Physiology and Pathophysiology of Hormone Secretion from Islets of Langerhans

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How many surrealists does it take to change a lightbulb?

Fish.

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ABSTRACT

Diabetes is a metabolic disorder stemming from the improper regulation of blood glucose levels by hormones secreted by pancreatic islets of Langerhans. **Paper I** describes the mechanism by which glucose can regulate glucagon secretion, independent of membrane potential, from pancreatic α-cells, via modulation of endoplasmic reticulum Ca^{2+} handling. **Paper II** shows that the GLP-1 metabolite $GLP\text{-}1^{(9\text{-}36)}$ acts directly on α-cells to inhibit glucagon secretion, via activation of G_i-coupled glucagon receptors. **Paper III**, shows that the islet autoantigen tetraspanin-7 regulates β-cell transmembrane Ca^{2+} influx and the Ca^{2+} sensitivity of exocytosis. **Paper IV** demonstrates that α-cells and their neighbouring δ-cells exhibit a novel paracrine signalling loop. δ-cells react to the activity of adjacent α-cells, secreting somatostatin, to prevent glucagon hypersecretion. This mechanism becomes sensitised following exposure to hypoglycaemia, leading to excessive intra-islet somatostatin secretion, impairing glucose counter-regulation. Together, these papers reveal novel mechanisms governing the regulation of islet hormone secretion, which may lead to improvements in therapies for diabetes.

Keywords: Islet; diabetes; glucagon; insulin; somatostatin; incretin; pancreas; hypoglycaemia; tetraspanin-7; GLP-1; endoplasmic reticulum.

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SAMMANFATTNING PÅ SVENSKA

Blodsockerreglering upprätthålls genom verkan av hormoner som utsöndras av celler i de Langerhanska öarna. Öarna består mestadels av insulinfrisättande β-celler, glukagonfrisättande a-celler och somatostatinfrisättande δ-celler; insulin sänker blodsockret, glukagon höjer blodsockret och somatostatin hämmar utsöndringen av insulin och glukagon i förebyggande syfte. Sockersjuka (Diabetes) är en metabolisk störning som kännetecknas av kroniskt högt blodsocker (hyperglykemi), där den normala blodsockerregleringen är satt ur spel. Den kategoriseras generellt som typ 1- eller typ 2. Typ 1-diabetes uppstår när ens egna immunceller förstorar de insulinproducerande β-cellerna, och de som lever med sjukdomen måste behandlas med insulin för att sänka sina blodsockernivåer. Vid typ 2-diabetes bidrar felaktig druvsockerreglering av insulin samt insulinresistens till fel regleringen av blodsocker. Det blir alltmer uppenbart att dysreglering av både glukagon och somatostatin också bidrar till utvecklingen av sjukdomen.

Det finns fortfarande många okända mekanismer som avgör vilka öceller som regleras och blir dysregulerade under diabetes. En djupare förståelse för cellöarnas funktion och

dysfunktion kommer förhoppningsvis att leda till förbättringar av diabetesbehandlingen.

Mekanismen för vilken glukagonutsöndring regleras av blodsockernivåer är fortfarande oklar, men det är väletablerat att en ökning av α-cellens cytoplasmatiska Ca^{2+}-koncentration utlöser glukagonexocytos. I den första studien visade vi att det endoplasmatiska retikulumet kan modulera glukagonutsöndring som svar på förändringar i den extracellulära glukoskoncentrationen, via modulering av cytoplasmatisk Ca^{2+}, på ett membranpotentialoberoende sätt.

Hormonet GLP-1 sänker blodsockernivån genom att höja av insulinfrisättning och hämning av glukagonfrisättning efter en måltid. Det mesta av GLP-1 når dock ön i form av metaboliten $GLP\text{-}1^{(9\text{-}36)}$ på grund av nedbrytning av dipeptidyl peptidase 4. I den andra studien visade vi att $GLP\text{-}1^{(9\text{-}36)}$ kan minska glukagonutsöndringen genom att direkt interagera med G_i-kopplade α-cell glukagonreceptorer, utan att påverka insulin- eller somatostatinsekretionen.

Tetraspanin-7 är en viktig β-cellsautoantigen vid typ 1-diabetes, men funktionen av tetraspanin-7 i β-celler är inte helt klarlagd. I den tredje studien visade vi att detta membranbundna protein spelar en roll för att reglera Ca^{2+}-

känsligheten för exocytos och transmembran Ca^{2+}-inflöde av β-celler.

Återkommande hypoglykemi är en farlig sekundär komplikation av typ 1-diabetes och beror på otillräcklig glukagonutsöndring för att återställa den normala blodsockernivån. I den fjärde studien visade vi att α-celler och deras angränsande δ-celler uppvisar en parakrin signalslinga. δ-celler svarar på aktiviteten hos intilliggande a-celler och utsöndrar somatostatin för att förhindra glukagonhyperutsöndring. Denna mekanism blir sensibiliserad efter hypoglykemisk exponering, vilket leder till överdriven intra-ö somatostatinfrisättning in i ön , vilket förvärrar motregleringen av druvsocker i blodet under efterföljande insulinkänning (hypoglikemi).

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Acreman, S., Ma, J., Denwood, G., Gao, R., Rorsman, P., Zhang, Q. The endoplasmic reticulum plays a key role in α-cell intracellular Ca^{2+} dynamics and glucose-regulated glucagon secretion. *Manuscript under revision, iScience.*

II. Gandasi, N.R., Gao, R., Kothegala, K., Pearce, A., Santos, C., Acreman, S., Basco, D., Benrick, A., Chibalina, M.V., Clark, A., Guida, C., Harris, M., Johnson, P.R.V., Knudsen, J.G., Ma, J., Miranda, C., Shigeto, M., Tarasov, A.I., Yeung, H.Y., Thorens, B., Wernstedt Asterholm, I., Zhang, Q., Ramracheya, R., Ladds, G., Rorsman, P. GLP-1 metabolite $GLP\text{-}1^{(9\text{-}36)}$ is a systemic inhibitor of mouse and human pancreatic islet glucagon secretion. *Manuscript under revision, Diabetologia.*

III. McLaughlin, K., Acreman, S., Nawaz, S., Cutteridge, J., Clark, A., Knudsen, J.G., Denwood, G., Spigelman, A.F., Manning Fox, J.E., Singh, S.P., MacDonald, P.E., Hastoy, B., Zhang, Q. Loss of tetraspanin-7 expression reduces pancreatic β-cell exocytosis Ca^{2+} sensitivity but has limited effect on systemic metabolism. *Diabetic Medicine* 2022; 39(12):e14984. doi: 10.1111/dme.14984.

IV. Gao, R., Acreman, S., Ma, J., Miranda, C., Dou, H., Zhao, R., Maghera, J., Ellis, C., Dickerson, M., Tarasov, A., Clark, A., Yang, T., Gilon, P., Macdonald, P.E., Jacobson, D.A., Rorsman, P., Zhang, Q. A mechanism for rapid cross-talk between pancreatic α- and δ-cells and its role in hypoglycaemia-induced glucagon secretory failure. *Manuscript.*

CONTENTS

ABBREVIATIONS

$[ATP]_i$	Intracellular adenosine triphosphate concentration
$[Ca^{2+}]_{ER}$	ER Calcium concentration
$[Ca^{2+}]_i$	Cytoplasmic Calcium concentration
ADP	Adenosine diphosphate
Ai95D	Mouse line carrying $Rosa26^{GCaMP6f}$ gene
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
Ca_v channel	Voltage-sensitive Ca^{2+} channel
CICR	Calcium-induced calcium release
CREB	cAMP response element binding protein
DPP4	Dipeptidyl peptidase IV
DREADD	Designer G_q-coupled receptor exclusively activated by designer drugs
EPAC2	Exchange protein directly activated by cAMP 2
ER	Endoplasmic reticulum
FBS	Foetal bovine serum
Floxed	LoxP-flanked
G6P	Glucose-6-phosphate
GCaMP6f	GCaMP-fast variant
Gcg-GCaMP6f	Mouse line expressing GCaMP6f specifically in glucagon-expressing cells
Gcg-$hM3Dq^+$	Mouse line expressing the hM3Dq DREADD receptor specifically in glucagon-expressing cells
GcgR	Glucagon receptor
GIP	Glucose-dependant inhibitory peptide
GIRK	G protein-coupled inwardly rectifying potassium channels
GK	Glucokinase
GLP-1	Glucagon-like peptide 1
GLP-1R	GLP-1 receptor

$Glu^{Cre/ERT2}$	Mouse line expressing glucagon expressing cell-specific tamoxifen-inducible Cre-recombinase
GLUT	Glucose transporter
H134R	Mouse line expressing channelrhodopsin-2
HLA	Human leukocyte anitgen
HLA	Human leukocyte antigen
HVA	High voltage activated
iGluRs	Ionotrpic glutamate receptors
IP	Intraperitoneal
IP_3	Inositol triphosphate
ITT	Insulin tolerance test
J60	JHU37160 compound
K_{ATP} Channel	ATP-sensitive K^+ channel
K_m	Michaelis constant
KD	Knockdown
KO	Knockout
KRB	Krebs ringer buffer
LVA	Low voltage activated
Na_v Channel	Voltage-sensitive Na^+ channel
PI	Propidium Iodide
PKA	Protein kinase A
PLC	Phospholipase C
RIM	Regulating synaptic membrane exocytosis protein
RNA	Ribonucelic acid
RyR	Ryanodine receptor
SERCA	Sarco/endoplasmic reticulum Ca^{2+} ATPase
SGLT	Sodium/glucose co-transporter
SNAP25	Synaptosomal-Associated Protein, 25kDa
SNARE	Soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor
SOC	Store-operated channel
SRB	Sulforhodamine B
SST^{Cre}	Mouse line expressing Cre-recombinase specifically in somatostatin-expressing cells

SST-GCaMP6f	Mouse line expressing GCaMP6f specifically in somatostatin-expressing cells
SSTR	Somatostatin receptor
STIMs	stromal interaction molecules
Syn1	syntaxin-1
SYT7	Synaptotagmin-7
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TASK-1	2-pore domain K^+ channel-related acid-sensitive K^+ channel-1
Tspan7	Tetraspanin-7
$Tspan7^{y/-}$	Mouse line with a whole-body KO of the *Tspan7* gene
TTX	Tetrodotoxin
Ucn3	Urocortin3
VAMP2	Vesicle associated membrane protein-2
WT	Wild type

1 INTRODUCTION

1.1 Islets of Langerhans

Whole body blood glucose is regulated by the islets of Langerhans [1]. Located in the pancreas, these micro-organs make up around 1% of the pancreas and are primarily composed of β-, α-, and δ-cells, which secrete insulin, glucagon, and somatostatin, respectively [2]–[4]. Insulin is the only endogenously produced glucose lowering hormone [5]; released when blood sugar levels are high, it stimulates glucose clearance from the plasma by increasing uptake in adipose, hepatic, and skeletal muscular tissue [1]. In opposition to this, glucagon raises blood sugar when levels are low, such as during fasting, by mechanisms that include stimulation of gluconeogenesis and glycogenolysis in the liver [1]. Somatostatin acts to inhibit release of both glucagon and insulin, preventing over-secretion of these two hormones [6], [7]. Properly regulated, these hormones maintain systemic euglycaemia, within a narrow physiological range; however, disturbances to the secretory function of islet cells leads to the development of diabetes, a metabolic disorder characterised by chronically elevated blood glucose levels [8]–[10].

1.2 Islet architecture depends on species

β-cells are the most abundant of the islet cell types, making up approximately 70% of total pancreatic endocrine cells in rodents and 60% in humans. α- and δ-cells, by comparison, make up 20% and 5%, respectively, in rodents or 30% and 10%, respectively, in humans [2]–[4]. Islet architecture is dependent on species; in rodents, β-cells form the core of the islet and are surrounded by clusters of α- and δ-cells on the periphery [11] whereas, in human, non-β-cells tend to be more scattered throughout the islet, though α- and δ-cells still cluster together [2]–[4]. The structure of islets is important for proper functional responses to stimuli; the interactions between neighbouring cells, both heterogenous and homogenous, facilitate paracrine signalling and hormone secretion as well as the synchronisation of β-cells, in mouse islets [12].

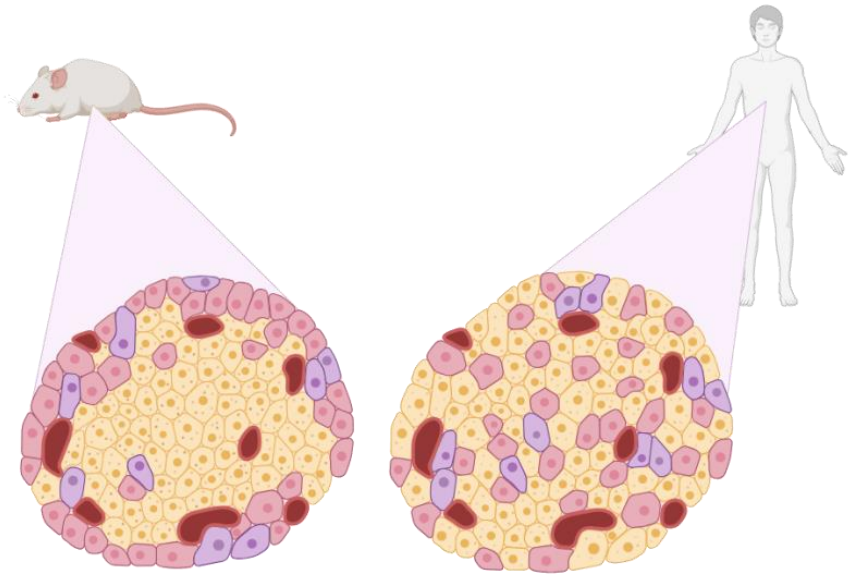


Figure 1: Representation of α-, β-, and δ-cell arrangement in rodent (left) and human (right) islets. A-cells are shown in red, β-cells are shown in yellow, and δ-cells are shown in purple. Created with BioRender.com

1.3 Islet cells exhibit electrical activity in response to changes in plasma glucose

Islet cells share many features with neurons, in particular, membrane electrical excitability [13]. The electrical activity of each islet cell type is modulated in respond to glucose and other metabolic changes, with each exhibiting a unique

electrophysiological signature according to their specific roles [14], [15]. The activity of these cells can also be altered by a number of different signals both external and internal to the islet [16]–[21].

1.4 The β-cell

As the most abundant of the pancreatic endocrine cells, the mechanisms governing the β-cell response to glucose, and consequent modulation of insulin secretion, are well characterised.

1.4.1 Intrinsic regulation of β-cell insulin secretion

1.4.1.1 From plasma glucose to ATP

Glucose sensing in β-cells begins with uptake of glucose into the cytoplasm by glucose transporter (GLUT) proteins; these catalyse the passive transport, or facilitated diffusion, of glucose across plasma membranes. In rodent β-cells, the primary subtype expressed is GLUT2, which has a relatively low affinity for glucose, (K_m ~ 17 mmol/l) and a high transport capacity [22], [23]. The presence of this transporter is important for the β-cell response to glucose, with

homozygous knockout (KO) of the gene in mice (*Slc2a2*$^{-/-}$) results in a flat response to glucose over 6 mM and diminished glucose clearance [24], [25]. Humans, by comparison, predominantly express the high affinity GLUTs 1 and 3, allowing for higher glucose uptake at low glucose levels and a consequent lower threshold for insulin secretion [26]–[28]. Interestingly, despite the low expression levels in human β-cells, mutations in the *Slc2a2* gene (GLUT2) can lead to neonatal diabetes in humans [29].

Once inside the β-cell, glucose undergoes glycolysis, the first step of which is conversion to glucose-6-phosphate (G6P), and subsequently pyruvate, which can then enter the Krebs cycle, undergoing oxidative metabolism, to generate ATP. In β-cells, conversion is catalysed by the enzyme hexokinase 4, also known as glucokinase (GK), which has a low affinity for glucose, does not saturate at physiological glucose concentrations (V_{max} > 20 mM), and is not inhibited by 6GP [30], [31]. The conversion of glucose to G6P is the rate limiting step for entry into the Krebs cycle and the catalysis of the conversion by GK allows changes in ATP production to continue above euglycaemic glucose levels. Consequently, as glucose increases, the cellular ATP:ADP ratio increases; it is half-maximal at 3 mM glucose and saturating at ~20 mM [32], producing a gradual change in insulin secretion. Mice

with a heterozygous KO of the glucokinase gene, specifically in β-cells, develop diabetes within 10 weeks, while homozygous KOs exhibit severe hyperglycaemia that quickly becomes fatal, within 7 days of birth [33]. Rapid sensing of the extracellular glucose concentration, and consequent modulation of metabolism, is key to allowing the β-cell to respond to glycaemic changes.

1.4.1.2 Intracellular ATP controls membrane excitability via modulation of K_{ATP} channel conductance

As the rate of glucose metabolism increases, β-cell cytoplasmic ATP:ADP also increases, closing ATP-sensitive K^+-channels (K_{ATP} channels), plasma membrane-bound channels that selectively facilitate the efflux of K^+ ions down the concentration gradient [34]. The β-cell K_{ATP} channel is composed of 8 subunits, 4 Kir6.2, which bind ATP and form the pore of the channel, and 4 SUR1, which are sensitive to modulators such as sulphonylureas and diazoxide [35]–[39]. The activity of these channels governs the β-cell membrane potential, determining electrical activity and insulin secretion.

In mouse β-cells, at low glucose concentrations (<3mM), when the ATP:ADP ratio is low, K_{ATP} channels are open and membrane conductance is dominated by these channels [40]. The membrane potential is, therefore, close to the K^+ reversal

potential (-70 mV) and no electrical activity is generated [41]. As the extracellular glucose concentration increases, K_{ATP} channels close, depolarising the membrane to the point at which action potentials can be generated [42], [43].

1.4.1.3 Membrane depolarisation activates Ca_v and Na_v channels

Action potential generation requires more than just closure of K_{ATP} channels, these only account for ~15 mV depolarisation [40]. The action potential upstroke, in mouse β-cells, is predominantly driven by ion flow through voltage-activated Ca^{2+} (Ca_v) channels [41], [44], [45]. Several Ca_v channel subtypes are present in mouse β-cells: high voltage activated (HVA) L-, R-, and P/Q-type [46], [47], with pharmacological blockade or KO of L-type Ca_v channels resulting in potent inhibition of insulin secretion [47], [48].

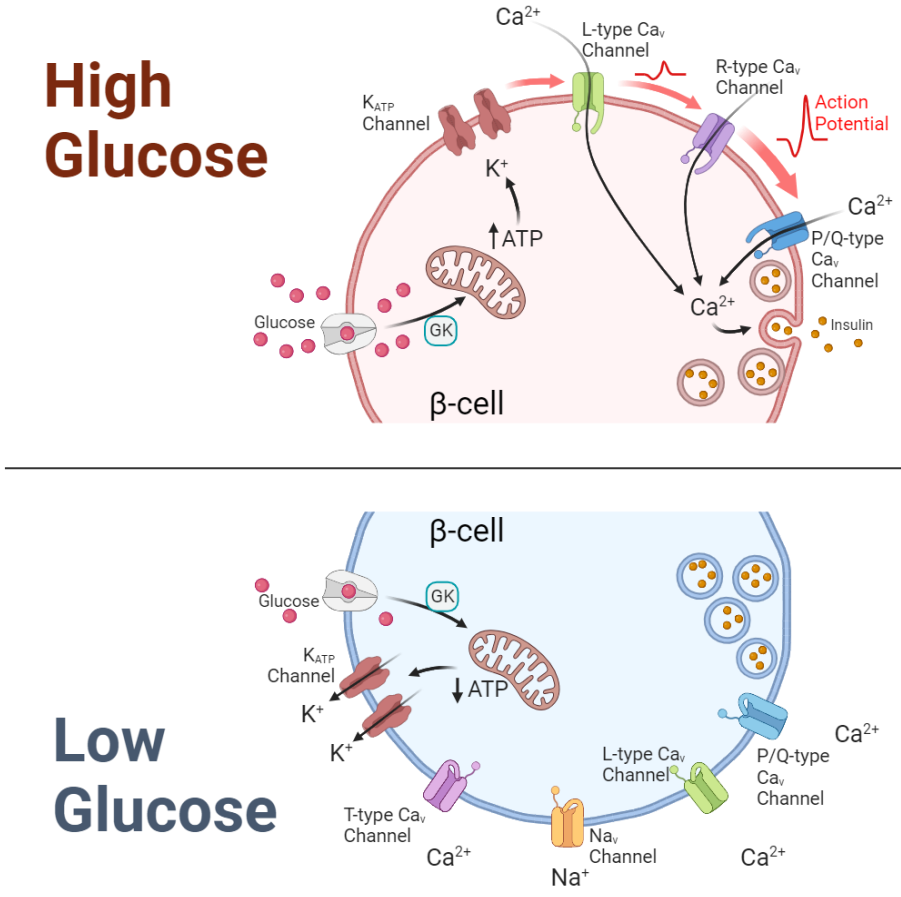


Figure 2: Glucose-regulation of insulin secretion in mouse β-cells. At high extracellular glucose concentrations (<6 mM; upper panel), K_{ATP} channel closure depolarises the membrane opening R-, P/Q-, and L-type Ca_v channels. The resultant Ca^{2+} influx triggers exocytosis of insulin-containing vesicles. Whereas, at low glucose (>6 mM; lower panel), K_{ATP} channels are open, the membrane is polarised and Cav channels remain closed, inhibiting the secretion of insulin. Created with BioRender.com

The contribution of voltage-gate ion channels to the action potential is slightly different in human β-cells. In addition to

L- and P/Q-type, human β-cells express low voltage activated (LVA) T-type Ca_v channels [49]. Unlike rodent β-cells, tetrodotoxin (TTX)-sensitive voltage-gated Na^+ (Na_v) currents are involved in the generation of action potentials in human β-cells [50], [51]. Opening transiently (~2 ms) and inactivating quickly, reactivating when the membrane returns to the resting potential [52], Na_v channels play an important role at glucose concentrations just above the threshold for triggering electrical activity [49], [53]. Consequently, in human β-cells, K_{ATP} channel closure triggers Ca^{2+} flux through T-type Ca_v channels. Depolarisation is further carried by Na_v and L-type Ca_v channels until, at the peak of the action potential (>−20 mV), P/Q-type Ca_v channels open and the resultant Ca^{2+} influx triggers exocytosis of insulin granules [49].

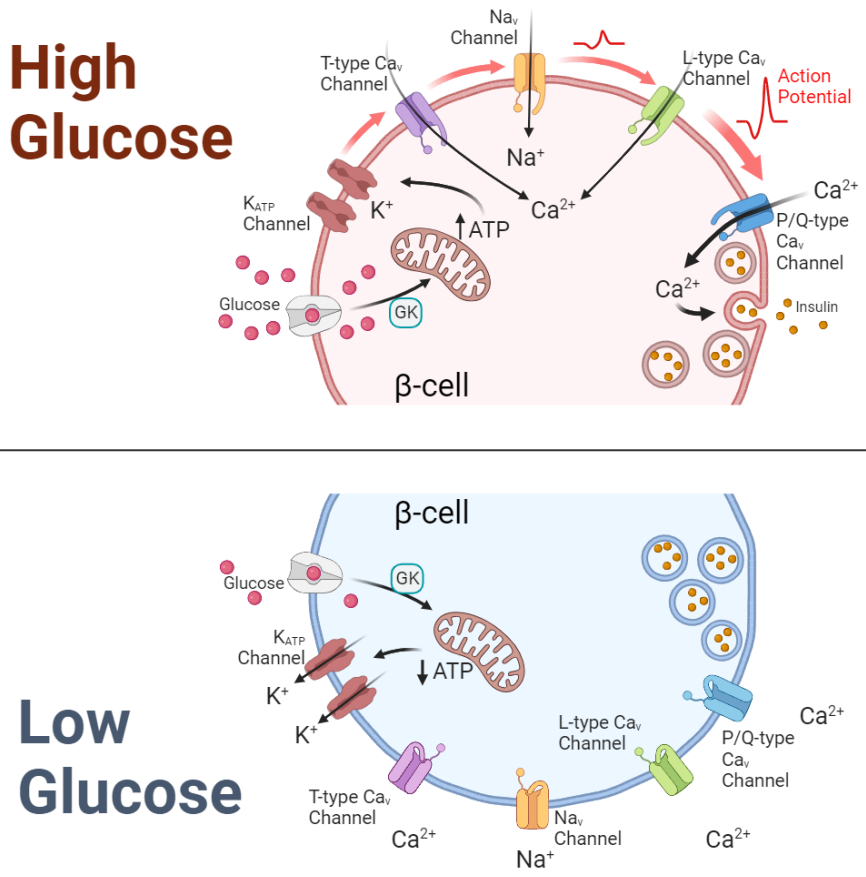


Figure 3: Glucose-regulation of insulin secretion in human β-cells. At high extracellular glucose concentrations (<6 mM; upper panel), K_{ATP} channel closure depolarises the membrane opening T-type Ca_v channels, which subsequently activate Na_v, and L-type Ca_v channels. Finally, at positive membrane potentials, P/Q-type Ca_v channels open, triggering exocytosis of insulin-containing vesicles. Whereas, at low glucose (>6 mM; lower panel), K_{ATP} channels are open, the membrane is polarised and voltage-gated channels remain closed, inhibiting the secretion of insulin. Created with BioRender.com

1.4.1.4 Exocytosis

Exocytosis in β-cells is Ca^{2+}-dependant, similar to that of neurons, and occurs at distinct sites co-localised with Ca_v channels [54]–[56]. The rapid accumulation of Ca^{2+} to trigger exocytosis is facilitated by the association of L-type Ca_v channels and the SNARE complex (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) – exocytotic machinery of insulin granules. This connection can be disrupted with the application of soluble peptides containing the interaction site and results in the abolishment of depolarization-evoked insulin secretion [55], [57]. A 'readily releasable pool' of insulin-containing vesicles is 'docked' at these sites, ready for exocytosis [58]. Docking occurs with conformational change of the membrane-associated SNARE syntaxin-1 (Syn1), from a closed to an open conformation, allowing the association between this and the other SNARE proteins: the membrane-bound SNAP25 (Synaptosomal-Associated Protein, 25kDa) and, the vesicle-bound, VAMP2 (vesicle associated membrane protein-2) [59]. It is interesting to note that both loss and overexpression of Syn1 has a detrimental effect on exocytosis, implying that the stoichiometry of SNARE protein expression plays a role in the modulation of exocytosis [60], [61]. Other data suggest that Syn1 can modulate L-type Ca_v channel activity, altering Ca^{2+} influx

[62]. The final step in exocytosis of insulin granules, the fusion of the vesicular and plasma membranes, is triggered by Ca^{2+} binding to synaptotagmin-7 (SYT7) [63]; KO of this protein in mice results in impaired insulin secretion and glucose intolerance [64], [65].

1.4.2 Other mechanisms regulating β-cell insulin secretion

In addition to the effects of glucose, numerous other signals play a role in the regulation of β-cell activity, acting in consort with intrinsic glucose sensing to modulate insulin secretion.

1.4.2.1 Electrical coupling

In mouse islets, gap junctions connect β-cells, formed by connexin-36, and these allow the propagation of action potentials across islets, facilitating the synchronisation of Ca^{2+} oscillations [66]–[68]. Loss of connexin-36 also deregulates *in vivo* insulin secretory dynamics, inhibiting both the first and second phases of glucose stimulated-insulin secretion [69]. In human islets, β-cells are also connected by gap junctions formed by connexin-36 [70], yet synchronisation across the whole islet does not occur, except in response to certain signals such as GLP-1 [71]. Synchronicity across human islets is limited to smaller β-cell

clusters and is likely due to the differences in the distribution of β- and non β-cells between species [3], [72].

1.4.2.2 Paracrine factors and incretins

Somatostatin is a potent inhibitor of insulin and allows δ-cell control of β-cell activity, exerting inhibition to prevent over-secretion of insulin [7], [73]. Somatostatin activates $G\alpha_i$-coupled somatostatin receptors (SSTR), suppressing insulin secretion by: decreasing adenylyl cyclase activity and cytoplasmic cAMP levels [74]; suppressing electrical activity by hyperpolarising the membrane, through opening of G protein-coupled inwardly rectifying K^+ (GIRK) channels [75] and activation of Na^+/K^+ ATPases [76]; inhibition of Ca^{2+} influx through Ca_v channels [77]; and de-priming of docked vesicles [78]. Pharmacological studies have identified SSTR5 and RNAseq data have implicated SSTR3 as the specific subtypes present on mouse β-cells [79]–[81]. By comparison, in human β-cells SSTR5, 2, and 3 mediate the actions of somatostatin [75].

α-cell glucagon and the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are potent stimulators of insulin secretion [82]–[84]. The incretins play an important role in the augmentation of the post-prandial insulin secretory response [85], increasing insulin section by three or four times that of glucose alone

[82]. GLP-1 is produced by L-cells of the gut in response to food intake, although there is some data to suggest that it may also be produced by α-cells [86]–[88]. Glucagon is able to activate β-cells by binding to glucagon receptors (GcgRs) or GLP-1 receptors (GLP-1Rs) [89], [90] present on β-cells [81], [91]. Loss of GcgR signalling impairs the glucose-stimulated insulin response [92], but can be partially compensated for by the presence of GLP-1Rs [89].

Activation of both GcgRs and GLP-1Rs by glucagon and/or GLP-1 leads to an increase in cAMP production, via the G-protein-mediated stimulation of adenylyl cyclases [93]–[95]. This subsequently activates protein kinase A (PKA) and exchange protein directly activated by cAMP 2 (EPAC2) [96]. In turn, priming of vesicles for exocytosis is enhanced via: binding of EPAC2 to Rim2 [97]–[101]; modulation of electrical excitability, through the closure of K_{ATP} channels [102] and opening of Ca_v channels [103]; and enhanced ryanodine receptor (RyR)-mediated Ca^{2+}-induced Ca^{2+} release (CICR) [104]–[106].

1.5 The α-cell

A great many similarities are shared between α- and β-cells. Most striking are the molecular identities of α-cell K_{ATP} and Ca_v channels, which are identical to that of the β-cell, though there are differences in abundance [80], [81]. Unlike β-cells, α-cells are active at hypoglycaemic glucose concentrations (<3 mM glucose), at the point where glucagon-induced gluconeogenesis is required to restore euglycaemia, and this pattern of activity has been used to distinguish α-cells within the islet [107].

1.5.1 Hypotheses describing the glucose regulation of glucagon secretion

Similarities with β-cells and the presence of α-cell electrical activity have centred hypotheses on the mechanisms of glucose modulation of glucagon secretion around the regulation of electrical activity. Unlike the well-established β-cell stimulation-secretion coupling model, there is no current consensus on precise mechanisms that regulate glucagon secretion. Hypotheses differ as to whether high glucose depolarises α-cells, through K_{ATP} channels [108], [109] and Na^+/glucose cotransporter (SGLT) 2-dependent mechanisms [110], [111]; or repolarises α-cells, via the modulation of store-operated channels (SOCs) [112] and 2-

pore domain K^+ channel-related acid-sensitive K^+ channel-1 (TASK-1) [113]. I will therefore describe the knowns and unknowns of the mechanisms regulating the α-cell response to glucose.

Different from β-cells, rodent α-cells have been shown to express SGLTs 1 and 2, which play an important role facilitating glucose transport into the cell [110], [114]–[116], in addition to GLUTs. SGLTs transport glucose and Na^+ ions into the cell, utilising the Na^+ electrochemical gradient [110]. The depolarising effect of this Na^+ entry has therefore been hypothesised to elicit generation of action potentials and consequently modulate glucagon secretion, a concept supported by mathematic modelling [110], [111]. Dapagliflozin, a SGLT2 inhibitor, has been demonstrated to stimulate glucagon secretion from isolated mouse and human islets at high glucose [110]. However, the non-metabolisable glucose analogues, 3-O-methyl-D-glucose and 2-deoxyglucose, which retains Na^+ entry without affecting ATP concentrations, does not inhibit low-glucose-stimulated glucagon secretion [117], [118]. Other data has suggested that the glucagon-suppressing effect of dapagliflozin is mediated via somatostatin, as suppression of δ-cell SGLTs can stimulate glucagon [119]. Application of the SSTR2

antagonist, CYN154806, eliminates the effect of the glucagon response to SGLT inhibition [120].

Inside the α-cell, glucose conversion to G6P, as with β-cells, remains the rate limiting step for the metabolism of glucose and is required for glucose suppression of glucagon secretion [114], [118], [121]. While these two cell types express glucokinase, α-cells also express the high affinity hexokinase 1 and are therefore able to produce ATP at low concentrations of glucose as well as sense metabolic changes by increasing ATP production [80], [81], [121], [122].

1.5.2 K_{ATP} and Ca_v channels: The same, but different

Changes in the ATP:ADP ratio alter K_{ATP} channel activity in α-cells [123]. These channels are of the same molecular identity as those of β-cells, however, they have much lower basal activity, ~0.1 nS at 1 mM glucose, likely due to the higher cellular ATP:ADP ratio at this concentration [109]. Islet α-cells are also equipped with L-, P/Q-, and N-type HVA Ca_v channels, as well as T-type LVA Ca_v channels [48], [107]–[109], [124] and Na_v channels [51]. At low glucose, T- and, subsequently, L-type Ca_v channels open, driving the upstroke of the action potential. Interestingly, unlike in human islets [124], blockade of L-type channels in mouse

islets has minimal effect on the glucagon secretory response to hypoglycaemia, despite carrying the majority of the Ca^{2+} current [48], [108], [125]. Na_v channels also contribute to the upstroke of the action potential, blockade with tetrodotoxin reduces both glucagon secretion and α-cell action potential amplitude, different to mouse β-cells [51]. Upon reaching positive membrane potentials, in both rodents and human, P/Q-type Ca_v channels (the exocytosis-relevant Ca^{2+} channels) open, triggering glucagon secretion [109], [124], [125].

The K_{ATP} hypothesis of glucose-inhibition of glucagon secretion states that as the extracellular glucose concentration increases, the α-cell K_{ATP} conductance drops to ~0.075 nS, in both human and mouse islets, due to channel closure [109], [121], [123], [126]. As a result, the membrane is depolarised by approximately 10 mV [127]. This inactivates Na_v channels, significantly reducing action potential amplitude and consequently, P/Q-type Ca_v channels are unable to open [108], [124], [126]. By this mechanism, glucose is able to attenuate glucagon secretion.

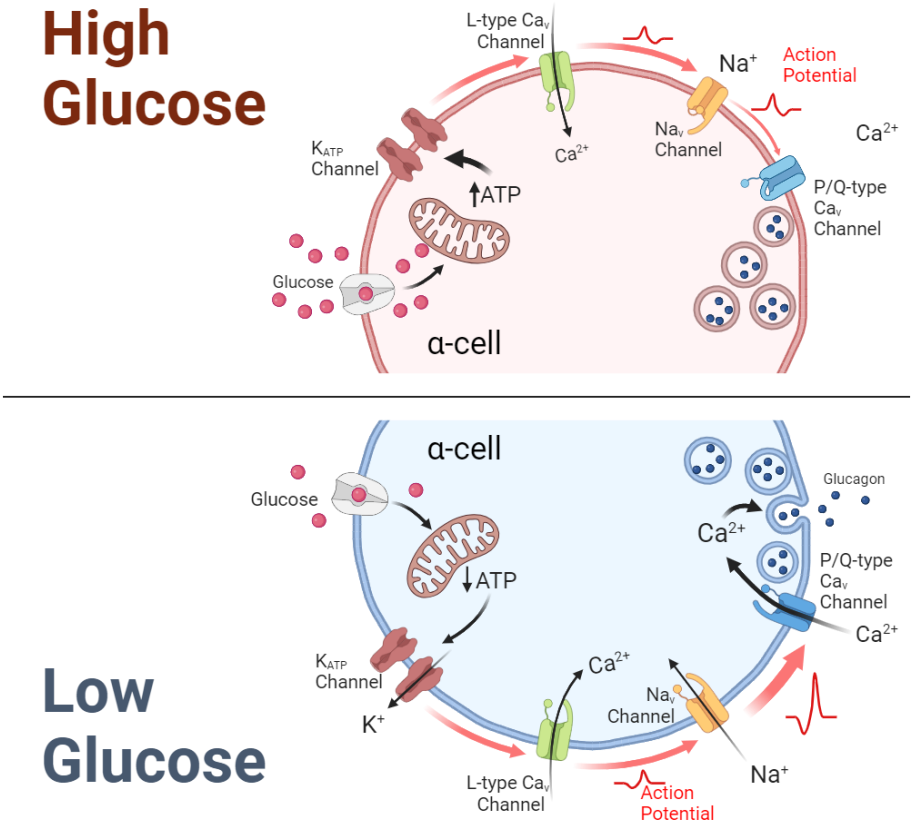


Figure 4: The K_{ATP} hypothesis of glucose inhibition of glucagon secretion. At high glucose (upper panel), K_{ATP} channels are closed and the resting membrane potential is slightly above the reversal potential for K+. T- and L-type Ca_v channels open, driving the upstroke of the action potential. However, Nav channels are inactivated, attenuating action potential height, preventing P/Q-type Ca_v channels opening. Consequently, glucagon secretion is low. At low glucose (lower panel), not all K_{ATP} channels are closed, maintaining a more depolarised membrane potential. This allows the reactivation of Na_v channels. Consequently, action potential height is sufficient to open P/Q-type Ca_v channels, stimulating exocytosis and glucagon secretion. Created with BioRender.com

1.5.2.1 Hyperpolarisation hypothesis

Several laboratories have published data indicating that glucose inhibits the secretion of glucagon by hyperpolarising α-cells, resulting in the suppression of Ca_v channel opening [113], [128]–[130]. This is in contrast to other data demonstrating a depolarising effect of glucose on α-cells, as previously discussed [51], [109], [121], [123], [127]. It is possible that hyperpolarisation may be due to the action of somatostatin, which can hyperpolarise α-cells by activating GIRK channels [75], [109], [131].

α-cells express K^+ channels, other than K_{ATP} channels, some of which are involved in repolarisation after an action potential and others that are active at all physiological voltages [132]. TASK-1 is an outwardly rectifying K^+ channel that can modulate the α-cell membrane potential, with blockade stimulating action potential firing and glucagon secretion, and have therefore been considered to limit excitability at high glucose [113], [130].

An alternative explanation for the mechanism by which glucose hyperpolarises α-cells is centred on the endoplasmic reticulum (ER) Ca^{2+} store. The sarco/endoplasmic Ca^{2+} ATPase (SERCA) pumps Ca^{2+} into the ER, in an ATP-dependant manner. This inactivates stromal interaction molecules (STIMs), closing Orai1 Ca^{2+} channels on the plasma

membrane, and repolarising the membrane [112], [133], [134]. When intracellular ATP ($[ATP]_i$) is low, the ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) drops, due to leakage from the organelle, and the consequent Ca^{2+} flux through Orai1 channels induces membrane depolarisation and α-cell electrical activity [112]. Currently, no direct measurements of these channels have been taken in α-cells, due to the challenging technical nature of the technique and the low expression of Orai1 channels.

1.5.3 Other mechanisms regulating α-cell activity

In addition to the intrinsic mechanisms regulating α-cell glucose sensing, external factors, such as β- or δ-cell paracrine signalling, incretins, and neurotransmitters, are also integral to the proper glucagon secretory response [131], [135]–[140].

1.5.3.1 Autocrine factors

Positive autocrine signalling is an important mechanism for the hypoglycaemic response, amplifying further glucagon secretion. α-cells express GcgRs and glucagon can elevate the intracellular cAMP concentration, a key messenger in the control of glucagon secretion [141], [142], promoting exocytosis [143]. Similarly, α-cells also produce and release

glutamate, an excitatory neurotransmitter, and this is able to activate ionotropic glutamate receptors (iGluRs) on the α-cell, depolarising the membrane, opening Ca_v channels, and stimulating secretion [144]–[146].

1.5.3.2 Paracrine factors and incretins

Both β- and δ-cells have been suggested to inhibit α-cell secretory function. Somatostatin, in particular, is a powerful inhibitor of glucagon release [73], [147]. Acting via SSTR2 [75], [79], it suppresses cAMP generation by inhibition of adenylyl cyclase [74], as well as suppressing electrical activity by activation of GIRK channels, hyperpolarising the membrane [75], [131]. While insulin has been seen to be an inhibitor of glucagon, [135], [148], recently, and in light of conflicting effects on electrical activity [139], [149], effects have been proposed to be mediated via somatostatin. Knockout of the δ-cell insulin receptor eliminates the effects of exogenous insulin on glucagon secretion [119]. There is also debate as to whether GLP-1 acts directly on α-cells, or via paracrine mechanisms, given their low GLP-1R expression [19], [136], [149]–[151]. Furthermore, blockade of SSTR2 can negate the inhibitory effect of GLP-1 on glucagon secretion [152], [153], though there is some evidence that a direct effect may be mediated via PKA [19], [150], [151]. What is clear is that GLP-1 is a strong inhibitor of glucagon secretion. It is likely that

the glucagonostatic effects are exerted by a combination of paracrine and direct mechanisms. Intestinal L-cells secrete GLP-1 in two forms: GLP-$1^{(7-37)}$ and GLP-$1^{(7-36)}$, with the latter making up 80% released [154]. GLP-$1^{(7-36)}$, however, is quickly degraded, only having a half-life of approximately 2 mins in circulation to GLP-$1^{(9-36)}$ by dipeptidyl peptidase IV (DPP4) [155], [156]. There are conflicting data as to whether GLP-$1^{(9-36)}$ has active properties and is able to influence islet function [157]–[159], therefore a deeper understanding of this peptide is required to allow a deeper view of islet function. By comparison, GIP stimulates glucagon secretion by enhancing α-cell cAMP, similar to its effect on β-cells [20], [138].

1.5.3.3 Adrenaline

The secretion of glucagon can also be controlled by neurotransmitters [139]. Adrenaline is released in response to stressors such as hypoglycaemia, as well as infection and the fight-or-flight reaction [160]. In human and rodent islets, adrenaline directly stimulates glucagon secretion via activation of G_s-coupled β-adrenergic receptors and increased cAMP [19], [161] switching the exocytosis-relevant Ca_v channel from the P/Q- to the L-type, and the G_q-linked α_1-adrenoceptors stimulate lysosomal and ER Ca^{2+} release via inositol triphosphate (IP_3) receptors [139], [161], [162]. The inhibitors isradipine and xestospongin C, of the L-type Ca_v

channel and IP_3 receptor respectively, are able to attenuate glucagon secretion in response to adrenaline [139], [162]. Together, the activation of G_s- and G_q-coupled receptors produce a powerful stimulatory effect on glucagon secretion.

1.6 The δ-cell

δ-cells are understudied, compared to β- or α-cells, due to both their relatively low abundance in islets, only making up 2-10% of the islet [2], [3], and the difficulties in studying them [163]. However, they are very important for proper islet function, setting the glycaemic level and preventing over secretion of both glucagon and insulin [73], [164]. Unlike other islet cells, δ-cells exhibit a unique, neuron-like morphology with filopodia-like extensions that can be more than 20 μm long [165]. This allows contact between a single δ-cell and multiple other islet cells, both proximal and distal, furnishing them with a wide signalling network. Despite the difficulties in studying these cells, the intrinsic glucose-response mechanisms have been established [14], [166]–[169]. δ-cells are electrically excitable [167] and glucose-stimulated somatostatin secretion is associated with the firing of action potentials [166]. Like β-cells, δ-cell secretion increases with glycaemia [73], however, the glucose level at

which mouse δ-cells are activated is much lower than that of β-cells, 3 mM versus 7 mM respectively [170].

1.6.1 Glucose regulation of somatostatin secretion

Similar to α-cells, δ-cell utilise GLUT transporters 1 and 3 in addition to SGLTs [119]. As with other endocrine cells, glucokinase activity is the rate limiting step for glucose conversation to G6P and therefore dictates the somatostatin secretory response [166]. Although, interestingly, δ-cell SGLTs are able to cause a rapid increase in the intracellular Na^+ concentration and trigger CICR, through activation of intracellular Na^+-Ca^{2+} exchange, without evoking electrical activity [168]. Glucose metabolism is able to stimulate δ-cell somatostatin secretion via either membrane potential-dependent [14], similar to that of β-cells, and independent pathways [167].

1.6.1.1 Membrane dependant regulation

δ-cells expresses K_{ATP}-channels of the same molecular identity as found in β- and α-cells [81], [91], [166], [171]. They are open in the absence of glucose [14] and, as with β-cells, closure of these channels triggers δ-cell electrical activity, Ca^{2+} influx, and secretion [166], [169], [171]. However, δ-cell K_{ATP} channel conductance at 10 mM glucose is 1/3 of that in

β-cells and, consequently, somatostatin secretion can be initiated at lower glucose concentrations than insulin secretion [14].

Mouse δ-cells are equipped with L-type and R-type voltage-gated Ca^{2+}-channels [14], [166]. While blockade of L-type Ca^{2+} channels with isradipine has no effect on glucose-stimulated somatostatin secretion, although Ca^{2+} oscillation frequency is reduced, elimination of Ca^{2+} entry via R-type channels, by KO or pharmacological inhibition, reduces secretion by >70% [166]. Human δ-cells, by comparison, express L-type, P/Q-type, and T-type Ca_v channels, all of which are essential for somatostatin secretion [169]. In δ-cells, a TTX-sensitive Na^+ current also contributes to the upstroke of the action potential, but inhibition does not significantly affect somatostatin secretion [14], [15][169]. Closure of K_{ATP} channels depolarises the membrane to the activation potential of T-type channels, which then further drives the action potential upstroke, activating L-type Ca_v channels, with the assistance of Na_v channels and, finally, P/Q-type, in human, or R-type, in mice, Ca_v channels open. In human δ-cells, this triggers exocytosis [169]. Surprisingly, the influx of transmembrane through R-type Ca_v channels alone is unable to directly induce somatostatin secretion in mouse δ-cells, despite the importance of Ca_v channels [166]. Instead

secretion requires Ca^{2+} release from the ER, via ryanodine receptors, in response to influx through R-type Ca_v channels to elicit exocytosis of somatostatin-containing vesicles [166].

1.6.1.2 Membrane independent regulation

Loss of K_{ATP} channels, by KO of *SUR1*, does not significantly affect somatostatin secretion and the blocking of K_{ATP} channels with tolbutamide is not as effective as glucose at stimulating secretion of somatostatin, despite eliciting electrical activity to the same extent [167]. This implies that a parallel membrane-independent mechanism is present in δ-cells. It is driven by a glucose dependant rise in intracellular cAMP, enhancing both CICR, via activation of EPAC2, and exocytosis directly, via PKA-dependant mechanisms, and can be blocked by the GK-inhibitor mannoheptulose [166]–[168]. The combination of these membrane-dependant and -independent mechanisms form the intrinsic pathways through which δ-cell somatostatin secretion is regulated by glucose.

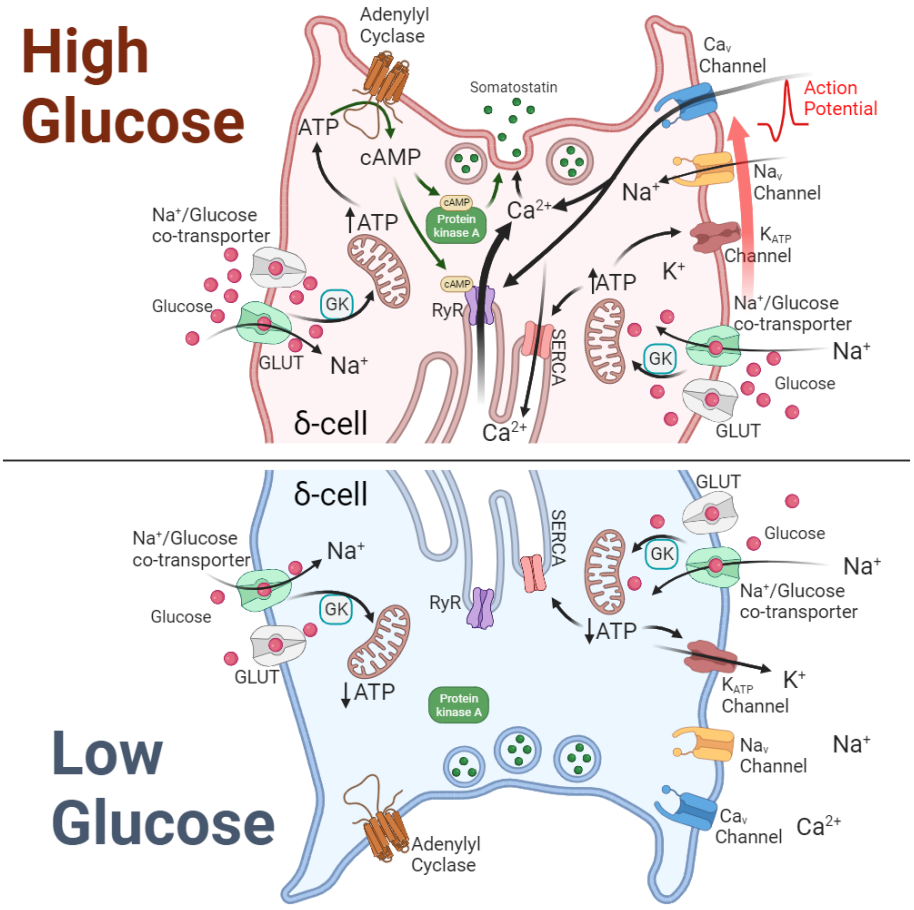


Figure 5: Intrinsic regulation of glucose-stimulated somatostatin secretion. At high glucose (upper panel), GLUTs and SGLTs transport glucose into the cell where it is converted to G6P by GK, before entering the Krebs cycle, and generating ATP. High ATP concentration is converted to cAMP by adenylyl cyclase. The increase in cAMP activates PKA which stimulates secretion, and primes ryanodine receptors for CICR. In parallel, ATP closes K_{ATP} channels and this, along with Na^+ entry, through SGLTs, depolarises the membrane, activating Na_v and Ca_v channels. The resultant transmembrane Ca^{2+} influx triggers CICR from the ER, stimulating exocytosis. ATP activation of SERCA also allows the refilling of ER Ca^{2+}. At low glucose (lower panel), low ATP results in low cAMP production; PKA and RyRs are not stimulated. Furthermore, K_{ATP} channels are open, polarising the membrane and inhibiting electrical activity. Created with BioRender.com

1.6.2 Other mechanisms regulating δ-cell activity

As with other islet cells, δ-cell activity, and somatostatin secretion, may be regulated by external factors in parallel to intrinsic glucose sensing.

1.6.2.1 Electrical coupling

δ-cells are connected to β-cells by gap junctions formed of connexin-36 [172]. Stimulation of islets expressing β-cell specific channelrhodopsin-2, a light sensitive cation channel, triggered β-cell electrical activity that propagated to δ-cells, and, in turn, suppressed α-cells activity [172]. It has therefore been proposed that synchronous pulses of insulin and somatostatin, secreted under the stimulation of glucose, is underpinned by electrical coupling between β-and δ-cells [173]. This coupling also contributes to β-cell inhibition of α-cells [172].

1.6.2.2 Paracrine signalling

In addition to gap junction-mediated activation, paracrine signals from β-cells are able to affect δ-cell activity. Much rodent islet data seem to indicate that urocortin3 (ucn3), a peptide hormone co-released with insulin by β-cells, rather than insulin, mediates this paracrine interaction [73], [174], [175]; KO of ucn3 reduces somatostatin secretion by half [175].

Similarly, α-cell factors are enhancers of δ-cell activity, with both GcgRs and GLP-1Rs expressed in δ-cells [80], [81]. Application of exogeneous glucagon stimulates somatostatin, with blockade of glucagon signalling, using glucagon-specific antibodies or antagonism of GcgRs or GLP-1Rs, reducing somatostatin release both in isolated islets and perfused mouse pancreas [174], [176]. In addition to glucagon, α-cell glutamate may be able to activate δ-cell iGluRs, depolarising the membrane, and this has been proposed to stimulate somatostatin secretion at low glucose concentrations [177].

As with the other islet cells, the incretins GLP-1 and GIP can modulate somatostatin secretion, although the effect of GIP on δ-cells is significantly lower than the effect on β-cells or of GLP-1 [178]. The glucose independent activation of the G_s-coupled GLP-1Rs on the δ-cell is likely, as in β-cells, to raise cAMP concentrations, thereby enhancing CICR and exocytosis [179]. The strong stimulatory effect on somatostatin has led to the suggestion that the actions of GLP-1 on α-cells are mediated via δ-cells, with SSTR2 blockade or KO eliminating the glucagonostatic effect of the incretin [120], [152]

1.7 Diabetes and therapy

Diabetes is a metabolic disorder underpinned by a lack of proper glycaemic regulation and characterised by sustained high glucose levels. Diabetes stems from a lack of insulin secretion and/or signalling and, while heterogenous, most cases can be broadly categorised into, insulin dependant, type 1 diabetes (T1D) or, insulin resistant, type 2 diabetes (T2D). The International Diabetes Federation estimated over 537 million people worldwide in 2021 were living with the disorder and, of those, T2D accounts for 90% [180].

The number of people affected by diabetes has been increasing over the last decade and is projected to increase further in the future [181]. A lack of sufficient insulin secretion, a resistance to insulin, or a combination of the two underlies T2D [182]. While development of T2D diabetes has a strong genetic component, lifestyle factors are generally seen the primary factor generally attributed to [183] and lifestyle management interventions have been shown to be an effective for many [184].

T1D results from autoimmune destruction of β-cells and, as a consequence, the body does not produce sufficient insulin, becoming unable to reduce glucose secretion. Patients with the disorder are therefore dependant on application of

exogenous insulin, first used as a treatment in 1922 [185], and must be carefully managed to maintain glycaemia within the normal range. In recent year, further improvements have been made in treatments, with continuous glucose monitoring and automatic insulin delivery devices aiming to maintain tighter control on euglycaemia.

1.7.1 Autoimmunity in T1D

Genetics plays an important role in T1D susceptibility. While fewer than 15% of affected patients have a first degree relative with T1D [186], [187], development of the disorder is twice as likely in monozygotic twins than in dizygotic twins [188]. T1D is an autoimmune disease, over 90% of patients express auto-antibodies that are detectable prior to the presentation of clinical symptoms [189], [190]. Specific human leukocyte antigens (HLAs) haplotypes and/or excessive HLA expression are associated with a risk of developing T1D [191]–[193]. HLAs are important for the immune system, involved in distinguishing between self and non-self, presenting antigens for recognition. In T1D, this process becomes dysregulated and β-cell auto-antigens, such as insulin [194] and glutamic acid decarboxylase [195], are presented to the immune system, stimulating islet inflammation, leukocytes infiltration, and, ultimately, β-cell destruction [196]. Different environmental triggers, such as

viral infection, have been posited to stimulate T1D autoimmunity, yet there remains much uncertainty.

The presence of autoantibodies directed towards β-cell targets can be used to diagnose T1D before clinical onset [197] and these have the potential for use as therapeutic targets. The major autoantigens have been identified as insulin [194], [198], glutamate decarboxylase [195], tyrosine phosphatase-related islet antigen 2 [199], and zinc transporter-8 [200], [201]. A further major autoantigen, a 38-kDa glycosylated membrane protein, was found in between 19 and 38% of patients with T1D or between 14 and 35% of prediabetic subjects [202]–[204]. This was later identified as tetraspanin-7 (Tspan7), using autoantibodies isolated from patients with T1D [205]. Tspan7 is a member of the 4-transmembrane domain protein family and is primarily expressed in the central nervous system, lung, and pancreatic islets [80], [81], [205]–[207]. Tspan proteins are involved in the organisation and distribution of proteins, both internal and external, aiding the formation of microdomains as well as regulating, processes such as cellular adhesion, migration, contractility, and morphology by influencing protein interactions [208], [209]. KD of *Tspan7* in dispersed mouse and human β-cells has revealed that the protein is involved in Ca^{2+} handling and

modulation of L-type Ca_v channel activity [210]. However, the full role of Tspan7 in islet cells remains enigmatic.

1.7.2 Secondary complications of diabetes

1.7.2.1 The contribution of α- and δ-cells to diabetes pathophysiology

The focus of diabetes treatment and research has previously centred on the β-cell. This is due to the loss of insulin signalling through resistance, impaired secretion, or a combination of the two that is central to development of the disorder. It is becoming more evident that dysregulation of glucagon and somatostatin play a key role in disease progression, driving secondary complications [9], [109], [211]–[215]. Glucagon becomes dysregulated in diabetes [216], secretion is inhibited during hypoglycaemia and potentiated during hyperglycaemia. Blocking glucagon action, with glucagon receptor antagonists, has been shown to be capable of restoring normoglycaemia in β-cell ablated animal models [217]–[221].

1.7.2.2 Hyperglycaemia

Hyperglycaemia is clinically classed as glucose concentrations above 11mM, or over 7mM when fasted, and is one of the primary diagnostic criteria for diabetes [222], [223]. As, under normal circumstances, insulin signalling

triggers glucose uptake, lowering glycaemia, the consequence of loss of this signalling is that patients with diabetes, if left untreated, experience chronic hyperglycaemia. In the short term, symptoms include thirst, frequent urination, weakness, tiredness, and blurred vision, while longer term complications can include cardiovascular disease, peripheral neuropathy, diabetic retinopathy, and nephropathy [224]. These secondary complications can severely reduce quality of life and contribute to diabetes-related mortality [225]. One of the cellular consequences of impaired hyperglycaemia is the dysregulation of α-cell function, with glucagon secretion occurring at high blood glucose concentrations, where it should be inhibited, further exacerbating hyperglycaemia [226]. The mechanisms are under investigation and have been suggested to occur by SGLT-dependent processes such as mitochondrial acidification [227]; increased K_{ATP}-channel activity in α-cells, possibly as a consequence of impaired glucose metabolism, which can be attenuated with low-dose sulfonylureas [109], [228]; or α-cell resistance to somatostatin [229].

1.7.2.3 Hypoglycaemia

A further consequence of glucagon dysregulation is hypoglycaemia, which can be extremely dangerous, and is currently a major limiting factor in the treatment of the

disorder [230], [231]. During episodes of low blood glucose concentrations (<3.3mM), patients present with symptoms such as dizziness, sweating, heart palpitations, confusion, and hunger. Severe hypoglycaemia can cause seizures, coma, and, in extreme cases, death [232]. This can become more prevalent later in disease progression, with hypoglycaemia begetting hypoglycaemia [233], [234]. It has been estimated that 4-10% of patients with T1D eventually die of hypoglycaemia [235].

Hypoglycaemia may be attributed to defective glucagon secretion at low glucose, impaired counter regulation, which is inadequate to restore normoglycaemia [230], [236]–[238]. At 1 mM glucose, T2D α-cells secrete glucagon at less than half the rate of non-diabetic α-cells, despite having at least double the glucagon content [109], [168]. The specific mechanisms by which glucagon becomes dysregulated is under investigation. It has been hypothesised that defective glucagon secretion is attributable to a loss of α-cell identity, through adoption of β-cell-typical features, when β-cell mass becomes depleted [239], [240], or to inhibition of α-cell activity by artificially elevated circulating insulin levels during hypoglycaemia, as a result of insulin therapy [241]. These factors are thought to contribute to the modulation of glucagon secretion to a more insulin-like pattern. However,

given that the precise intrinsic mechanisms that regulate α-cell control of glucagon secretion in response to glucose are yet to be fully established, it is difficult to decipher changes underlying dysregulation in diabetes.

It is not only intrinsic α-cell changes that can become altered during diabetes. Patients with T2D, as well as T1D, demonstrate an increase in direct δ-cell to α-cell contacts, partially due to loss of β-cells [242]–[244]. Inevitably, this alters the communication between α- and δ-cells and data, such as that demonstrating that δ-cells are glucose-blind islets from donors with T2D [168], is beginning to illuminate role that altered somatostatin secretion plays in glucagon failure during hypoglycaemia. This has led to promising studies investigating therapeutic interventions targeting somatostatin control of glucagon secretion, which has shown that antagonism of SSTR2 is able to restore counter regulation in diabetic animals [215], [245]–[247]. Further understanding of islet cell physiology, their interactions, and the pathophysiology of diabetes can form the foundation to the development of new therapies, which will improve treatment for those living with diabetes, enhancing their quality of life.

2 THESIS PAPERS

2.1 Aims:

The overall aim of this thesis was to further establish the mechanisms governing the regulation of hormone secretion from islets of Langerhans.

2.1.1 Specific aims:

I. Determine the role of transmembrane and ER Ca^{2+} on the intrinsic mechanisms of glucose regulation of glucagon secretion.
II. Determine the effect and pathways of GLP-$1^{(9-36)}$ on α-cell glucagon secretion.
III. Investigate the impact of *Tspan7* KO on β-cell function and secretion to further illuminate the role of the Tspan7 protein.
IV. Investigate the mechanisms of α-δ-cell crosstalk and how defects in this crosstalk contribute to recurrent hypoglycaemia.

2.1.2 Papers:

I. The endoplasmic reticulum plays a key role in α-cell intracellular Ca^{2+} dynamics and glucose-regulated glucagon secretion.

II. GLP-1 metabolite GLP-$1^{(9-36)}$ is a systemic inhibitor of mouse and human pancreatic islet glucagon secretion.

III. Loss of tetraspanin-7 expression reduces pancreatic β-cell exocytosis Ca^{2+} sensitivity, but has limited effect on systemic metabolism.

IV. A mechanism for rapid cross-talk between pancreatic α- and δ-cells and its role in hypoglycaemia-induced glucagon secretory failure.

2.2 Methods:

Papers I-IV contain detailed descriptions of the methods used for the separate studies. A short summary of key methodologies is provided below.

2.2.1 Mouse models

A number of mouse models were used to derive the data within each of the papers. Use of these models allows genetic manipulation and control of environmental and genetic factors that are not possible in human, as well as providing increased tissue availability. While similarities in genetics and physiology to humans make them useful models, differences remain that require validation of mouse data in human tissue to ensure translatability of conclusions.

All research performed as part of this thesis was approved by, and conformed to the rules set by, the relevant authorities presiding over the studies. The three Rs of replacement, reduction, and refinement were implemented to ensure the most ethical animal use and to minimise suffering.

2.2.1.1 Wild type

C57B/6J black mice were used for wild-type (WT) experiments, such as hormone secretion, immunofluorescent

staining, insulin tolerance tests, and electrophysiological studies.

2.2.1.2 Gcg- and SST-GCaMP6f

Ca^{2+} is an important signal in cells of the pancreatic islet, triggering the exocytosis of hormone-containing vesicles from α-, β-, and δ-cells. Two mouse lines were generated expressing GCaMP6-fast variant (GCaMP6f), a quick-responding green fluorescent Ca^{2+} sensor, with which to measure the changes in the intracellular cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) [248]. To achieve cell-specific expression we used the Cre-LoxP approach. The LoxP-flanked (floxed) GCaMP6f mouse line, $Rosa26^{GCaMP6f}$ (Ai95D) [249] was combined with mouse lines possessing the genetically encoded Cre-recombinase, the presence of which results in excision of the STOP cassette, inducing production of GCaMP6f. For α-cell specific expression, the Ai95D line was crossed with a mouse line expressing tamoxifen-inducible Cre recombinase in glucagon-expressing cells ($Glu^{Cre/ERT2}$) [250], creating the Gcg-GCaMP6f line. The application of tamoxifen induces translocation of the Cre-recombinase to the nucleus of the cell, where it can induce transcription of the target gene. For δ-cell specific expression, Ai95D mice were crossed with SST^{Cre} mice, those with transcription of the Cre-recombinase gene driven by

the somatostatin promoter [91], creating the SST-GCaMP6f line. As a result, islets from the SST-GCaMP6f and Gcg-GCaMP6f mouse lines express GCaMP6f exclusively in δ- or α-cells, respectively. Both lines demonstrated a high recombination rate and clear function. The advantage of this model is primarily the specificity of reporter localisation. It also eliminates the need for loading of islets with Ca^{2+} dyes, improving efficiency and reproducibility. As a result, fewer mice are required.

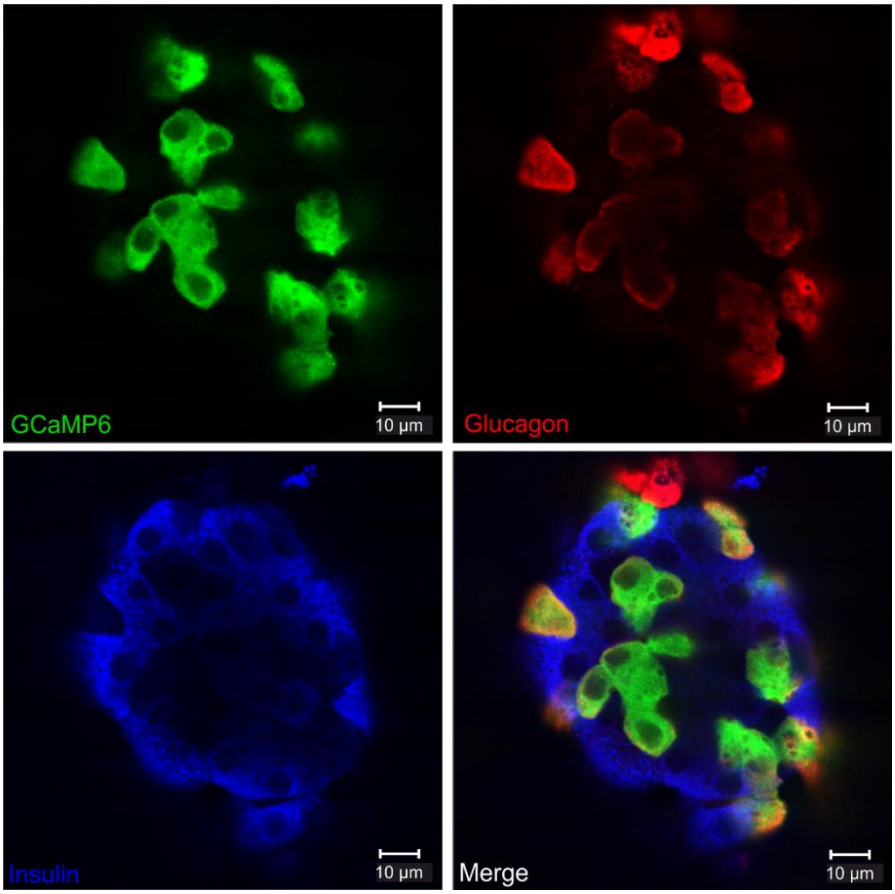


Figure 6: Image of an immunostained Gcg-GCaMP6f islet for GCaMP6f (Green), glucagon (Red), and insulin (blue) to show cell specificity of GCaMP6f. Top left: Green channel, GCaMP6f. Top right: Red channel, Glucagon. Bottom left: Blue channel, insulin. Bottom right: Merge of green, red, and blue channels.

2.2.1.3 Sst-tdRFP

Given the scarcity of δ-cells within the islet, the Sst-tdRFP mouse model was utilised [167], [251], which expresses the tdTomato red fluorescent protein specifically in

somatostatin-producing cells for identification. This model was generated using the Cre-LoxP approach, by breeding of SST^{Cre} mice with those expressing a floxed tdTomato at the *Rosa26* locus. This allows the identification of δ-cells within the islet by red fluorescence when observed under a fluorescence microscope and therefore allows targeted manipulation with techniques such as electrophysiology. This improves experimental efficiency, by eliminating cell targeting errors, and reduces the number of mice required.

2.2.1.4 Gcg-ChR2 and Gcg-hM3Dq$^+$

To allow specific, temporally precise, activation of α-cells, without the potential off-target effects that can arise from pharmacological stimulation of endogenous targets, two mouse models were employed: Gcg-ChR2 and Gcg-hM3Dq$^+$. Islets from the Gcg-ChR2 mouse line express Channelrhodopsin-2 (H134R), a light activated cation channel, and an enhanced yellow fluorescent protein specifically in α-cells. This allows the triggering of Ca^{2+} influx into α-cells, and consequent glucagon secretion, when exposed to 470 nM light. Islets from the Gcg-hM3Dq$^+$ mouse line express the designer G_q-coupled receptor exclusively activated by designer drugs (DREADD), hM3Dq, specifically in α-cells. Using the otherwise biologically inert agonist JHU37160 (J60), activation of the hM3Dq receptor stimulates

glucagon secretion by IP_3-midiated Ca^{2+} mobilisation. Both lines utilised the Cre-LoxP expression system. The Gcg-ChR2 line was generated by the crossing of a Gcg-iCre mouse line [252] with a ChR2-expressing mouse line. Gcg-hM3Dq were generated by crossing the $Glu^{Cre/ERT2}$ mouse line with a DREADD-expressing mouse line [253].

2.2.1.5 Tspan7 KO and GLP-1 receptor KO

As well as the above mouse models, KO models we also used to investigate the roles of Tspan7 and the target receptor for $GLP\text{-}1^{(9\text{-}36)}$. In paper II, experiments were performed on islets isolated from mice with a whole-body KO of the GLP-1R [254], generated by homologous recombination in mouse embryonic stem cells, and their wildtype littermates were used as controls. In paper III, islets or islet cells were isolated from mice with a whole-body KO of the *Tspan7* gene ($Tspan7^{y/-}$) [255] were utilised for experiments and their wildtype littermates were used as controls. These mice were kindly donated by Dr Luca Murru (CNR Institute of Neuroscience, Milano, Italy) and generated by homologous recombination in mouse embryonic stem cells.

2.2.2 Mouse islet isolation

After euthanasia, a mixture of Liberase, a collagenase, and thermolysin, a neutral protease was infused through the bile

duct of the mouse to inflate the pancreas. The pancreas was subsequently excised and digested at 37˚C, in a water bath for 12-15 minutes. At this point, ice-cold Hanks' balanced salt solution, supplemented with 0.2% bovine serum albumin, was added to inactivate the enzymes and the pancreas was shaken manually, to dissociate the tissue. Islets were handpicked out from the exocrine debris, under a stereo microscope, using a pipette and cultured in RPMI 1640 containing 10% foetal bovine serum (FBS), 1% penicillin/streptomycin, and supplemented with 5 mM glucose in a tissue culture incubator at 37˚C, 5% CO_2 before experimentation.

2.2.3 Human islets

Human pancreatic islets were obtained from human cadaveric donors isolated by the Oxford Human Islet Transplantation facility, the Nordic Network for Clinical Islet Transplantation, or the ADI Isletcore at the University of Alberta, with appropriate consent for use in research. Isolated islets were cultured in RPMI 1640 containing 10% FBS, 1% penicillin/streptomycin, and supplemented with glucose in a tissue culture incubator at 37˚C, 5% CO_2 before experimentation.

2.2.4 Hormone secretion

For hormone secretion experiments, islets were size-matched into groups and placed in microfuge tubes in RPMI, as cultured. These were then cultured at 37˚C, 5% CO_2 before replacement of the cell culture medium with the pre-incubation buffer. This consisted of Krebs Ringer buffer (KRB; 140 mM NaCl, 3.6 mM KCl, 0.5 mM $MgSO_4$, 2.6 mM $CaCl_2$, 0.5 mM NaH_2PO_4, 2 mM $NaHCO_3$, and 5 mM HEPES with pH adjusted to 7.4, using NaOH), supplemented with 3 mM glucose, and islets were incubated for 1 hour at 37˚C in a humidified chamber. The pre-incubation buffer was then replaced with the experimental buffer, KRB, supplemented with glucose and pharmacological agents, as indicated, and the islets were incubated again for 1 hour at 37˚C in the humidified chamber. After incubation, tubes were centrifuged at 900x gravity, the supernatant was removed and supplemented with aprotinin before storage at -80˚C, and acidic ethanol was added to the islet pellet, which was then sonicated and stored at -20˚C. Insulin and glucagon were measured by enzyme-linked immunosorbent assay and somatostatin was determined by radioimmunoassay.

For *in vitro* hypoglycaemic precondition experiments, islets were cultured on the first day for the durations as indicated in the manuscript IV at either 2 mM (hypoglycaemia) or 6 mM

(euglycaemia), in the presence or absence of the GcgR blocker glucagon amide, (preconditioning phase) and subsequently cultured at 6 mM glucose overnight to recover. On the second day a static incubation test was performed to assess the hormone secretory function of the islets. For Gcg-hM3Dq^{+} islets, J60 was added to induce glucagon secretion in the absence of hypoglycaemia during the preconditioning phase. In Gcg-ChR2 islets, light at 470 nm was flashed onto the islets (as indicated), during the preconditioning phase, at 0.1 Hz to stimulate Ca^{2+} entry and induce glucagon secretion in the absence of hypoglycaemia.

2.2.5 Electrophysiology and patch imaging

Cell capacitance, Ca^{2+} current, and membrane potential were assessed using the perforated patch or standard whole-cell patch clamp techniques on individual cells within intact islets. Cellular identity was determined by electrophysiological fingerprinting [15] in WT islets or the presence of red fluorescence in SST-tdRFP islets.

Patch imaging experiments investigating α-cells used WT islets and the Ca^{2+} indicator fluo-4 was supplemented into the patch solution, with fluorescence of this dye used to determine $[Ca^{2+}]_I$, in addition to the experimental concentration of ATP. Experiments investigating α-δ-cell

signalling used SST-GCaMP6f islets. For this, propidium iodide (PI) or sulforhodamine B (SRB) was supplemented into the pipette solution, to visualise the α-cells, and determine proximity to neighbouring δ-cells. GCaMP6f fluorescence was used to monitor δ-cell $[Ca^{2+}]_i$ in response to activation of adjacent α-cells and their exocytosis, induced by application of a depolarising pulse. Identity of patched α-cells was confirmed by electrophysiological fingerprinting [15] before the experimental protocol was applied.

2.2.6 Imaging

2.2.6.1 Calcium

Imaging of $[Ca^{2+}]_i$ was either performed on GCaMP6f-expressing islets or islets loaded with a Ca^{2+}-sensitive dye and used a confocal scanning microscope or a widefield fluorescence microscope, according to the more appropriate method for the experimental design.

Simultaneous imaging of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_i$ was either performed on GCaMP6f-expressing islets or islets loaded with the Ca^{2+}-sensitive dye Calbryte 520 and infected with an adenovirus containing the ER-Ca^{2+} reporter RCEPIA1*er*. Islets were placed in a custom imaging chamber on a wide field microscope and imaged simultaneously for both reporters.

Chambers were perfused with KRB supplemented with glucose and pharmacological agents, as indicated during the experiment, and functional identification of cells was performed at the end of the experiment by infusion of either adrenaline or by raising the K^+ concentration 70 mM to stimulate the cells, as appropriate.

For puff imaging experiments, an SST-GCaMP6f islet was held by a suction pipette in a chamber, perfused with KRB, and imaged by wide-field fluorescence microscopy. Using a microprocessor-controlled injector, 69 nl of testing solution was applied to the islets at a rate of 46 nl/s and the δ-cell $[Ca^{2+}]_i$ response was measure by the change in GCaMP6f fluorescence.

2.2.6.2 ATP:ADP

Simultaneous imaging of ATP:ADP and $[Ca^{2+}]_i$ was performed on islets infected with an adenovirus containing the reporter of ATP:ADP, PERCEVAL, and loaded with a Ca^{2+}-sensitive dye, CalBryte-630. Islets were placed in a custom imaging chamber on a wide field microscope and imaged simultaneously for PERCEVAL in the green channel and Calbryte-630 in the red channel. The chamber was perfused with KRB supplemented with glucose and pharmacological agents, as indicated during the experiment, and functional identification of cells was performed at the end of the

experiment by infusion of adrenaline, to identify α-cells, and carbonyl cyanide p-trifluoro methoxyphenylhydrazone, to confirm PERCEVAL function.

2.2.7 *In vivo* tests

Insulin tolerance tests provide a good simulacrum for iaterogenic hypoglycaemia, which has been considered as the current limiting factor in diabetes therapy [231]. Insulin tolerance tests (ITTs) were performed by intraperitoneal (IP) injection of insulin, or the pharmacological agent of interest, to subject animals. Blood was subsequently collected from the tail vein and measured for insulin or glucose.

2.2.7.1 Hypoglycaemic pre-conditioning

Hypoglycaemic preconditioning experiments, *in vivo*, were conducted by first inducing hypoglycaemia by IP injection of insulin or saline, as a control (with or without compounds of interest), before being allowed to recover for 24 hours. Mice subsequently, on the second day, underwent an ITT, with blood sampled for plasma hormone and glucose content. For Gcg-hM3Dq^{+} mice, glucagon secretion in the absence of hypoglycaemia was induced by the IP injection of J60, on the first day, before recovering for 24 hours prior to ITT.

3 RESULTS AND DISCUSSION

3.1 Paper I: The endoplasmic reticulum plays a key role in α-cell intracellular Ca^{2+} dynamics and glucose-regulated glucagon secretion

3.1.1 Transmembrane calcium is not required for the α-cell response to glucose

It has been long established that α-cell electrical activity is Ca^{2+}-dependant [107] and these cells express L-, N-, and P/Q-type Ca_vs [48], [108], [109], [124]. Dissecting the Ca^{2+} current by progressive pharmacological blockade of Ca_v channel subtypes revealed that L-type Ca_v channels carry the largest proportion of the transmembrane Ca^{2+} current and membrane potential recordings showed they are responsible for pace-making in mouse α-cell electrical activity, as they are in human [124]. It is therefore surprising that isradipine (an L-type Ca_v channel blocker) had no effect on glucagon secretion and did not inhibit α-cell Ca^{2+} activity; although this is consistent with previous studies [19], [48], [108], [162], [256], [257]. However, opening of K_{ATP} channels with diazoxide eliminated the glucose sensitivity and suppressed the Ca^{2+}

activity of α-cells, an effect that has been attributed to its potent repolarising effect. Although the two reagents both inhibit α-cell electrical activity, application of diazoxide was also associated with an increase in α-cell ATP:ADP, likely mediated by the inhibition of cellular ATPases [258], [259]. This indicates the presence of an ATP-dependent regulatory mechanism operating in the absence of α-cell electrical activity. To test this, α-cells were voltage-clamped at resting membrane potential, -70 mV and $[Ca^{2+}]_i$ was monitored. $[Ca^{2+}]_i$ oscillations were present in voltage clamped α-cells at 1 mM ATP but absent in the presence of 3 mM ATP, with no apparent transmembrane Ca^{2+} current under either condition. From these collective data, it was deduced that α-cell Ca^{2+} activity can originate from, and be modulated by, an intracellular source.

3.1.2 Ca^{2+} handling by the endoplasmic reticulum is key to the α-cell response to glucose

The ER performs a wide range of biological functions, is sensitive to the metabolic state of the cell and, as the largest intracellular store of Ca^{2+}, plays an important role in maintaining Ca^{2+} homeostasis [260]. Not only can the ER release Ca^{2+} into the cytoplasm, such as in the δ-cell response

to glucose [167], but also act to rapidly sequester cytosolic Ca^{2+}. It was therefore hypothesised that the ER was responsible for the Ca^{2+} activity present in the absence of transmembrane Ca^{2+}.

Emptying of the ER and elimination of Ca^{2+} uptake, by cyclopiazonic acid-mediated inhibition of SERCA, rendered islet glucagon secretion insensitive to changes in extracellular glucose. Ca^{2+} oscillations were also significantly suppressed. It was also clear from $[Ca^{2+}]_{ER}$ imaging experiments, using the low affinity ER-bound Ca^{2+} indicator RCEPIA1*er*, that reduction of the extracellular glucose concentration stimulated the release of ER luminal Ca^{2+} into the cytosol, both in the presence and absence of membrane electrical activity. Blockade of ER Ca^{2+} releasing RyRs, but not IP_3 receptors, prevented ER Ca^{2+} release and low glucose-stimulated glucagon secretion. Suppressing of Ca^{2+} oscillations was also observed, in a manner identical to SERCA blockade. This places them as a key determinant of the α-cell glucose response, as is the case in the adrenaline response [162].

P/Q-type Ca_v channels have been previously demonstrated to be key to α-cells exocytosis [108] and $[Ca^{2+}]_{ER}$ imaging showed that ω-agatoxin IVA, an antagonist of these channels,

prevented ER Ca^{2+} release. This indicates a close link between the P/Q-type Ca_v channel and ER Ca^{2+} release. It was therefore hypothesised that RyRs may respond to Ca^{2+} influx through P/Q-type Ca_v channels, stimulating glucagon secretion through CICR. It was observed that, indeed, ryanodine exerted a inhibitory effect similar to ω-agatoxin IVA and when applied together the effect on secretion was not additive.

These data expand our understanding of the pathways through which glucose is able to modulate α-cell activity in mouse islets. However, given the species differences already known (as discussed above) it will be important to validate these findings in human islets. Cyclic AMP has also been indicated as a secondary messenger linking glucose to glucagon secretion [142], in addition to its role in paracrine and adrenal signalling [19], [20], [74], [138], [161]. It would therefore be interesting to investigate the relationship between cAMP and ER Ca^{2+}, especially given the potentiating effect of cAMP on CICR via RyRs [106], [167], [261].

3.1.3 A model for ER-mediated glucose regulation of α-cell glucagon secretion

From our data we propose a model for the glucose regulation of glucagon secretion mediated by the ER. At high glucose,

ATP:ADP is high and, consequently, SERCA activity is high, sequestering Ca^{2+} from the cytoplasm, into the ER lumen, reducing availability for exocytosis. High ATP:ADP also closes K_{ATP} channels, depolarising the membrane and inactivating Na_v channels, lowering action potential height. This limits P/Q-type Ca_v channel activity and subsequent CICR, restricting exocytosis. At low glucose, ATP:ADP is low and, consequently, SERCA activity is low, reducing Ca^{2+} sequestration into the ER and allowing buildup of $[Ca^{2+}]_i$. Due to partial closure of K_{ATP} channels, action potentials generation occurs to height allowing opening of P/Q-type Ca_v, facilitating Ca^{2+} influx into the cytosol. This influx triggers release of ER Ca^{2+} through RyRs, sensitised by an increase in cytoplasmic cAMP, stimulates glucagon secretion.

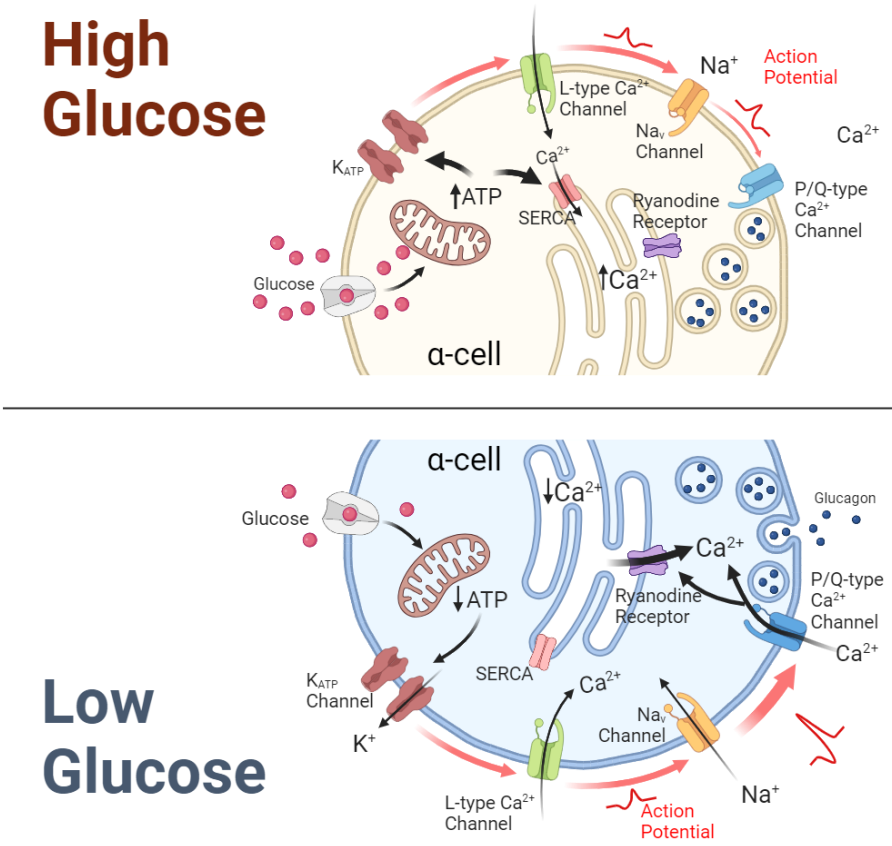


Figure 7: A model for ER-mediated glucose regulation of glucagon secretion. At high glucose concentrations (upper panel), a high concentration of ATP closes K_{ATP} channels, depolarising the membrane and leading to a reduction in action potential height due to inactivation of voltage-gated ion channels. Cytosolic Ca^{2+} influx is sequestered into the ER by ATP-driven SERCA activity. As a consequence, glucagon secretion is low. At low glucose (lower panel), K_{ATP} channels are partially open, polarising the membrane. Action potentials are generated, reaching a voltage where P/Q-type Ca_v channels are able to open and the resultant Ca^{2+} influx triggers CICR via RyRs, which triggers exocytosis. As a consequence, glucagon secretion is high. Created with BioRender.com

3.2 Paper II: GLP-1 metabolite GLP-1$^{(9-36)}$ is a systemic 1 inhibitor of mouse and human pancreatic islet glucagon secretion

3.2.1 GLP-1$^{(9-36)}$ inhibits glucagon but not insulin or somatostatin

GLP-1 is a strong potentiator of insulin secretion and inhibitor of glucagon secretion, as mentioned above [137]. However, GLP-1$^{(7-36)}$ is quickly degraded to GLP-1$^{(9-36)}$ by DDP4, with only between 10 and 15% of the GLP-1$^{(7-36)}$ secreted by intestinal L-cells reaching islets [137]. Given the low amounts of GLP-1$^{(7-36)}$ that reach the α-cells, it was hypothesised that GLP-1$^{(9-36)}$ exerts a glucagonostatic effect, as data suggests it may be able to inhibit hepatic glucose production, independent of insulin modulation [157], although there is variability in responses reported [158], [159].

Secretion of glucagon from both mouse and human isolated islets at low glucose was inhibited by the application of both GLP-1$^{(9-36)}$ and GLP-1$^{(7-36)}$, in a dose dependant manner. While GLP-1$^{(7-36)}$ stimulated the secretion of somatostatin, and insulin, as has been previously demonstrated [152], [262], GLP-1$^{(9-36)}$ directly inhibited glucagon secretion without

significantly alterations in insulin or somatostatin secretion. Previous data gathered on the action of GLP-$1^{(9-36)}$ on glycaemic hormone regulation has been performed *in vivo* in humans, and are therefore subject to a host of confounding factors, which may explain the variation observed [158], [159], [178]. The published data indicates that this is the first assessment of GLP-$1^{(9-36)}$ directly on isolated islets, in the absence of endogenous GLP-$1^{(9-36)}$ that may obscure results *in vivo*.

3.2.2 GLP-$1^{(9-36)}$ acts via the glucagon receptor

The GLP-1R is expressed by α-, β-, and δ-cells, although expression is much lower in α-cells [80], [81] and there has been some dispute as to whether GLP-$1^{(7-36)}$ is able to act directly on α-cells [19], [136], [149]–[151]. It was therefore considered that it was possible that the two GLP-1 isoforms acted upon different receptors. In GLP-1R KO islets, and in WT islets treated with the GLP-1R antagonist exendin$^{(9-39)}$, the glucagonostatic effects of both GLP-$1^{(9-36)}$ and GLP-$1^{(7-36)}$ isoforms were retained. However, GLP-$1^{(7-36)}$ signalling could be abolished in GLP-1R KO islets with inhibition of DDP4, using sitagliptin [263]. This implies that the GLP-1R-independant effects of GLP-$1^{(7-36)}$ occur only after degradation to GLP-$1^{(9-36)}$.

The GLP-1 and glucagon receptors share substantial homology, with glucagon able to activate β-cells by binding to either GcgRs or GLP-1Rs [89]. REMD2.59, a competitive GcgR antagonist that is unable to bind to GLP-1Rs [264], prevented GLP-$1^{(9-36)}$-induced suppression of glucagon secretion. Tests were undertaken to determine whether the GcgRs may be the target for GLP-$1^{(9-36)}$ in islets and observed that GLP-$1^{(9-36)}$ was able to induce dose-dependant increases in cAMP activity in GcgR-expressing HEK cells. GcgRs can interact with either G_i- or G_s-proteins [265], [266]. The sensitivity of the GLP-$1^{(9-36)}$ response, in intact islets, to pertussis toxin, demonstrates that it is G_i-coupled GcgR activation that mediates the α-cell response to this peptide. However, the mechanisms governing the preferential binding to G_i- over G_s-proteins and how this interaction differs in the presence of high intra-islet glucagon remain illusive.

3.2.3 Activation of the GcgR by GLP-$1^{(9-36)}$ leads to undocking of granules and inhibition P/Q-type Ca_v channels

In α-cells, docking of glucagon granules can be stimulated by cAMP, priming them for exocytosis, [19], [139], [162] with Ca^{2+} entry through the P/Q-type Ca_v channel triggering secretion [108], [109]. It was established that GLP-$1^{(9-36)}$ activation of G_i-

coupled GcgR inhibits adenylyl cyclase; prompting further characterisation of the downstream mechanisms of cAMP suppression. The consistency of the glucagonostatic effect of GLP-$1^{(9\text{-}36)}$ in response to a wide range of stimuli, including membrane depolarisation by 70 mM K^+; amino acids; and the β-adrenoceptor agonist isoprenaline, implies that it is mediated at the point of exocytosis. Docking of glucagon-containing vesicles was inhibited and undocking was stimulated, resulting in a net reduction in granule availability for exocytosis, in both mouse and human islets. Reversal could be achieved by application of pertussis toxin or the antagonism of the GcgR, but not the GLP-1R. In T2D islets, this ability to reduce granule docking was abolished, an effect that merits further research.

In addition to reducing the number of docked granules, GLP-$1^{(9\text{-}36)}$ is also able to modulate Ca^{2+} handing. It suppresses K^+-induced increases in $[Ca^{2+}]_i$. This suppression likely occurs via inhibition of the P/Q-type Ca_v channel, through which Ca^{2+} ion flow triggers exocytosis, as ω-agatoxin IVA was as inhibitory in combination with GLP-$1^{(9\text{-}36)}$ as GLP-$1^{(9\text{-}36)}$ alone. GLP-$1^{(9\text{-}36)}$ is therefore able to suppress glucagon secretion by a combining a reduction in docked granules and restriction of P/Q Ca^{2+} influx, but this is lost in T2D, contributing to the phenotype of the disorder.

3.2.4 *In vivo*, GLP-1$^{(9-36)}$ suppresses glucagon secretion in response to insulin-induced hypoglycaemia

Previous investigations have presented conflicting data on the *in vivo* effects of GLP-1$^{(9-36)}$. However, these have focussed on euglycaemic blood glucose [157]–[159]. Investigations were undertaken to examine how the peptide affected glucagon secretion after insulin induced hypoglycaemia *in vivo*. Here, correlating with *in vitro* data, addition of exogenous GLP-1$^{(9-36)}$ was able to modestly reduce glucagon secretion and, again, was not effective in the presence of REMD2.59. It was interesting to note that plasma glucose did not appear to be affected.

3.3 Paper III: Loss of tetraspanin-7 expression reduces pancreatic β-cell exocytosis Ca^{2+} sensitivity but has limited effect on systemic metabolism

3.3.1 KO of Tspan7 reduces the Ca^{2+} sensitivity of exocytosis in mouse β-cells

Recently identified as an autoantigen for T1D, Tspan7 is expressed in islet β-cells [81], [91], [205], [210] and, while it has been demonstrated to be involved in regulation of a wide range of cellular functions, including exocytosis, in other cell types [255], [267], [268], its role in insulin secretion is not fully understood. As an autoantigen, Tspan7 may be useful as a predictor of T1D and as a potential therapeutic target in at-risk patients. It is therefore important to understand its role in the β-cell to both aid in the optimisation of those therapies and predict any potential side effects that may arise.

First, the cellular localisation of the Tspan7 protein was investigated. The Tspan7 protein was found to be located in both the cytoplasm and in dense-core vesicles of in β- and α-cells, co-localising with both insulin and glucagon, respectively.

KD of the *Tspan7* gene has previously been shown induce an increase in intracellular Ca^{2+} via modulation of β-cell L-type Ca_v channels, with which Tspan7 associates [210]. Despite this effect on $[Ca^{2+}]_i$, no differences were found in glucose tolerance or glucose stimulated insulin secretion between *Tspan7* KO mice and their littermate controls. When a closer inspection of the electrical activity of the β-cells was performed, there was no difference between KO and control or KO cells at 1mM glucose, with both electrically silent and the membrane polarised at -80 mV. At hyperglycaemic glucose concentrations, the KO cells were more depolarised, potentially due to an increase in L-type Ca_v channel conductance [210], yet the firing frequency and peak height of action potentials was unchanged. The KO cells responded slightly faster to increasing the glucose concentration to 20 mM. However, this did not translate to any enhanced secretion, with KO and control islets secreting insulin identically. While there was an increase in Ca^{2+} influx, similar to that of the L-type Ca_v channel agonist, bayK 8644 [269], the Ca^{2+} required for exocytosis in KO β-cells was 33% more than in the controls, implying that Ca^{2+} sensitivity was reduced.

3.3.2 Human β-cells are affected by TSPAN7 KD in a manner similar to mouse islets and have changes in gene expression

Psuedo-islets are made by dispersing and reaggregating islets, retaining the function of intact islets [270], allowing for efficient treatment of individual cells, such as with shRNA to create a genetic KD. Secretory response to glucose from TSPAN7 KD was identical to control pseudo-islets but reduced in response to application of a high concentration of K^+. When exocytosis was examined in KD and control cells, influx of Ca^{2+} was increased in KD cells, while exocytosis in response to depolarisation was identical.

Subsequent investigations into gene expression changes in human β-cells revealed a reduction in the transcription of the Ca^{2+}-sensitive exocytosis-regulating protein SYT7 and an increase in *CACNA1A*, which encodes the P/Q-type Ca_v channel, the exocytosis-relevant channel. With the importance of SYT7, as a Ca^{2+} sensor, to β-cell exocytosis, [60], [61], [63], diminished expression of the protein most likely plays a key role in the loss of Ca^{2+} sensitivity observed in KO β-cells. This may be a result of Ca^{2+}-dependant transcriptional regulation but also potentially implies a role for Tspan7 in the assembly of exocytotic machinery. Genes

involved in apoptosis were also upregulated, potentially due to the proapoptotic effects of elevated cytosolic Ca^{2+} consequent to enhanced Ca_v channel activity. However, this did not appear to significantly affect β-cells, with islets exhibiting normal insulin content.

The two alterations in β-cell Ca^{2+} handling observed in this study – loss of exocytosis Ca^{2+} sensitivity and increase in transmembrane Ca^{2+} influx – appear to balance out, resulting in a similar secretory phenotype across KD and control cells. Clearly, further questions remain regarding the role of Tspan7 in the β-cell, as well as other islet cells, particularly given its involvement in Ca^{2+} regulation.

3.4 Paper IV: A Mechanism for Rapid Cross-talk between Pancreatic α- and δ-cells and its Role in Hypoglycaemia-induced Glucagon Secretory Failure

Recurrent hypoglycaemia is a major complication facing patients with diabetes and stems from dysregulation of proper glucose counter-regulation. Proper islet function requires somatostatin [164], [271]. However, excessive somatostatin secretion has been implicated as a major causative factor of recurrent hypoglycaemia, resulting in inhibition of proper glucagon secretion and somatostatin receptor antagonists have been shown to restore some glucagon response [215], [245]–[247]. While the communication between δ- and β-cells has been quite extensively studied [16], [172], [175], [272], much less is known about how α- and δ-cells interact. In this paper, we dissected the mechanisms underlying the α-δ-cell interaction within islets and studied how it becomes altered in response to hypoglycaemia.

3.4.1 α-cells are under tonic inhibition by δ-cells

The close relationship between α- and δ-cells can be seen when islets undergo immunofluorescence staining for

somatostatin and glucagon; almost all δ-cells are adjacent to at least one α-cell, with more than half of α-cells adjacent to a δ-cell. δ-cell intrinsic activity is glucose dependent, and exhibit low measurable activity during hypoglycaemia, unlike α-cells [166]–[168]. Much previous dissection of the δ-α interaction has therefore been focussed on suppression of glucagon secretion at euglycaemic concentrations and higher [73], [74], [273]. Antagonism of the somatostatin receptor with CYN at hypoglycaemic glucose concentrations, however, not only enhanced glucagon secretion and α-cell calcium activity in isolated islets, but also prevented spontaneous repolarisation of the plasma membrane. This builds upon data demonstrating that α-cells are under tonic inhibition by δ-cells at all glucose concentrations [176], [274].

Somatostatin secretion is lowest during hypoglycaemia. However, SSTR blockers have a strong stimulatory effect on glucagon secretion at low glucose. It is possible that somatostatin secretion is specifically targeted towards α-cells. It was therefore important to not only understand how α-cells are able to stimulate δ-cells, but how the δ-cells were able to specifically respond to active α-cells and whether any coupling was electrical or paracrine in nature. β-cells are coupled to δ-cells both electrically, via gap junctions, and in a paracrine manner [16], [172], [175], [272]. Depolarisation of

α-cells, using electrophysiology in the patch-imaging setup, lead to activation of δ-cells in proportion with exocytosis and not with the extend of depolarisation. Furthermore, the gap junction permeable dye propidium iodide, supplemented into the pipette-filling solution, was not able to permeate into neighbouring δ-cells. Together these indicate that α-cells are are able to activate δ-cells at low glucose concentrations directly and are connected to δ-cells via paracrine signalling, not via gap junctions.

3.4.2 Glucagon and glutamate mediate the α- to δ-cell connection

It has long been clear that, glucagon is able enhance somatostatin secretion [275], [276] and our data support this. Interestingly, glutamate, also secreted by α-cells [145], enhanced both δ-cell Ca^{2+} activity and somatostatin secretion. We observed that inhibitors of both the GcgR and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), a glutaminergic ion channel expressed by δ-cells [81], [91], [177], were able to suppress δ-cell Ca^{2+} activity and somatostatin secretion at 1 mM glucose. Other α-cell secretory factors postulated to stimulate δ-cell activity, such as GLP-1 and GRPP, did not appear to be involved in modulation of δ-cell activity during hypoglycaemia [80], [81].

Rapid local activation of glutamate, or an AMPAR agonist, onto islets elicited an immediate δ-cell Ca^{2+} response. By comparison, puffing glucagon did not elicit a direct islet response. Furthermore, δ-cell activation during patch imaging could be eliminated with the non-competitive AMPAR inhibitor CP465022. It can therefore be surmised that glutamate is responsible for the fast activation of δ-cells. As ion channels, ionotropic glutamate receptors are able to respond much faster than, G-protein related, GcgRs, which activate downstream PKA-, EPAC2-, and G_q-dependent signalling pathways that trigger somatostatin secretion by RyR- and/or IP_3R-mediated ER Ca^{2+} mobilisation [165], [273].

3.4.3 The α-δ-cell connection is enhanced by hypoglycaemia

On average patients with diabetes experience one or two episodes of symptomatic hypoglycaemia per week and a severe episode once per year [277]. A severe hypoglycaemic episode was the strongest predictor for future hypoglycaemic events [233], [278] with a reduced response to further hypoglycaemia evident after only 24 hours [279]. This was simulated, both *in vitro* and *in vivo* by exposing mice, or islets, to a hypoglycaemic episode before performing functional assessments in response to a second episode. *In vivo*, glucagon secretion, in response to insulin-induced

hypoglycaemia, was suppressed and, as a result, plasma glucose remained below the control levels in mice that has previously experienced hypoglycaemia. Blockade of somatostatin signalling, was able to alleviate this phenotype, as has been shown previously [215], [245]. In patch imaging experiments, this enhanced δ-cell response to α-cell activation and also reduced the secretion of glucagon, correlating with a left shift in the sensitivity of δ-cells to glucagon and an increased response to glutamate. Through specific activation of α-cells, using the αhM3Dq^{+} and αChR2^{+} models, and antagonism of glucagon receptors, with glucagon amide and REMD, during the conditioning period it was eveident that increased δ-cell sensitivity was specifically due to the secretion of glucagon, not to the reduction in the extracellular glucose concentration *per se* or other external factors. Furthermore, hypoglycaemic treatment also lead to an increase in the length of δ-cell filopodia, highly dynamic neuron-like extensions [165], [237]. This likely increases contact between δ- and α- cells, already increased in patients with diabetes due to loss β-cells [243], [244], increasing the strength of the paracrine interaction.

What remains unclear is the precise mechanism through which activation of glucagon receptors is able to induce an increase in the δ-cell response to α-cell secretory factors. In

hepatocytes, glucagon secretion stimulates phosphorylation of cAMP response element binding (CREB) protein, causing translocation of the protein to the nucleus, where it acts as a transcription factor [280]. Targets of phosphorylated CREB include BDNF, mTOR, somatostatin, and the glucagon receptor [281]–[283]. BDNF has been implicated in the dendritic growth [284] and causes AMPAR translocation to the membrane in neurons [285]. It is therefore possible that CREB phosphorylation may play a role in the sensitisation of δ-cells to α-cell activity.

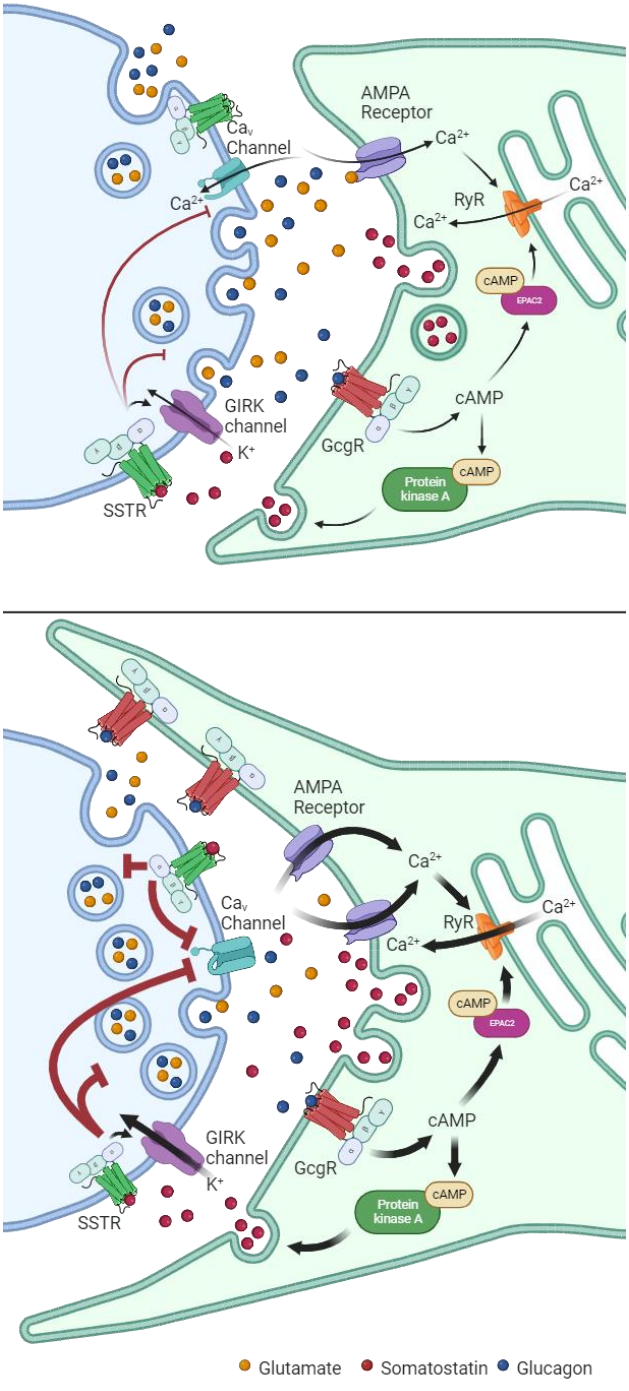
Understanding these mechanisms may aid the design of novel therapies to prevent the development of recurrent hypoglycaemia that could greatly improving the quality of life for patients suffering with the disorder.

3.4.4 A model for the α-δ-cell crosstalk and the suppression of glucagon secretion during recurrent hypoglycaemia

During hypoglycaemia, α-cells become activated; glucagon and glutamate are secreted, activating δ-cell GcgRs and AMPARs, respectively. Upon activation, AMPARs open, allowing the influx of Ca^{2+} into the cytosol, and GcgRs stimulate either adenylyl cyclases ($G_{\alpha s}$), increasing cAMP production, and/or PLC ($G_{\alpha q}$). Elevated intracellular cAMP

levels stimulate PKA-mediated exocytosis and Epac2-mediated ER-Ca^{2+} release via RyRs, while PLC activation triggers IP_3R-mediated ER-Ca^{2+} release. PKA-mediated exocytosis together with the combination of Ca^{2+} influx via AMPAR, which may act to depolarise the membrane, and GcgR-mediated ER Ca^{2+} release enhances somatostatin secretion. This somatostatin activates α-cell SSTRs, inhibiting cAMP production and Ca^{2+} influx, suppressing secretion of glucagon.

After an episode of hypoglycaemia, δ-cells become sensitised to the α-cell secretory factors glucagon and glutamate, lowering the threshold for activation. δ-cell filopodia also extend, enhancing the surface area of δ-α-cell contact and, consequently, δ-cell control of neighbouring α-cells is enhanced. During subsequent hypoglycaemic episodes, enhanced somatostatin secretion across a greater surface area of contact effectively shuts down the glucagon secretory response, exacerbating hypoglycaemia.


AMPA
Receptor
Ca2+
Cav
Channel
Ca2+
RyR
Ca2+
Ca2+
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EPAC2
GIRK
channel
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GcgR
Protein
kinase A
cAMP
SSTR
AMPA
Receptor
Ca2+
Cav
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RyR
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cAMP
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channel
K+
cAMP
GcgR
Protein
kinase A
cAMP
SSTR
Glutamate
Somatostatin
Glucagon

Figure 8: A model for the mechanisms of α-δ-cell crosstalk and the metabolic memory. During hypoglycaemia (upper panel), α-cells secrete glucagon and glutamate. Glucagon activates GcgRs that increase δ-cell cAMP concentrations, activating PKA and leading to increased exocytosis. cAMP also activates EPAC2, which sensitises RyRs to Ca^{2+}. Glutamate activates AMPARs, leading to an increase in Ca^{2+} influx that triggers CICR via RyRs. As a result, somatostatin is secreted, inhibiting α-cell secretion. During subsequent hypoglycaemic events (lower panel), δ-cell filopodia have extended and the δ-cell is sensitised to α-cell factors. This inhibits glucagon secretion, exacerbating hypoglycaemia. Created with BioRender.com

4 CONCLUSIONS

In this thesis, I have illuminated novel mechanisms by which the hormones secreted by the cells that make up the islets of Langerhans are regulated. In particular, it has been demonstrated that:

I. The ER is key to the α-cell response to glucose. It can regulate intracellular Ca^{2+}, and consequent exocytosis, in response to changes in glucose and, while it's actions are independent of membrane electrical activity, CICR can be triggered by influx of Ca^{2+} through P/Q-type Ca_v channels.

II. GLP-$1^{(9-36)}$ acts to inhibit glucagon secretion, at low glucose, by acting directly on α-cell GcgRs, leading to a reduction in docked granules and closure of P/Q-type Ca_v channels.

III. KO of *Tspan7* increased the expression of *CACNA1A*, encoding the membrane-bound P/Q-type Ca_v channel,

and SYT7, encoding the exocytosis-related Ca^{2+} sensor synaptotagmin 7, resulting in changes to β-cell Ca^{2+} handling and a reduction in the sensitivity of exocytosis to Ca^{2+}.

IV. At low glucose, α-cell secretion of glucagon and glutamate stimulates activation of δ-cells and the secretion of somatostatin, which subsequently inhibits glucagon secretion, in a paracrine feedback loop. Hypoglycaemia sensitises δ-cells to the α-cell factors, enhancing somatostatin secretion, which, during subsequent hypoglycaemic episodes, results in the suppression of glucagon secretion and exacerbation of hypoglycaemia.

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REFERENCES

[1] J. M. Berg, J. L. Tymoczko, and L. Stryer, *Biochemistry*, 7th ed., I. New York (United States): W.H. Freeman, 2012.

[2] M. Brissova *et al.*, "Assessment of Human Pancreatic Islet Architecture and Composition by Laser Scanning Confocal Microscopy," *J. Histochem. Cytochem.*, vol. 53, no. 9, pp. 1087–1097, Sep. 2005, doi: 10.1369/jhc.5C6684.2005.

[3] O. Cabrera, D. M. Berman, N. S. Kenyon, C. Ricordi, P. O. Berggren, and A. Caicedo, "The unique cytoarchitecture of human pancreatic islets has implications for islet cell function," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 103, no. 7, pp. 2334–2339, 2006, doi: 10.1073/pnas.0510790103.

[4] D. J. Steiner, A. Kim, K. Miller, and M. Hara, "Pancreatic islet plasticity: Interspecies comparison of islet architecture and composition," *Islets*, vol. 2, no. 3, pp. 135–145, 2010, doi: 10.4161/isl.2.3.11815.

[5] P. Rorsman and E. Renström, "Insulin granule dynamics in pancreatic beta cells," *Diabetologia*, vol. 46, no. 8, pp. 1029–1045, 2003, doi: 10.1007/s00125-003-1153-1.

[6] W. Y. Fujimoto, J. W. Ensinck, and R. H. Williams, "Somatostatin inhibits insulin and glucagon release by monolayer cell cultures of rat endocrine pancreas," *Life Sci.*, vol. 15, no. 11, pp. 1999–2004, Dec. 1974, doi: 10.1016/0024-3205(74)90050-2.

[7] K. G. M. M. Alberti *et al.*, "INHIBITION OF INSULIN SECRETION BY SOMATOSTATIN," *Lancet*, vol. 302, no. 7841, pp. 1299–1301, Dec. 1973, doi: 10.1016/S0140-6736(73)92873-0.

[8] L. Orci *et al.*, "Hypertrophy and hyperplasia of somatostatin-containing D-cells in diabetes.," *Proc. Natl. Acad. Sci.*, vol. 73, no. 4, pp. 1338–1342, Apr. 1976, doi: 10.1073/pnas.73.4.1338.

[9] R. H. Unger and A. D. Cherrington, "Glucagonocentric restructuring of diabetes: A pathophysiologic and therapeutic makeover," *J. Clin. Invest.*, vol. 122, no. 1, pp. 4–12, 2012, doi: 10.1172/JCI60016.

[10] F. G. Banting, Best, Collip, Campbell, and Fletcher, "Pancreatic Extracts in Treatment of Diabetes Mellitus," *J.*

Nerv. Ment. Dis., vol. 59, no. 1, p. 73, Jan. 1924, doi: 10.1097/00005053-192401000-00013.

[11] L. Orci and R. H. Unger, "Functional Subdivision of Islets of Langerhans and Possible Role of D Cells," *Lancet*, vol. 306, no. 7947, pp. 1243–1244, 1975, doi: 10.1016/S0140-6736(75)92078-4.

[12] A. Wojtusciszyn, M. Armanet, P. Morel, T. Berney, and D. Bosco, "Insulin secretion from human beta cells is heterogeneous and dependent on cell-to-cell contacts," *Diabetologia*, vol. 51, no. 10, pp. 1843–1852, 2008, doi: 10.1007/s00125-008-1103-z.

[13] F. M. Ashcroft and P. Rorsman, "Elecrophysiology of Pancreatic Islet Cells," in *The Electrophysiology of Neuroendocrine Cells*, H. Scherübl and J. Hescheler, Eds. Boca Raton, FL, USA: CRC Press, 1995, pp. 207–243.

[14] S. O. Göpel, T. Kanno, S. Barg, and P. Rorsman, "Patch-clamp characterisation of somatostatin-secreting δ-cells in intact mouse pancreatic islets," *J. Physiol.*, vol. 528, no. 3, pp. 497–507, 2000, doi: 10.1111/j.1469-7793.2000.00497.x.

[15] L. J. B. Briant *et al.*, "Functional identification of islet cell types by electrophysiological fingerprinting," *J. R. Soc. Interface*, vol. 14, no. 128, 2017, doi: 10.1098/rsif.2016.0999.

[16] C. Li, P. Chen, J. Vaughan, K. F. Lee, and W. Vale, "Urocortin 3 regulates glucose-stimulated insulin secretion and energy homeostasis," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 104, no. 10, pp. 4206–4211, 2007, doi: 10.1073/pnas.0611641104.

[17] J. N. Walker, R. D. Ramracheya, Q. Zhang, P. R. V Johnson, M. Braun, and P. Rorsman, "Regulation of glucagon secretion by glucose: paracrine, intrinsic or both?," *Diabetes, Obes. Metab.*, vol. 13, pp. 95–105, Oct. 2011, doi: 10.1111/j.1463-1326.2011.01450.x.

[18] F. C. Schuit and D. G. Pipeleers, "Differences in adrenergic recognition by pancreatic A and B cells," *Science (80-.).*, vol. 232, no. 4752, pp. 875–877, 1986, doi: 10.1126/science.2871625.

[19] Y. Z. de Marinis *et al.*, "GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+ channel-dependent exocytosis," *Cell Metab.*, vol. 11, no. 6, pp. 543–553, Jun. 2010, doi: 10.1016/j.cmet.2010.04.007.

[20] L. L. Baggio and D. J. Drucker, "Biology of Incretins: GLP-1 and GIP," *Gastroenterology*, vol. 132, no. 6, pp. 2131–2157, 2007, doi: 10.1053/j.gastro.2007.03.054.

[21] K. Kawai, C. Yokota, S. Ohashi, Y. Watanabe, and K. Yamashita, "Evidence that glucagon stimulates insulin secretion through its own receptor in rats," *Diabetologia*, vol. 38, no. 3, pp. 274–276, 1995, doi: 10.1007/BF00400630.

[22] B. Thorens, "GLUT2, glucose sensing and glucose homeostasis," *Diabetologia*, vol. 58, no. 2, pp. 221–232, 2015, doi: 10.1007/s00125-014-3451-1.

[23] J. H. Johnson, C. B. Newgard, J. L. Milburn, H. F. Lodish, and B. Thorens, "The high K(m) glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence," *J. Biol. Chem.*, vol. 265, no. 12, pp. 6548–6551, 1990, doi: 10.1016/s0021-9258(19)39181-1.

[24] M. T. Guillam, P. Dupraz, and B. Thorens, "Glucose uptake, utilization, and signaling in GLUT2-null islets.," *Diabetes*, vol. 49, no. 9, pp. 1485–1491, Sep. 2000, doi: 10.2337/diabetes.49.9.1485.

[25] M.-T. Guillam *et al.*, "Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2," *Nat. Genet.*, vol. 17, no. 3, pp. 327–330, Nov. 1997, doi: 10.1038/ng1197-327.

[26] A. De Vos *et al.*, "Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression," *J. Clin. Invest.*, vol. 96, no. 5, pp. 2489–2495, 1995, doi: 10.1172/JCI118308.

[27] A. Pingitore, I. Ruz-Maldonado, B. Liu, G. C. Huang, P. Choudhary, and S. J. Persaud, "Dynamic Profiling of Insulin Secretion and ATP Generation in Isolated Human and Mouse Islets Reveals Differential Glucose Sensitivity," *Cell. Physiol. Biochem.*, vol. 44, no. 4, pp. 1352–1359, 2017, doi: 10.1159/000485532.

[28] L. J. McCulloch, M. van de Bunt, M. Braun, K. N. Frayn, A. Clark, and A. L. Gloyn, "GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: Implications for understanding genetic association signals at this locus," *Mol. Genet. Metab.*, vol. 104, no. 4, pp. 648–653, 2011, doi: 10.1016/j.ymgme.2011.08.026.

[29] F. H. Sansbury *et al.*, “SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion,” *Diabetologia*, vol. 55, no. 9, pp. 2381–2385, Sep. 2012, doi: 10.1007/s00125-012-2595-0.

[30] M. D. Meglasson and F. M. Matschinsky, “New perspectives on pancreatic islet glucokinase.,” *Am. J. Physiol.*, vol. 246, no. 1 Pt 1, 1984.

[31] F. M. Matschinsky, “Regulation of Pancreatic β-Cell Glucokinase,” *Diabetes*, vol. 51, no. suppl_3, pp. S394–S404, Dec. 2002, doi: 10.2337/diabetes.51.2007.S394.

[32] S. J. Ashcroft, L. C. Weerasinghe, and P. J. Randle, “Interrelationship of islet metabolism, adenosine triphosphate content and insulin release.,” *Biochem. J.*, vol. 132, no. 2, pp. 223–231, 1973, doi: 10.1042/bj1320223.

[33] Y. Terauchi *et al.*, “Pancreatic β-cell-specific targeted disruption of glucokinase gene: Diabetes mellitus due to defective insulin secretion to glucose,” *J. Biol. Chem.*, vol. 270, no. 51, pp. 30253–30256, 1995, doi: 10.1074/jbc.270.51.30253.

[34] F. M. Ashcroft, D. E. Harrison, and S. J. H. Ashcroft, “Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells,” *Nature*, vol. 312, no. 5993, pp. 446–448, Nov. 1984, doi: 10.1038/312446a0.

[35] L. Aguilar-Bryan *et al.*, “Cloning of the β Cell High-Affinity Sulfonylurea Receptor: a Regulator of Insulin Secretion,” *Science (80-.).*, vol. 268, no. 5209, pp. 423–426, Apr. 1995, doi: 10.1126/science.7716547.

[36] S.-L. Shyng and C. G. Nichols, “Octameric Stoichiometry of the KATP Channel Complex,” *J. Gen. Physiol.*, vol. 110, no. 6, pp. 655–664, Dec. 1997, doi: 10.1085/jgp.110.6.655.

[37] H. Sakura, C. Ämmälä, P. A. Smith, F. M. Gribble, and F. M. Ashcroft, “Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic β-cells, brain, heart and skeletal muscle,” *FEBS Lett.*, vol. 377, no. 3, pp. 338–344, 1995, doi: 10.1016/0014-5793(95)01369-5.

[38] S. J. Tucker *et al.*, “Molecular determinants of KATP channel inhibition by ATP.,” *EMBO J.*, vol. 17, no. 12, pp. 3290–6, Jun. 1998, doi: 10.1093/emboj/17.12.3290.

[39] F. M. Gribble, S. J. Tucker, and F. M. Ashcroft, “The essential

role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide.," *EMBO J.*, vol. 16, no. 6, pp. 1145–52, Mar. 1997, doi: 10.1093/emboj/16.6.1145.

[40] P. A. Smith, F. M. Ashcroft, and P. Rorsman, "Simultaneous recordings of glucose dependent electrical activity and ATP-regulated K + -currents in isolated mouse pancreatic β-cells," *FEBS Lett.*, vol. 261, no. 1, pp. 187–190, Feb. 1990, doi: 10.1016/0014-5793(90)80667-8.

[41] S. O. Göpel, T. Kanno, S. Barg, J. Galvanovskis, and P. Rorsman, "Voltage-gated and resting membrane currents recorded from B-cells in intact mouse pancreatic islets," *J. Physiol.*, vol. 521, no. 3, pp. 717–728, Dec. 1999, doi: 10.1111/j.1469-7793.1999.00717.x.

[42] S. Misler, W. M. Gee, K. D. Gillis, D. W. Scharp, and L. C. Falke, "Metabolite-regulated ATP-sensitive K+ channel in human pancreatic islet cells," *Diabetes*, vol. 38, no. 4, pp. 422–427, 1989, doi: 10.2337/diab.38.4.422.

[43] J. C. Henquin and H. P. Meissner, "Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells," *Experientia*, vol. 40, no. 10, pp. 1043–1052, 1984, doi: 10.1007/BF01971450.

[44] P. Rorsman and G. Trube, "Calcium and delayed potassium currents in mouse pancreatic beta-cells under voltage-clamp conditions.," *J. Physiol.*, vol. 374, no. 1, pp. 531–550, May 1986, doi: 10.1113/jphysiol.1986.sp016096.

[45] F. M. Ashcroft and P. Rorsman, "Electrophysiology of the pancreatic β-cell," *Prog. Biophys. Mol. Biol.*, vol. 54, no. 2, pp. 87–143, 1989, doi: 10.1016/0079-6107(89)90013-8.

[46] P. Gilon, J. Yakel, J. Gromada, Y. Zhu, J. C. Henquin, and P. Rorsman, "G protein-dependent inhibition of L-type Ca2+ currents by acetylcholine in mouse pancreatic B-cells," *J. Physiol.*, vol. 499, no. 1, pp. 65–76, 1997, doi: 10.1113/jphysiol.1997.sp021911.

[47] V. Schulla *et al.*, "Impaired insulin secretion and glucose tolerance in β cell-selective Cav1.2 Ca2+ channel null mice," *EMBO J.*, vol. 22, no. 15, pp. 3844–3854, 2003, doi: 10.1093/emboj/cdg389.

[48] S. O. Göpel *et al.*, "Capacitance measurements of exocytosis in mouse pancreatic α-, β- and δ-cells within intact islets of Langerhans," *J. Physiol.*, vol. 556, no. 3, pp. 711–726, 2004,

doi: 10.1113/jphysiol.2003.059675.
[49] M. Braun *et al.*, "Voltage-gated ion channels in human pancreatic β-cells: Electrophysiological characterization and role in insulin secretion," *Diabetes*, vol. 57, no. 6, pp. 1618–1628, 2008, doi: 10.2337/db07-0991.
[50] T. D. Plant, "Na+ currents in cultured mouse pancreatic B-cells," *Pfl*gers Arch. Eur. J. Physiol.*, vol. 411, no. 4, pp. 429–435, Apr. 1988, doi: 10.1007/BF00587723.
[51] Q. Zhang *et al.*, "Na+ current properties in islet α- and β-cells reflect cell-specific Scn3a and Scn9a expression," *J. Physiol.*, vol. 592, no. 21, pp. 4677–4696, 2014, doi: 10.1113/jphysiol.2014.274209.
[52] L. S. Milescu, T. Yamanishi, K. Ptak, M. Z. Mogri, and J. C. Smith, "Real-Time Kinetic Modeling of Voltage-Gated Ion Channels Using Dynamic Clamp," *Biophys. J.*, vol. 95, no. 1, pp. 66–87, Jul. 2008, doi: 10.1529/biophysj.107.118190.
[53] D. W. Barnett, D. M. Pressel, and S. Misler, "Voltage-dependent Na+ and Ca2+ currents in human pancreatic islet β-cells: evidence for roles in the generation of action potentials and insulin secretion," *Pflügers Arch. Eur. J. Physiol.*, vol. 431, no. 2, pp. 272–282, 1995, doi: 10.1007/BF00410201.
[54] K. Bokvist, L. Eliasson, C. Ammälä, E. Renström, and P. Rorsman, "Co-localization of L-type Ca2+ channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic B-cells.," *EMBO J.*, vol. 14, no. 1, pp. 50–57, Jan. 1995, doi: 10.1002/j.1460-2075.1995.tb06974.x.
[55] S. Barg *et al.*, "Fast exocytosis with few Ca2+ channels in insulin-secreting mouse pancreatic B cells," *Biophys. J.*, vol. 81, no. 6, pp. 3308–3323, 2001, doi: 10.1016/S0006-3495(01)75964-4.
[56] C. Ammälä, L. Eliasson, K. Bokvist, O. Larsson, F. M. Ashcroft, and P. Rorsman, "Exocytosis elicited by action potentials and voltage-clamp calcium currents in individual mouse pancreatic B-cells.," *J. Physiol.*, vol. 472, no. 1, pp. 665–688, Dec. 1993, doi: 10.1113/jphysiol.1993.sp019966.
[57] O. Wiser *et al.*, "The voltage sensitive Lc-type Ca2+ channel is functionally coupled to the exocytotic machinery," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 96, no. 1, pp. 248–253, 1999,

doi: 10.1073/pnas.96.1.248.

[58] C. S. Olofsson *et al.*, "Fast insulin secretion reflects exocytosis of docked granules in mouse pancreatic B-cells," *Pflügers Arch.*, vol. 444, no. 1–2, pp. 43–51, May 2002, doi: 10.1007/s00424-002-0781-5.

[59] R. Regazzi *et al.*, "VAMP-2 and cellubrevin are expressed in pancreatic β-cells and are essential for Ca2+ - but not for GTPγS-induced insulin secretion," *EMBO J.*, vol. 14, no. 12, pp. 2723–2730, 1995, doi: 10.1002/j.1460-2075.1995.tb07273.x.

[60] M. Ohara-Imaizumi *et al.*, "Imaging analysis reveals mechanistic differences between first- and second-phase insulin exocytosis," *J. Cell Biol.*, vol. 177, no. 4, pp. 695–705, May 2007, doi: 10.1083/jcb.200608132.

[61] P. P. L. Lam *et al.*, "Transgenic Mouse Overexpressing Syntaxin-1A as a Diabetes Model," *Diabetes*, vol. 54, no. 9, pp. 2744–2754, Sep. 2005, doi: 10.2337/diabetes.54.9.2744.

[62] Y. Kang *et al.*, "Syntaxin-3 and syntaxin-1A inhibit L-type calcium channel activity, insulin biosynthesis and exocytosis in beta-cell lines," *Diabetologia*, vol. 45, no. 2, pp. 231–241, Feb. 2002, doi: 10.1007/s00125-001-0718-0.

[63] B. R. Gauthier and C. B. Wollheim, "Synaptotagmins bind calcium to release insulin," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 295, no. 6, 2008, doi: 10.1152/ajpendo.90568.2008.

[64] N. Gustavsson *et al.*, "Impaired insulin secretion and glucose intolerance in synaptotagmin-7 null mutant mice," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 10, pp. 3992–3997, 2008, doi: 10.1073/pnas.0711700105.

[65] Q. Zhou *et al.*, "Architecture of the synaptotagmin-SNARE machinery for neuronal exocytosis," *Nature*, vol. 525, no. 7567, pp. 62–67, 2015, doi: 10.1038/nature14975.

[66] V. Serre-Beinier *et al.*, "Cx36 preferentially connects beta-cells within pancreatic islets.," *Diabetes*, vol. 49, no. 5, pp. 727–734, May 2000, doi: 10.2337/diabetes.49.5.727.

[67] M. a Ravier *et al.*, "Loss of Connexin36 Channels Alters β-Cell Coupling, Islet Synchronization of Glucose-Induced Ca2+ and Insulin Oscillations, and Basal Insulin Release," *Diabetes*, vol. 54, no. 6, pp. 1798–1807, Jun. 2005, doi: 10.2337/diabetes.54.6.1798.

[68] R. M. Santos, L. M. Rosario, A. Nadal, J. Garcia-Sancho, B. Soria, and M. Valdeolmillos, "Widespread synchronous [Ca2+]i oscillations due to bursting electrical activity in single pancreatic islets," *Pflügers Arch. Eur. J. Physiol.*, vol. 418, no. 4, pp. 417–422, 1991, doi: 10.1007/BF00550880.

[69] W. S. Head, M. L. Orseth, C. S. Nunemaker, L. S. Satin, D. W. Piston, and R. K. P. Benninger, "Connexin-36 gap junctions regulate in vivo first- and second-phase insulin secretion dynamics and glucose tolerance in the conscious mouse," *Diabetes*, vol. 61, no. 7, pp. 1700–1707, 2012, doi: 10.2337/db11-1312.

[70] V. Serre-Beinier *et al.*, "Cx36 makes channels coupling human pancreatic β-cells, and correlates with insulin expression," *Hum. Mol. Genet.*, vol. 18, no. 3, pp. 428–439, Feb. 2009, doi: 10.1093/hmg/ddn370.

[71] D. J. Hodson *et al.*, "Lipotoxicity disrupts incretin-regulated human β cell connectivity," *J. Clin. Invest.*, vol. 123, no. 10, pp. 4182–4194, 2013, doi: 10.1172/JCI68459.

[72] I. Quesada *et al.*, "Glucose Induces Opposite Intracellular Ca 2+ Concentration Oscillatory Patterns in Identified α- and β-Cells Within Intact Human Islets of Langerhans," *Diabetes*, vol. 55, no. 9, pp. 2463–2469, Sep. 2006, doi: 10.2337/db06-0272.

[73] A. C. Hauge-Evans *et al.*, "Somatostatin Secreted by Islet δ-Cells Fulfills Multiple Roles as a Paracrine Regulator of Islet Function," *Diabetes*, vol. 58, no. 2, pp. 403–411, Feb. 2009, doi: 10.2337/db08-0792.

[74] A. D. Elliott, A. Ustione, and D. W. Piston, "Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic α-cell by lowering cAMP," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 308, no. 2, pp. E130–E143, 2015, doi: 10.1152/ajpendo.00344.2014.

[75] B. Kailey *et al.*, "SSTR2 is the functionally dominant somatostatin receptor in human pancreatic β- and α-cells," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 303, no. 9, pp. 1107–1116, 2012, doi: 10.1152/ajpendo.00207.2012.

[76] M. T. Dickerson *et al.*, "Gi/o protein-coupled receptor inhibition of beta-cell electrical excitability and insulin secretion depends on Na+/K+ ATPase activation," *Nat. Commun.*, vol. 13, no. 1, pp. 1–18, 2022, doi: 10.1038/s41467-

022-34166-z.

[77] W. H. Hsu, H. Xiang, A. S. Rajan, D. L. Kunze, and A. E. Boyd, "Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca2+ entry through voltage-dependent Ca2+ channels in the beta cell," *J. Biol. Chem.*, vol. 266, no. 2, pp. 837–843, 1991, doi: 10.1016/s0021-9258(17)35249-3.

[78] E. Renström, W. G. Ding, K. Bokvist, and P. Rorsman, "Neurotransmitter-induced inhibition of exocytosis in insulin-secreting β cells by activation of calcineurin," *Neuron*, vol. 17, no. 3, pp. 513–522, 1996, doi: 10.1016/S0896-6273(00)80183-X.

[79] M. Z. Strowski, R. M. Parmar, A. D. Blake, and J. M. Schaeffer, "Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: An in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice," *Endocrinology*, vol. 141, no. 1, pp. 111–117, 2000, doi: 10.1210/endo.141.1.7263.

[80] A. E. Adriaenssens *et al.*, "Transcriptomic profiling of pancreatic alpha, beta and delta cell populations identifies delta cells as a principal target for ghrelin in mouse islets," *Diabetologia*, vol. 59, no. 10, pp. 2156–2165, 2016, doi: 10.1007/s00125-016-4033-1.

[81] M. R. DiGruccio *et al.*, "Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets," *Mol. Metab.*, vol. 5, no. 7, pp. 449–458, Jul. 2016, doi: 10.1016/j.molmet.2016.04.007.

[82] M. A. Nauck *et al.*, "Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses," *J. Clin. Endocrinol. Metab.*, vol. 63, no. 2, pp. 492–498, 1986, doi: 10.1210/jcem-63-2-492.

[83] E. Samols, G. Marri, and V. Marks, "PROMOTION OF INSULIN SECRETION BY GLUCAGON," *Lancet*, vol. 286, no. 7409, pp. 415–416, Aug. 1965, doi: 10.1016/S0140-6736(65)90761-0.

[84] K. Miyawaki *et al.*, "Glucose intolerance caused by a defect in the entero-insular axis: A study in gastric inhibitory polypeptide receptor knockout mice," *Proc. Natl. Acad. Sci.*, vol. 96, no. 26, pp. 14843–14847, Dec. 1999, doi:

10.1073/pnas.96.26.14843.
[85] J. J. Holst and J. Gromada, “Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans,” *Am. J. Physiol. - Endocrinol. Metab.*, vol. 287, no. 2 50-2, 2004, doi: 10.1152/ajpendo.00545.2003.
[86] C. Ørskov, A. Wettergren, and J. J. Holst, “Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day,” *Scand. J. Gastroenterol.*, vol. 31, no. 7, pp. 665–670, 1996, doi: 10.3109/00365529609009147.
[87] P. Marchetti *et al.*, “A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets,” *Diabetologia*, vol. 55, no. 12, pp. 3262–3272, 2012, doi: 10.1007/s00125-012-2716-9.
[88] N. M. Whalley, L. E. Pritchard, D. M. Smith, and A. White, “Processing of proglucagon to GLP-1 in pancreatic α-cells: Is this a paracrine mechanism enabling GLP-1 to act on β-cells?,” *J. Endocrinol.*, vol. 211, no. 1, pp. 99–106, 2011, doi: 10.1530/JOE-11-0094.
[89] B. Svendsen *et al.*, “Insulin Secretion Depends on Intra-islet Glucagon Signaling,” *Cell Rep.*, vol. 25, no. 5, pp. 1127-1134.e2, 2018, doi: 10.1016/j.celrep.2018.10.018.
[90] K. Moens, D. Flamez, C. Van Schravendijk, Z. Ling, D. Pipeleers, and F. C. Schuit, “Dual glucagon recognition by pancreatic β-cells via glucagon and glucagon-like peptide 1 receptors,” *Diabetes*, vol. 47, no. 1, pp. 66–72, 1998, doi: 10.2337/diab.47.1.66.
[91] A. E. Adriaenssens *et al.*, “A Transcriptome-Led Exploration of Molecular Mechanisms Regulating Somatostatin-Producing D-Cells in the Gastric Epithelium,” *Endocrinology*, vol. 156, no. 11, pp. 3924–3936, Nov. 2015, doi: 10.1210/en.2015-1301.
[92] H. Sørensen *et al.*, “Glucagon receptor knockout mice display increased insulin sensitivity and impaired β-cell function,” *Diabetes*, vol. 55, no. 12, pp. 3463–3469, 2006, doi: 10.2337/db06-0307.
[93] M. E. Capozzi *et al.*, “β Cell tone is defined by proglucagon peptides through cAMP signaling,” *JCI insight*, vol. 4, no. 5, pp. 1–15, 2019, doi: 10.1172/jci.insight.126742.
[94] J. Gromada, J. J. Holst, and P. Rorsman, “Cellular regulation

of islet hormone secretion by the incretin hormone glucagon-like peptide 1," *Pflugers Arch. Eur. J. Physiol.*, vol. 435, no. 5, pp. 583–594, 1998, doi: 10.1007/s004240050558.

[95] O. Dyachok, Y. Isakov, J. Sågetorp, and A. Tengholm, "Oscillations of cyclic AMP in hormone-stimulated insulin-secreting β-cells," *Nature*, vol. 439, no. 7074, pp. 349–352, 2006, doi: 10.1038/nature04410.

[96] S. Dalle *et al.*, "Glucagon Promotes cAMP-response Element-binding Protein Phosphorylation via Activation of ERK1/2 in MIN6 Cell Line and Isolated Islets of Langerhans," *J. Biol. Chem.*, vol. 279, no. 19, pp. 20345–20355, 2004, doi: 10.1074/jbc.M312483200.

[97] E. Renström, L. Eliasson, and P. Rorsman, "Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells," *J. Physiol.*, vol. 502, no. 1, pp. 105–118, 1997, doi: 10.1111/j.1469-7793.1997.105bl.x.

[98] L. Eliasson *et al.*, "SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic b-cells," *J. Gen. Physiol.*, vol. 121, no. 3, pp. 181–197, 2003, doi: 10.1085/jgp.20028707.

[99] T. Shibasaki, Y. Sunaga, K. Fujimoto, Y. Kashima, and S. Seino, "Interaction of ATP Sensor, cAMP Sensor, $Ca2+$ Sensor, and Voltage-dependent $Ca2+$ Channel in Insulin Granule Exocytosis," *J. Biol. Chem.*, vol. 279, no. 9, pp. 7956–7961, 2004, doi: 10.1074/jbc.M309068200.

[100] N. Ozaki *et al.*, "cAMP-GEFII is a direct target of cAMP in regulated exocytosis," *Nat. Cell Biol.*, vol. 2, no. 11, pp. 805–811, 2000, doi: 10.1038/35041046.

[101] Y. Kashima *et al.*, "Critical Role of cAMP-GEFII·Rim2 Complex in Incretin-potentiated Insulin Secretion," *J. Biol. Chem.*, vol. 276, no. 49, pp. 46046–46053, 2001, doi: 10.1074/jbc.M108378200.

[102] P. E. Light, J. E. Manning Fox, M. J. Riedel, and M. B. Wheeler, "Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism," *Mol. Endocrinol.*, vol. 16, no. 9, pp. 2135–2144, 2002, doi: 10.1210/me.2002-0084.

[103] J. Gromada, K. Bokvist, W.-G. Ding, J. J. Holst, J. H. Nielsen, and P. Rorsman, "Glucagon-Like Peptide 1(7-36) Amide Stimulates Exocytosis in Human Pancreatic β-Cells by Both

Proximal and Distal Regulatory Steps in Stimulus-Secretion Coupling," *Diabetes*, vol. 47, no. 1, pp. 57–65, Jan. 1998, doi: 10.2337/diab.47.1.57.

[104] G. Kang and G. G. Holz, "Amplification of exocytosis by Ca2+-induced Ca2+ release in INS-1 pancreatic β cells," *J. Physiol.*, vol. 546, no. 1, pp. 175–189, 2003, doi: 10.1113/jphysiol.2002.029959.

[105] G. Kang *et al.*, "Epac-selective cAMP Analog 8-pCPT-2′-O-Me-cAMP as a Stimulus for Ca2+-induced Ca2+ Release and Exocytosis in Pancreatic β-Cells," *J. Biol. Chem.*, vol. 278, no. 10, pp. 8279–8285, Mar. 2003, doi: 10.1074/jbc.M211682200.

[106] G. Kang, O. G. Chepurny, and G. G. Holz, "cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca 2+ -induced Ca 2+ release in INS-1 pancreatic β-cells," *J. Physiol.*, vol. 536, no. 2, pp. 375–385, Oct. 2001, doi: 10.1111/j.1469-7793.2001.0375c.xd.

[107] P. Rorsman and B. Hellman, "Voltage-activated currents in guinea pig pancreatic α2 Cells: Evidence for Ca2+-dependent action potentials," *J. Gen. Physiol.*, vol. 91, no. 2, pp. 223–242, 1988, doi: 10.1085/jgp.91.2.223.

[108] P. E. MacDonald *et al.*, "A KATP channel-dependent pathway within α cells regulates glucagon release from both rodent and human islets of langerhans," *PLoS Biol.*, vol. 5, no. 6, pp. 1236–1247, 2007, doi: 10.1371/journal.pbio.0050143.

[109] Q. Zhang *et al.*, "Role of KATP channels in glucose-regulated glucagon secretion and impaired counterregulation in type 2 diabetes," *Cell Metab.*, vol. 18, no. 6, pp. 871–882, 2013, doi: 10.1016/j.cmet.2013.10.014.

[110] C. Bonner *et al.*, "Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion," *Nat. Med.*, vol. 21, no. 5, pp. 512–517, May 2015, doi: 10.1038/nm.3828.

[111] M. G. Pedersen, I. Ahlstedt, M. F. El Hachmane, and S. O. Göpel, "Dapagliflozin stimulates glucagon secretion at high glucose: Experiments and mathematical simulations of human A-cells," *Sci. Rep.*, vol. 6, no. January, pp. 1–9, 2016, doi: 10.1038/srep31214.

[112] Y. J. Liu, E. Vieira, and E. Gylfe, "A store-operated mechanism determines the activity of the electrically

excitable glucagon-secreting pancreatic α-cell," *Cell Calcium*, vol. 35, no. 4, pp. 357–365, 2004, doi: 10.1016/j.ceca.2003.10.002.

[113] P. K. Dadi, B. Luo, N. C. Vierra, and D. A. Jacobson, "TASK-1 potassium channels limit pancreatic α-cell calcium influx and glucagon secretion," *Mol. Endocrinol.*, vol. 29, no. 5, pp. 777–787, 2015, doi: 10.1210/me.2014-1321.

[114] H. Heimberg, A. De Vos, D. Pipeleers, B. Thorens, and F. C. Schuit, "Differences in glucose transporter gene expression between rat pancreatic α- and β-cells are correlated to differences in glucose transport but not in glucose utilization," *Journal of Biological Chemistry*, vol. 270, no. 15. pp. 8971–8975, 1995, doi: 10.1074/jbc.270.15.8971.

[115] C. S. Hummel, C. Lu, D. D. F. Loo, B. A. Hirayama, A. A. Voss, and E. M. Wright, "Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2," *Am. J. Physiol. - Cell Physiol.*, vol. 300, no. 1, pp. 14–21, 2011, doi: 10.1152/ajpcell.00388.2010.

[116] T. Suga *et al.*, "SGLT1 in pancreatic α cells regulates glucagon secretion in mice, possibly explaining the distinct effects of SGLT2 inhibitors on plasma glucagon levels," *Mol. Metab.*, vol. 19, no. October 2018, pp. 1–12, 2019, doi: 10.1016/j.molmet.2018.10.009.

[117] R. Cheng-Xue *et al.*, "Tolbutamide controls glucagon release from mouse islets differently than glucose: Involvement of KATP channels from both α-cells and δ-cells," *Diabetes*, vol. 62, no. 5, pp. 1612–1622, 2013, doi: 10.2337/db12-0347.

[118] X. Q. Dai *et al.*, "Heterogenous impairment of α cell function in type 2 diabetes is linked to cell maturation state," *Cell Metab.*, vol. 34, no. 2, pp. 256-268.e5, 2022, doi: 10.1016/j.cmet.2021.12.021.

[119] E. Vergari *et al.*, "Insulin inhibits glucagon release by SGLT2-induced stimulation of somatostatin secretion," *Nat. Commun.*, vol. 10, no. 1, pp. 1–11, 2019, doi: 10.1038/s41467-018-08193-8.

[120] C. Saponaro *et al.*, "The GLP1R Agonist Liraglutide Reduces Hyperglucagonemia Induced by the SGLT2 Inhibitor Dapagliflozin via Somatostatin Release," *Cell Rep.*, vol. 28, no. 6, pp. 1447-1454.e4, 2019, doi: 10.1016/j.celrep.2019.07.009.

[121] D. Basco *et al.*, "A-Cell Glucokinase Suppresses Glucose-Regulated Glucagon Secretion," *Nat. Commun.*, vol. 9, no. 1, 2018, doi: 10.1038/s41467-018-03034-0.
[122] H. Heimberg *et al.*, "The glucose sensor protein glucokinase is expressed in glucagon-producing α-cells," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 93, no. 14, pp. 7036–7041, 1996, doi: 10.1073/pnas.93.14.7036.
[123] K. Bokvist *et al.*, "Characterisation of sulphonylurea and ATP-regulated K+ channels in rat pancreatic A-cells.," *Pflugers Arch.*, vol. 438, no. 4, pp. 428–36, Sep. 1999, doi: 10.1007/s004249900076.
[124] R. D. Ramracheya *et al.*, "Membrane potential-dependent inactivation of voltage-gated ion channels in α-cells inhibits glucagon secretion from human islets," *Diabetes*, vol. 59, no. 9, pp. 2198–2208, 2010, doi: 10.2337/db09-1505.
[125] Y. Z. De Marinis *et al.*, "Enhancement of glucagon secretion in mouse and human pancreatic alpha cells by protein kinase C (PKC) involves intracellular trafficking of PKCalpha and PKCdelta.," *Diabetologia*, vol. 53, no. 4, pp. 717–29, Apr. 2010, doi: 10.1007/s00125-009-1635-x.
[126] S. O. Göpel, T. Kanno, S. Barg, X. G. Weng, J. Gromada, and P. Rorsman, "Regulation of glucagon release in mouse α-cells by K(ATP) channels and inactivation of TTX-sensitive Na+ channels," *J. Physiol.*, vol. 528, no. 3, pp. 509–520, 2000, doi: 10.1111/j.1469-7793.2000.00509.x.
[127] P. Rorsman, R. D. Ramracheya, N. J. G. Rorsman, and Q. Zhang, "ATP-regulated potassium channels and voltage-gated calcium channels in pancreatic alpha and beta cells: Similar functions but reciprocal effects on secretion," *Diabetologia*, vol. 57, no. 9, pp. 1749–1761, 2014, doi: 10.1007/s00125-014-3279-8.
[128] E. M. Allister *et al.*, "UCP2 regulates the glucagon response to fasting and starvation," *Diabetes*, vol. 62, no. 5, pp. 1623–1633, 2013, doi: 10.2337/db12-0981.
[129] J. E. Manning Fox, A. V. Gyulkhandanyan, L. S. Satin, and M. B. Wheeler, "Oscillatory membrane potential response to glucose in islet β-cells: A comparison of islet-cell electrical activity in mouse and rat," *Endocrinology*, vol. 147, no. 10, pp. 4655–4663, 2006, doi: 10.1210/en.2006-0424.
[130] P. K. Dadi, N. C. Vierra, and D. A. Jacobson, "Pancreatic β-

cell-specific ablation of TASK-1 channels augments glucose-stimulated calcium entry and insulin secretion, improving glucose tolerance," *Endocrinology*, vol. 155, no. 10, pp. 3757–3768, 2014, doi: 10.1210/en.2013-2051.

[131] Y. Yoshimoto, Y. Fukuyama, Y. Horio, A. Inanobe, M. Gotoh, and Y. Kurachi, "Somatostatin induces hyperpolarization in pancreatic islet α cells by activating a G protein-gated K+ channel," *FEBS Lett.*, vol. 444, no. 2–3, pp. 265–269, 1999, doi: 10.1016/S0014-5793(99)00076-9.

[132] J. Gromada *et al.*, "ATP-sensitive K+ channel-dependent regulation of glucagon release and electrical activity by glucose in wild-type and SUR1-/- mouse α-cells," in *Diabetes*, 2004, vol. 53, no. SUPPL. 3, pp. 181–189, doi: 10.2337/diabetes.53.suppl_3.S181.

[133] E. Vieira, A. Salehi, and E. Gylfe, "Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic alpha cells.," *Diabetologia*, vol. 50, no. 2, pp. 370–379, 2007, doi: 10.1007/s00125-006-0511-1.

[134] I. Derler, I. Jardin, and C. Romanin, "Molecular mechanisms of STIM/Orai communication," *Am. J. Physiol. - Cell Physiol.*, vol. 310, no. 8, pp. C643–C662, 2016, doi: 10.1152/ajpcell.00007.2016.

[135] H. Maruyama, A. Hisatomi, L. Orci, G. M. Grodsky, and R. H. Unger, "Insulin within islets is a physiologic glucagon release inhibitor.," *J. Clin. Invest.*, vol. 74, no. 6, pp. 2296–2299, Dec. 1984, doi: 10.1172/JCI111658.

[136] K. Moens *et al.*, "Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells," *Diabetes*, vol. 45, no. 2, pp. 257–261, 1996, doi: 10.2337/diabetes.45.2.257.

[137] J. J. Holst, "The physiology of glucagon-like peptide 1," *Physiol. Rev.*, vol. 87, no. 4, pp. 1409–1439, 2007, doi: 10.1152/physrev.00034.2006.

[138] S. Gremlich *et al.*, "Cloning, Functional Expression, and Chromosomal Localization of the Human Pancreatic Islet Glucose-Dependent Insulinotropic Polypeptide Receptor," *Diabetes*, vol. 44, no. 10, pp. 1202–1208, Oct. 1995, doi: 10.2337/diab.44.10.1202.

[139] J. Gromada *et al.*, "Adrenaline stimulates glucagon secretion

in pancreatic A-cells by increasing the Ca2+ current and the number of granules close to the L-type Ca2+ channels," *J. Gen. Physiol.*, vol. 110, no. 3, pp. 217–228, 1997, doi: 10.1085/jgp.110.3.217.

[140] J. E. Gerich, M. A. Charles, and G. M. Grodsky, "Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas," *J. Clin. Invest.*, vol. 54, no. 4, pp. 833–841, 1974, doi: 10.1172/JCI107823.

[141] G. Tian, S. Sandler, E. Gylfe, and A. Tengholm, "Glucose- and hormone-induced cAMP oscillations in α- and β-cells within intact pancreatic islets," *Diabetes*, vol. 60, no. 5, pp. 1535–1543, 2011, doi: 10.2337/db10-1087.

[142] Q. Yu, H. Shuai, P. Ahooghalandari, E. Gylfe, and A. Tengholm, "Glucose controls glucagon secretion by directly modulating cAMP in alpha cells," *Diabetologia*, vol. 62, no. 7, pp. 1212–1224, 2019, doi: 10.1007/s00125-019-4857-6.

[143] X. Ma *et al.*, "Glucagon stimulates exocytosis in mouse and rat pancreatic α-cells by binding to glucagon receptors," *Mol. Endocrinol.*, vol. 19, no. 1, pp. 198–212, 2005, doi: 10.1210/me.2004-0059.

[144] O. Cabrera *et al.*, "Glutamate Is a Positive Autocrine Signal for Glucagon Release," *Cell Metab.*, vol. 7, no. 6, pp. 545–554, Jun. 2008, doi: 10.1016/j.cmet.2008.03.004.

[145] S. Uehara *et al.*, "Metabotropic Glutamate Receptor Type 4 Is Involved in Autoinhibitory Cascade for Glucagon Secretion by α-Cells of Islet of Langerhans," *Diabetes*, vol. 53, no. 4, pp. 998–1006, Apr. 2004, doi: 10.2337/diabetes.53.4.998.

[146] M. Hayashi *et al.*, "Secretory granule-mediated co-secretion of L-glutamate and glucagon triggers glutamatergic signal transmission in islets of Langerhans," *J. Biol. Chem.*, vol. 278, no. 3, pp. 1966–1974, 2003, doi: 10.1074/jbc.M206758200.

[147] J. E. Gerich *et al.*, "Inhibition of pancreatic glucagon responses to arginine by somatostatin in normal man and in insulin dependent diabetics," *Diabetes*, vol. 23, no. 11, pp. 876–880, 1974, doi: 10.2337/diab.23.11.876.

[148] D. Kawamori *et al.*, "Insulin Signaling in α Cells Modulates Glucagon Secretion In Vivo," *Cell Metab.*, vol. 9, no. 4, pp. 350–361, Apr. 2009, doi: 10.1016/j.cmet.2009.02.007.

[149] I. Franklin, J. Gromada, A. Gjinovci, S. Theander, and C. B. Wollheim, "Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release.," *Diabetes*, vol. 54, no. 6, pp. 1808–15, Jun. 2005, doi: 10.2337/diabetes.54.6.1808.

[150] R. D. Ramracheya *et al.*, "GLP-1 suppresses glucagon secretion in human pancreatic alpha-cells by inhibition of P/Q-type Ca 2+ channels," *Physiol. Rep.*, vol. 6, no. 17, pp. 1–17, 2018, doi: 10.14814/phy2.13852.

[151] W. G. Ding, E. Renström, P. Rorsman, K. Buschard, and J. Gromada, "Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate Ca2+-induced secretion in rat α-cells by a protein kinase A- mediated mechanism," *Diabetes*, vol. 46, no. 5, pp. 792–800, 1997, doi: 10.2337/diabetes.46.5.792.

[152] A. Ørgaard and J. J. Holst, "The role of somatostatin in GLP-1-induced inhibition of glucagon secretion in mice," *Diabetologia*, vol. 60, no. 9, pp. 1731–1739, 2017, doi: 10.1007/s00125-017-4315-2.

[153] J. De Heer, C. Rasmussen, D. H. Coy, and J. J. Holst, "Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas," *Diabetologia*, vol. 51, no. 12, pp. 2263–2270, 2008, doi: 10.1007/s00125-008-1149-y.

[154] C. Ørskov, L. Rabenhøj, A. Wettergren, H. Kofod, and J. J. Holst, "Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans," *Diabetes*, vol. 43, no. 4, pp. 535–539, 1994, doi: 10.2337/diab.43.4.535.

[155] C. F. Deacon, M. A. Nauck, M. Toft-Nielsen, L. Pridal, B. Willms, and J. J. Holst, "Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects," *Diabetes*, vol. 44, no. 9, pp. 1126–1131, 1995, doi: 10.2337/diab.44.9.1126.

[156] C. F. Deacon, A. H. Johnsen, and J. J. Holst, "Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo.," *J. Clin. Endocrinol. Metab.*, vol. 80, no.

3, pp. 952–957, Mar. 1995, doi: 10.1210/jcem.80.3.7883856.
[157] D. Elahi *et al.*, "GLP-1 (9-36) amide, cleavage product of GLP-1 (7-36) amide, is a glucoregulatory peptide," *Obesity*, vol. 16, no. 7, pp. 1501–1509, 2008, doi: 10.1038/oby.2008.229.
[158] J. J. Meier *et al.*, "The glucagon-like peptide-1 metabolite GLP-1-(9-36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 290, no. 6, pp. 1118–1123, 2006, doi: 10.1152/ajpendo.00576.2005.
[159] T. P. Vahl, B. W. Paty, B. D. Fuller, R. L. Prigeon, and D. A. D'Alessio, "Effects of GLP-1-(7-36)NH2, GLP-1-(7-37), and GLP-1-(9-36)NH2 on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans," *J. Clin. Endocrinol. Metab.*, vol. 88, no. 4, pp. 1772–1779, 2003, doi: 10.1210/jc.2002-021479.
[160] A. J. M. Verberne, W. S. Korim, A. Sabetghadam, and I. J. Llewellyn-Smith, "Adrenaline: Insights into its metabolic roles in hypoglycaemia and diabetes," *Br. J. Pharmacol.*, vol. 173, no. 9, pp. 1425–1437, 2016, doi: 10.1111/bph.13458.
[161] E. Vieira, Y. J. Liu, and E. Gylfe, "Involvement of α1 and β-adrenoceptors in adrenaline stimulation of the glucagon-secreting mouse α-cell," *Naunyn. Schmiedebergs. Arch. Pharmacol.*, vol. 369, no. 2, pp. 179–183, 2004, doi: 10.1007/s00210-003-0858-5.
[162] A. Hamilton *et al.*, "Adrenaline stimulates glucagon secretion by Tpc2-Dependent ca2+ mobilization from acidic stores in pancreatic a-Cells," *Diabetes*, vol. 67, no. 6, pp. 1128–1139, 2018, doi: 10.2337/db17-1102.
[163] D. G. Pipeleers and M. A. Pipeleers-Marichal, "A method for the purification of single A, B and D cells and for the isolation of coupled cells from isolated rat islets," *Diabetologia*, vol. 20, no. 6, pp. 654–663, 1981, doi: 10.1007/BF00257436.
[164] J. L. Huang *et al.*, "Paracrine signaling by pancreatic δ cells determines the glycemic set point in mice," *bioRxiv*, p. 2022.06.29.496132, Jan. 2022, doi: 10.1101/2022.06.29.496132.
[165] R. Arrojo e Drigo *et al.*, "Structural basis for delta cell paracrine regulation in pancreatic islets," *Nat. Commun.*, vol. 10, no. 1, 2019, doi: 10.1038/s41467-019-11517-x.

[166] Q. Zhang *et al.*, "R-type Ca2+-channel-evoked CICR regulates glucose-induced somatostatin secretion," *Nat. Cell Biol.*, vol. 9, no. 4, pp. 453–460, 2007, doi: 10.1038/ncb1563.

[167] G. Denwood *et al.*, "Glucose stimulates somatostatin secretion in pancreatic δ-cells by cAMP-dependent intracellular Ca2+ release," *J. Gen. Physiol.*, vol. 151, no. 9, pp. 1094–1115, Sep. 2019, doi: 10.1085/jgp.201912351.

[168] E. Vergari *et al.*, "Somatostatin secretion by Na+-dependent Ca2+-induced Ca2+ release in pancreatic delta cells," *Nat. Metab.*, vol. 2, no. 1, pp. 32–40, 2020, doi: 10.1038/s42255-019-0158-0.

[169] M. Braun *et al.*, "Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic delta cells," *Diabetologia*, vol. 52, no. 8, pp. 1566–1578, 2009, doi: 10.1007/s00125-009-1382-z.

[170] G. M. Noguchi and M. O. Huising, "Integrating the inputs that shape pancreatic islet hormone release," *Nat. Metab.*, vol. 1, no. 12, pp. 1189–1201, 2019, doi: 10.1038/s42255-019-0148-2.

[171] A. Berts, A. Ball, S. Dryselius, E. Gylfe, and B. Hellman, "Glucose Stimulation of Somatostatin-Producing Islet Cells Involves Oscillatory Ca2+ Signaling," *Endocrinology*, vol. 137, no. 2, pp. 693–697, 1996, doi: 10.1210/endo.137.2.8593819.

[172] L. J. B. Briant, T. M. Reinbothe, I. Spiliotis, C. Miranda, B. Rodriguez, and P. Rorsman, "Δ-Cells and Β-Cells Are Electrically Coupled and Regulate Α-Cell Activity Via Somatostatin," *J. Physiol.*, vol. 596, no. 2, pp. 197–215, 2018, doi: 10.1113/JP274581.

[173] B. Hellman, A. Salehi, E. Gylfe, H. Dansk, and E. Grapengiesser, "Glucose generates coincident insulin and somatostatin pulses and antisynchronous glucagon pulses from human pancreatic islets," *Endocrinology*, vol. 150, no. 12, pp. 5334–5340, 2009, doi: 10.1210/en.2009-0600.

[174] K. Murakami, H. Taniguchi, M. Tamagawa, K. Ejiri, and S. Baba, "Modulation of somatostatin release by endogenous glucagon and insulin: Physiological relationship between A,B and D cells in rat pancreatic islets.," *Endocrinol. Jpn.*, vol. 29, no. 5, pp. 503–508, 1982, doi: 10.1507/endocrj1954.29.503.

[175] T. van der Meulen *et al.*, "Urocortin3 mediates somatostatin-dependent negative feedback control of insulin secretion," *Nat. Med.*, vol. 21, no. 7, pp. 769–776, Jul. 2015, doi: 10.1038/nm.3872.
[176] B. Svendsen and J. J. Holst, "Paracrine regulation of somatostatin secretion by insulin and glucagon in mouse pancreatic islets," *Diabetologia*, vol. 64, no. 1, pp. 142–151, Jan. 2021, doi: 10.1007/s00125-020-05288-0.
[177] A. Muroyama *et al.*, "A novel variant of ionotropic glutamate receptor regulates somatostatin secretion from δ-cells of islets of Langerhans," *Diabetes*, vol. 53, no. 7, pp. 1743–1753, 2004, doi: 10.2337/diabetes.53.7.1743.
[178] D. Elahi *et al.*, "Pancreatic alpha- and beta-cell responses to GIP infusion in normal man.," *Am. J. Physiol.*, vol. 237, no. 2, 1979, doi: 10.1152/ajpendo.1979.237.2.e185.
[179] H. C. Fehmann *et al.*, "The effects of glucagon-like peptide-i (glp-i) on hormone secretion from isolated human pancreatic islets," *Pancreas*, vol. 11, no. 2, pp. 196–200, 1995, doi: 10.1097/00006676-199508000-00014.
[180] "IDF Diabetes Atlas, 10th edn," Brussels, Belgium, Dec. 2021. doi: 10.1136/bmjopen-2016-013806.
[181] H. Sun *et al.*, "IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045," *Diabetes Res. Clin. Pract.*, vol. 183, p. 109119, 2022, doi: 10.1016/j.diabres.2021.109119.
[182] M. Roden and G. I. Shulman, "The integrative biology of type 2 diabetes," *Nature*, vol. 576, no. 7785, pp. 51–60, 2019, doi: 10.1038/s41586-019-1797-8.
[183] M. I. McCarthy, "Genomics, Type 2 Diabetes, and Obesity," *N. Engl. J. Med.*, vol. 363, no. 24, pp. 2339–2350, 2010, doi: 10.1056/nejmra0906948.
[184] M. E. J. Lean *et al.*, "Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial," *Lancet Diabetes Endocrinol.*, vol. 7, no. 5, pp. 344–355, 2019, doi: 10.1016/S2213-8587(19)30068-3.
[185] Banting, Best, Collip, Campbell, and Fletcher, "Pancreatic Extracts in Treatment of Diabetes Mellitus," *J. Nerv. Ment. Dis.*, vol. 59, no. 1, p. 73, Jan. 1924, doi: 10.1097/00005053-192401000-00013.

[186] J.-M. Anaya, Y. Shoenfeld, A. Rojas-Villarraga, R. Levy, and R. Cervera, *Autoimmunity. From Bench to Bedside.*, 1st ed. Bogota (Colombia): El Rosario University Press, 2013.

[187] M. J. Redondo, A. K. Steck, and A. Pugliese, "Genetics of type 1 diabetes," *Pediatr. Diabetes*, vol. 19, no. 3, pp. 346–353, 2018, doi: 10.1111/pedi.12597.

[188] M. J. Redonclo *et al.*, "Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: Prospective twin study," *Br. Med. J.*, vol. 318, no. 7185, pp. 698–702, 1999, doi: 10.1136/bmj.318.7185.698.

[189] A. N. Gorsuch *et al.*, "Evidence for a long prediabetic period in type I (insulin-dependent) diabetes mellitus.," *Lancet (London, England)*, vol. 2, no. 8260–61, pp. 1363–5, Dec. 1981, doi: 10.1016/s0140-6736(81)92795-1.

[190] R. A. Insel *et al.*, "Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association," *Diabetes Care*, vol. 38, no. 10, pp. 1964–1974, 2015, doi: 10.2337/dc15-1419.

[191] J. Nerup *et al.*, "HL-A ANTIGENS AND DIABETES MELLITUS," *Lancet*, vol. 304, no. 7885, pp. 864–866, Oct. 1974, doi: 10.1016/S0140-6736(74)91201-X.

[192] J. A. Noble, A. M. Valdes, M. Cook, W. Klitz, G. Thomson, and H. A. Erlich, "The role of HLA class II genes in insulin-dependent diabetes mellitus: Molecular analysis of 180 Caucasian, multiplex families," *Am. J. Hum. Genet.*, vol. 59, no. 5, pp. 1134–1148, 1996.

[193] S. J. Richardson *et al.*, "Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes," *Diabetologia*, vol. 59, no. 11, pp. 2448–2458, 2016, doi: 10.1007/s00125-016-4067-4.

[194] J. M. Jasinski and G. S. Eisenbarth, "Insulin as a primary autoantigen for type 1A diabetes," *Clin. Dev. Immunol.*, vol. 12, no. 3, pp. 181–186, 2005, doi: 10.1080/17402520500078204.

[195] S. Baekkeskov *et al.*, "Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase," *Nature*, vol. 347, no. 6289, pp. 151–156, Sep. 1990, doi: 10.1038/347151a0.

[196] A. Willcox, S. J. Richardson, A. J. Bone, A. K. Foulis, and N.

G. Morgan, “Analysis of islet inflammation in human type 1 diabetes,” *Clin. Exp. Immunol.*, vol. 155, no. 2, pp. 173–181, 2009, doi: 10.1111/j.1365-2249.2008.03860.x.

[197] W. E. Winter, D. L. Pittman, and I. Jialal, “Practical Clinical Applications of Islet Autoantibody Testing in Type 1 Diabetes,” *J. Appl. Lab. Med.*, vol. 7, no. 1, pp. 197–205, 2022, doi: 10.1093/jalm/jfab113.

[198] J. P. Palmer *et al.*, “Insulin Antibodies in Insulin-Dependent Diabetics Before Insulin Treatment,” *Science (80-.).*, vol. 222, no. 4630, pp. 1337–1339, Dec. 1983, doi: 10.1126/science.6362005.

[199] M. A. Payton, C. J. Hawkes, and M. R. Christie, “Relationship of the 37,000- and 40,000-Mr tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512),” *J. Clin. Invest.*, vol. 96, no. 3, pp. 1506–1511, 1995, doi: 10.1172/JCI118188.

[200] J. Ilonen *et al.*, “Patterns of β-cell autoantibody appearance and genetic associations during the first years of life,” *Diabetes*, vol. 62, no. 10, pp. 3636–3640, 2013, doi: 10.2337/db13-0300.

[201] J. M. Wenzlau *et al.*, “The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 104, no. 43, pp. 17040–17045, 2007, doi: 10.1073/pnas.0705894104.

[202] H.-J. Aanstoot *et al.*, “Identification and characterization of glima 38, a glycosylated islet cell membrane antigen, which together with GAD65 and IA2 marks the early phases of autoimmune response in type 1 diabetes,” *J. Clin. Invest.*, vol. 97, no. 12, pp. 2772–2783, 1996, doi: 10.1172/JCI118732.

[203] U. Roll *et al.*, “Peptide mapping and characterisation of glycation patterns of the glima 38 antigen recognised by autoantibodies in Type I diabetic patients,” *Diabetologia*, vol. 43, no. 5, pp. 598–608, 2000, doi: 10.1007/s001250051349.

[204] F. Winnock *et al.*, “Autoantibodies to a 38-kDa Glycosylated Islet Cell Membrane–Associated Antigen in (Pre)type 1 Diabetes,” *Diabetes Care*, vol. 24, no. 7, pp. 1181–1186, Jul. 2001, doi: 10.2337/diacare.24.7.1181.

[205] K. A. McLaughlin *et al.*, “Identification of tetraspanin-7 as a

target of autoantibodies in type 1 diabetes," *Diabetes*, vol. 65, no. 6, pp. 1690–1698, 2016, doi: 10.2337/db15-1058.

[206] H. T. Maecker, S. C. Todd, and S. Levy, "The tetraspanin superfamily: molecular facilitators," *FASEB J.*, vol. 11, no. 6, pp. 428–42, May 1997, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/9194523.

[207] C. Lindskog, A. Asplund, M. Engkvist, M. Uhlen, O. Korsgren, and F. Ponten, "Antibody-based proteomics for discovery and exploration of proteins expressed in pancreatic islets.," *Discov. Med.*, vol. 9, no. 49, pp. 565–78, Jun. 2010, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/20587347.

[208] C. M. Termini and J. M. Gillette, "Tetraspanins function as regulators of cellular signaling," *Front. Cell Dev. Biol.*, vol. 5, no. APR, pp. 1–14, 2017, doi: 10.3389/fcell.2017.00034.

[209] F. Berditchevski, "Complexes of tetraspanins with integrins: More than meets the eye," *J. Cell Sci.*, vol. 114, no. 23, pp. 4143–4151, 2001, doi: 10.1242/jcs.114.23.4143.

[210] M. T. Dickerson *et al.*, "Tetraspanin-7 regulation of L-type voltage-dependent calcium channels controls pancreatic β-cell insulin secretion," *J. Physiol.*, vol. 598, no. 21, pp. 4887–4905, Nov. 2020, doi: 10.1113/JP279941.

[211] R. H. Unger, E. Aguilar-Parada, W. A. Müller, and A. M. Eisentraut, "Studies of pancreatic alpha cell function in normal and diabetic subjects," *J. Clin. Invest.*, vol. 49, no. 4, pp. 837–848, Apr. 1970, doi: 10.1172/JCI106297.

[212] P. Shah, A. Vella, A. Basu, R. Basu, W. F. Schwenk, and R. A. Rizza, "Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetees mellitus," *J. Clin. Endocrinol. Metab.*, vol. 85, no. 11, pp. 4053–4059, 2000, doi: 10.1210/jc.85.11.4053.

[213] P. Shah, A. Basu, R. R. Basu, and R. A. Rizza, "Impact of lack of suppression of glucagon on glucose tolerance in humans," *Am. J. Physiol. - Endocrinol. Metab.*, vol. 277, no. 2 40-2, pp. 283–290, 1999, doi: 10.1152/ajpendo.1999.277.2.e283.

[214] C. J. Greenbaum, R. L. Prigeon, and D. A. D'Alessio, "Impaired β-Cell Function, Incretin Effect, and Glucagon Suppression in Patients With Type 1 Diabetes Who Have Normal Fasting Glucose," *Diabetes*, vol. 51, no. 4, pp. 951–957, Apr. 2002, doi: 10.2337/diabetes.51.4.951.

[215] J. T. Y. Yue, M. C. Riddell, E. Burdett, D. H. Coy, S. Efendic, and M. Vranic, "Amelioration of hypoglycemia via somatostatin receptor type 2 antagonism in recurrently hypoglycemic diabetic rats," *Diabetes*, vol. 62, no. 7, pp. 2215–2222, 2013, doi: 10.2337/db12-1523.

[216] W. A. Müller, G. R. Faloona, and R. H. Unger, "Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance," *Am. J. Med.*, vol. 54, no. 1, pp. 52–57, 1973, doi: 10.1016/0002-9343(73)90083-1.

[217] M.-Y. Wang *et al.*, "Glucagon receptor antibody completely suppresses type 1 diabetes phenotype without insulin by disrupting a novel diabetogenic pathway," *Proc. Natl. Acad. Sci.*, vol. 112, no. 8, pp. 2503–2508, Feb. 2015, doi: 10.1073/pnas.1424934112.

[218] N. Damond *et al.*, "Blockade of glucagon signaling prevents or reverses diabetes onset only if residual β-cells persist," *Elife*, vol. 5, no. APRIL2016, pp. 1–18, 2016, doi: 10.7554/eLife.13828.

[219] R. Wei *et al.*, "Antagonistic Glucagon Receptor Antibody Promotes α-Cell Proliferation and Increases β-Cell Mass in Diabetic Mice," *iScience*, vol. 16, pp. 326–339, 2019, doi: 10.1016/j.isci.2019.05.030.

[220] M. Y. Wang *et al.*, "Glucagon blockade restores functional β-cell mass in type 1 diabetic mice and enhances function of human islets," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 118, no. 9, pp. 1–8, 2021, doi: 10.1073/pnas.2022142118.

[221] J. Pettus *et al.*, "Glucagon receptor antagonist volagidemab in type 1 diabetes: a 12-week, randomized, double-blind, phase 2 trial," *Nat. Med.*, vol. 28, no. 10, pp. 2092–2099, Oct. 2022, doi: 10.1038/s41591-022-02011-x.

[222] D. O. F. Diabetes, "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 33, no. SUPPL. 1, 2010, doi: 10.2337/dc10-S062.

[223] World Health Organization and International Diabetes Federation, "Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia." 2006.

[224] J. Pirart, "Diabetes Mellitus and Its Degenerative Complications: A Prospective Study of 4,400 Patients Observed Between 1947 and 1973," *Diabetes Care*, vol. 1, no. 4, pp. 252–263, Jul. 1978, doi: 10.2337/diacare.1.4.252.

[225] K. Asadollahi, N. Beeching, and G. V. Gill, "Hyperglycaemia and mortality," *J. R. Soc. Med.*, vol. 100, no. 11, pp. 503–507, 2007, doi: 10.1258/jrsm.100.11.503.

[226] W. A. Müller, G. R. Faloona, E. Aguilar-Parada, and R. H. Unger, "Abnormal Alpha-Cell Function in Diabetes," *N. Engl. J. Med.*, vol. 283, no. 3, pp. 109–115, Jul. 1970, doi: 10.1056/NEJM197007162830301.

[227] J. G. Knudsen *et al.*, "Dysregulation of Glucagon Secretion by Hyperglycemia-Induced Sodium-Dependent Reduction of ATP Production," *Cell Metab.*, vol. 29, no. 2, pp. 430-442.e4, 2019, doi: 10.1016/j.cmet.2018.10.003.

[228] I. I. Spiliotis, R. Chalk, S. Gough, and P. Rorsman, "Reducing hyperglucagonaemia in type 2 diabetes using low-dose glibenclamide: Results of the LEGEND-A pilot study," *Diabetes, Obes. Metab.*, vol. 24, no. 8, pp. 1671–1675, 2022, doi: 10.1111/dom.14740.

[229] M. Omar-Hmeadi, P. E. Lund, N. R. Gandasi, A. Tengholm, and S. Barg, "Paracrine control of α-cell glucagon exocytosis is compromised in human type-2 diabetes," *Nat. Commun.*, vol. 11, no. 1, pp. 1–11, 2020, doi: 10.1038/s41467-020-15717-8.

[230] J. E. Gerich, M. Langlois, C. Noacco, J. H. Karam, and P. H. Forsham, "Lack of glucagon response to hypoglycemia in diabetes: Evidence for an intrinsic pancreatic alpha cell defect," *Science (80-.).*, vol. 182, no. 4108, pp. 171–173, 1973, doi: 10.1126/science.182.4108.171.

[231] P. E. Cryer, "Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes.," *Diabetologia*, vol. 45, no. 7, pp. 937–48, Jul. 2002, doi: 10.1007/s00125-002-0822-9.

[232] P. E. Cryer, "The barrier of hypoglycemia in diabetes," *Diabetes*, vol. 57, no. 12, pp. 3169–3176, 2008, doi: 10.2337/db08-1084.

[233] E. B. Schroeder, S. Xu, G. K. Goodrich, G. A. Nichols, P. J. O'Connor, and J. F. Steiner, "Predicting the 6-month risk of severe hypoglycemia among adults with diabetes: Development and external validation of a prediction model," *J. Diabetes Complications*, vol. 31, no. 7, pp. 1158–1163, Jul. 2017, doi: 10.1016/j.jdiacomp.2017.04.004.

[234] P. E. Cryer, S. N. Davis, and H. Shamoon, "Hypoglycemia in

Diabetes," *Diabetes Care*, vol. 26, no. 6, pp. 1902–1912, Jun. 2003, doi: 10.2337/diacare.26.6.1902.

[235] P. E. Cryer, "Severe hypoglycemia predicts mortality in diabetes," *Diabetes Care*, vol. 35, no. 9, pp. 1814–1816, 2012, doi: 10.2337/dc12-0749.

[236] P. E. Cryer, "Hypoglycemia in Type 1 Diabetes Mellitus," *Endocrinol. Metab. Clin. North Am.*, vol. 39, no. 3, pp. 641–654, Sep. 2010, doi: 10.1016/j.ecl.2010.05.003.

[237] M. F. Brereton, E. Vergari, Q. Zhang, and A. Clark, "Alpha-, Delta- and PP-cells: Are They the Architectural Cornerstones of Islet Structure and Co-ordination?," *J. Histochem. Cytochem.*, vol. 63, no. 8, pp. 575–591, 2015, doi: 10.1369/0022155415583535.

[238] S. R. Heller, M. Peyrot, S. K. Oates, and A. D. Taylor, "Hypoglycemia in patient with type 2 diabetes treated with insulin: It can happen," *BMJ Open Diabetes Res. Care*, vol. 8, no. 1, pp. 1–8, 2020, doi: 10.1136/bmjdrc-2020-001194.

[239] E. Bru-Tari *et al.*, "Pancreatic alpha-cell mass in the early-onset and advanced stage of a mouse model of experimental autoimmune diabetes," *Sci. Rep.*, vol. 9, no. 1, pp. 1–12, 2019, doi: 10.1038/s41598-019-45853-1.

[240] K. Furuyama *et al.*, "Diabetes relief in mice by glucose-sensing insulin-secreting human α-cells," *Nature*, vol. 567, no. 7746, pp. 43–48, Mar. 2019, doi: 10.1038/s41586-019-0942-8.

[241] B. Raju and P. E. Cryer, "Loss of the Decrement in Intraislet Insulin Plausibly Explains Loss of the Glucagon Response to Hypoglycemia in Insulin-Deficient Diabetes: Documentation of the Intraislet Insulin Hypothesis in Humans," *Diabetes*, vol. 54, no. 3, pp. 757–764, Mar. 2005, doi: 10.2337/diabetes.54.3.757.

[242] G. Kilimnik *et al.*, "Altered islet composition and disproportionate loss of large islets in patients with type 2 diabetes," *PLoS One*, vol. 6, no. 11, 2011, doi: 10.1371/journal.pone.0027445.

[243] K. Saito, N. Yaginuma, and T. Takahashi, "Differential volumetry of A, B and D cells in the pancreatic islets of diabetic and nondiabetic subjects.," *Tohoku J. Exp. Med.*, vol. 129, no. 3, pp. 273–83, Nov. 1979, doi: 10.1620/tjem.129.273.

[244] J. Rahier, R. M. M. Goebbels, and J. C. Henquin, "Cellular composition of the human diabetic pancreas," *Diabetologia*, vol. 24, no. 5, pp. 366–371, May 1983, doi: 10.1007/BF00251826.

[245] N. Karimian *et al.*, "Somatostatin receptor type 2 antagonism improves glucagon counterregulation in biobreeding diabetic rats," *Diabetes*, vol. 62, no. 8, pp. 2968–2977, 2013, doi: 10.2337/db13-0164.

[246] J. T. Y. Yue, E. Burdett, D. H. Coy, A. Giacca, S. Efendic, and M. Vranic, "Somatostatin receptor type 2 antagonism improves glucagon and corticosterone counterregulatory responses to hypoglycemia in streptozotocin-induced diabetic rats," *Diabetes*, vol. 61, no. 1, pp. 197–207, 2012, doi: 10.2337/db11-0690.

[247] E. Leclair *et al.*, "Glucagon responses to exercise-induced hypoglycaemia are improved by somatostatin receptor type 2 antagonism in a rat model of diabetes," *Diabetologia*, vol. 59, no. 8, pp. 1724–1731, 2016, doi: 10.1007/s00125-016-3953-0.

[248] T. W. Chen *et al.*, "Ultrasensitive fluorescent proteins for imaging neuronal activity," *Nature*, vol. 499, no. 7458, pp. 295–300, 2013, doi: 10.1038/nature12354.

[249] L. Madisen *et al.*, "Transgenic Mice for Intersectional Targeting of Neural Sensors and Effectors with High Specificity and Performance," *Neuron*, vol. 85, no. 5, pp. 942–958, Mar. 2015, doi: 10.1016/j.neuron.2015.02.022.

[250] A. M. Ackermann, J. Zhang, A. Heller, A. Briker, and K. H. Kaestner, "High-fidelity Glucagon-CreER mouse line generated by CRISPR-Cas9 assisted gene targeting," *Mol. Metab.*, vol. 6, no. 3, pp. 236–244, 2017, doi: 10.1016/j.molmet.2017.01.003.

[251] S. Chera *et al.*, "Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers," *Nature*, vol. 514, no. 7253, pp. 503–507, 2014, doi: 10.1038/nature13633.

[252] H. E. Parker *et al.*, "Predominant role of active versus facilitative glucose transport for glucagon-like peptide-1 secretion," *Diabetologia*, vol. 55, no. 9, pp. 2445–2455, Sep. 2012, doi: 10.1007/s00125-012-2585-2.

[253] H. Zhu *et al.*, "Cre-dependent DREADD (Designer

Receptors Exclusively Activated by Designer Drugs) mice.," *Genesis*, vol. 54, no. 8, pp. 439–46, Aug. 2016, doi: 10.1002/dvg.22949.

[254] L. A. Scrocchi *et al.*, "Glucose intolerance but normal satiety in mice with a null mutation in the glucagon–like peptide 1 receptor gene," *Nat. Med.*, vol. 2, no. 11, pp. 1254–1258, Nov. 1996, doi: 10.1038/nm1196-1254.

[255] L. Murru *et al.*, "Pharmacological Modulation of AMPAR Rescues Intellectual Disability-Like Phenotype in Tm4sf2-/y Mice," *Cereb. Cortex*, vol. 27, no. 11, pp. 5369–5384, 2017, doi: 10.1093/cercor/bhx221.

[256] S. Vignali, V. Leiss, R. Karl, F. Hofmann, and A. Welling, "Characterization of voltage-dependent sodium and calcium channels in mouse pancreatic A- and B-cells," *J. Physiol.*, vol. 572, no. 3, pp. 691–706, 2006, doi: 10.1113/jphysiol.2005.102368.

[257] S. J. Le Marchand and D. W. Piston, "Glucose Decouples Intracellular Ca2+ Activity from Glucagon Secretion in Mouse Pancreatic Islet Alpha-Cells," *PLoS One*, vol. 7, no. 10, pp. 1–10, 2012, doi: 10.1371/journal.pone.0047084.

[258] E. Belisle and A. J. Kowaltowski, "Opening of mitochondrial K+ channels increases ischemic ATP levels by preventing hydrolysis," *J. Bioenerg. Biomembr.*, vol. 34, no. 4, pp. 285–298, 2002, doi: 10.1023/A:1020256502583.

[259] P. P. Dzeja *et al.*, "Targeting nucleotide-requiring enzymes: implications for diazoxide-induced cardioprotection," *Am. J. Physiol. Circ. Physiol.*, vol. 284, no. 4, pp. H1048–H1056, Apr. 2003, doi: 10.1152/ajpheart.00847.2002.

[260] E. Zampese and P. Pizzo, "Intracellular organelles in the saga of Ca2+ homeostasis: different molecules for different purposes?," *Cell. Mol. Life Sci.*, vol. 69, no. 7, pp. 1077–1104, Apr. 2012, doi: 10.1007/s00018-011-0845-9.

[261] J. Gromada *et al.*, "Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization," *Diabetes*, vol. 44, no. 7, pp. 767–774, Jul. 1995, doi: 10.2337/diabetes.44.7.767.

[262] K. J. Hare, T. Vilsbøll, M. Asmar, C. F. Deacon, F. K. Knop, and J. J. Holst, "The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its

glucose-lowering action," *Diabetes*, vol. 59, no. 7, pp. 1765–1770, 2010, doi: 10.2337/db09-1414.

[263] C. Guida *et al.*, "Sitagliptin and Roux-en-Y gastric bypass modulate insulin secretion via regulation of intra-islet PYY," *Diabetes, Obes. Metab.*, vol. 20, no. 3, pp. 571–581, 2018, doi: 10.1111/dom.13113.

[264] H. Yan *et al.*, "Fully Human Monoclonal Antibodies Antagonizing the Glucagon Receptor Improve Glucose Homeostasis in Mice and Monkeys," *J. Pharmacol. Exp. Ther.*, vol. 329, no. 1, pp. 102–111, Apr. 2009, doi: 10.1124/jpet.108.147009.

[265] K. E. Mayo *et al.*, "International Union of Pharmacology. XXXV. The glucagon receptor family," *Pharmacol. Rev.*, vol. 55, no. 1, pp. 167–194, 2003, doi: 10.1124/pr.55.1.6.

[266] C. Weston *et al.*, "Modulation of glucagon receptor pharmacology by receptor activity-modifying protein-2 (RAMP2)," *J. Biol. Chem.*, vol. 290, no. 38, pp. 23009–23022, 2015, doi: 10.1074/jbc.M114.624601.

[267] S. Bassani *et al.*, "The X-Linked Intellectual Disability Protein TSPAN7 Regulates Excitatory Synapse Development and AMPAR Trafficking," *Neuron*, vol. 73, no. 6, pp. 1143–1158, 2012, doi: 10.1016/j.neuron.2012.01.021.

[268] M. E. Hemler, "Tetraspanin functions and associated microdomains," *Nat. Rev. Mol. Cell Biol.*, vol. 6, no. 10, pp. 801–811, 2005, doi: 10.1038/nrm1736.

[269] P. Lebrun and I. Atwater, "Effects of the calcium channel agonist, BAY K 8644, on electrical activity in mouse pancreatic B-cells," *Biophys. J.*, vol. 48, no. 6, pp. 919–930, Dec. 1985, doi: 10.1016/S0006-3495(85)83855-8.

[270] Y. Yu *et al.*, "Bioengineered human pseudoislets form efficiently from donated tissue, compare favourably with native islets in vitro and restore normoglycaemia in mice," *Diabetologia*, vol. 61, no. 9, pp. 2016–2029, 2018, doi: 10.1007/s00125-018-4672-5.

[271] N. Li *et al.*, "Ablation of somatostatin cells leads to impaired pancreatic islet function and neonatal death in rodents article," *Cell Death Dis.*, vol. 9, no. 6, 2018, doi: 10.1038/s41419-018-0741-4.

[272] M. Braun *et al.*, "γ-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic β-cells,"

Diabetes, vol. 59, no. 7, pp. 1694–1701, 2010, doi: 10.2337/db09-0797.

[273] L. J. Klaff and G. J. Taborsky, "Pancreatic Somatostatin is a Mediator of Glucagon Inhibition by Hyperglycemia," *Diabetes*, vol. 36, no. 5, pp. 592–596, May 1987, doi: 10.2337/diab.36.5.592.

[274] P. Rorsman and M. O. Huising, "The somatostatin-secreting pancreatic δ-cell in health and disease," *Nat. Rev. Endocrinol.*, vol. 14, no. 7, pp. 404–414, 2018, doi: 10.1038/s41574-018-0020-6.

[275] R. H. Unger and L. Orci, "Possible Roles of the Pancreatic D-cell in the Normal and Diabetic States," *Diabetes*, vol. 26, no. 3, pp. 241–244, Mar. 1977, doi: 10.2337/diab.26.3.241.

[276] G. S. Patton, R. Dobbs, L. Orci, W. Vale, and R. H. Unger, "Stimulation of pancreatic immunoreactive somatostatin (IRS) release by glucagon," *Metabolism*, vol. 25, no. 11, p. 1499, Nov. 1976, doi: 10.1016/S0026-0495(76)80177-1.

[277] P. E. Cryer, "Hypoglycemia: The limiting factor in the management of IDDM," *Diabetes*, vol. 43, no. 11, pp. 1378–1389, 1994, doi: 10.2337/diab.43.11.1378.

[278] S. R. Heller and P. E. Cryer, "Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans," *Diabetes*, vol. 40, no. 2, pp. 223–226, 1991, doi: 10.2337/diab.40.2.223.

[279] A. M. Arbelaez, W. J. Powers, T. O. Videen, J. L. Price, and P. E. Cryer, "Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition a mechanism for hypoglycemia-associated autonomic failure," *Diabetes*, vol. 57, no. 2, pp. 470–475, 2008, doi: 10.2337/db07-1329.

[280] S. Herzig *et al.*, "CREB regulates hepatic gluconeogenesis through the coactivator PGC-1," *Nature*, vol. 413, no. 6852, pp. 179–183, 2001, doi: 10.1038/35093131.

[281] G. A. Gonzalez and M. R. Montminy, "Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133," *Cell*, vol. 59, no. 4, pp. 675–680, Nov. 1989, doi: 10.1016/0092-8674(89)90013-5.

[282] X. Zhang *et al.*, "Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues," *Proc. Natl. Acad.*

Sci. U. S. A., vol. 102, no. 12, pp. 4459–4464, 2005, doi: 10.1073/pnas.0501076102.

[283] B. Mayr and M. Montminy, "Transcriptional regulation by the phosphorylation-dependent factor CREB," *Nat. Rev. Mol. Cell Biol.*, vol. 2, no. 8, pp. 599–609, Aug. 2001, doi: 10.1038/35085068.

[284] S. Cohen-Cory, A. H. Kidane, N. J. Shirkey, and S. Marshak, "Brain-derived neurotrophic factor and the development of structural neuronal connectivity," *Dev. Neurobiol.*, vol. 70, no. 5, pp. 271–288, 2010, doi: 10.1002/dneu.20774.

[285] M. V. Caldeira *et al.*, "Brain-derived neurotrophic factor regulates the expression and synaptic delivery of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons," *J. Biol. Chem.*, vol. 282, no. 17, pp. 12619–12628, 2007, doi: 10.1074/jbc.M700607200.