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Integrating the inputs that shape pancreatic islet hormone release

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Abstract

The pancreatic islet is a complex mini organ composed of a variety of endocrine cells and their support cells that work in concert to tightly control blood glucose homeostasis. Changes in glucose concentration are commonly regarded as the chief signal controlling insulin-secreting beta cells, glucagon-secreting alpha cells, and somatostatin-secreting delta cells. However, each of these cell types is highly responsive to a multitude of endocrine, paracrine, nutritional, and neural inputs, which collectively shape the final endocrine output of the islet. Here we review the principal inputs for each islet cell type and the physiological circumstances in which these signals arise through the prism of the insights generated by transcriptomes of each of the major endocrine cell types. A comprehensive integration of the factors that influence blood glucose homeostasis is essential if we are to succeed in improving therapeutic strategies to better manage diabetes.

Introduction

Over the past four decades, the number of adults with diabetes has nearly quadrupled with over 420 million individuals estimated to be affected by the disease worldwide[1]. As these numbers are expected to continue to rise, it is evident that improved therapeutic strategies to manage diabetes are necessary. Diabetes is a disease of chronically high blood glucose stemming principally from insulin impairment. However, defects in glucagon secretion – inappropriate hyperglucagonaemia as well as impaired counterregulation – are also inextricably intertwined with the etiology of diabetes[2]. This places the source of insulin and glucagon – the pancreatic islets – in the crosshairs of researchers' attempts to understand and ameliorate the disease. A better appreciation for the mechanisms controlling islet hormone secretion is imperative to developing better strategies for dealing with diabetes.

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Author Contributions

G.M.N. wrote the article together with M.O.H.

Data availability Statement

The dataset analysed in this study is available in the GEO repository under accession number GSE90766.

The pancreatic islets are a heterogeneous mixture of endocrine cells and non-endocrine support cells that maintain homeostatic blood glucose levels via balanced hormone secretion. The beta cells make up (50–75%) of the islet cell mass in humans, and 60–80% in mice [Figure 1][3–5], and are the sole source of insulin in the body[6]. Insulin release, triggered by increased blood glucose[7,8], lowers glycaemia through the net effect of decreased glycogenolysis and gluconeogenesis at the liver and skeletal muscle and increased uptake of glucose in the liver, skeletal muscle, and adipose tissue[9,10] [Figure 2]. Insulin further stimulates nutrient uptake and triglyceride (TG) synthesis in adipocytes. Collectively these insulin actions restore normoglycaemia following a meal. Alpha cells are the second most abundant islet cell type, accounting for approximately 15–20% and of the endocrine cells in mice, and 25–35% in humans [Figure 1] [3–5]. Alpha cells secrete glucagon as a counterregulatory signal in response to hypoglycaemia, and is additionally potentiated (amplified) by adrenergic stimulation and circulating amino acids. Glucagon increases hepatic glucose production primarily via increased glycogenolysis and gluconeogenesis [Figure 2][11]. Delta cells make up 5–10% of the islet[3] and release somatostatin dose-dependently in response to high glucose[12,13]. While insulin and glucagon are true hormones that are released into the circulation to elicit effects on target cells distant from site of release, somatostatin instead provides local inhibitory control over alpha and beta cells[14–16]. Nevertheless, this local regulation helps determine the homeostatic set point for plasma glucose[17].

However, beyond glucose, multiple levels of paracrine, endocrine, neuronal, and nutritional inputs collectively determine islet cell activity. In this review, we focus on emerging themes with regards to control of islet endocrine function. Part of this discussion will incorporate insights gained from islet cell transcriptomes that have provided a wealth of information on the inputs that do, and do not, impinge on the islet cell types they were long thought to act upon. It is not our intent to cover all possible inputs that have been attributed to islets over many years. We refer the interested reader to comprehensive descriptions of important topics such as species differences[10,18], islet innervation[19], and islet cell receptors[20–22] that have been published elsewhere. Additionally, non-endocrine islet cells such as macrophages, endothelial, and stellate cells make important contributions to the islet as a functional unit [Box 1] that we will not address in detail.

Goal of Review

Our goal with this review is to focus on areas where recent insights challenge us to reconsider traditional views of the physiological mechanisms that control islet hormone release, and we discuss specific differences between rodent and human islets where appropriate. One major theme is the renewed appreciation for amino acids as significant contributors to nutrient-stimulated alpha cell secretion. With regards to intra-islet crosstalk, increased evidence of alpha cell-mediated beta cell potentiation now compels us to reconsider the view of glucagon as a predominantly counterregulatory hormone in favor of a model where glucagon also makes significant physiological contributions to glucose-stimulated insulin secretion. Additionally, delta cells have emerged as physiologically important modulators of insulin and glucagon secretion. Finally, we discuss how the inputs that coordinate insulin and glucagon release from healthy islets are affected by diabetes and

how a better understanding of the physiological inputs into the healthy islets may be leveraged towards improved management in disease.

The complexities of studying islet endocrine cells—Multiple layers of nutrient, paracrine, endocrine, and neuronal signals modulate the islet cell hormone secretion that is triggered by glucose levels. The mammalian islet is highly vascularised[23], allowing for both rapid sensing of changes in nutritional status or circulating hormones, and for swift delivery of insulin or glucagon to peripheral tissues. Islets are also tightly innervated by autonomic neurons[23] which supports sympathetic and parasympathetic modulation of insulin, glucagon, and somatostatin release [Figure 2]. Briefly, the net effect of sympathetic stimulation is an increase in glucagon release and a decrease in insulin and somatostatin release[19,24]. Net parasympathetic signaling activates both insulin and glucagon secretion while decreasing somatostatin. Interestingly, islet cells also synthesise a number of classic neurotransmitters such as GABA, acetylcholine, and serotonin for intra-islet signaling independent of innervation (detailed below)[25–27]. And while mouse islets have historically been suggested to be more highly innervated than human islets[28], there are also reports showing prominent autonomic innervation of humans islets[23].

The plethora of input signals that target the islet as a functional unit has made it a challenge to distinguish direct versus indirect mechanisms that modulate alpha and beta cell activity. In recent years, islet cell type-specific reporter mice[29] and antibodies[30], supported by advances in RNA-Seq approaches, have made it possible to disentangle how multiple layers of external and intra-islet signals affect each individual cell type. These efforts have generated comprehensive, high-quality bulk and single cell transcriptomes of mouse and human islet cells[31–34]. Advances in functional imaging and electrophysiology have similarly made characterising islet responses much more cell-type specific: Genetically-encoded calcium indicators have improved upon traditional calcium dyes by enabling targeted functional imaging of populations of a single cell type[33]. In parallel, patch-clamp recordings aggregated across hundreds of islet cells provide us with cell type-specific electrophysiological fingerprints[35]. And Patch-Seq provides a unique approach to validate single cell transcriptomes with direct functional correlates acquired by patch-clamp measurements on the same single cell[36,37]. These technical advances now make it more feasible than ever to distinguish direct versus indirect actions on islet cells with single cell resolution.

As detailed in this review, the collective inputs that influence islet secretion are sufficiently similar between mice and humans that mouse models, with their ease of experimental manipulation, offer unparalleled advantages in understanding islet biology. Nevertheless, species differences do exist. Mouse islets are organised as a core of beta cells surrounded by a mantle of alpha and delta cells [Figure 1][3]. This same architecture is seen in islets from young humans, but adult human islets exhibit a variable assortment of islet architecture from the rodent-like mantle-core organisation to a more intermingled distribution of alpha, beta, and delta cells – a setup well suited for paracrine signaling through the interstitial space[10,38,39]. And while many islet paracrine signals are shared between species, although some such as islet amyloidogenic polypeptide (IAPP) and peptide YY (PYY) are notably enriched in mouse over human islets[22,40]. Morphologically, human delta cells are relatively

compact while mouse delta cells have long, neuron-like projections, which may help them overcome the distance from mantle to core when releasing somatostatin to inhibit beta cell activity[15]. In spite of these differences, mouse and human islets share responses to many external factors and intra-islet paracrine signals that shape the final islet output.

Nutrient stimulation of alpha, beta and delta cells—Most textbooks offer the glucose-centric view that insulin secretion is triggered when glucose values rise over a threshold of 5 mM (7 mM in mice)[41]. Meanwhile, alpha cells release most glucagon under hypoglycemic conditions, and demonstrate modest glucagon secretion under hyperglycemic conditions. However, insulin and glucagon play a large role in the metabolism of not only carbohydrates, but also of lipids (free fatty acids)[9,42] and proteins (amino acids)[9,43] as detailed in this section.

Glucose-Stimulated Insulin and Somatostatin Secretion: Glucose is arguably the single most important signal that controls insulin release, although glucose fluctuations in healthy subjects are relatively modest and would not by themselves elicit robust insulin secretion. Full insulin secretion *in vivo* requires glucose stimulation that is potentiated by the combined actions of other nutrients, endocrine, and paracrine factors. Glucose-stimulated insulin secretion (GSIS) is initiated when beta cells sense increases in blood glucose via glucose transporters (GLUT1 in humans and Glut2 in mice)[44,45] [Figure 3]. This glucose serves as a substrate for glycolysis and oxidative phosphorylation, generating ATP and leading to an accompanying drop in ADP levels. This shift in ATP/ADP ratio closes ATP-sensitive potassium channels (K_{ATP} channels), which causes membrane depolarisation. This in turn opens L-type voltage-gated Ca^{2+} channels (VGCCs), leading to a Ca^{2+} influx and calcium-induced calcium release that triggers exocytosis of insulin secretory granules[18]. While L-type VGCCs are responsible for the majority of Ca^{2+} currents in mouse beta cells, in humans P/Q-type VGCCs are about equally as involved as L-type channels[41].

The delta cell signal transduction for glucose-stimulated somatostatin secretion (GSSS) shares many features with beta cells[15] albeit with a few distinctions. K_{ATP} channel closure and subsequent membrane depolarisation is required, but in contrast with beta cells, calcium-induced calcium release plays a larger role in GSSS than in GSIS[46]. Additionally, delta cells have been suggested to be electrically coupled to beta cells[47]. The propagation of depolarisation from glucose-activated beta cells to delta cells may help potentiate somatostatin release. However, delta cells are active at lower glucose concentrations than beta cells, possibly due to a difference in K_{ATP} activity[48]. Moreover, somatostatin secretion in response to hyperglycemia is synchronous with insulin secretion, albeit with a 30 second to 2 minute delay[49]. Such a delay is not readily reconciled with a model where delta cells operate in lock-step with beta cells mediated solely through gap junction-mediated coordination and suggests an important paracrine component to the coordination between beta and delta cells.

Glucose-Mediated Glucagon Secretion: The mechanism underlying glucose-mediated alpha cell activation remains incompletely understood. Alpha cells express analogous machinery to that used for GSIS in beta cells and similarly rely on K_{ATP} channels and VGCCs for secretion, yet alpha cells are active at low as opposed to high glucose. While

there is no consensus paradigm for glucagon secretion, one current model is that increasing glucose induces a K_{ATP}-driven depolarisation to inactivate voltage-gated Na^+ channels[50]. By driving Na^+ channels to a non-conducting state, alpha cells are unable to reach the membrane potential necessary for VGCC-opening and cease to secrete glucagon. Conversely, under hypoglycemic conditions, alpha cell K_{ATP} channels operate at a low level that holds alpha cells in an electrically active state and causes small depolarisations that open P/Q-type VGCCs. While this model of alpha cell activation helps explain some of the dynamics of glucagon release, a full explanation of alpha cell activity in response to hypoglycemia likely involves a combination of alpha cell-intrinsic, paracrine, endocrine, and neural factors. Indeed, when stripped of the paracrine influence of delta cells, alpha cell glucagon release is uniformly increased across a gradient of glucose concentrations[51]. This suggests that paracrine factors such as somatostatin have significant influence on the glucose-responsiveness of alpha cells, which we will revisit in more detail.

Amino Acids: Beta cells are sensitive to circulating amino acids and insulin signaling promotes both amino acid uptake and protein synthesis in skeletal muscle[9]. Beta cells express high levels of cationic amino acid transporters (CATs) and sodium-coupled neutral amino acid transporters (SNATs) [Figure 3c][32,33]. The mechanisms by which amino acids stimulate insulin secretion vary[18]. Many amino acids including arginine, lysine, leucine, and glutamine depolarise beta cells upon import, either directly if they carry a positive charge[52], or due to sodium co-transport[53]. The ensuing depolarisation triggers Ca^{2+} influx to stimulate insulin release[54]. In parallel, amino acids such as alanine, glutamic acid and glutamine can fuel components of mitochondrial metabolism, thus increasing the ATP/ADP ratio[55]. Other amino acids such as glycine potentiate insulin secretion via its ionotropic glycine receptor (GlyR) on the beta cell surface[56] [Figure 3c]. Furthermore, paracrine interactions contribute to the effects of amino acids on insulin.

Alpha cells are highly sensitive to increases in amino acids, and are stimulated by 17 of the 20 natural amino acids[57]. This largely explains the post-prandial spike in glucagon in response to a normal mixed meal[58]. It is vital that glucagon is released along with insulin in response to amino acids as the two hormones will synergistically increase amino acid uptake in response to protein ingestion while effectively countering each other's actions on carbohydrates. Glucagon signaling in the liver increases hepatic utilisation of amino acid substrates in gluconeogenesis[43], leading to a decrease in circulating amino acids. This important safety mechanism ensures normoglycemia during protein-rich, carbohydrate-low diets[58]. The liver-alpha cell axis that connects alpha cells, amino acids, and the liver is one of the primary mediators of amino acid homeostasis, highlighting the important role of glucagon in the post-prandial state.

The exact mechanism by which most amino acids directly stimulate glucagon secretion is less characterised than in beta cells, but likely involves similar mechanisms. Arginine is a potent stimulator of glucagon secretion that directly depolarises the alpha cell upon cellular transport into the cell[59]. Other amino acids induce glucagon secretion following import via abundantly expressed CATs and SNATs [Figure 3c] followed by use as metabolic substrate[32,33]. Glycine, signaling through ionotropic GlyR, can increase intracellular Ca^{2+} and stimulate exocytosis in alpha cells independent of amino acid transporters[60].

Much like peripheral insulin resistance causes hyperglycemia, which in turn contributes to beta cell hyperplasia (at least in mice), a similar relationship is emerging between hepatic glucagon signaling, amino acid levels and alpha cell mass. Chronically elevated amino acids can influence the total capacity for glucagon secretion by stimulating alpha cell proliferation through mTOR signaling[61]. Indeed, in glucagon receptor knockout mice or mice treated with glucagon receptor antagonists, interrupted hepatic glucagon signaling causes a marked accumulation of serum amino acids that triggers a remarkable alpha cell hyperplasia[62]. This expansion is largely driven by glutamine and alanine, which activate mTor signaling in alpha cells through SNAT5 (Slc38a5) in mice and an as of yet unknown amino acid transporter in humans[61,63]. These drastic alpha cell phenotypes are supported by clinical evidence. Hyperaminoacidemia is accompanied by hyperglucagonemia in patients with impaired liver function[64,65]. Interestingly, the liver-alpha cell axis appears to function independently of glucose levels as a comparison between diabetic and non-diabetic patients with non-alcoholic fatty liver disease revealed no correlation of glucose levels to elevated amino acids and glucagon[64,65].

Lipids: Insulin regulates lipid metabolism by promoting glucose uptake for conversion into triglycerides, while simultaneously inhibiting lipolysis[9]. The net effect of this is to promote glucose storage as triglycerides in adipocytes. Beta cell sensitivity to circulating fatty acids in addition to glucose is therefore important to nutrient balance. Beta cell secretion is stimulated by fatty acids of varying chain length and saturation level[66,67]. These effects are mediated by fatty acid receptor signaling as well as by signaling downstream of intracellular fatty acid metabolism[68]. The primary receptor for circulating fatty acids expressed by mammalian beta cells is free fatty acid receptor 1 (FFAR1/Ffar1; a.k.a. GPR40), a $G_{\alpha q}$-coupled G protein-coupled receptor (GPCR)[33,69] [Figure 3b]. FFAR1 supports medium- to long-chain saturated fatty acid as well as unsaturated fatty acid signaling. Most studies on FFAR1 have focused on the actions of palmitate – one of the most abundant circulating saturated fatty acids[69,70]. The $G_{\alpha q}$ signaling cascade activates phospholipase C (PLC) and induces 1,4,5 inositol-triphosphate (IP_3) formation. This mobilises Ca^{2+} from the ER, which in beta cells triggers insulin secretion[71]. Acute Ffar1/FFAR1 activation on the minute-to-hour timescale in mouse[72] and human[73] islets increases insulin secretion at high glucose levels, and the strength of insulin secretion increases with the length of the FFA chain[74]. Conversely, chronic (multi-day) palmitate exposure induces dissociation between insulin granules and VGCCs that drive secretion, which results in insulin secretion being decreased by more than 50%[73,75]. Separate of IP_3-mediated activation, fatty acids that diffuse into beta cells can be converted into triglycerides and diacylglycerol that feed into GSIS amplification pathways or directly induce insulin exocytosis[68], independent of fatty acid receptors. Indeed, palmitate increases the calcium currents and increases the readily-releasable pool of insulin-containing granules[76].

The degree to which free fatty acids directly affect glucagon secretion has been debated for decades. Early studies suggested that alpha cells were actually inhibited by free fatty acids[77], but there is growing evidence of an activating role[78,79]. Similar to beta cells, human alpha cells express the activating receptor, FFAR1[32]. In both mice and humans, glucagon release is directly stimulated by long-chain fatty acids via the resulting downstream increase in

cytosolic Ca^{2+}[78,79]. Glucagon's role is primarily to modulate hepatic lipid catabolism. Glucagon signaling at the liver increases fatty acid beta oxidation[42] and decreases lipoprotein synthesis and secretion[80,81]. At the adipose tissue glucagon stimulates lipolysis, although this is balanced by simultaneous lipolysis inhibition by insulin[9]. Alpha cell sensitivity to circulating free fatty acids is thus an important contributor to whole body lipid metabolism.

In both mice and humans, delta cells preferentially express free fatty acid receptor 4 (FFAR4/Ffar4; a.k.a. GPR120) [Figure 3b][33], which is a $G_{\alpha i}$-coupled GPCR. $G_{\alpha i}$ signaling inhibits adenylyl cyclase, which catalyses the formation of cyclic AMP (cAMP). As cAMP potentiates hormone secretion in islet cells, activation of Ffar4 in delta cells inhibits somatostatin secretion by 50%[79,82]. Together, these fatty acid receptor profiles illustrate a system where, in the presence of high circulating free fatty acids, the combined activation of insulin and glucagon is augmented by disabling the inhibitory actions of delta cells to handle this increased lipid load.

Paracrine Signaling in the Islet

Although nutrients are significant inputs to stimulate insulin and glucagon release, the islet itself is a rich source of signals that engage in intra-islet crosstalk. Paracrine signaling provides an additional layer of control over islet endocrine output that is essential for maintaining establishing and maintaining the homeostatic glucose setpoint. In particular, we will focus on recent developments in the paracrine actions of alpha cells on beta cells – and vice versa – as well as the overarching role of intra-islet delta cell signaling.

Alpha Cell-Mediated Beta Cell Activation

It would seem intuitive for beta and alpha cell activity to be mutually suppressive given that insulin and glucagon are functional antagonists, at least when it comes to maintaining euglycemia. Yet, while beta cell activity suppresses alpha cells (mediated at least in part via delta cells as will be discussed later), glucagon from alpha cells has long been known to stimulate insulin secretion[83]. While an arrangement where alpha cells stimulate beta cells, beta cells inhibit alpha cells, but stimulate delta cells, and delta cells inhibit both may appear counterintuitive (Figure 4a), modeling studies validate that this is the most stable way to organise a 3-node interaction[84]. It is increasingly apparent that beta cells require alpha cell inputs for full insulin secretion and establishing normoglycemia[85]. This realisation warrants a paradigm shift in our thinking of alpha cells as not only mediators of counterregulatory hepatic glucose production, but also as an important local source beta cell potentiation. The following section highlights local alpha cell secretions that potentiate beta cell activation, namely glucagon as well as acetylcholine and corticotropin-releasing hormone.

The Traditional Incretin Effect

Incretins are classically defined as enteroendocrine hormones that potentiate GSIS. The two main incretins are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 is produced by L-cells in the intestinal ileum and derives from the same precursor protein as the glucagon that is released by pancreatic alpha cells. In L-cells,

proglucagon is cleaved by proprotein convertase 1/3 (PCSK1/3) to produce GLP-1, while alpha cells express PCSK2 that produces glucagon instead. GIP is released from K cells in the duodenum and jejunum[86].

Together, incretins are believed to be responsible for as much as half of the insulin response to a carbohydrate meal[87,88]. Beta cells express relatively high levels of the GLP-1 receptor (GLP1R/Glp1r), while the GIP receptor (GIPR/Gipr) is more broadly expressed in multiple islet cells [Figure 3b]. Both receptors are $G_{\alpha s}$-coupled GPCRs that potentiate insulin secretion primarily through adenylyl cyclase activation and the resulting cAMP-mediated signaling cascade[86,89]. In concert with the cAMP pathway, activation of the PLC/protein kinase C (PKC) may contribute to beta cell stimulation by incretins[90]. This potentiation of GSIS is known as the incretin effect, whereby oral glucose consumption results in a markedly higher insulin response compared to intravenous administration in which glucose bypasses the GI tract. Incretins also stimulate an expansion of beta cell mass via proliferation, which further augments total insulin secretory capacity[91]. However, the incretin effect is observed during the cephalic phase of a meal, before nutrients enter the gastro-intestinal tract. This constitutes a disconnect with regards to the source of the GLP-1 that potentiates GSIS long before food reaches the ileum where most L cells are located. A resolution of this conundrum may necessitate a re-evaluation of the physiological role of glucagon and incretin receptors and how they interact to potentiate GSIS.

Intra-Islet Glucagon Signaling

Glucagon has been known to be able to potentiate GSIS in a manner reminiscent of the incretin effect since the 1960s[83], but the importance of this local action of glucagon for islet function is only now coming into full view. Mammalian beta cells express relatively high levels of glucagon receptor (GCGR/Gcgr) [Figure 3b], as well as GLP1R and GIPR. All these receptors belong to the same family of GPCRs that share similar cAMP-mediated downstream signaling mechanisms. Glucagon and GLP-1 are both derived from proglucagon, and signal via receptors that share 47% sequence homology[92], which points to the potential for cross-reactivity between glucagon and GLP-1 and their receptors in the islet.

Indeed, multiple groups have now independently demonstrated that proglucagon-derived peptides produced by alpha cells activate beta cells via either GCGR or GLP1R and that these actions are required for normal GSIS in humans and mice[93–98]. The majority of circulating GLP-1 is derived from the GI tract, and knocking out gut-derived GLP-1 is impairs oral glucose tolerance[99]. However, islet-specific GLP-1 signaling, without any contribution from the gut, is shown to be necessary for normal glucose handling[94]. Separate studies have demonstrated that simultaneously knocking down or blocking both GLP1R and GIPR in beta cells severely reduces glucose-stimulated insulin secretion and glucose tolerance[93,95]. Glucagon is the predominant alpha cell ligand in mouse islets that engages both GCGR and GLP1R on beta cells to mediate most of this intra-islet signaling[93,95]. While alpha cells can produce GLP-1 under certain circumstances[100], wild type mouse islets under normal circumstances secrete relatively little GLP-1[95].

The activation of GLP1R and other $G_{\alpha s}$-coupled GPCRs such as GCGR and CRHR1 (see below) on beta cells in response to locally released, alpha cell-derived hormones represents a paradigm shift in our understanding of how insulin release can be potentiated. For instance, amino acids such as arginine and glutamine may elicit insulin secretion at least in part by stimulating glucagon release, which then indirectly promotes insulin release in a paracrine fashion, and not by direct stimulation of beta cells as was previously thought[93]. Such a scenario likely requires beta cell triggering by glucose, which is going to be present along with amino acids in most mixed-meal settings. A number of groups are pursuing GLP1R/GCGR dual agonists that may prove to be a superior method for amplifying insulin in treating type 2 diabetes given these mechanistic discoveries[101,102]. Together, these latest series of observations suggest that we need to reconsider our definition of GSIS: instead of reflecting the direct stimulatory effect of glucose on beta cells, GSIS from intact islets reflects the combined effects of glucose stimulation plus paracrine amplification via locally released glucagon and other alpha cell-derived products [Figure 4a]. These observations also reconcile the traditional view of glucagon as a counterregulatory hormone with the long-known ability of glucagon to potentiate GSIS[83].

Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH/Crh), originally discovered as the principal hypothalamic factor to initiate ACTH release from the anterior pituitary, is also expressed abundantly in human and rat alpha cells[32,103]. Interestingly, mouse alpha cells do not express Crh peptide or mRNA[32], although beta cells of mice, rats, and human all express corticotropin releasing hormone receptor 1 (CRHR1/Crhr1) [Figure 3b][104]. CRHR1 is a $G_{\alpha s}$-coupled GPCR that is related to the incretin receptors. Treating beta cells with CRH predictably induces a cAMP-mediated Ca^{2+} influx[105], potentiates GSIS, protects against cytokine-induced beta cell apoptosis, promotes beta cell proliferation, and stimulates the expression of an immediate early gene signature[106]. While the physiological contribution of CRH derived from alpha cells remains untested, it is another alpha cell-derived peptide that is poised to potentiate GSIS.

Acetylcholine

In mice, acetylcholine originates from parasympathetic neurons innervating the islet[19] [Figure 2]. In human islets, acetylcholine is released locally from alpha cells[26,107] [Figure 4a]. Irrespective of whether acetylcholine is of neural or paracrine origins, acetylcholine potentiates insulin secretion from mouse and human islets via the $G_{\alpha q}$-coupled muscarinic 3 cholinergic receptor (CHRM3/Chrm3) [Figure 3b][26,107–109]. Acetylcholine's effect on alpha cells is species-dependent. Human alpha cells do not respond to exogenous cholinergic as they synthesize their own acetylcholine[107]. Meanwhile, mouse alpha cells can be activated by cholinergic stimulation[110], although the underlying mechanism may be indirect as cholinergic cholinergic receptors are enriched in beta and delta cells (Figure 3). In contrast to its stimulation of beta cells, acetylcholine inhibits glucose-induced somatostatin secretion from delta cells. Parasympathetic tone is known to inhibit somatostatin release[24], and in mouse islets, cholinergic-mediated inhibition is prevented by pertussis toxin, suggesting the involvement of $G_{\alpha i}$-mediated signaling[111]. This is in line with the selective expression by mammalian delta cells of the $G_{\alpha i}$-coupled muscarinic 4 cholinergic receptor (CHRM4/

Chrm4) [Figure 3b]. These observations support a model where cholinergic signals (derived from parasympathetic innervations or from alpha cells in humans islets) amplifies insulin release directly and indirectly by inhibiting delta cells [Figure 2, 4a]. It should be noted that, in direct contrast with acetylcholine-mediated delta cell inhibition, other groups have reported IP_3-mediated stimulatory effects of cholinergic agonists on somatostatin secretion from mouse[46] and human[107] islets. Another way to use transcriptomes for decrypting signaling targets beyond just receptor profiles is to interrogate synthesis and degradation pathways for signaling molecules. Delta cells fit the profile of a target of cholinergic signaling given their receptor expression and the preferential expression of the enzyme acetylcholinesterase[33], which, in neurons, breaks down acetylcholine at the postsynaptic membrane[112]. The coordinated actions of acetylcholine on beta and delta cells during normoglycemia may therefore contribute to the maintenance of basal insulin release between meals.

Beta Cell-Mediated Alpha Cell Inhibition

There is general agreement in the field that beta cell-derived products contribute to alpha cell silencing at high glucose. Insulin, serotonin, GABA, and zinc are some of the beta cell factors proposed to directly inhibit alpha cells. Beta cells also secrete the peptide hormone urocortin 3 at high glucose, which potentiates delta cell glucose-stimulated somatostatin secretion. Given that somatostatin is a powerful inhibitor of glucagon secretion, urocortin 3-mediated stimulation of somatostatin release represents an indirect mechanism by which beta cells may suppress glucagon release during high glucose. Many papers over the years have favoured one signal over the other for reasons we will review here. It is plausible that there is redundancy or additivity among these beta cell-derived signals in their ability to suppress alpha cells, or that they play similar roles in distinct physiological settings.

Insulin—While it is difficult to disentangle which of several beta cell-derived paracrine signal may be principally responsible for suppressing alpha cells at high glucose, multiple groups have reported decrements in glucagon upon direct insulin administration[113–116]. Human and mouse alpha cells express the insulin receptor (INSR/Insr)[32,33], which can maintain K_{ATP} channels in the open configuration via PI3K/AKT signaling when activated. Open K_{ATP} channels drive alpha cells to a hyperpolarised state that prevents glucagon granule exocytosis [Figure 4a][115]. Mice with an alpha cell-specific deletion of Insr exhibit increased glucagon secretion, and as a result develop hyperglycemia and glucose intolerance[114]. However, this effect is milder than expected given the amount of insulin present locally within the islet and is by itself insufficient to explain beta cell-mediated alpha cell inhibition. Indeed, knocking out the insulin receptor in delta cells results in lower somatostatin release and alpha cell insensitivity to insulin indicating 1) insulin also has a paracrine effect potentiating delta cells, and 2) that the inhibition of alpha cells by insulin is mediated at least in part indirectly via somatostatin[117].

Serotonin—Serotonin is a neurotransmitter derived from tryptophan that regulates mood and anxiety in the brain. Beta cells express all of the components for serotonin production – tryptophan hydroxylase (TPH1,TPH2) and DOPA decarboxylase (DDC) – and vesicular loading – Vesicular monoamine transporter 1 and 2 (SLC18A1 and SLC18A2)[118,119]. Beta

cell serotonin synthesis is more pronounced in females and is further enhanced during pregnancy and old age[119,120]. In humans, serotonin from beta cells is released at high glucose and acts in a paracrine manner, inhibiting neighbouring alpha cells via the $G_{\alpha i}$-coupled serotonin receptor 1F (HTR1F)[27,32]. Early clinical studies where healthy human volunteers were administered serotonin antagonists reported increased glucagon secretion[121].

GABA—GABA is the classic inhibitory neurotransmitter of the CNS, and while it plays a role in islet signaling, neurogenic GABA's contribution appears to be minor. Beta cells are able to synthesise GABA at some of the highest concentrations outside the CNS, with islet tissue content measurements in the millimolar range; comparable to local insulin concentrations at basal glucose[25,122]. Glutamic acid decarboxylase 1 (GAD1), the enzyme that synthesises GABA, is highly enriched in beta cells and is a major Type 1 Diabetes autoantigen[32,33]. In rodents, GABA inhibits alpha cells through ionotropic GABA A receptors[123–125] [Figure 3b], although metabotropic GABA B1 receptor expression is also detectable. GABA from beta cells has been proposed to be the reason alpha cells are silenced at high glucose concentrations[124], but GABA treatment by itself does not fully suppress rodent glucagon secretion[126]. The fact that GABA A receptors are difficult to detect on human alpha cells and that the application of GABA elicits little electrophysiological response[127] argue against GABA as a significant contributor to alpha cell inhibition during hyperglycemia [Figure 4a].

Zinc—Insulin secretory granules in the beta cells are highly enriched for zinc ions (Zn^{2+}) to facilitate the formation of the insulin crystal[128]. Zn^{2+} co-released with insulin has therefore been considered as another beta cell product poised to inhibit alpha cells[129,130]. Zn^{2+} is taken up by the zinc transporter Znt8 (SLC30A8), which is abundantly expressed by both alpha and beta cells[32,33,131] [Figure 3c]. However, whole body, beta-specific, and alpha-specific Znt8 deletion does not affect glucagon secretion[131,132], indicating that Zn^{2+} is unlikely to contribute to beta cell-dependent inhibition of glucagon under high glucose.

Delta Cell-Mediated Islet Hormone Coordination

The delta cells have emerged as important inhibitory modulators of alpha and beta cell activity and physiological metabolism. Somatostatin is an important local factor controlling and coordinating the amount and timing of insulin and glucagon release from the islet, which ultimately contributes to setting homeostatic blood glucose levels.

Somatostatin—Somatostatin has important inhibitory functions in the GI tract as well as the anterior pituitary gland. Since delta cell-derived somatostatin accounts for only 5–10% of systemic circulating somatostatin content, its predominant function is likely as a paracrine regulator[15,16]. Delta cells are active throughout the majority of the physiological range of glucose with secretion starting as low as 3 mM glucose and increasing in a linear, dose-dependent manner towards 20 mM[12,15,133]. This large range of activity may have direct implications in the inhibition of both alpha and beta cells.

There are five somatostatin receptor (SSTR/Sstr) isoforms – all of which are $G_{\alpha i}$-coupled GCPRs[134]. The most abundant form expressed by mouse beta cells is Sstr3 [Figure 3b][31,33,135]. The predominant SSTR(s) on human beta cells remains unclear as some combination of SSTR1, SSTR2, SSTR3 and SSTR5 are expressed[32] and impact insulin secretion to some degree[136,137]. In addition to preventing hormone secretion by decreasing adenylyl cyclase activity, somatostatin receptor signaling simultaneously activates G protein-gated inwardly-rectifying K^+ (GIRK) channels that can counteract glucose-mediated membrane depolarisation[137] and inactivates VGCCs that are critical to insulin release[138]. Somatostatin released during high glucose provides inhibitory feedback that – under physiological conditions – does not fully shut down beta cells, but rather provides tonic inhibition. We have proposed that this arrangement is likely instrumental in preventing insulin release in excess of what is required to restore normoglycemia, and in doing so prevents insulin-induced hypoglycemia[17]. The local release of somatostatin during high blood glucose thus provides an additional layer of control to establish and stabilise blood glucose around its homeostatic setpoint.

Alpha cell activity is controlled by delta cells as the majority of the glucagonostatic effect of high glucose requires the paracrine actions of somatostatin. Somatostatin inhibits alpha cells primarily via SSTR2 in mouse and human islets with additional contribution of SSTR1 in humans[31,33,137,139]. While somatostatin-independent factors such as glucose contribute to inhibition, alpha cells are under constant tonic inhibition from delta cells. In mice, glucagon output increases across the full range of physiological blood glucose levels when either somatostatin is knocked out[140] or when islets are treated with Sstr2 antagonists or inhibitors of downstream somatostatin signaling[12,51,141].

Based on our understanding of the inhibition provided by delta cells, it is clear that somatostatin as a paracrine regulator ultimately dictates the total alpha and beta cell output. However, delta cell activity itself is dependent on paracrine inputs as well, chief among these the beta cell hormone urocortin 3.

Urocortin 3—The peptide urocortin 3 (UCN3/Ucn3) is the third most abundant hormone produced by beta cells, and is a member of the same peptide hormone family as CRH[14]. Urocortin 3 is packaged in the same secretory granules as insulin and co-released with insulin during GSIS. Delta cell GSSS is potentiated by urocortin 3 via the $G_{\alpha s}$-coupled GPCR, corticotropin releasing hormone receptor 2 (CRHR2/Crhr2)[14], which is selectively expressed by delta cells [Figure 3b]. Beta cell activation is required for full delta cell activity at high glucose as demonstrated by impaired somatostatin release in urocortin 3 knockout mice and islets treated with a Crhr2 antagonist[14]. Additionally, these same conditions – urocortin 3 knockout and Crhr2 antagonism – both demonstrate markedly increased GSIS, demonstrating that urocortin 3 attenuates insulin release by potentiating GSSS from delta cells in a classic negative feedback loop.

The physiological contribution of urocortin 3 to glucose homeostasis is best illustrated by the timing of its expression during development. *Ucn3* is one of the last beta cell genes that is turned on during beta cell maturation; full expression does not occur until around day 14 postpartum in [142] and at the end of the first trimester in human pancreas development[143].

The onset of urocortin 3 expression coincides with a general increase in plasma glucose levels that is correlated with a drop in circulating insulin levels[144]. Premature induction of urocortin 3 specifically in beta cells of transgenic mice from embryonic day 10.5 onwards results in prematurely elevated blood glucose relative to control littermates[14] demonstrating causality between the onset of urocortin 3 and the rise in blood glucose. Urocortin 3 thus establishes the homeostatic glucose setpoint by activating somatostatin-mediated feedback inhibition of insulin [Figure 4c].

Islet signaling changes in diabetes

The balance between insulin and glucagon release that is so effectively maintained by the integration of a multitude of signals that converge on healthy islets is severely disrupted in diabetes. Autoimmune attack in type 1 diabetes, or peripheral insulin resistance in type 2 diabetes, ultimately leads to beta dysfunction and death[6]. Alpha cells in type 1 diabetes exhibit an impaired counterregulatory response to hypoglycemia[145], and conversely in type 2 diabetes they aggravate hyperglycemia by inappropriate post-prandial glucagon secretion [Figure 4d][146].

There is evidence that these many clinical manifestations are tied to a breakdown of the paracrine crosstalk that so tightly regulates islet function in healthy islets. The impaired alpha cell counterregulatory response has been attributed to autonomic failure, where adrenergic stimulation that assists in potentiating hypoglycemic glucagon secretion is lost[147,148]. A paracrine explanation (that need not be mutually-exclusive) is that somatostatin is elevated in response to hypoglycemia in STZ-induced diabetic rats[149], likely contributing to impaired counterregulatory glucagon release. Indeed, blockade of the Sstr2 that is selectively expressed by alpha cells suffices to restore counterregulation in rats[150].

In another example where paracrine crosstalk breaks down in diabetes, impaired urocortin 3 signaling likely contributes to dysregulated insulin, somatostatin, secretion in diabetes. In Type 1 Diabetes, the majority of beta cells are destroyed and no longer can serve as a source of local urocortin 3 [Figure 4b]. Pre-diabetic (type 2) human, NHP, and mouse beta cells selectively down regulate urocortin 3 expression[14,151,152]. The ensuing effects of the loss of urocortin 3 on GSSS in human and NHP islets have not been established, but islets from moderately diabetic leptin-deficient mice demonstrate a loss of urocortin 3 and consequently release little somatostatin at high glucose, despite the fact that delta cells remain in normal, or even increased numbers in diabetes[14]. Restoring urocortin 3 expression in diabetic mice exacerbates hyperglycemia, in line with the re-activation of beta cell inhibition by somatostatin and similar to the premature embryonic urocortin 3 induction[14]. The mechanism responsible for the loss of urocortin 3 expression that precipitates the breakdown of local crosstalk early in diabetes is not known, which illustrates that a better understanding of paracrine communication between islet cells is vital to improved therapeutic options.

At the nutrient level, elevated blood glucose in diabetes provokes many functional changes in beta cells. Hyperglycemia contributes to beta cell proliferation in mice and ultimately leads to glucotoxicity and beta cell dysfunction. In the dysfunctional state, the loss of urocortin 3 likely removes much of the somatostatin inhibition, as stated above[14].

Unrestrained from paracrine signals including somatostatin, alpha cells release glucagon that stimulates hepatic glucose production and aggravates hyperglycemia [Figure 4d]. With hepatic insulin resistance, individuals can also develop impaired glucagon signaling, which inhibits gluconeogenesis and leads to an accumulation of circulating amino acids. This sets off a vicious cycle of hyperaminoacidemia and hyperglucagonemia, with neither signal efficiently correcting the other[64]. Initial insulin compensation in type 2 diabetes can lead to fatty infiltration where the islet microenvironment becomes filled with adipocytes[23]. While fatty acids will contribute to the compensatory increase in insulin release via potentiating secretion and increasing beta cell mass, extended exposure to fatty acids can lead to lipotoxic stress, beta cell dysfunction and decreased insulin secretion[153]. Without intervention, these initially compensatory mechanisms to increase insulin output cause a state of partial or complete beta cell dysfunction.

The value of transcriptomics and pathways for drug discovery

Transcriptomics supported by rigourous validation experiments via imaging and hormone secretion have rapidly advanced our grasp of islet function. Such increased mechanistic understanding can inform direct translational progress. RNA-Seq performed on islet cells from diabetic donors has allowed for direct comparisons between the healthy and diseased islet [34,154]. Two examples of the insights generated by these islet cell transcriptomes are clarifications of the islet mechanisms of action of the hunger hormone, ghrelin[33], and, separately, the receptor GPR119:

The mechanism of ghrelin-mediated insulin suppression

Ghrelin release occurs in the fasted state and inhibits insulin release from rodent and human pancreata[155–157]. For years, these insulinostatic actions had been attributed to direct inhibition of beta cells by ghrelin[155,158], even though the growth hormone secretagogue receptor (GHSR/Ghsr) that mediates ghrelin's signal is a GPCR normally associated with an activating $G_{\alpha q}$ subunit[159]. As ghrelin's inhibitory actions were sensitive to pertussis toxin, it was proposed that GHSR couples to an inhibitory $G_{\alpha i}$ subunit in beta cells[160]. However, islet cell transcriptomes from multiple groups resolved this conundrum by demonstrating that GHSR is selectively expressed by delta cells [Figure 3b], and the resulting $G_{\alpha q}$-mediated somatostatin release from delta cells silences beta cells in a $G_{\alpha i}$-mediated fashion[31,33,161]. Coupling transcriptomics to functional assays has expanded our ability to interrogate which islet cell type expresses the requisite receptors in order to respond to each respective signal that impinges on the islet.

GPR119 stimulates insulin secretion indirectly via alpha cells

GPR119 is a $G_{\alpha s}$-coupled GPCR that binds lipids and lipid metabolites. The receptor has been a drug target of interest for diabetes since the discovery that GPR119 activation enhances both GSIS and incretin release[162,163]. Early GPR119 research found its expression to be largely limited to the pancreatic islets and GI tract, and inaccurately built a case for GPR119 as a beta cell-specific insulinotropic receptor. Following these observation, multiple companies generated small molecule agonists for GPR119[164]. The receptor proved to be highly druggable with a number of molecules eliciting improved glucose clearance, but

efforts have stalled recently in the face of common challenges related to safety and efficacy. Moreover, drug developers may have been targeting the wrong cell type – comprehensive islet transcriptomes clearly demonstrate relatively selective expression of GPR119 by alpha cells [Figure 3b][33,40]. This has been validated by a subsequent study demonstrating that GPR119 activation in mouse and human islets improves glucagon release during hypoglycemia[165]. The improved GSIS that is observed in response to GPR119 agonism is likely due to the actions of glucagon potentiating GSIS from beta cells that we reviewed earlier.

Conclusions

While many aspects that contribute to the regulation of pancreatic islets in health and disease remain unresolved, a brief survey of recent trends in islet research shows that in taking a small step back to appreciate the islet beyond the beta cells and glucose, the diabetes field is taking significant strides forward towards a more comprehensive understanding. Shining a light on alpha and delta cells, the intra-islet crosstalk they engage in, and non-canonical nutrient signaling may turn out to be key in tackling diabetes. Especially in the context of drug discovery, these interactions cannot be ignored. The availability of comprehensive bulk and single cell transcriptomes for each islet cell type will continue to facilitate the delineation between direct and indirect effects of hormones, nutrients, and neurotransmitters. Similarly, much of the work done to date on stem cells for a cure for Type 1 Diabetes has focused on generating insulin-secreting beta cell-like cells. However, it has become clear that beta cells require input from neighboring alpha and delta cells for mature beta cell function. Future studies should be cognizant of these interactions as Occam's Razor (the simplest explanation is likely correct) applied to the pancreatic islet must account for the islet as an interactive unit where multiple endocrine and non-endocrine cell types coordinate the overall release of insulin and glucagon from the islet.

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Competing Interests

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Box 1.

The contributions of non-canonical endocrine cells and non-endocrine cells to proper islet function.

What follows are brief descriptions of the various other cells within the islet and their relation to intra-islet signaling and diabetes.

PP/gamma cells

Pancreatic polypeptide (PP) cells are the fourth islet endocrine cell type, which comprise <5% of human and <2% of mouse islet mass[167]. PP cells are found in the islet and sparingly throughout the gastrointestinal tract, and release PP in response to meals[168]. PP regulates satiety and decreases appetite and food intake in rodents and humans with no apparent paracrine effect on insulin and glucagon levels[169].

Epsilon cells

Epsilon cells are the fifth endocrine cell type and are defined by the expression of ghrelin, classically known as the “hunger hormone”. The epsilon cells increase in number throughout development reaching as high as 30% of the islet mass before decreasing to <5% in neonatal and <1% in adult islets[170]. Whether they play a role in both developing and adult islets is currently undefined.

Endothelial cells

Endothelial cells that make up the microvasculature of the islet are essential to proper endocrine function as the islet cells require high blood flow and blood volume to effectively sense nutrients and distribute their hormones. Beta cells (and to some extent alpha cells) produce a number of angiogenic and angiostatic factors that target endothelial cell receptors including VEGF-A and Angiopoietin 1[171]. Defects in beta cell-endothelial cell crosstalk in mice result in impaired GSIS and angiogenesis is vital for successful integration of transplanted islets[172].

Pericytes

Pericytes associate closely with islet capillaries, and dynamically regulate blood flow by constricting or dilating capillaries in response to signals from beta cells, endothelial cells and peripheral nerves[173]. The pericytes exhibit a certain amount of plasticity as vascular damage to islets during type 1 diabetes increases pericyte density as a possible healing response. Conversely, in type 2 diabetes the opposite occurs and vascular coverage decreases[174]. This likely contributes to impaired diabetic GSIS due to compromised in blood flow. Interestingly, pericytes also regulate beta cell function independent from their role in controlling vasculature. Beta cell insulin content and expression are reduced and GSIS is impaired when pericytes are ablated suggesting that pericytes sustain beta cell maturity in a paracrine fashion[175].

Glial/Schwann cells

Glial cells are peripheral neuronal cells that have been shown to both penetrate the core of islets and form a peripheral sheath around the islet mantle. This sheath becomes more

dense in response to injury such as stress or autoimmune attack during type 1 diabetes, reflecting a protective role of the glial cells[173]. The glial cells also serve a paracrine role: glial-derived neurotrophic factor increases beta cell mass and insulin content, which improves glucose tolerance[176].

Resident macrophages

The islet also contains resident macrophages, which, under non-inflammatory conditions, contribute to endocrine cell development in mice by supporting normal alpha and beta cell expansion[177,178].

Stellate cells

Fibroblasts and myofibroblasts are uncommon in healthy islets, but contribute to fibrosis seen in pancreatic diseases like pancreatitis and pancreatic cancer. Stellate cells are quiescent myofibroblast-like cells that secrete fibrous extracellular matrix proteins upon activation. Stellate cells are primarily responsible for fibrosis that is occasionally observed in type 2 diabetes, which has been linked to reduced insulin expression and apoptosis among beta cells[179].

Acinar cells

The exocrine pancreatic acinar tissue releases digestive enzymes and is affected not just by nutrient status following food intake, but also by local signaling from the endocrine islet. Insulin potentiates amylase release while somatostatin and pancreatic polypeptide both inhibit exocrine secretion[180].

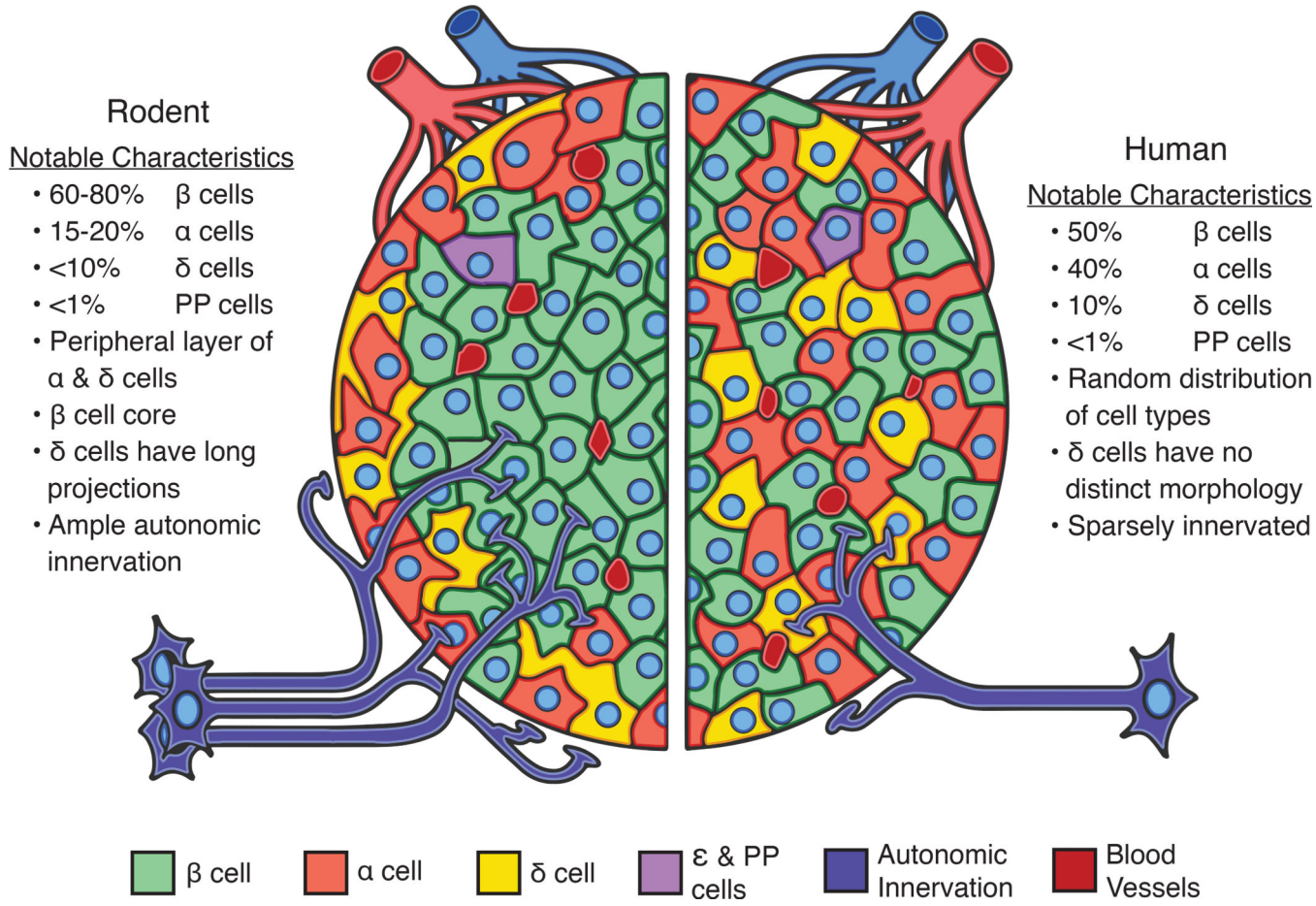

Figure 1. Comparative architecture of pancreatic islets of mice and humans.

Pancreatic islets of mice and humans differ in important ways, but also share many features in common. These shared features make mouse islets useful experimental models to study many aspects of human islet biology. The relative proportions of endocrine cell types in mouse (left) and human islets (right) are similar with beta cells (β; green) comprising the majority of the islet cell mass followed by alpha (α; light red) and delta cells (δ; yellow). Other islet endocrine cells such as pancreatic polypeptide and epsilon cells (PP and ε; purple) are more sparse in number. Human islets occur in a wide variety of sizes and conformations that range from highly structured to more random distributions of cells. Mouse islets exhibit a more uniform architecture with alpha and delta cells at the islet periphery surrounding a beta cell core. Islets in both species are vascularised (dark red) and innervated (dark blue) for rapid sensing of changing energy needs, although mouse islets are more densely innervated than humans.

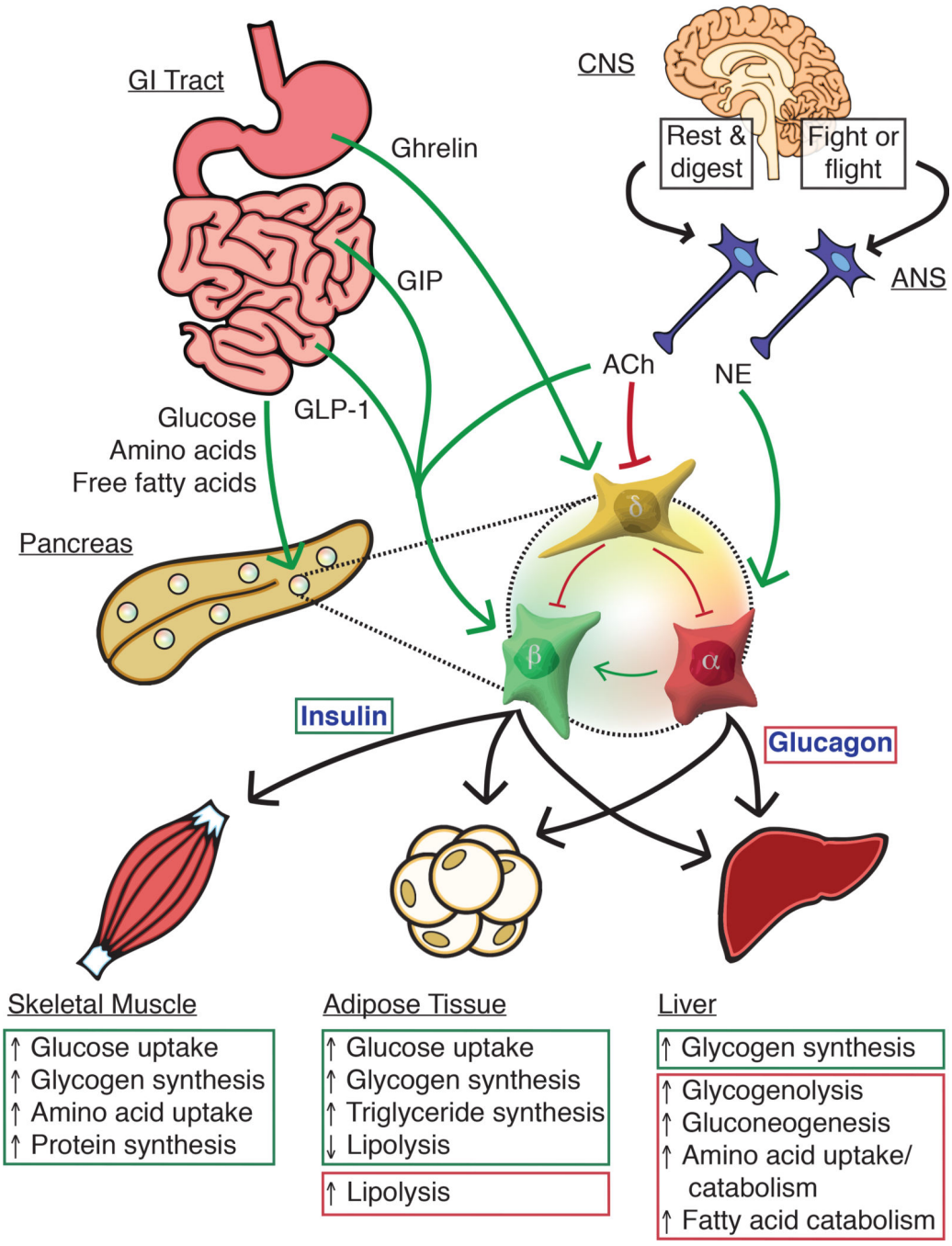

Figure 2. Inter-organ signaling from nutrient sensing to islet-mediated metabolic effects.

Nutrition-related signals from the gastrointestinal (GI) tract combine with neuronal input from the autonomic nervous system (ANS) to direct insulin and glucagon secretion from pancreatic islets. Changes in blood glucose levels are sensed by alpha, beta, and delta cells, which respond by restoring blood glucose to homeostatic levels. Alpha cells release glucagon at low glucose to increase hepatic glucose production. During hyperglycemia, beta cells lower blood glucose by releasing insulin to increase glucose storage in the liver, skeletal muscle, and adipose tissue. Insulin release is amplified by the incretin hormones

GLP-1 and GIP from the small intestine as well as by glucagon from neighbouring alpha cells. Delta cells secrete somatostatin across a range of glucose levels, but most prominently in response to hyperglycemia. Amino acids and free fatty acids (FFAs) stimulate both alpha and beta cells, and the peripheral effects of both glucagon and insulin result in reduced circulating amino acids and FFAs. The central nervous system (CNS) can augment islet secretion in conditions such as the “rest and digest” state where direct insulin secretion is further facilitate by a suppression of somatostatin secretion by acetylcholine (ACh) associated with parasympathetic innervation. Glucagon secretion is increased during the “fight or flight” response by norepinephrine (NE) released by sympathetic nerves.

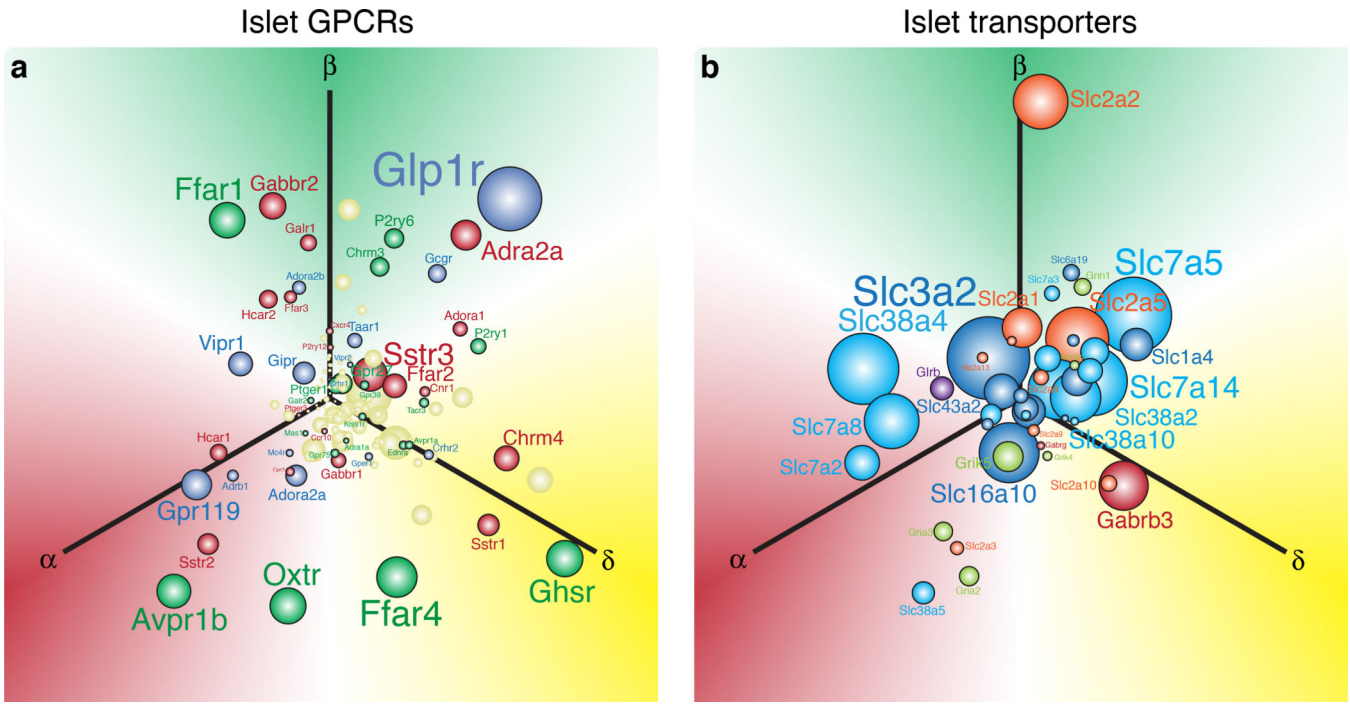

Figure 3. Visualisation of the abundance and selectivity of GPCR and transporter gene expression in alpha, beta, and delta cells.

We used the natural log of the normalised expression values for a gene (ln[RPKM]) to plot the relative position of that gene along three axes representing alpha, beta, and delta cells. These expression values are derived from transcriptomes of FACS-purified mouse alpha, beta, and delta cells described elsewhere[33]. **a)** Each of these three individual gene expression values are converted into x and y vectors and then consolidated into a single set of x, y coordinates that represents the overall selectivity of the expression of that gene. The origin represents equal expression (*i.e.* no enrichment) in each of the three islet cell types, whereas. placement in any direction along one of the axes reflects enrichment in the corresponding cell type. Sphere and font sizes are proportional to abundance of the gene based on the highest RPKM value for that gene in alpha, beta, or delta cells. **b)** The top 150 most abundant G protein-coupled receptors (GPCR) of the islet cells are color coded in accordance with the predominant signaling cascade associated with each receptor. Blue genes are $G_{\alpha s}$-coupled, green are $G_{\alpha q}$, red are $G_{\alpha i}$, and yellow is 'unknown' or ambiguous based on receptor classifications from IUPHAR (International Union of Basic and Clinical Pharmacology)[166]. **c)** Non-GPCR receptors and transporters are colour-coded according to the class of signaling molecules utilised by each receptor/transporter, following IUPHAR classification for solute carriers.

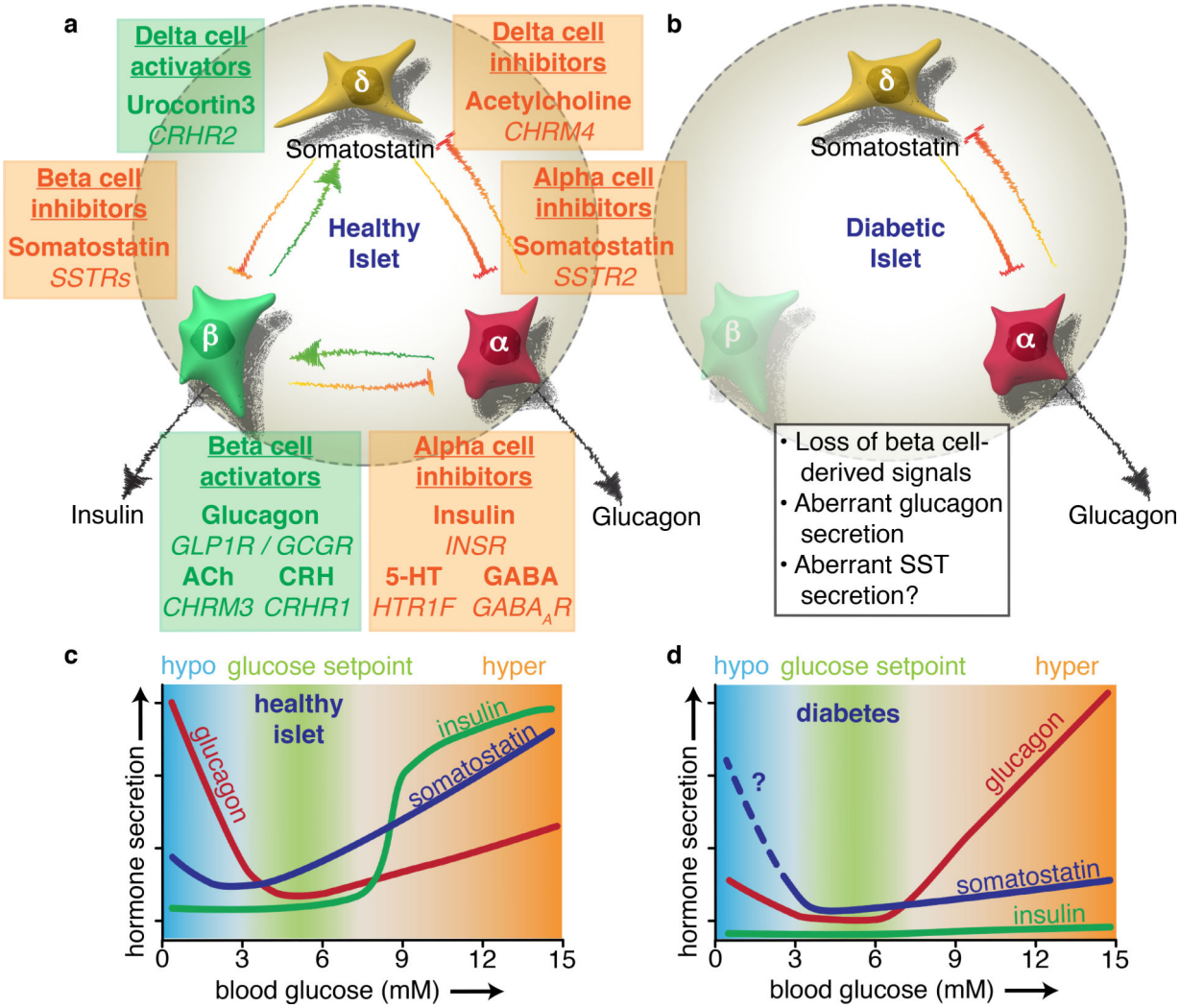

Figure 4. Diabetes disrupts the extensive paracrine signaling network of the islet.

Alpha, beta, and delta cells influence each other's secretion via intra-islet crosstalk. **a)** Coloured text boxes (green for activating, orange for inactivating) denote the target cell type of paracrine signaling (underlined), the signal molecule involved (bold), and the target receptor gene (italicised). Each box is placed in between the target cell and the source of the signal. Beta cells initiate a negative feedback loop in high glucose whereby they release urocortin 3 to activate delta cells. The resulting somatostatin (SST) release feeds back to mediate insulin release, providing tonic inhibition that establishes the homeostatic glucose setpoint. Beta cells also experience paracrine activation from alpha cells, which release glucagon, acetylcholine (ACh), and corticotropin-releasing hormone (CRH), which all potentiate GSIS. Beta cell-derived products such as insulin, serotonin (5-HT), and GABA – in addition to urocortin 3-induced somatostatin release – all contribute to silence alpha cells during hyperglycemia. **b)** The onset of diabetes results in a loss of multiple paracrine signals. Due to autoimmune destruction, type 1 diabetic islets effectively lose all beta cell signals. In type 2 diabetes, urocortin 3 is severely downregulated in beta cells, blunting glucose-stimulated somatostatin secretion. **c)** The physiological impact of paracrine

signaling can be visualised with glucose curves for each islet hormone. The homeostatic glucose set point is maintained by glucagon raising blood glucose during hypoglycaemia and insulin lowering glucose during hyperglycaemia. Somatostatin contributes as a fine-tuning mechanism via paracrine inhibition of both alpha and beta cells. **d)** The absence of beta cell-derived products in diabetes results in inappropriately high glucagon secretion during high glucose, which exacerbates hyperglycaemia. Glucagon counterregulation at low glucose is also impaired, possibly due to aberrant somatostatin secretion, although this is not yet fully understood. Based in part on Ref[17].