A selective non-peptide somatostatin receptor 5 (SST5) agonist effectively decreases insulin secretion in the K<sub>ATP</sub> HI mouse model and human HI islets

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Introduction

• Congenital hyperinsulinism (HI) is the most common cause of persistent hypoglycemia in infants and children.
• Inactivating mutations of β-cell K<sub>ATP</sub> channels cause the most common and severe form of HI, known as K<sub>ATP</sub>-HI.
• Children with K<sub>ATP</sub>-HI are typically unresponsive to diazoxide, the only drug with regulatory approval for HI.
• Octreotide, an SST-selective peptide agonist that inhibits insulin secretion, is used off label as second line therapy, but poor efficacy and SST-mediated side effects limit its use in infants.
• Crinetics has ongoing discovery and development efforts aimed at finding a compound to treat HI. They have identified potent and selective nonpeptide SST agonists with sub-nanomolar EC<sub>50</sub>s in cell-based assays of receptor activation.
• We characterized the ability of the selective SST5 nonpeptide agonist CRN02481 to suppress insulin secretion and prevent fasting hypoglycemia in the Sur<sup>2</sup>- HI mouse model of K<sub>ATP</sub>-HI, and to suppress insulin secretion from healthy human islets and from HI islets.

Methods

• In vitro studies: Sur<sup>2</sup>- and Sur<sup>1</sup>- HI mouse islets were isolated and cultured for 72 hrs. For static incubations, islets were treated with CRN02481 (100 nM) or vehicle and stimulated with glucose or a physiological amino acids mixture (AAMS) for 1.5 hours. Supernatant was collected to measure insulin by homogenous Time Resolved Fluorescence Immunoassay (TRF). For intracellular Ca<sup>2+</sup> measurements, islets were pre-incubated with the Fura 2 fluorescent probe, treated with CRN02481 (500 nM) or vehicle and then exposed to increasing concentrations of glucose or AAMS. Intracellular Ca<sup>2+</sup> was calculated as the ratio of excitation of Fura 2 at 334 and 390 nm. Normal human islets (Prodo Labs, CA) were loaded in a perfusion system and treated with 3, 6, and 16.7 mM glucose, and 16.7 mM glucose + 100 μM tolbutamide <sup>•</sup> increasing concentrations of CRN02481. Insulin was quantified by ELISA (Mercodia, Uppsala, Sweden).
• In vivo studies: Sur<sup>2</sup>- and Sur<sup>1</sup>- HI mice received CRN02481 (30 mg/kg/day) or PBS by gavage (n=7/group) while fasting. Glucose tolerance tests (GTT) were performed after an overnight fast (16 hrs) and with 2 g/kg dextrose by intraperitoneal injection.

Results

CRN02481 increases plasma glucose and decreases insulin secretion in both Sur<sup>2</sup>- and Sur<sup>1</sup>- mice

CRN02481 decreases insulin secretion in both normal and HI human pancreatic islets

Conclusions

• The somatostatin receptor agonist CRN02481 (SST5 selective) effectively decreases insulin secretion in the Sur<sup>2</sup>- K<sub>ATP</sub>-HI mouse model and in both normal and HI human islets.
• Selective targeting of specific SST5 somatostatin receptors by non-peptide agonists is a viable option for the development of HI therapeutics.

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Figure 1: Treatment with SST5 agonists inhibits abnormal insulin secretion from HI islets. The most common mutations in HI are highlighted in red. GAK, K<sub>ATP</sub> channel; SUR1, SUR2A.

Figure 2: CRN02481 inhibits fuel-stimulated insulin secretion and calcium flux in mouse islets

Figure 3: Perifusion of primary isolated islets to assess glucose/insulin release in both Sur<sup>2</sup>- and Sur<sup>1</sup>- islets stimulated with glucose (10 – 25 mM) or K<sub>Cl</sub> (30 mM or 60 mM) or Sur<sup>1</sup>- islets stimulated with AAMS (a = 1.2 mM) and (b) (50 μM). (a) A, B: AUCs for CRN02481+vehicle control, * p<0.05, ** p<0.01,

Figure 4: Intracellular Ca<sup>2+</sup> measurement of primary isolated perifused islets in Sur<sup>2</sup>- and Sur<sup>1</sup>- islets treated with CRN02481 (30 mg/kg/day) or PBS by gavage (n=7/group) while fasting. Glucose tolerance tests (GTT) were performed after an overnight fast (16 hrs) and with 2 g/kg dextrose by intraperitoneal injection.

Figure 5: Fasting evaluation of Sur<sup>1</sup>- treated by gavage with PBS control or CRN02481 after overnight fast demonstrating (A) plasma glucose, (B) plasma insulin, and (C) insulin/glucose ratio at times denoted. Fasting evaluation of Sur<sup>2</sup>- treated by gavage with PBS control or CRN02481 after overnight fast demonstrating (A) plasma glucose, (B) plasma insulin, and (C) insulin/glucose ratio at times denoted. Glucose tolerance test (GTT) in (D) Sur<sup>1</sup>- mice and (E) Sur<sup>2</sup>- mice under the same conditions. (F) Plasma insulin levels by Sur<sup>2</sup>- mice at dosed times during GTT. GITT in (G) Sur<sup>1</sup>- mice treated with PBS control or CRN02481 by gavage and (H) AUC calculation. (I) Plasma insulin levels by Sur<sup>2</sup>- mice at dosed times during GTT. AUC = area under the curve (AUC) calculation. (J) Plasma insulin levels by Sur<sup>2</sup>- mice at dosed times during GTT. AUC = area under the curve (AUC) calculation. (K) Plasma insulin levels by Sur<sup>2</sup>- mice at dosed times during GTT. AUC = area under the curve (AUC) calculation. (L) Plasma insulin levels by Sur<sup>2</sup>- mice at dosed times during GTT. AUC = area under the curve (AUC) calculation.