groups over all phases of secretion (n=4 donors). Interestingly, we did observe diminished somatostatin secretion at elevated glucose upon GJD2 knockdown (n=2 donors).
Conclusion: These results suggest an important role for gap junctions in coordinating [Ca^{2+}] dynamics in human islets, which may influence second-phase insulin secretion pulsatility. However, there may also be a role for gap junctions independent of β-cell function. Thus, further study of human gap junctions would provide deeper understanding of necessary structural elements that facilitate the proper function of the human islet, which will help in studying and potentially treating diabetes.
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Semaphorin signalling alters the function of islet alpha and beta cells

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Background and aims: The Centre for Artificial Intelligence in Precision Medicine (CAIPM) aims to develop small molecules for use in precision medicine. Type 2 Diabetes (T2D) gene targets were identified using AI, employing a neural network (NN) approach, and the role of these targets in islet function is then characterised to identify therapeutic potential. Our NN identified *SEMA3E*, encoding the signalling peptide semaphorin3E (SEMA3E), and *NRP1*, encoding the semaphorin-associated co-receptor neuropilin-1 (NRP1). SEMA3E acts, via the receptor plexin D1 (PLXND1), to direct cytoskeletal remodelling in diverse physiological processes, including angiogenesis and axon growth, signalling of which can be modulated by NRP1. Previous studies have shown that SEMA3E levels are increased in diabetic conditions. However, the role of the SEMA3E/PLXND1/NRP1 signalling pathway in pancreatic islet function remains to be established.
Materials and methods: T2D-related genes were identified by NN interrogation of UK BioBank sequencing data. The role of these genes was assessed by confocal imaging of intracellular Ca^{2+} and hormone secretion experiments in mouse and human islets.
Results: The NN presented a strong association between T2D and the genes *SEMA3E* (p=3.17 x10^{-14}) and *NRP1* (p=3.03 x10^{-11}). Physiological interrogation revealed that the response to changes in glucose when exposed to SEMA3E (20 nM) is altered. The frequency of beta-cell Ca^{2+} oscillations in response to 10 mM glucose was reduced by >40% (p=0.05) and this correlated with a significant reduction in stimulated insulin secretion in both mouse (25% reduction; p<0.05) and human (47% reduction; p<0.005) islets. By comparison, inhibition of NRP1 lead to a 71% reduction in glucagon secretion (p<0.05) from mouse islets, suggesting differential roles of NRP1 in alpha- and beta-cells.
Conclusion: Our data support the importance of SEMA3E/PLXND1/NRP1 in islet function. Antagonism of the SEMA3E signalling pathway, to potentiate insulin secretion, may be a promising avenue for small molecule development to rescue deficiency in T2D. Further dissection of the associated pathway(s) will clarify therapeutic potential for patients living with T2D, carrying semaphorin signalling-associated risk alleles.
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Characterisation of the secretion of glucagon from the pancreas of GCGRKO alpha cells in mice

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Background and aims: The pancreatic hormone glucagon is of utmost importance for our understanding of diabetes and other metabolic disorders, however, the mechanisms involved in regulating glucagon secretion are still uncertain. Intrinsic nutrient sensing mechanisms and paracrine factors from neighboring cells seem essential for an appropriate secretion of glucagon from the pancreatic α-cells. Thus, this study aimed to investigate the role of inhibitory paracrine factors in glucose-regulated glucagon secretion
Materials and methods: The secretion of pancreatic hormones from an α-cell hyperplastic model (the glucagon receptor knockout mouse - GCGRKO) was investigated using the isolated perfused mouse pancreas. Both wild-type (WT) and GCGRKO pancreases were perfused with varying glucose concentrations (3.5 and 10mM), as well as exogenous somatostatin receptor (SSTR) 2, 3, and 5 antagonists.
Results: Glucagon secretion was reduced equally (~50%) in both WT ((139.1 ± 55fmol/min vs. 254.4 ± 69fmol/min, P=0.021) and GCGRKO mice (3669 ± 889fmol/min vs. 6309 ± 1365fmol/min, P=0.0045) after increasing glucose from 3.5 to 10mM, while somatostatin secretion was stimulated. Blockage of SSTR-2, 3, and 5 by specific antagonists increased the secretion of glucagon relatively more in WT (685.5 ± 125fmol/L vs. 294.3 ± 82fmol/L, P=0.007) than in GCGRKO pancreases (10710 ± 1378fmol/L vs. 6963 ± 927fmol/L, P=0.067), however, it did not prevent glucose-induced suppression of glucagon secretion in either of the models (WT: 195.9 ± 44.4 fmol/min vs. 267.8 ± 34.5fmol/min, P=0.04/ GCGRKO: 5527 ± 1199fmol/min vs. 7347 ± 1375fmol/min, P=0.05).
Conclusion: This study provides evidence that the altered islet composition in GCGRKO mice causes a slight attenuation of the tonic inhibitory effects of SST on glucagon secretion, while the paracrine regulatory axis driven by SST remains intact. Furthermore, we show that SST may not be essential for glucose to inhibit glucagon secretion in mice.
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Ghrelin and LEAP2 affect insulin and glucagon secretion in mouse but not in human islets, and their effects in mouse islets are mediated by somatostatin

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Background and aims: Ghrelin, which activates the growth hormone secretagogue receptor 1a (GHS-R1a), stimulates appetite ("hunger hormone") and impairs glucose homeostasis. Conversely, liver-expressed antimicrobial peptide-2 (LEAP2), which acts as an endogenous antagonist and inverse agonist of GHS-R1a, counteracts the effects of ghrelin and improves glucose homeostasis. The mechanisms of control of insulin (INS) and glucagon (GCG) secretion by ghrelin and LEAP2 are debated or poorly understood. Since δ-cells strongly express GHS-R1a,

ghrelin and LEAP2 are expected to affect somatostatin (SST) secretion. This study aims to explore the effects of ghrelin and LEAP2 on pancreatic hormone secretion in mice and humans, to test the efficacy of two other GHSR inverse agonists, $LEAP2_{(38-47)}$ (a fragment of LEAP2 upregulated in Roux-en-Y gastric bypass) and PF-5190457 (PF), and to determine whether their effects are mediated directly or indirectly via SST.

Materials and methods: The experiments were performed on islets from control ($Sst^{+/+}$) and SST knockout ($Sst^{-/-}$) mice (both strains on C57BL/6N background), and human islets (IIDP). Islets were incubated for 1h in a medium containing a 6 mM amino acid mixture (2 mM alanine, 2 mM arginine and 2 mM glutamine) and various glucose concentrations (1 mM (G1), 7 mM (G7), or 15 mM (G15)), ghrelin (acyl-ghrelin) or LEAP2 (both tested at 0.1, 1, 10 or 100 nM), LEAP2(38-47) or PF (both tested at 0.1, 1, 10, 100 nM or 1 µM).

Results: As expected, glucose stimulated INS and SST secretion, and inhibited GCG release from $Sst^{+/+}$ mouse and human islets. $Sst^{-/-}$ islets showed similar INS secretion but higher GCG secretion compared to $Sst^{+/+}$ islets. In $Sst^{+/+}$ islets, ghrelin dose-dependently inhibited GCG release in G1 (by 35% to 50% with 0.1 nM and 100 nM, respectively; P<0.01), whereas only 100 nM ghrelin inhibited INS secretion in G15 (by 20%; P<0.05). LEAP2, at 100 nM only, increased GCG secretion in G1 (by 45%; P<0.01), and increased INS secretion in G15 (by 19%; P<0.05). [Ghrelin] ≥1 nM and [LEAP2] ≥10 nM stimulated and inhibited (P<0.01), respectively, SST secretion from $Sst^{+/+}$ islets. Neither ghrelin nor LEAP2 affected INS or GCG secretion from $Sst^{-/-}$ islets demonstrating that the effects they exerted in $Sst^{+/+}$ islets were mediated by SST. $LEAP2_{(38-47)}$ and PF failed to stimulate GCG and INS release from mouse $Sst^{+/+}$ islets. In human islets, neither ghrelin nor LEAP2 affected pancreatic hormone secretion, except for a 60% stimulation of SST release by 100 nM ghrelin in G15 (P<0.01).

Conclusion: In mouse islets, ghrelin exhibits dose- and glucose-dependent inhibitory effects on GCG and INS secretion, while LEAP2 stimulates GCG secretion in G1 and INS secretion in G15. Notably, ghrelin stimulates SST secretion, whereas LEAP2 inhibits it. The effects of ghrelin and LEAP2 on INS and GCG secretion are indirect, mediated by SST, which is compatible with the high expression of GHS-R1a in δ-cells. $LEAP2_{(38-47)}$ and PF do not reproduce the inverse agonistic effect of LEAP2. In human islets, neither ghrelin nor LEAP2 affect INS or GCG secretion. This suggests that modulation of pancreatic hormone secretion by both peptides contributes less to glucose homeostasis in humans than in mice.

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Urocortin-3 regulates glucagon secretion through an inhibitory beta to δ to alpha cell axis in mice, but not in rats

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Background and aims: The secretion of glucagon from the pancreatic α-cells is regulated by nutrients (glucose, lipids, and amino acids) and by paracrine factors secreted from neighboring cells (β- and δ-cells). We recently identified that urocortin-3 (UCN-3) from the β-cells inhibits its glucagon secretion and stimulates somatostatin secretion. Therefore, UCN-3 might be the paracrine β-cell factor that inhibits the secretion of glucagon, a role that has previously been attributed to insulin but has been difficult to reproduce.

Materials and methods: The secretion of pancreatic hormones (glucagon and somatostatin) was investigated using the isolated mouse and rat pancreas perfusion, in response to exogenous UCN-3, and/or specific SST-receptor (SSTR) and Corticotropin Releasing Hormone Receptor 2 (CRHR2) antagonists. Specific anti-CRHR2 and anti-SST antibodies were used to identify CRHR2 localization in rat, mouse, and human pancreatic islets.

Results: Urocortin-3 potently stimulates somatostatin (177.7±29.3fmol/min vs 52.8±9.1fmol/min, P>0.01) and inhibits glucagon secretion (122.4±34.28 fmol/min vs 222.4±63.27 fmol/min, P=0.050) in mice, but not in rats (SST: 269.4±78.8 fmol/min vs 223.1±40.48 fmol/min, P=0.73; Glucagon: 480.6±56.28 fmol/min vs 820.1±191.5, P=0.24). The inhibitory actions of UCN-3 on glucagon secretion can be prevented by antagonizing SST receptors (89.83±7.81 fmol/min vs 99.46±8.36fmol/min, P>0.99). The UCN-3 stimulatory actions on SST secretion could be prevented by co-infusing CRHR2a (62.63±7.3 fmol vs 72.97±20.27 fmol/min, P=0.83). Immunostaining of CRHR2 supports the expression of the receptor in mouse and human δ-cells, but not in rat δ-cells, in line with our perfusion data.

Conclusion: This study supports that in mice, but not in rats, activation of β-cells initiates a β- to δ- to α-cell axis driven by UCN-3. Activation of CRHR2 in δ-cells stimulates somatostatin secretion which in turn inhibits glucagon secretion through SST receptors. Whether a similar pathway is active in the human pancreatic islet is currently being tested, but histology data supports this hypothesis. Thus, we propose that UCN-3 is the factor that drives β-cell regulated glucagon secretion.

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Regulation of alpha cell glucagon exocytosis by TMEM55A

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Background and aims: Diabetes is associated with the dysfunction of glucagon-producing pancreatic islet α cells, although molecular mechanisms underlying the regulation of glucagon secretion remain unclear. Using the patch-seq technique we previously found that expression of *TMEM55A*, a lipid phosphatase which converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol-5-phosphate (PI5P), is positively correlated with α cell glucagon exocytosis. We aim to explore whether TMEM55A regulates exocytosis in α cells, and the functional mechanism by which this occurs.

Materials and methods: Human islets were isolated in the Alberta Diabetes Institute IsletCore. Electrical recordings were performed on dispersed human or mouse islet cells following transfection with scrambled siRNA control or si-*TMEM55A* duplexes. Glucagon secretion was measured using an islet perifusion system with intact human or mouse islets. Confocal microscopy was used to quantify F-actin intensity using primary α cells and αTC1-9 cells. We made recombinant wide-type (WT) and loss of function (C107S) TMEM55A through site-directed mutagenesis on the phosphatase domain, CX5R. Malachite green phosphatase assay was used to test the phosphatase activity of TMEM55A *in vitro*.

Results: We found that knockdown of TMEM55A, quantified by qRT-PCR, western blotting, and immunostaining, decreased α cell exocytosis compared with scrambled siRNA (n = 28, 29 cells, from 5 donors). This was consistent with the direction of correlation from our previous patch-seq studies and occurred without affecting calcium currents. We also found that direct intracellular dialysis of PIP2, the substrate of TMEM55A, decreases α cell exocytosis (n = 23, 18 cells, from 4 donors) and that PI5P, the product of TMEM55A, highly increases α cell exocytosis (n = 13, 15 cells, from 3 donors). Furthermore, intracellular dialysis of PI5P rescued the effect of TMEM55A knockdown (n = 14, 16 cells, from 3 donors). Glucagon secretion was increased from