A selective non-peptide somatostatin receptor 5 (SST5) agonist effectively decreases insulin secretion in the K_{ATP}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_{ATP} channels cause the most common and severe form of HI, known as K_{ATP}HI.
- Children with K_{ATP}HI are typically unresponsive to diazoxide, the only drug with regulatory approval for HI.
- Octreotide, an SST2-selective peptide agonist that inhibits insulin secretion, is used off label as second line therapy, but poor efficacy and SST2-mediated side effects limit its use in infants.
- Crinetics has ongoing discovery and development efforts aimed at finding a compound to treat HI. These efforts have yielded potent and selective nonpeptide SST5 agonists with sub-nanomolar EC_{50}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 *Sur1*$^{-/-}$ mouse model of K_{ATP}HI, and to suppress insulin secretion from healthy human islets and from HI islets.

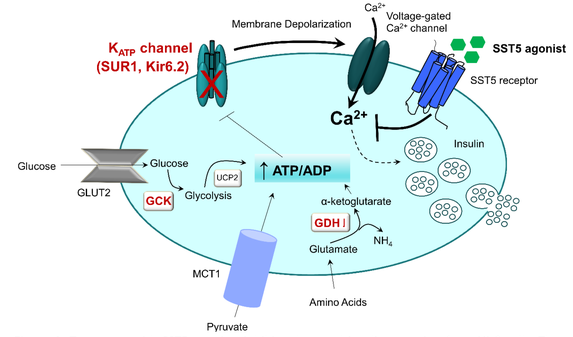

Figure 1: Treatment with SST5 agonists inhibits abnormal insulin secretion from HI β-cells. The most common mutations in HI are highlighted in red (GCK, GDH, K_{ATP} channel- SUR1 & Kir6.2).

Methods

- *In vitro studies*: *Sur1*$^{-/-}$ and *Sur1*$^{+/+}$ 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 (AAM) for 1.5 hours. Supernatant was collected to measure insulin by Homogeneous Time Resolved Fluorescence (HTRF). For perifusions, islets were treated with CRN02481 (500 nM) or vehicle and then stimulated with a glucose ramp (0 to 25 mM) or AAM ramp (0 to 12 mM) followed by KCl (30 mM). Fractions were collected at a rate of 1 mL/min and were assessed for secreted insulin by HTRF. For intracellular Ca^{2+} measurement, 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 AAM. Intracellular Ca^{2+} 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 perifusion system and treated with 3, 16.7 mM glucose, and 16.7 mM glucose + 100 µM tolbutamide +/- increasing concentrations of CRN02481. Insulin was quantified by ELISA (Mercodia, Uppsala. Sweden).
- *In vivo studies*: *Sur1*$^{-/-}$ and *Sur1*$^{+/+}$ mice received CRN02481 (30 mg/kg/day) or PBS by gavage (n=7/group) then fasting glucose, glucose tolerance, and plasma insulin were assessed 1 hour post dose. Glucose tolerance tests (GTT) were completed after an overnight fast (16 hrs) and with 2 g/kg dextrose by intraperitoneal injection.

Results

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

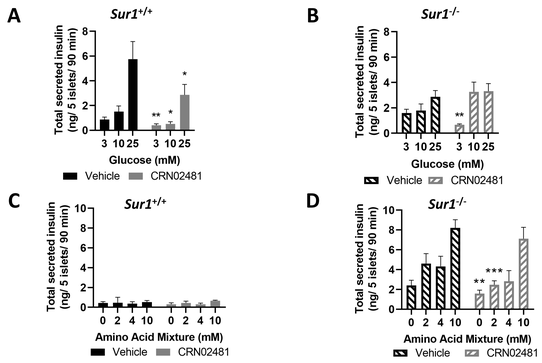

Figure 2: Total secreted insulin after batch incubation of **(A)** *Sur1*$^{+/+}$ or **(B)** *Sur1*$^{-/-}$ isolated pancreatic islets stimulated with denoted glucose concentrations and treated with vehicle control or CRN02481 (100 nM). Secreted insulin from batch incubation of **(C)** *Sur1*$^{+/+}$ or **(D)** *Sur1*$^{-/-}$ isolated pancreatic islets stimulated with denoted AAM concentrations and treated with vehicle control or CRN02481 (100 nM). p-values calculated with ANOVA test (Comparison to Vehicle control: *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001). n=4-5

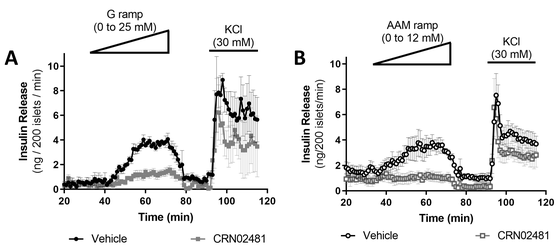

Figure 3: Perifusion of primary isolated islets to assess kinetic insulin release in **(A)** *Sur1*$^{+/+}$ islets stimulated with a glucose ramp (0 – 25 mM) and KCl (30 mM) or **(B)** *Sur1*$^{-/-}$ islets stimulated with (AAM ramp (0 – 12 mM) and KCl (30 mM). n=3

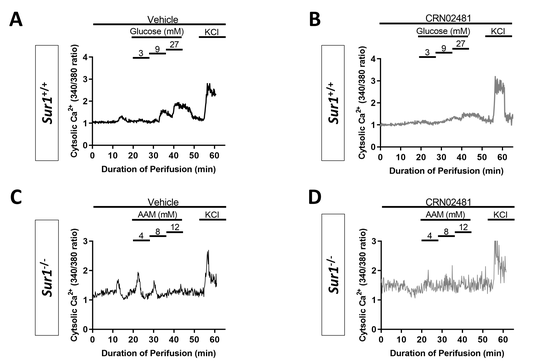

Figure 4: Intracellular Ca^{2+} measurement of primary isolated pancreatic islets in *Sur1*$^{+/+}$ islets treated with glucose steps (9 mM, 15 mM, 27 mM), KCl (30 mM), and **(A)** vehicle or **(B)** 500 nM CRN02481. *Sur1*$^{-/-}$ islets treated with amino acid (AA) steps (4 mM, 8 mM, and 12 mM), KCl (30 mM) and **(C)** vehicle or **(D)** 500 nM CRN02481. n=3-6

CRN02481 increases plasma glucose and decreases insulin secretion in both *Sur1*$^{+/+}$ and *Sur1*$^{-/-}$ mice

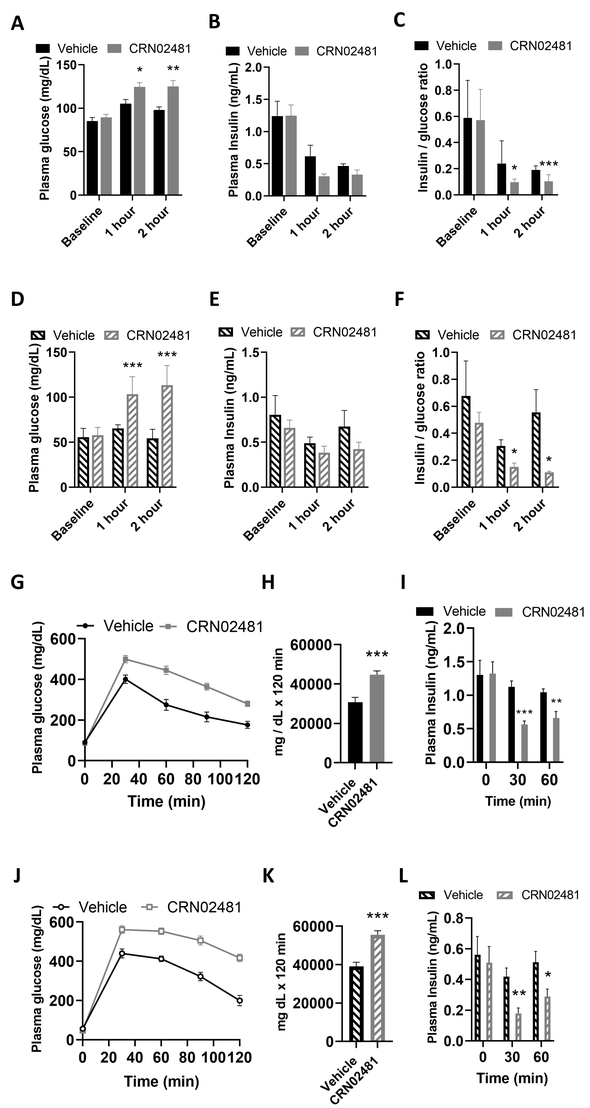

Figure 5: Fasting evaluation of *Sur1*$^{+/+}$ treated by gavage with PBS control or CRN02481 after overnight fast assessing **(A)** plasma glucose, **(B)** plasma insulin, and **(C)** insulin / glucose ratio at times denoted. Fasting evaluation of *Sur1*$^{-/-}$ treated by gavage with PBS control or CRN02481 after overnight fast assessing **(D)** plasma glucose, **(E)** plasma insulin, and **(F)** insulin / glucose ratio at times denoted. Glucose tolerance test (GTT) in **(G)** *Sur1*$^{+/+}$ mice and **(H)** area under the curve (AUC) calculation. **(I)** Plasma insulin levels for *Sur1*$^{+/+}$ mice at denoted times during GTT. GTT in **(J)** *Sur1*$^{-/-}$ mice treated with PBS control or CRN02481 by gavage and **(K)** AUC calculation. **(L)** Plasma insulin levels for *Sur1*$^{-/-}$ mice at denoted times during GTT. p-values calculated with ANOVA test (Comparison to vehicle control: *, p < 0.05; **, p < 0.01; ***, p < 0.001) n=7.

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

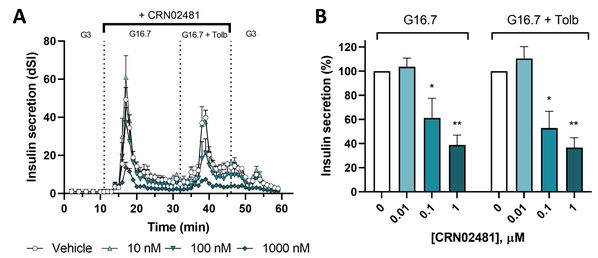

Figure 6: Human islets were treated with 3 mM glucose (G3), 16.7 mM glucose (G16.7), and 16.7 mM glucose + 100 µM tolbutamide (G16.7 + Tolb) +/- increasing concentrations of CRN02481. The dynamic stimulation index (dSI) was calculated as stimulated insulin levels/basal insulin levels. **(A)** Mean dSI ± range (n=2 technical replicates) from one representative donor. **(B)** Insulin secretion 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 secretion in the vehicle group is 100%. Graph shows mean percent secretion ± SEM (n=4 independent donors). P-value calculated with ANOVA test (compared to vehicle: *, p < 0.05; **, p < 0.01).

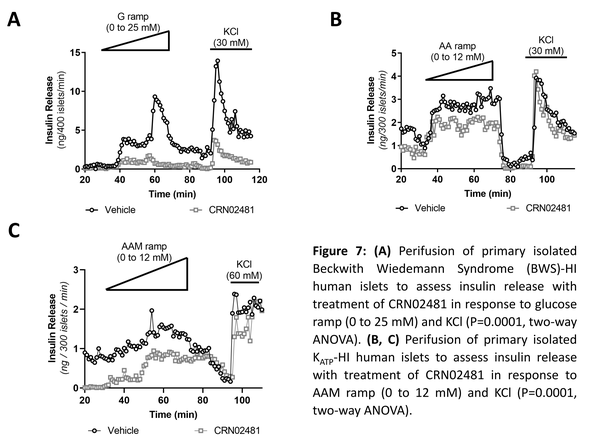

Figure 7: (A) Perifusion of primary isolated Beckwith Wiedemann Syndrome (BWS)-HI human islets to assess insulin release with treatment of CRN02481 in response to glucose ramp (0 to 25 mM) and KCl (P=0.0001, two-way ANOVA). **(B, C)** Perifusion of primary isolated K_{ATP}-HI human islets to assess insulin release with treatment of CRN02481 in response to AAM ramp (0 to 12 mM) and KCl (P=0.0001, two-way ANOVA).

Conclusions

- The somatostatin receptor agonist CRN02481 (SST5 selective) effectively decreases insulin secretion in the *Sur1*$^{-/-}$ K_{ATP}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.

Acknowledgements:

This work is supported by a research grant from Crinetics Pharmaceuticals to DDL

Children's Hospital of Philadelphia℠
Congenital Hyperinsulinism Center