Selective somatostatin 5 (SST5) and somatostatin 2 (SST2) nonpeptide agonists potently suppress glucose- and tolbutamide-stimulated dynamic insulin secretion from isolated human islets

Elizabeth Rico-Bautista, Jian Zhao, Mi Chen, Ana Karin Kusnetzow, Yun Fei Zhu, and Stephen F. Betz
Crinetics Pharmaceuticals, San Diego, CA.

Poster #8684

Congenital hyperinsulinism (HI) is the most common cause of persistent hypoglycemia in newborns and infants and arises from dysregulated insulin secretion. Rapid recognition and treatment are vital to prevent seizures, permanent developmental delays, coma, or even death. Very few medical options exist to treat congenital HI patients: the K_{ATP} channel activator diazoxide, the injectable somatostatin receptor peptide agonists octreotide and lanreotide, or chronic glucose infusions. However, side effects and/or limited efficacy render these therapies inadequate for many patients.

Somatostatin is a 14-amino acid peptide hormone with a broad spectrum of biological actions, which are regulated through five somatostatin receptor subtypes (SST1-SST5). Somatostatin's common physiological role is to down-regulate secretion of other hormones in various tissues. Its role in the maintenance of euglycemia is to regulate insulin and glucagon secretion from pancreatic β- and α-cells, respectively. Somatostatin regulates insulin secretion by decreasing the intracellular levels of cAMP, inhibition of voltage-gated calcium channels (VGCC), activation of the G protein-activated inward rectifier K^+ channel (GIRK), and direct inhibition of insulin exocytosis.

Several studies have evaluated the effect of somatostatin, somatostatin peptide analogs, and a limited number of nonpeptide somatostatin receptor agonists on insulin secretion in static assays using isolated human islets. However, the lack of highly selective agonists has made the interpretation of the contribution of SST receptor sub-types difficult to discern. Our programs for the treatment of hyperinsulinism, acromegaly, and other indications have led to the development of selective nonpeptide SST2, SST3, SST4, and SST5 agonists, possessing EC_{50}s < 1 nM in cell-based assays of receptor activation and selectivity > 130 times over the other members of the family. The ability of these selective nonpeptide agonists to regulate glucose- and tolbutamide-stimulated dynamic insulin secretion from human islets was evaluated using a perifusion system (Biorep, FL).

We found that selective SST2 and SST5 agonists potently suppressed dynamic insulin secretion in contrast to SST3 or SST4 selective agonists. Importantly, SST5 agonists were shown to have a greater effect than selective SST2 agonists or diazoxide, demonstrating their potential utility in human conditions such as congenital HI. In addition, SST5 activation is also known to have a smaller effect on glucagon secretion and is also less prone to agonist-driven desensitization than SST2 activation. Taken together, these studies support our program to identify, characterize, and develop potent, nonpeptide, orally-bioavailable, selective SST5 agonists with appropriate pharmaceutical and safety characteristics for the treatment of congenital HI.

Targeting SST5 or SST2 receptors in the β-cell results in suppression of insulin secretion

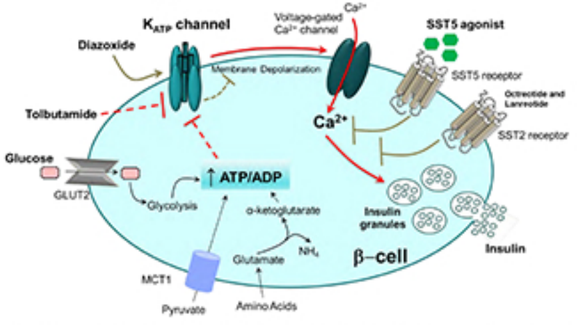

Figure 1. Insulin secretion is initiated by closing the K_{ATP} channel in β-cells (red dotted line) in response to glucose, pyruvate, amino acids or sulfonylureas (e.g., tolbutamide), leading to plasma membrane depolarization and influx of calcium through voltage-gated calcium channels resulting in insulin exocytosis. The inhibitory effect of diazoxide is mediated by binding to the SUR1 subunit in the K_{ATP} channel, inhibiting membrane depolarization (dotted brown line). Somatostatin (SST) agonists act on their specific receptors (SST5 and/or SST2) to inhibit insulin secretion downstream K_{ATP} channel.

Glucose and tolbutamide stimulate insulin secretion in a biphasic manner in islets from healthy donors

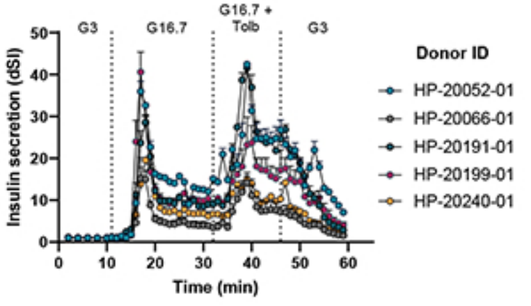

Figure 2. Human islets (Prodo Labs, CA) were loaded in a perifusion system (Biorep, FL) and treated over time with 3 mM glucose (G3), 16.7 mM glucose (G16.7) or 16.7 mM glucose + 100 µM tolbutamide (G16.7 + Tolb). Insulin secretion was quantified using an ELISA assay (Mercodia, Uppsala, Sweden) and the dynamic stimulation index (dSI) was calculated as stimulated insulin levels/basal insulin levels. Figure shows mean dSI ± range (n=2 technical replicates) for 5 representative donors.

Diazoxide inhibits glucose- but not tolbutamide-stimulated insulin secretion from human islets

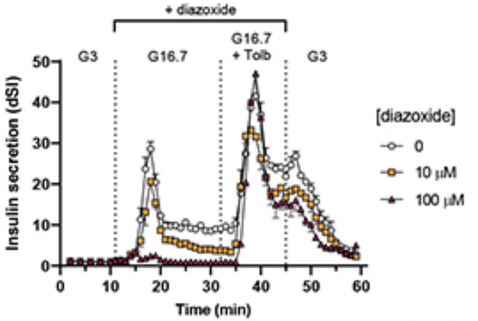

Figure 3. Human islets were treated as in Figure 2 but in the presence or absence of diazoxide (K_{ATP} channel opener). Figure shows mean dSI ± range (n=2 technical replicates) from one donor. Diazoxide suppresses GSIS in a concentration dependent manner, with a maximal effect (95% insulin secretion suppression) at 100 µM but has a diminished effect on tolbutamide-stimulated insulin secretion that is not concentration-dependent.

Somatostatin 14 (SS14) and nonpeptide SST5 and SST2 specific agonists suppress dynamic 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
Agonist 2	>10000	0.27	3300	1100	>10000

EC_{50} (nM)	hSST1	hSST2	hSST3	hSST4	hSST5
Agonist 3	1200	1300	0.9	120	5100
Agonist 4	ND	>10000	>10000	0.73	>10000

EC_{50} (nM)	hSST1	hSST2	hSST3	hSST4	hSST5
Agonist 5a	>10000	770	540	4700	0.41
Agonist 5b	>10000	17	530	4500	0.05

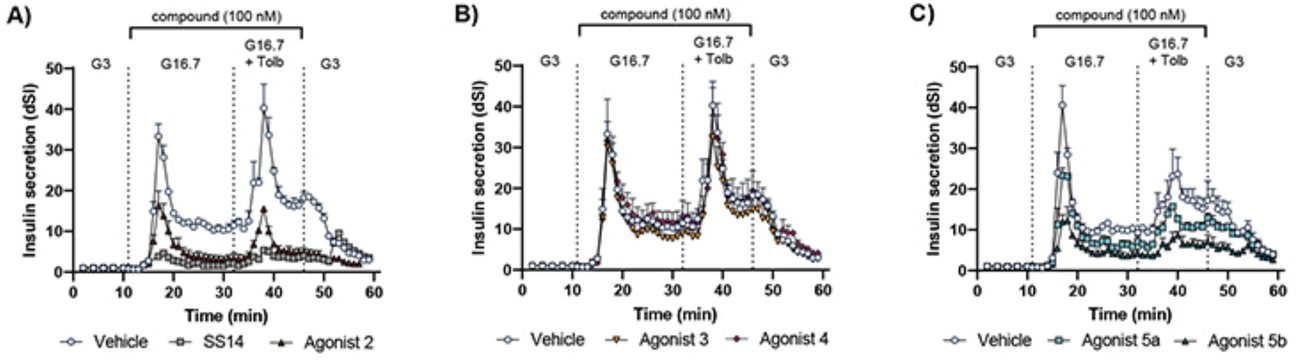

Figure 4. Human islets were treated as in Figure 2 but in the presence or absence of **A)** somatostatin (SS14) or Agonist 2 (SST2 specific), **B)** Agonist 3 or Agonist 4 (specific SST3 and SST4, respectively), and **C)** Agonists 5a and 5b (SST5 specific). All agonists were evaluated at 100 nM. Each profile is a representative from one donor and shows mean dSI ± range (n=2 technical replicates). Tables above show agonist activity at human SST receptors measuring G_i activation using the CisBio cAMP assay in CHOK cells stably expressing each human SST receptor.

Agonist 5b is as potent as SS14 but more potent than SST2-specific agonist on suppressing insulin secretion from human islets

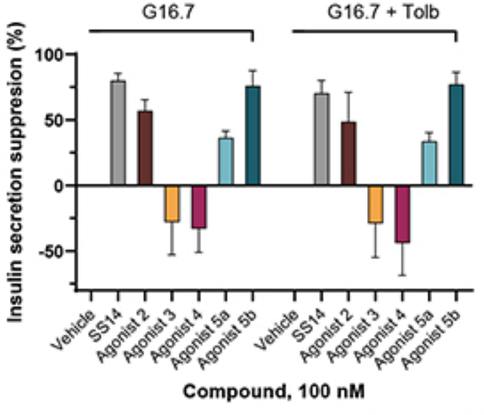

Figure 5. Insulin secretion suppression was calculated by comparing the insulin secreted at each condition to the insulin secreted in the vehicle group at G16.7 or G16.7 + Tolbutamide. Insulin suppression in the vehicle group is zero (0). Figure shows mean percent suppression ±SEM (n=2-5 independent donors).

Conclusions

We have developed several potent and selective nonpeptide SST5 and SST2 agonists that inhibit glucose- and tolbutamide-stimulated insulin secretion from human islets more potently than diazoxide, a well know inhibitor of insulin secretion.

- Stimulation of pancreatic β-cells with high glucose and tolbutamide results in the dynamic insulin secretion in a biphasic manner. The magnitude of the response varies among donors, as expected.
- Diazoxide at 100 µM inhibits glucose-stimulated insulin secretion but has a small effect on tolbutamide-stimulated insulin secretion.
- SS14, a potent agonist of all SST receptors, inhibits insulin secretion by ~75% in both conditions, glucose- and tolbutamide-stimulated secretion.
- The SST5-selective nonpeptide agonists 5a and 5b (100 nM) suppress 35 and 75% insulin secretion, respectively. These results agree with the established in vitro pharmacology, showing that agonist 5b is 8-fold more potent than agonist 5a and more importantly it is as effective as SS14 on insulin secretion suppression.
- The SST2-selective nonpeptide suppresses ~50% insulin secretion at 0.1 µM, while the SST3 and SST4-selective nonpeptide agonists have no effect on insulin secretion.

Crinetics Pharmaceuticals