Selective Nonpeptide Somatostatin Subtype 5 (sst5) Agonists Suppress Induced Insulin Secretion in Pancreatic Islets from both Rats and Healthy Human Donors.

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Hyperinsulinemia is a heterogeneous condition in which dangerously low blood sugar levels are caused by improperly regulated insulin secretion from pancreatic ß-cells. The most severe form of hyperinsulinemia arises from congenital hyperinsulinism (CHI), a set of genetic disorders in which the underlying pathology is driven by mutations in key genes that regulate insulin secretion. CHI is the most common cause of persistent hypoglycemia in newborns and infants, and prompt recognition and treatment are vital to prevent coma, long-term neurological complications, and even death. The neuropeptide somatostatin is an important modulator of hormonal signaling from the pancreas mediated by different somatostatin receptor (sst) subtypes. Glucagon secretion from a-cells is inhibited through sst2 receptor activation and insulin secretion from b-cells is inhibited through sst2, sst3, and sst5. The injectable peptide drugs octreotide and lanreotide are primarily agonists at sst2 and are often prescribed for these patients in an attempt to reduce insulin secretion, but their efficacy is limited, and they carry the risk of impairing glucagon secretion, an important defense mechanism against hypoglycemia. We hypothesize that an orally available selective sst5 agonist may be a useful new approach to managing hyperinsulinemia.

We launched an iterative medicinal chemistry program that led to the discovery of selective sst5 agonists, with multiple nonpeptide series possessing EC_{50}s < 1 nM in cell-based assays of receptor activation (these compounds also routinely possess similar potencies for the rat sst5 receptor). We have shown that these sst5 agonists potently suppress insulin and raise plasma glucose in multiple glycemic studies in rats. To explore the mechanism of selective sst agonism and its translation from rat studies to humans, we undertook a series of studies using pancreatic islets isolated from human donors and from naïve Sprague Dawley rats. Selective sst5 agonists were compared to selective sst2 and selective sst3 agonists as well as somatostatin peptides for their capacity to suppress insulin and/or glucagon from islets under various conditions including increasing glucose concentrations and the presence of a sulfonylurea (the increased insulin secretion mimics many CHI patients). In both human and rat islets, we found that selective sst5 agonists potently suppressed insulin secretion more effectively than selective sst2 or sst3 agonists, while having little effect on glucagon secretion, demonstrating their potential efficacy in the human condition. These studies support our program to identify and develop potent nonpeptide selective sst5 agonists with pharmaceutical and safety characteristics suitable for evaluation in human clinical trials.

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Hypothesis: An Oral Drug Selectively Targeting sst5 is the Optimal Strategy for Treating HI

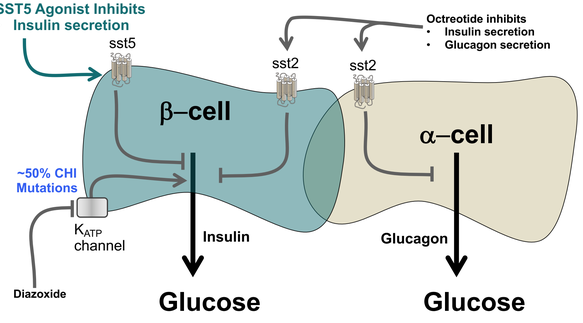

Figure 1. Depiction of pancreatic islet cells that regulate glucose

Peptide sst agonists inhibit glucose- and tolbutamide-stimulated insulin secretion from human islets.

EC_{50} (nM)	hsst1	hsst2	hsst3	hsst4	hsst5
SS14	0.8	0.13	0.16	0.07	0.063
Octreotide	>30000	0.057	7.9	470	2.5
Pasireotide	5.7	0.59	0.78	> 10000	0.076

Table 1. Agonist peptide activity at human sst receptors. G_i activation was measured using the CisBio cAMP assay in CHOK cells stably expressing each human sst receptor.

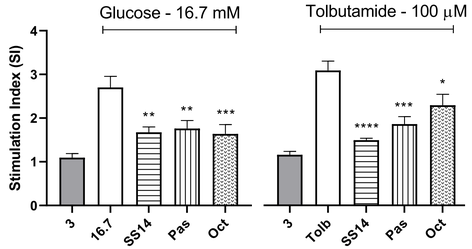

Figure 2. Human islets were treated with glucose or the sulfonylurea tolbutamide (K_{ATP} channel inhibitor) in the presence or absence of somatostatin peptide agonists (100 nM) for 90 min. Stimulation Index (SI) was calculated as stimulated insulin levels/basal insulin levels. Figure shows mean SI ± SEM (n = 6-7 independent experiments); *, p<0.05; **, p < 0.01; ***, p< 0.001; ****, p<0.0001.

Peptide sst agonists inhibit glucose-stimulated insulin secretion from rat islets.

EC_{50} (nM)	rsst1	rsst2	rsst3	rsst4	rsst5
SS14	ND	0.18	0.13	0.063	1.3
Octreotide	ND	0.081	15	120	2.5
Pasireotide	ND	2.5	0.49	ND	0.019

Table 2. Agonist peptide activity at rat sst receptors. G_i activation was measured using the CisBio cAMP assay in CHOK cells stably expressing the sst2-sst5 rat receptors. ND = not determined

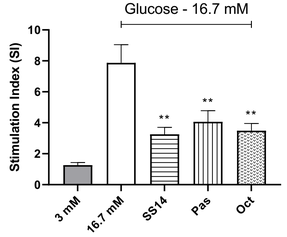

Figure 3. Rat islets were treated with glucose in the presence or absence of somatostatin agonists (100 nM) for 90 min. Figure shows mean SI ± SEM (n = 3-4 independent experiments). **, p < 0.01.

Nonpeptide selective-sst5 agonists inhibit glucose- and tolbutamide-stimulated insulin secretion from human islets

EC_{50} (nM)	hsst1	hsst2	hsst3	hsst4	hsst5
Agonist 1	>10000	440	39	5.7	0.39
Agonist 2	>10000	310	62	12	0.11
Agonist 3	>10000	340	140	4400	0.037
Agonist 4	89	0.040	4.1	0.34	2800

Table 3. Agonist peptide activity at human sst receptors. G_i activation was measured using the CisBio cAMP assay in CHOK cells stably expressing each human sst receptor.

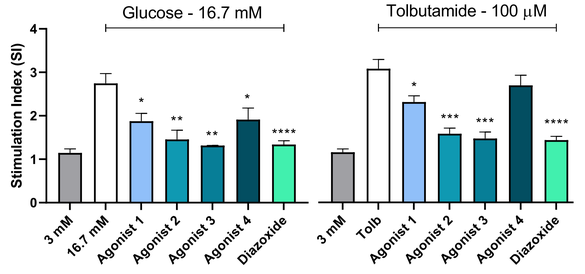

Figure 4. Human islets were treated as in Figure 2 but in the presence or absence of Crinetics sst nonpeptide agonists (100 nM) or a maximally effective dose of diazoxide (100 μM) for 90 min. Figure shows mean SI ± SEM (n = 2-7 independent experiments). *, p<0.05; **, p < 0.01; ***, p< 0.001; ****, p<0.0001.

Nonpeptide selective-sst agonists inhibit glucose-stimulated insulin secretion from rat islets

EC_{50} (nM)	rsst1	rsst2	rsst3	rsst4	rsst5
Agonist 1	ND	ND	22	ND	0.36
Agonist 4	ND	0.30	6.4	0.071	24

Table 4. Crinetics nonpeptide sst agonists activity at rat sst receptors. G_i activation was measured using the CisBio cAMP assay in CHOK cells stably expressing the sst2-sst5 rat receptors. ND = not determined

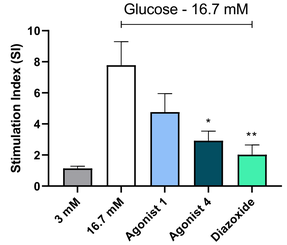

Figure 5. Rat islets were treated with glucose in the presence or absence of Crinetics nonpeptide sst agonists (100 nM) or a maximally effective dose of diazoxide (100 μM) for 90 min. Figure shows mean SI ± SEM (n = 4 independent experiments). *, p<0.05; **, p < 0.01.

Conclusions

We have developed several potent and selective sst5 agonists that inhibit glucose- and tolbutamide-stimulated insulin secretion from human and rat islets. This pharmacologic profile may be useful for the treatment of congenital hyperinsulinism.

- Glucose-stimulated insulin secretion is equally inhibited by peptide sst agonists (SS14, pasireotide, and octreotide) from human and rat islets (50-60% inhibition)
- Tolbutamide-induced insulin secretion from human islets is inhibited by peptides agonists: SS14 > pasireotide > octreotide, suggesting the participation of other sst receptors, in addition to sst5 and sst2.
- sst5-selective nonpeptide agonists (agonists 1-3) inhibit glucose- and tolbutamide-stimulated insulin secretion from human islets. Compounds 2 & 3 suppress insulin secretion to the same degree as a maximally effective concentration of diazoxide.
- A potent, sst2-selective nonpeptide agonist (agonist 4), has a limited effect (similar to octreotide) in glucose- and tolbutamide-stimulated insulin secretion from human islets.
- Agonist 4, however, demonstrates greater insulin suppression than agonist 1 in rat islets, suggesting that insulin secretion regulation by sst2 is more important in rat than in human islets.

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