous system (CNS). In the pituitary, somatostatin is a known inhibitor of growth hormone (GH) and thyroid-stimulating hormone (TSH) secretion *(2)*. It can suppress insulin and glucagon secretion in the pancreas *(3)*, and gastrin release from the gastric mucosa. It occurs naturally in two major forms: a tetradecapeptide (SRIF-14), and a 28 amino acid form (SRIF-28). SRIF exerts its biologic effects through at least five G protein–related SRIF receptors termed somatostatin receptors (SSTRs) 1–5 *(4,5)*. The SSTR genes lack introns, are defined by the presence of seven-membrane-spanning α-helical segments, and are most closely related to the opioid receptor family with approx 30% identity between the sequences of both receptor groups *(4)*. SSTRs function through a number of second-messenger pathways, including the inhibition of adenylyl cyclase activity, and stimulation of rectifying K^+ channel activity, while reducing Ca^{+2} channel conductance and enhancing tyrosine phosphatase activity *(4)*. The different SSTR subtypes are widely expressed in rodent and human tissues. All five subtypes are expressed in the CNS and the hypothalamus *(6,7)*. SSTRs have been identified in all somatostatin target tissues, including the anterior pituitary gland, the gastrointestinal tract, and the endocrine and exocrine parts of the pancreas.

SSTRs in Pituitary

In the rat pituitary, expression of both SSTR2 and SSTR5 occurs in somatotrophs, thyrotrophs, corticotrophs, lactotrophs, and gonadotrophs, and SSTR5 is expressed more abundantly than SSTR2 in all these cell subsets *(8)*. In humans, the fetal pituitary shows mRNA expression of SSTR1, 2, and 5, whereas SSTR3 and SSTR4 are not detected *(9)*. In the adult normal pituitary, SSTR1, 2, and 5 are generally expressed *(10,11)*, whereas SSTR4 is absent, and some studies report the expression of SSTR3 *(11)*.

Several groups studied the different SSTR subtype gene expression patterns in human pituitary adenomas using dif-

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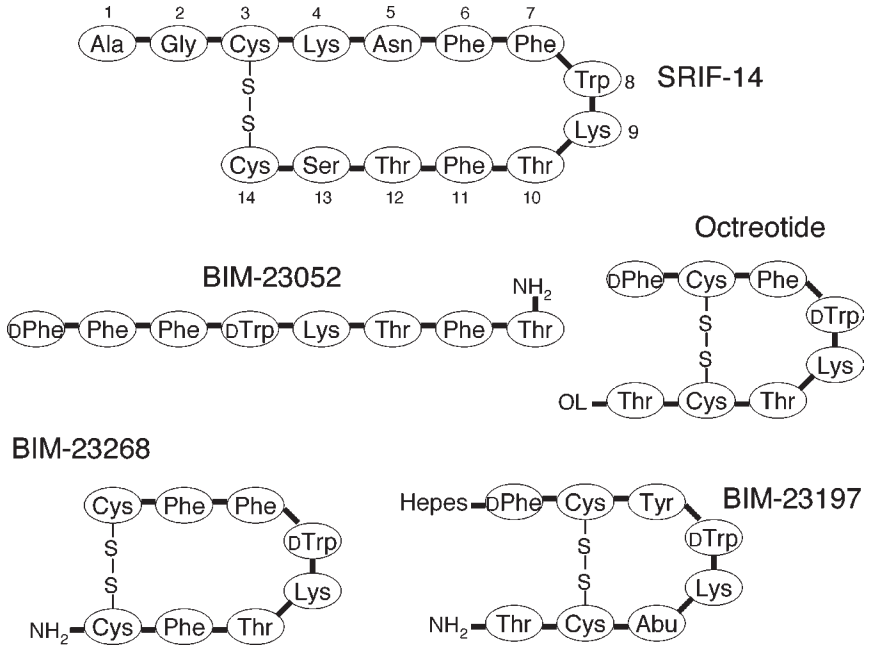

Fig. 1. Amino acid structure of SRIF-14, octreotide, BIM-23052, BIM-23268, and BIM-23197. The suggested pharmacophore essential for binding and biologic activity includes amino acids at positions 6–11 of the native SRIF. Abu, aminobutyric acid.

ferent methods. Greenman and Melmed *(12,13)* showed that almost all GH-secreting adenomas expressed SSTR2 (using RNase protection assay), SSTR3 (using reverse transcriptase polymerase chain reaction [RT-PCR]), and SSTR5 (using protection assay), whereas half of the tumors expressed SSTR1 (using protection assay), and none expressed SSTR4 (using RT-PCR). Prolactinomas expressed SSTR1, 3, and 5, but not SSTR2 or 4 *(12,13)*. Nonfunctioning pituitary adenomas did not express SSTR1, 4, and 5, 50% of nonfunctioning tumors expressed SSTR2, and all had SSTR3. Miller et al. *(10)* studied pituitary tumors with RT-PCR and showed expression of SSTR1, 2, and 5 in all nonfunctioning and PRL-, GH-, and adrenocorticotropic hormone–secreting adenomas, whereas SSTR3 and 4 were not detected in all pituitary adenoma types. Panetta and Patel *(11)* used RT-PCR followed by Southern blots to study mRNA expression of SSTR1–5 in secreting and nonsecreting adenomas. They demonstrated SSTR2 in most studied adenomas (some PRL tumors did not express SSTR2). SSTR1 was also detected in most tumors, while SST3, 4, and 5 were expressed in about 50% of the adenomas *(11)*. Jaquet et al. *(14)* using RT-PCR quantitative analysis, showed a large predominance of SSTR5 over SSTR2 mRNA in 10 studied prolactinomas, and also in 5 pure GH adenomas and 10 mixed GH-PRL tumors *(15)*. SSTR4 was not observed in any GH-secreting tumor *(15)*. In summary, SSTR2 and SSTR5 are expressed in almost all GH cell adenomas, and PRL-secreting tumors express SSTR5 more than SSTR2. SSTR4 is not detected in all pituitary adenoma subtypes, and SSTR1 and 3 are expressed in about 50% of tumors.

Somatostatin Analogs

Somatostatin-14 binds to SSTR1–4 better than SRIF-28, whereas human SSTR5 binds SRIF-28 with higher affinity. The two clinically available SRIF analogs, octreotide (SMS 201-995; Novartis, Basel, Switzerland) (Fig. 1) and lanreotide (BIM-23014; Ipsen, France), are cyclic (disulfide-bridged) octapeptides with high binding affinity for SSTR2 (IC_{50}: 0.56–0.75 n*M*), modestly low affinity for SSTR5 (IC_{50}: 5.2–7 n*M*), low affinity for SSTR3, and no binding for SSTR1 and 4 (Table 1). These cyclic peptides were shown to suppress and normalize GH secretion in 60% of patients with acromegaly *(16,17)*, whereas 40% of the treated patients were resistant or responded only partially to these analogs. As in the rat, in which these two analogs were tested for their ability to suppress GH, human GH (hGH) suppression is also regulated by SSTR2. However, in contrast to SSTR2, there are species-specific differences in ligand-binding affinity of SSTR5; in fact, human SSTR5 has a 160-fold lower affinity for octreotide than the rat receptor *(18)*.

The isolation and cloning of the five SSTRs have dramatically increased the research of SRIF analog chemistry and pharmacology. The key residues of native SRIF essential for binding and biologic activity were identified. These residues include Phe^6-Phe^7-Trp^8-Lys^9-Thr^{10}-Phe^{11} (Fig. 1; the positions follow that of SRIF-14). Several years ago, Biomeasure (Milford, MA) developed new SSTR2- and SSTR5-selective analogs *(19)*. These octapeptides were designed based on the known expression of SSTR2 and SSTR5 in

Table 1
SSTR Subtype Binding Affinity of Somatostatin Analogs

	Receptor subtype affinity (IC_{50}, n*M*)				
Compound	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Somatostatin-14	2.26	0.23	1.43	1.77	0.88
Somatostatin-28	1.85	0.31	1.3	ND[a]	0.4
Octreotide	1140	0.56	34	7030	7
Lanreotide	2330	0.75	107	2100	5.2
BIM-23190	4577	0.34	217	>1000	11.1
BIM-23197	5547	0.19	27	3897	9.8
BIM-23052	100	11.9	5.6	132	1.22
BIM-23268	18	15.1	62	16	0.37
L-817,818	3.3	52	64	82	0.4
BIM-23244	1020	0.29	133	>1000	0.67
BIM-23454	1000	31	50.5	301	139
BIM-23627	2757	6.4	44	423	86.5
SOM-230	9.3	1	1.5	>100	0.16
PTR-3173	>1000	3	>100	7	6
BIM-23A387	293	0.1	77	>1000	>1000

[a]ND, not determined.

the pituitary. These linear and cyclic octapeptides contained the smaller pharmacophore Phe/Tyr-D-Trp-Lys-Thr/Val/aminobutyric acid (Fig. 1) necessary for biologic activity and were modified at the N-terminus to increase their length of action. Compounds were evaluated in vitro for affinity to human SSTRs expressed in CHO-K_1 cells, and in vitro in rats for the inhibition of circulating GH *(20)*.

Peptides BIM-23190 and BIM-23197 are SSTR2-selective cyclic analogs (Fig. 1) with improved binding affinity for this receptor subtype (IC_{50}: 0.19–0.34 n*M*; Table 1) that were highly potent in suppressing GH in cultures of human fetal pituitaries, better than SRIF-14 and lanreotide *(9)*. BIM-23052, a linear octapeptide (Fig. 1), and BIM-23268 are SSTR5-specific SRIF analogs with high affinity for this receptor (IC_{50}: 0.37–1.22 n*M*; Table 1) and low affinity for SSTR2 (IC_{50}: 11.9–15.1 n*M*) that are also potent suppressors of hGH release. The structure of BIM-23268 is unique and differs from other cyclic octapeptide analogs in that the characteristic disulfide bridge of this cyclic peptide begins and ends at the NH_2 and COOH terminals (Fig. 1), rather than positions 2 and 7 *(9)*. Both SSTR2- and SSTR5-selective analogs suppressed human TSH, but PRL secretion in fetal human lactotrophs was reduced via selective binding to SSTR2 alone *(9)*.

These data illustrate the important role of both SSTR2 and SSTR5 in mediating human anterior pituitary hormone suppression by somatostatin. When these novel analogs were used in cultures of GH-secreting adenomas, both the SSTR2- and the SSTR5-selective compounds were more potent than octreotide and lanreotide in suppressing hGH *(21)*. Moreover, heterologous analog combinations containing both SSTR2- and SSTR5-preferential compounds were more potent in decreasing GH than analogs used alone *(21)*, and in suppressing GH and PRL in mixed adenomas taken from patients partially resistant to octreotide *(15)*. Thus, somatostatin suppression of GH secretion in normal (fetal) and in adenomatous cells is similar and mediated through ligand binding to both SSTR2 and SSTR5. By contrast, SSTR2-selective analogs did not suppress PRL from cultured prolactinomas, whereas the new SSTR5-selective analogs suppressed in vitro PRL secretion *(14,21)*. SSTR5 expression was correlated to PRL regulation *(14)*, but no additive effect on PRL suppression was achieved by cotreatment of cultured prolactinomas with a dopamine agonist and SSTR5-selective analogs *(14)*. Recently, researchers from Merck (Rahway, NJ) identified several nonpeptide agonists selective for each human SSTR subtype *(22,23)*. SSTR2-selective agonist was a potent inhibitor of rat GH (rGH) and glucagon *(22,23)*. An hSSTR5-selective compound (L-817,818; Table 1) suppressed rGH and insulin but not glucagon. None of the SSTR1-, 3-, and 4-selective agonists inhibited release of GH, glucagon, or insulin *(23)*. This supports the role of both SSTR2 and SSTR5 in GH regulation. SSTR1 probably regulates angiogenesis, hSSTR3 can induce apoptosis *(24)*, and SSTR5 might be involved in the cell cycle *(25)*. The physiologic role of SSTR4 is currently unknown.

BIM-23244 is a new SSTR2+5 bispecific analog with high affinity and selectivity for both SSTR2 (IC_{50}: 0.29 n*M*) and SSTR5 (IC_{50}: 0.67 n*M*; Table 1) *(26)*. This peptide can activate both receptors, and because of heterogeneous expres-

sion of SSTR2 and SSTR5 subtypes in GH-secreting adenomas, this bispecific analog can achieve a better control of GH hypersecretion compared to octreotide, especially in tumors partially responsive to octreotide *(26)*. However, a major concern of all SSTR5-selective analogs is inhibition of human insulin release. SSTR5-specific analogs suppress insulin secretion in cultured human pancreatic islets, whereas a group of SSTR2-selective analogs failed to inhibit human insulin release *(27)*.

In contrast to the SSTR-selective agonist analogs, a subset of SSTR2-selective antagonists was developed by Biomeasure. BIM-23454 and BIM-23627 are two unique peptides with moderately high affinity for SSTR2 (IC_{50}: 6–31 n*M*; Table 1) *(28)*. These antagonist peptides reversed the in vitro suppressive effect of somatostatin on rGH secretion, and induced a dose-dependent increase in serum GH when administered to rats *(28)*.

Recently, Novartis successfully produced a "universal" somatostatin ligand (SOM-230) that binds with high affinity to human SSTR1, 2, 3, and 5, but not to SSTR4 *(29)*. This novel cyclohexapeptide SRIF analog exhibits a 100- to 200-fold higher binding affinity to hSSTR1 (IC_{50}: 9.3 n*M*) compared with octreotide and lanreotide (Table 1), and a 40-fold higher binding affinity to hSSTR5 (0.16 n*M*). This unique binding profile was translated into improved rGH inhibitory profile in vitro, and prolonged duration in inhibiting rGH secretion in vivo with no significant impact on plasma glucose levels *(29)*. In addition, SOM-230 displayed long-lasting reduction of insulin-like growth factor-1 (IGF-1) levels in rats, which was far more effective compared to treatment with octreotide *(29)*. More important, SOM-230 had no diabetogenic effect in rats, despite the potential SSTR5-mediated insulin suppression. Preliminary studies with SOM-230 in cultured human fetal pituitaries and GH- and PRL-secreting adenomas demonstrated in vitro human GH and PRL suppression in both normal and adenomatous cells *(30, 31)*. Another new multispecific SRIF-compound, PTR-3173, was introduced by Peptor (Rehovot, Israel). This cyclic heptapeptide has a unique binding profile with affinity of 3–7 n*M* for human SSTR2, 4, and 5; however, it recognizes with very low affinity hSSTR1 and 3 (Table 1) *(32)*. This peptide suppressed in vitro rGH equally to octreotide, while not affecting glucagon and insulin secretion *(32)*. PTR-3173 is a potent inhibitor of hGH release from cultures of fetal pituitaries and GH-secreting adenomas (personal data). All these novel SRIF analogs should be studied in clinical trials to evaluate their therapeutic potential in humans. These studies will reveal not only the inhibitory profile on in vivo GH secretion, but also on IGF-1 release, insulin secretion, and glucose levels, and signs of biologic escape.

SSTR Heterodimerization and Hybrid Molecules

Rocheville et al. *(33)* demonstrated that expression of both SSTR1 and SSTR5 in transfected CHO-K_1 cells will allow SRIF-induced receptor homo- and heterodimerization after receptor internalization. This heterodimerization formed between SSTR1 and SSTR5 subtypes may define a new level of molecular cross talk among SSTR subtypes and possibly related receptor families. Such heterodimerization may improve the hormonal and antiproliferative effects induced by somatostatin ligand binding. Heterooligomerization between the dopamine D2 receptor and SSTR5 was also described recently *(34)*. This direct intramembrane physical interaction among receptors from different G protein–coupled receptor families creates a novel receptor complex with enhanced functional activity *(34)*. Similar oligomeric interactions among other members of these two receptor families may exist. This suggested type of molecular cross talk was used to plan and create a somatostatin-dopamine hybrid molecule by Biomeasure *(35)*. This hybrid molecule (BIM-23A387) retains the ability to bind both the somatostatin subtype 2 receptor (IC_{50}: 0.1 n*M*; Table 1) and the dopamine D2 receptor (22.1 n*M*). The new hybrid molecule was much more potent than either of the individual parent somatostatin or dopamine agonists in suppressing GH and PRL release from cultured human pituitary adenoma cells *(34)*. The tremendous functional synergy of the hybrid SRIF/dopamine molecule in terms of hormonal suppression cannot be explained only on the basis of receptor binding affinity, but probably reflects subreceptor intracellular modification induced by the receptor heterodimerization.

The concept of hybrid compounds that contain structural elements of different molecules can be extended to produce hybrids of SSTR-selective analogs with cytotoxic agents or radioisotopes. These molecules would selectively bind a specific receptor on tumor cells and after receptor internalization will destroy these SRIF-responsive tumor cells *(36)*. Thus, somatostatin analogs can serve as targeting vectors for specific cytotoxic drugs, which can be introduced to SSTR-expressing tumor cells with minimal side effects on adjacent nonexpressing cells. This receptor-specific cytotoxicity has profound effects on both angiogenic blood vessels and tumor cells.

Conclusion

In the last two decades, somatostatin analogs were used for the treatment of hormone (GH and TSH)-secreting pituitary adenomas, islet cell tumors, and carcinoid syndrome. However, almost half of treated patients were found resistant to the suppressive effects of the drugs currently used. With our improved understanding of SRIF and SSTR physiology and interactions, and with the increasing involvement of the industry in novel SRIF formulation production, well-characterized selective analogs for the various receptor subtypes were developed. Using these novel analogs in vitro in pituitary cell cultures resulted in new concepts and potential mechanisms for the treatment of pituitary hor-

mone hypersecretion. These concepts include ligand binding to both SSTR2 and SSTR5 to achieve greater efficacy in suppressing GH in acromegaly, binding to SSTR5 to suppress PRL in prolactinomas, and interaction among SSTR subtypes and receptors of other G protein–coupled receptor families. This interactive multireceptor system of ligand activation and cross talk at the membrane and intracellular levels may open the search for new therapeutic ideas, improve manipulation of pituitary hormone regulation, and enhance efficacy of medical therapy for pituitary hormone hypersecretion.

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