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The role of G protein-coupled receptors and receptor kinases in pancreatic β-cell function and diabetes

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Text pages: 135

Tables: 1

Figures: 3

References: 363

Words in abstract: 247

ABBREVIATIONS: AR, adrenergic receptor; α_2AR, α2-adrenergic receptor; β_2AR, β2-adrenergic receptor; CasR, calcium sensing receptor; CIC3, chloride channel 3; DAG, diacylglycerol; Epac2, exchange protein directly activated by cAMP 2; ECL, extracellular loop; GCGR, glucagon receptor; GWAS, genome wide association studies; GIP, gastric inhibitory peptide; GIPR, gastric inhibitory peptide receptor; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide-1 receptor; GSIS, glucose-stimulated insulin secretion; G6P, glucose-6-phosphate; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GIRK, G protein-gated inwardly rectifying potassium channel; HbA1c, hemoglobin A1C; HFD, high fat diet; IP3, inositol trisphosphate; ICL, intracellular loop; IDH1, isocitrate dehydrogenase; Munc13, mammalian uncoordinated-13; M3R, muscarinic receptor subtype 3; LCFA, long chain fatty acid; PC, prohormone converting enzyme; PI3Kγ, phosphoinositide 3-kinase γ; PKA, protein kinase A; PLCβ,

phospholipase Cβ; PTX, pertussis toxin; RGS, regulator of G-protein signaling; RH, RGS homology domain; SENP1, sentrin-specific protease 1; SCFA, short chain fatty acid; S1PR, spingosine-1 phosphate receptor; STZ, streptozotocin; TCA, tricarboxylic acid; TCF1, transcription factor 1; TM, transmembrane α-helices; T1D, Type 1 diabetes; T2D, Type 2 diabetes; VGCC, voltage gated calcium channels.

Abstract

Type 2 diabetes mellitus (T2D) has emerged as a major global health concern that has accelerated in recent years due to poor diet and lifestyle. Afflicted individuals have high blood glucose levels that stem from the inability of the pancreas to make enough insulin to meet demand. While medication can help to maintain normal blood glucose levels in individuals with chronic disease, many of these medicines are outdated, have severe side effects, and often become less efficacious over time necessitating the need for insulin therapy. G protein-coupled receptors (GPCRs) regulate many physiological processes including blood glucose levels. In pancreatic β-cells, GPCRs regulate β-cell growth, apoptosis, and insulin secretion which are all critical in maintaining sufficient β-cell mass and insulin output to ensure euglycemia. In recent years, new insight into the signaling of incretin receptors and other GPCRs have underscored the potential of these receptors as desirable targets in the treatment of diabetes. The signaling of these receptors is modulated by GPCR kinases (GRKs) that phosphorylate agonist activated GPCRs marking the receptor for arrestin binding and internalization. Interestingly, genome wide association studies (GWAS) using diabetic patient cohorts link the GRKs and arrestins with T2D. Moreover, recent reports show that GRKs and arrestins expressed in the β-cell serve a critical role in the regulation of β-cell function including β-cell growth and insulin secretion in both GPCR-dependent and independent pathways. In this review, we describe recent insight into GPCR signaling and the importance of GRK function in modulating β-cell physiology.

Significance Statement

Pancreatic β-cells contain a diverse array of GPCRs that have been shown to improve β-cell function and survival, yet only a handful have been successfully targeted in the treatment of diabetes. This review discusses recent advances in our understanding of β-cell GPCR pharmacology and regulation by GRKs while also highlighting the necessity of investigating islet enriched GPCRs that have largely been unexplored to unveil novel treatment strategies.

Table of Contents

I. Introduction

Diabetes mellitus is currently the largest worldwide epidemic affecting over 500 million people and costing nearly a trillion dollars a year. The International Diabetes Federation predicts that by 2045, close to 800 million people will be living with diabetes demarcating an unsustainable development to worldwide health and productivity (International Diabetes Federation, 2021).

Diabetes mellitus is a disease characterized by hyperglycemia or high levels of circulating glucose. This is typically the result of insufficient quantities of the pancreatic β-cell hormone insulin and its inability to activate target cells to maintain glucose homeostasis known as insulin resistance. There are various types of diabetes mellitus classified as Type 1 (T1D), Type 2 (T2D), rare monogenic forms of diabetes such as maturity onset diabetes of the young (MODY) that are due to genetic mutations, and temporary diabetes such as gestational diabetes due to pregnancy. T1D accounts for less than 10%, T2D for roughly 90%, and all other forms constitute only 1-2% of total cases (International Diabetes Federation, 2021; Reed et al., 2021; Flannick et al., 2016; Riddle et al., 2020).

In T2D, β-cell dysfunction and peripheral insulin resistance create an environment that limits glucose uptake in muscle and adipose and increases hepatic glucose production causing and exacerbating hyperglycemia (Reed et al., 2021; Kahn et al., 2014). The factors contributing to β-cell demise are both chronic and complex. These include genetic and environmental impacts that alter the ability of the β-cells to produce and secrete insulin. These stressors compound over many years leading to increased obesity and insulin resistance that then require higher insulin levels to

maintain healthy glucose levels. Continued exposure eventually develops into prediabetes and impaired glucose tolerance where the level of insulin is no longer sufficient to maintain normoglycemia due to the accumulation of fat and insulin resistance. Without intervention, prediabetes progresses into diabetes which is denoted by fasting blood sugar levels above 126 mg/dL (Page and Johnson, 2018; Johnson and Kushner, 2018; Bar-Tana, 2020). This disease is preventable in most cases so early detection is paramount to ensure interventions are implemented. Interventions include diet, exercise, and medicines to combat chronic hyperglycemia before severe diabetic complications develop such as cardiovascular disease, kidney disease (nephropathy), and liver failure (NAFLD, steatosis) that can result in death if left untreated (Reed et al., 2021; Mazzone et al., 2008; Ritz et al., 1999; Wilding, 2014; Tilg et al., 2017; Goldberg, 2001; Gross et al., 2005; Cole and Florez, 2020; Corkey, 2012a,b; Alonso-Magdalena et al., 2011).

Although all forms of diabetes culminate in hyperglycemia, the etiology of the disease can differ significantly, especially within T2D. Statistical analysis of data from a large cohort of patients identified 5 clusters of diabetes: severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), and mild age-related diabetes (MARD) where SAID represents T1D and the other forms are within T2D (Ahlqvist et al., 2018). This study highlights the importance of reclassifying T2D into subtypes based on individual pathologies. Effective treatment regiments will depend on whether the individual needs to lower insulin resistance and hyperlipidemia (weight loss) or increase pancreatic insulin output. This also helps to address the debate as to whether obesity

and insulin resistance precedes β-cell dysfunction and T2D or β-cell hyperstimulation and hyperinsulinemia causes obesity and insulin resistance leading to T2D. As is becoming apparent, likely both are true depending on genetic predispositions in specific populations and the environmental context patients endured that contributed to their diabetes progression making it essential to realize that individual T2D pathologies may differ (Esser et al., 2020; Shanik et al., 2008; Corkey, 2012a,b; Weir and Bonner-Weir, 2004; Kahn, 2003; Johnson, 2021). Nevertheless, in each case, the hallmark of the disease is hyperglycemia accompanied with insulin resistance and often obesity that if left untreated, can progress into life threatening complications.

The primary treatment for most people with T2D is lifestyle modifications including a healthy diet and increased exercise. For many, these changes will successfully help patients lose weight and achieve hemoglobin A1C (HbA1c) levels no longer in the diabetic range (Reed et al., 2021; Kahn et al., 2014). However, there is a significant population of individuals with T2D where these interventional strategies will be inadequate. These individuals require pharmacological help due to genetic predispositions, poor compliance, or the necessity for emergency intervention where immediate HbA1c reduction is needed to avoid dangerous complications such as in obesity or chronic hyperglycemia (Williams et al., 2022). The FDA approved drugs for diabetes include those that increase insulin output from the pancreas, decrease insulin resistance, or prevent reabsorption of glucose into the blood by the kidneys (Raz, 2013; Marin-Penalver et al., 2016; Artasensi et al., 2020).

Insulin insufficiency and reduced β-cell mass are major characteristics of T2D (Meier and Bonadonna, 2013). Of the available non-insulin FDA approved drugs for

T2D, only a handful act directly on the β-cell to increase insulin secretion and only agonists for the glucagon-like peptide-1 receptor (GLP-1R) have been shown to increase functional β-cell mass (Fusco et al., 2017; Tamura et al., 2015; Drucker 2018). Many reports have shown that sulfonylureas/meglitinides can actually reduce β-cell mass and induce β-cell hyperexcitability ultimately leading to ineffective treatment over time necessitating the need for insulin therapy (Maedler et al., 2005; Kahn et al., 2006; Del Guerra et al., 2005; Rosengren et al., 2008). However, exogenous insulin and other insulin inducing therapies can lead to weight gain and insulin resistance aggravating glycemic control and even worsening cardiovascular risk and other diabetic comorbidities (Heller, 2004; Russell-Jones and Khan, 2007; Apovian et al., 2019). It is therefore of utmost importance to further dissect the biology of β-cells and other hormone secreting islet cells to fully understand and implement therapeutic modalities that improve efficacy and clinical outcomes with limited side effects. The best options will likely include titrating the hormones controlling glucose levels coupled with strategies to improve insulin sensitivity such as exercise, weight loss, and better pharmacological agents.

G protein-coupled receptors (GPCRs) are the target of approximately 35% of FDA approved drugs but until recently only one GPCR, the GLP-1R, has been targeted on the β-cell for the treatment of T2D (Sriram and Insel, 2018; Drucker, 2018). In 2022, another incretin activated GPCR, the gastric inhibitory peptide receptor (GIPR), was approved by the FDA for treatment of T2D with tirzepatide, a GLP-1R/GIPR dual agonist (El et al., 2023; Chavda et al., 2022). These receptors have proven utility as cell surface drug targets that allow them high accessibility to pharmacologic agents. The

islets, and especially β-cells, express a multitude of GPCRs, some of which have been identified as potential targets to improve insulin output (Perreault et al., 2021; Husted et al., 2017).

In this review, we first discuss GPCRs that have robust biology in β-cells focusing on receptors that have been extensively investigated and have proven utility as drug targets such as the incretin receptors, fatty acid receptors, and muscarinic receptors. We then discuss the potential role of GPCR kinases (GRKs) in diabetes, a family of regulatory proteins that modulate GPCR signaling but are largely unexplored in islet biology. Finally, we provide a review of islet transcriptomic data describing the plethora of GPCRs expressed on islets with minimal functional data and a clear need for further analyses. Together, we provide a comprehensive and updated review of the potential for targeting GPCRs in islets to improve β-cell function in patients with diabetes.

A. Glucose stimulated insulin secretion (GSIS)

The prototypical mechanism of insulin secretion is mediated by glucose influx and metabolism in the β-cell that culminates in robust and rapid increases in intracellular calcium concentrations that initiate insulin granule exocytosis. This glucose-stimulated insulin secretion is biphasic in response to the rapid increase and persistent presence of glucose (square wave stimulation) and is sustained for as long as the stimulatory glucose concentrations are present. First phase insulin secretion is known as the triggering phase or the K_{ATP} channel-dependent phase. The triggering phase is characterized by a rapid peak of insulin secretion within 1-5 minutes following stimulation that is lessened to plateau at approximately half of the peak response by 10

minutes after stimulation. The triggering phase is necessary to reach interstitial concentrations of insulin quickly to combat rising glucose levels. The second phase of insulin secretion is referred to as the amplifying phase or the K_{ATP} channel independent-phase (Henquin et al. 2006; Henquin et al., 2002; Bratanova-Tochkova et al., 2002). The amplifying phase begins approximately 10 minutes following stimulation and is maintained for as long as the stimulatory signals are present. This phase of insulin secretion is characterized by a flat but steady release of insulin that in humans and rats gradually rises over time (Henquin et al., 2006; Curry et al., 1968; Berglund, 1980). Therefore, for prolonged durations with glucose stimulation, most of the insulin released is due to amplifying mechanisms of insulin secretion even though the amplitude of second phase insulin secretion is less than that of the triggering phase (Kalwat and Cobb, 2017; Skelin Klemen et al., 2017). Together, this biphasic secretory response of β-cells allows for rapid and sustained release of insulin to lower serum levels of glucose in a timely manner where only a tiny fraction of the β-cell insulin content is required to accomplish this feat.

The triggering phase of insulin secretion is well understood in β-cells (Skelin Klemen et al., 2017). Rising glucose concentrations enter the β-cell through a low affinity-high capacity GLUT2 transporter (GLUT1 in rodents) that allows for rapid equilibration of glucose levels within the physiological range. The glucose then binds glucokinase (hexokinase IV) to generate glucose-6-phosphate (G6P) and initiating a cascade of metabolic events to increase the production of ATP. Increasing levels of ATP are then able to bind the nucleotide binding pocket of the SUR1 subunit of the K_{ATP} channel prompting channel closure and membrane depolarization. This membrane

voltage change then activates voltage gated calcium channels (VGCCs) on the plasma membrane that rush calcium into the β-cell generating a spike in intracellular calcium concentrations that invoke insulin release. The pancreatic β-cell isoform of glucokinase, also present in the liver, is the rate limiting step of the triggering phase due to its approximately 10-fold higher K_m for glucose compared to other hexokinase isoforms found in other tissues. It also lacks the N-terminal domain found in other hexokinases that usually mediates product inhibition by high levels of G6P (Matschinsky and Wilson, 2019; Wilson, 2003). Thus, pancreatic β-cell glucokinase can phosphorylate high quantities of glucose quickly sparking the high level of glucose metabolism required for K_{ATP} channel inhibition and membrane depolarization. This phase of insulin secretion is largely dependent upon K_{ATP} channel dynamics and intracellular calcium (Henquin et al. 2006; Henquin et al., 2002; Bratanova-Tochkova et al., 2002).

Biphasic insulin secretion is dependent on the rapid and large increase in intracellular calcium that is mediated by the triggering phase of insulin secretion in response to square wave glucose stimulation. The amplifying phase of insulin secretion does not elicit a spike in intracellular calcium like the triggering phase but rather augments insulin secretion using the already established rise in intracellular calcium from the triggering phase (Henquin et al. 2006; Henquin et al., 2002; Bratanova-Tochkova et al., 2002; Campbell and Newgard, 2021). So, although it is referred to as the K_{ATP} channel-independent pathway, it does require K_{ATP} channel inhibition and the ensuing calcium influx to support insulin release. The amplifying mechanisms of second phase insulin secretion are not completely understood but include metabolic flux pathways of glucose metabolism that dictate the concentration of various metabolic

intermediates generated from the metabolism of glucose that are coupled to exocytic machinery to maintain release competency of insulin granules. Of these, tricarboxylic acid (TCA) cycle intermediates such as citrate, isocitrate, and succinate are among the best studied in their role of GSIS (Campbell and Newgard, 2021). Additionally, changes in β-cell lipid composition also influence GSIS (Prentki et al., 2013).

Anaplerosis refers to the ability of metabolic pathways to regenerate intermediates of the TCA cycle to combat carbon loss and maintain sufficient levels of energy metabolism. Post glucose stimulation, pyruvate levels are increased through glycolysis and serve as a foundation for anaplerotic reactions that replenish TCA cycle intermediates. In the β-cell, pyruvate enters the TCA mainly through its conversion to oxaloacetate mediated by the anaplerotic enzyme pyruvate carboxylase as opposed to its conversion to acetyl-CoA by pyruvate dehydrogenase (Campbell and Newgard, 2021). Inhibition of pyruvate carboxylase reduces GSIS highlighting the importance of pyruvate levels (Lu et al., 2002). Additionally, pyruvate is prevented from its conversion to lactate in β-cells due to the β-cell specific downregulation of lactate dehydrogenase ensuring pyruvate enters the TCA cycle (Schuit et al., 2012). This accumulation of pyruvate regenerates the TCA cycle allowing for intermediates to exit the mitochondria and be used in cytosolic reactions that increase the cellular redox state or the concentration specific metabolites that increase the release of insulin through various mechanisms. For example, pretreatment of β-cells with the TCA cycle intermediate isocitrate increases insulin exocytosis. Isocitrate is converted to α-ketoglutarate by the cytosolic isoform of isocitrate dehydrogenase (IDH1) simultaneously generating NADPH from NADP. The NADPH can then be used in glutathione redox reactions that activate

glutaredoxin and ultimately result in the reduction/activation of sentrin-specific protease 1 (SENP1), a protein necessary for granule release competency increasing insulin release (Ferdaoussi et al., 2015). β-cells treated with siRNA against IDH1 have reduced potentiation of GSIS by isocitrate implying its role as a metabolic coupling factor to insulin secretion (Ronnebaum et al., 2006). The export of citrate and isocitrate is made possible by the anaplerotic reactions of pyruvate and glutamine that replenish the TCA cycle (Campbell and Newgard, 2021; Prentki et al., 2013).

Multiple studies utilizing knockout of different TCA cycle, NADH shuttle, and pentose shunt pathway enzymes impair GSIS revealing the importance of glucose metabolism and its connection to insulin secretion-independent of the K_{ATP} channel (Zhang et al., 2021a; Spegel et al., 2013; Goehring et al., 2011). Often, they converge on the exocytic machinery proteins such as SENP1, mammalian uncoordinated-13 (Munc13), and snare proteins to enhance release competency of insulin granules (Ferdaoussi et al., 2015; Zhao et al., 2014; Gaisano, 2017). Why so many metabolic coupling mechanisms exist to influence insulin secretion remains unknown but clearly, it illustrates the importance of insulin in regulating whole-body glucose homeostasis and the necessity of tightly regulating insulin secretion from the β-cell. Importantly, β-cell GSIS and glucose utilization are also modulated by lipids, gut microbiota metabolites, amino acids, and hormones such as GLP-1 and GIP in the islet microcirculation (Fig. 1). The effects of these compounds are predominately adjudicated by β-cell expressed GPCRs that transmit the extracellular milieu of small molecules and peptide hormones to an intracellular secretory response (Oliveira et al., 2021; Riddy et al., 2018; Oh and Olefsky, 2016; Husted et al., 2017).

II. GPCR Signaling

A. Overview of GPCRs

GPCRs are ubiquitous integral membrane proteins expressed in virtually all tissues and cell types. GPCRs transmit the ability of extracellular stimuli such as neurotransmitters, peptide hormones, and even photons of light to mediate an intracellular response. GPCRs represent the largest class of cell surface receptors comprised of over 800 members and represent about 35% of drug targets approved by the FDA (Pierce et al., 2002; Sriram and Insel, 2018). However, only approximately 30% of potentially druggable GPCRs are targeted by these FDA approved molecules highlighting the untapped therapeutic potential of this class of receptors for numerous diseases (Congreve et al., 2020).

GPCRs are subdivided into five classes based on primary sequence similarities, ligand binding profiles, and molecular architecture (Foord et al., 2005). The vast majority of GPCRs are class A (rhodopsin-like) with over 700 members in humans. Approximately half of these include the taste and odorant receptors that are activated by various pheromones, odorants, and tastants. The other half includes receptors that are activated by diverse ligands including catecholamines, neurotransmitters, and other small molecule ligands. Some of the most well studied members include the adrenergic, muscarinic, and serotonin receptors. Class B GPCRs (secretin receptor-like) include 15 members and are activated by large peptide hormones such as GLP-1, GIP, vasopressin, and angiotensin. The binding of these hormones is in part mediated by the large N-terminus of these GPCRs (Congreve et al., 2020; Hollenstein et al., 2014). Class C GPCRs (glutamate receptor-like) include 22 members and have the unique

requirement for dimerization to initiate signaling (Levitz et al., 2016; El Moustaine et al., 2012). Class C GPCRs often have a large N-terminus such as the calcium sensing receptor and some taste receptors (Brauner-Osborne et al., 2007). The class F GPCRs (frizzled/smoothened) has 11 members including smoothened and the frizzled receptors that participate in Wnt signaling. These receptors are critical in the regulation of tissue homeostasis and tumorigenesis and are therefore targets in some cancers (Gurney et al., 2012). Finally, there are the 33 adhesion GPCRs (aGPCRs) that harbor a hybrid structure that utilizes an extracellular domain that interacts with the extracellular environment. These receptors mediate cell adhesion, positioning, and orientation during development and immune responses through the ability to couple the physical environment to intracellular signaling (Langenhan et al., 2013).

B. GPCR structure/signaling

The overall architecture and activation mechanism of GPCRs are largely conserved among the different GPCR classes even though there is relatively little sequence homology. The characteristic structure of a GPCR contains seven transmembrane α-helices (TM1-7) stabilized by intramolecular contacts that help to stabilize an inactive form of the receptor. These transmembrane helices are connected by three extracellular loops (ECLs) and three intracellular loops (ICLs) (Kobilka, 2007). The ECLs in conjunction with the extracellular half of the transmembrane α-helices form the orthosteric binding site for the plethora of ligands that can bind the receptors. Some ligands interact specifically with the ECL domains whereas others interact with the transmembrane core in the plasma membrane (Wingler and Lefkowitz, 2020; Manglik et

al., 2015; Siu et al., 2013; Lin et al., 2021). The ICLs are less variable and aid in the binding of transducer proteins that control the signaling of the receptor. However, the size of the ICLs can vary, especially ICL3, and participate in controlling the activation status of the receptor (Pao and Benovic, 2005; Rosenbaum et al., 2007). Finally, GPCRs have a variable cytoplasmic C-terminus that can range in length from 25-150 amino acids. This helps to mediate binding to various intracellular effector proteins that influence GPCR signaling and trafficking (Lagerstrom and Schioth, 2008).

Although there is ample structural diversity aside from the transmembrane core of GPCRs, the activation mechanisms are similar. Ligand binding to the receptor induces conformational changes that disrupt intramolecular contacts of transmembrane helices that allow for the outward movement of TM5 and TM6. These conformational changes promote heterotrimeric G-protein binding and the exchange of GDP for GTP on the associated G-protein α subunit. The GTP bound Gα and G$\beta\gamma$ subunits then dissociate from the activated receptor and interact with effector molecules to mediate changes in intracellular signaling. Gα subunits regulate adenylyl cyclase, phospholipase Cβ (PLCβ), and RhoGEFs whereas G$\beta\gamma$ subunits can regulate various effectors including G protein-gated inwardly rectifying potassium channels (GIRK), and phosphoinositide 3-kinase γ (PI3Kγ).

The heterotrimeric G-proteins consist of Gα, Gβ, and Gγ subunits. There are 16 Gα, 5 Gβ, and 14 Gγ subunits. In the cell, Gβ and Gγ subunits are constitutively associated as a G$\beta\gamma$ dimer. The Gα proteins are divided into four subfamilies. The Gα_s family has two members and these primarily activate adenylyl cyclase to increase cAMP levels and subsequent PKA activation. The Gα_i family is the largest class with eight

members and functions to inhibit adenylyl cyclase and oppose $G\alpha_s$ activity. The $G\alpha_q$ family has four members that activate PLCβ to catalyze the production of diacylglycerol (DAG) and inositol trisphosphate (IP3) increasing cytosolic calcium concentrations. Finally, the $G\alpha_{12/13}$ family has two members that activate RhoGEFs to regulate cell adhesion and cytoskeletal rearrangements (Milligan and Kostenis, 2006). The Gα subunits have an intrinsic GTPase activity allowing them to hydrolyze GTP to GDP inactivating themselves. For some Gα subunits, the hydrolysis of GTP is accelerated by regulator of G-protein signaling (RGS) proteins that can bind Gα proteins and stimulate GTPase activity (Lambert et al., 2010). Additionally, mutant forms of Gα that prevent GTP hydrolysis exist that have aberrant constitutive active intracellular signaling including $G\alpha_q$-Q209L (Katoh et al., 1998; Lapadula and Benovic, 2021).

C. Regulation by GRKs and arrestins

GPCR mediated G-protein activation continues while the agonist is bound to the receptor and, if left unchecked, overactivation can become problematic to cell physiology. To combat this, activated GPCRs are phosphorylated on residues residing in their intracellular loops and C-tail by various kinases, most notably the GPCR kinases (GRKs). This phosphorylation event promotes the recruitment of adaptor proteins called arrestins, which by steric hindrance inhibit further G-protein activation. This is referred to as desensitization or tachyphylaxis, where the activity of the receptor is diminished even in the continued presence of its activating ligand (Moore et al., 2007). There are four mammalian arrestins. Two of the arrestins, rod arrestin and cone arrestin (aka arrestin-1 and arrestin-4, respectively) are found exclusively in the visual system within the retina

while the two non-visual arrestins, β-arrestin1 (arrestin-2) and β-arrestin2 (arrestin-3), are ubiquitously expressed. Following receptor phosphorylation by GRKs, β-arrestins are recruited to agonist-occupied GPCRs and promote receptor trafficking to clathrin coated pits via the ability to bind directly to AP-2 and clathrin. This induces β-arrestin mediated internalization of the agonist bound receptor into endosomes. Some internalized receptors continue to signal from the endosome and are subsequently directed towards degradation or recycling pathways. Degradation mechanisms include β-arrestin mediated ubiquitination of the receptor designated for lysosomal degradation, while recycling pathways return the receptor to the plasma membrane in its inactive form to become available again for ligand binding. The desensitization and internalization of the receptor mediated by GRKs and β-arrestins provides the ability to tightly regulate GPCR signaling in response to various ligands (Moore et al., 2007; Gurevich and Gurevich, 2019; Reiter and Lefkowitz, 2006).

III. GPCRs regulating β-cell insulin dynamics

A. The Incretin effect: GLP-1R and GIPR

Following a meal, nutrient metabolites including glucose, amino acids, and free fatty acids from the breakdown of food products stimulate enteroendocrine cells of the intestine to secrete insulinotropic hormones that serve critical glucoregulatory functions. These intestinal hormones are called the incretins and they are responsible for reducing postprandial increases in serum glucose concentrations. The incretins, glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP) mediate what is known as the incretin effect. The incretin effect describes the markedly improved insulin secretory

response following oral ingestion of food as opposed to intravenous infusions that bypass the digestive system. This manifests in a more robust control of postprandial glucose mobilization than with glucose alone that is dependent upon the exposure of the intestine to nutrients (carbohydrates/glucose, proteins/amino acids, and lipids/free fatty acids) (Boer and Holst, 2020; Nauck and Meier, 2018; Drucker, 2006).

B. GLP-1R

GLP-1 is secreted from the L cells of the jejunum, ileum, and colon in the distal intestine. It originates from the 160 amino acid precursor peptide, proglucagon, that can be processed into several metabolic hormones including GLP-1, GLP-2, and glucagon (Campbell and Drucker, 2015). The glucose-dependent potentiation of insulin secretion by GLP-1 is mediated by its binding and activation of the GLP-1R on β-cells (MacDonald et al., 2002). The GLP-1R is a class B GPCR characterized by a large extracellular domain that can accommodate large peptide agonists. It is a G_s-coupled receptor and therefore its activation increases the cellular level of cAMP through increased adenylyl cyclase activity. The increased cAMP level is then able to activate protein kinase A (PKA) sensitizing the β-cell to calcium induced exocytosis. Phosphoproteomic analysis of GLP-1 activated β-cells showed more than 5000 phosphorylated proteins of which the vast majority are uncharacterized. The K_{ATP} channel subunits, Kir6.2 and SUR1, as well as exocytic proteins are phosphorylated by PKA to enhance insulin secretion (Tang et al., 2017; Light et al., 2002). Interestingly, PKA does not account for the entirety of the GLP-1 induced secretory response as PKA inhibition did not completely abrogate insulin secretion after GLP-1 treatment. Indeed,

an alternate cAMP-dependent/PKA-independent pathway has been elucidated that is controlled by the Exchange protein directly activated by cAMP 2 (Epac2) (Holz, 2004). In addition, studies have shown that the GLP-1R can also couple to G_q and activate PLCβ (Montrose-Rafizadeh et al., 1999).

The activation of Epac2 by cAMP induces calcium induced calcium release from ER proteins such as the IP3 and ryanodine receptors. The activation of the ryanodine receptor has also been shown to be mediated by Epac2 activation of PLC and subsequent IP3 production. It also synergizes with glucose metabolism and PKA activation to maintain K_{ATP} channel inhibition preventing β-cell hyperpolarization (Holz, 2004; Holz et al., 1999; Tsuboi et al., 2003). Finally, it has been proposed that Epac2 regulates chloride channel 3 (CIC3) on insulin granules that are critical in maintaining electroneutrality as the vesicular H^+-ATPase (i.e., proton pump) reduces the pH by interacting with granular SUR subunits. This is consistent with the increased proinsulin levels and reduced insulin secretion seen in secretory granules of β-cells with CIC3 knockout due to defective granular acidification (Leech et al., 2010; Deriy et al., 2009). GLP-1 stimulation also improves the viability of β-cells as streptozotocin (STZ) treated rodents were protected from STZ toxicity. This is due to reduced apoptosis and increased β-cell neogenesis providing the islet not only with enhanced secretory capacity but also improved β-cell mass in response to GLP-1R activation (Li et al., 2003; Xu et al., 2006). Together, these GLP-1 signaling pathways help to ensure the islet has sufficient β-cell mass and insulin secretory capacity, both of which are lost in T2D.

C. GIPR

GLP-1 has received the most attention due to the ability of pharmacological levels of the hormone to improve glycemic control and other metrics in patients with T2D. However, GIP in healthy individuals accounts for most of the incretin effect although its physiology is less well understood than GLP-1. The incretin effect accounts for approximately 70% of the increased insulin response following a meal (Gasbjerg et al., 2019). Of the insulin in circulation following an oral glucose load, 44% was due to GIP, 22% due to GLP-1 and 33% due to glucose (Nauck and Meier, 2019). Over time, patients with T2D have a reduced incretin effect and are much less responsive to endogenous amounts of the hormones. Supraphysiological levels of GLP-1 have a robust potentiation effect on GSIS (Nauck et al., 1993a). Disappointingly, high levels of GIP have not been shown to have effects similar to high GLP-1 and the interest in GIP as a therapeutic modality has dwindled (Nauck et al., 1993b). However, recent progress in incretin biology has revealed that dual- and tri-agonist compounds (GIP, GLP-1, and GCG) can have synergistic effects on glycemic control. So, although GIP alone had no effect on improving glycemic control, its combination with GLP-1 can have potent glycemic and weight control.

The GIP incretin is processed from the 153 amino acid precursor peptide proGIP. The release of GIP occurs from the intestinal K cells of the duodenum and occurs in a manner similar to GLP-1 release. The augmentation of insulin release elicited by GIP is mediated by its activation of the GIP receptor on the pancreatic β-cell (Boer and Holst, 2020). The GIPR is a class B GPCR known for the ability to bind large peptide hormones. This receptor is coupled to G_s and its activation leads to the increased

production of cAMP within the cell (Gabe et al., 2018). The β-cell tone mediated by increased cAMP levels augments insulin secretion and GIPR activation contributes to this (Capozzi et al., 2019). It was reported that the GIPR can also increase cAMP levels from continued signaling in endosomes (Ismail et al., 2016). Additionally, the GIPR is constitutively internalized by β-arrestin1 and 2 and recycled back to the plasma membrane even in the absence of GIP, although its activation by GIP triggers rapid internalization, desensitization, and less receptor recycling (Mohammad et al., 2014). For these reasons, both GIPR activation and antagonism have been proposed as having therapeutic benefit due to the unique stimulatory and antagonist trafficking properties of the GIPR (Boer and Holst, 2020; Nauck and Meier, 2018).

The signaling mechanisms by which GIPR enhances insulin secretion other than increased cAMP are poorly understood. Some studies have shown GIPR knockout mice have reduced meal-stimulated insulin secretion and ablated insulin secretion in response to GIP while having similar glycemic control to wild type mice due in part to the upregulation of other insulinotropic mechanisms such as GLP-1R and GPR119 signaling (Pamir et al., 2003; Flock et al., 2011). Surprisingly, however, GIPR activation on the β-cell had a profound effect on the expression of transcription factor 1 (TCF1) and its mRNA (encoded by *Tcf7* in mice, *TCF7* in humans) as GIPR knockout mice had significantly diminished levels of Tcf7 mRNA associated with a much higher sensitivity to STZ induced β-cell apoptosis. TCF1 is known to promote thymocyte survival and it was found that TCF1 in β-cells increases the expression of anti-apoptotic genes that can prevent β-cell apoptosis. Loss of Tcf7 also caused age related and high fat feeding induced glucose intolerance. Thus, the GIPR-TCF1 axis, absent in GLP-1R signaling,

represents a novel signaling paradigm in which GIP administration could improve diabetic β-cell survival and long-term function. These effects were mediated by extracellular signal-regulated kinase 1/2 (ERK1/2) activation but not by cAMP levels suggesting that β-arrestin mediated signaling may underlie the GIP induced expression of Tcf7/TCF7 (Campbell et al., 2016).

D. GCGR

The proglucagon proprotein is well conserved across species and gives rise to various metabolic hormones often referred to as proglucagon derived peptides (PGDPs). In the pancreatic α-cells, proglucagon processing by PC2 produces bioactive glucagon, a 29 amino acid peptide that has substantial glucoregulatory capabilities throughout the body. Mice with PC2 knockout are unable to create mature glucagon (Campbell and Drucker, 2015). The secretion of glucagon is less understood than that of insulin from β-cells. Glucagon is released in response to hypoglycemic glucose concentrations and the presence of amino acids, both indicators of a fasting state. However, glucagon is also released in normal individuals following the ingestion of a mixed nutrient meal suggesting that it harnesses postprandial glucose regulation in combination with insulin (Carr et al., 2010; Alsalim et al., 2016). Moreover, patients with T2D have extremely high levels of glucagon, that when diminished is detrimental to prognostic outcomes (Muller et al., 1970). The release of glucagon from α-cells is also controlled in a paracrine manner where other components of the endocrine pancreas and the incretin hormones modulate glucagon release. For example, both insulin and GLP-1 inhibit glucagon release whereas GIP enhances it in a fasting state.

Somatostatin release from adjacent pancreatic δ-cells also inhibits glucagon release (El et al., 2021; Vergari et al., 2019).

Whole animal glucagon receptor (GCGR) knockout mice have improved glucose tolerance, higher glucagon levels, and healthy β-cell mass. They also exhibit better insulin sensitivity, much lower fed and fasted glucose levels, and reduced hepatic glucose output (Parker et al., 2002; Gelling et al., 2003; Lasher et al., 2022). These effects are largely mediated by loss of glucagon activation of hepatic GCGRs as hepatocyte targeted GCGR knockout displays a similar phenotype to the whole animal knockout (Longuet et al., 2013; Kim et al., 2018). The GCGR is another class B GPCR like GLP-1R and GIPR. In the liver, activation of GCGR stimulates G_s activity and adenylyl cyclase production of cAMP. The subsequent activation of PKA induces the transcription of genes coding for critical gluconeogenic enzymes thus augmenting glucose production. Additionally, PKA initiates a cascade of phosphorylation events that inhibit glycogenesis and activate glycogenolysis. Some reports have also described a role for GCGR/G_q coupling that modulates intracellular calcium concentrations that may also reinforce these outcomes. The net effect is a mobilization of the hepatocyte to output glucose in a fasted state (Janah et al., 2019; Xu and Xie, 2009).

The prevailing dogma is that insulin decreases glucose levels in a fed state and glucagon increases glucose levels in a fasted state. While this is true, it does not account for the insulinotropic effect glucagon has on β-cell insulin secretion and the increased release of α-cell glucagon in healthy subjects after a mixed nutrient meal. The pancreatic islets express GCGRs on their α- and β-cells. GCGR couples to G_s in these cell types but GCGR/G_s activation in the islet serves an opposing role to its anti-

hypoglycemic effects mediated by the liver. Mice overexpressing the GCGR on β-cells treated with glucagon exhibited a 4-fold increase in insulin secretion. This was accompanied by improved β-cell mass and insulin content (Gelling et al., 2009). Furthermore, antagonizing the GCGR on β-cells inhibited glucose promoted insulin secretion (Zhang et al., 2021b). Interestingly, a robust glycemic effect was seen in mice given exogenous glucagon following nutrient ingestion as opposed to the glucose lowering effect in a fasted state. The increased insulin secretion and glucose reduction observed in the fed state was not apparent in mice with specific β-cell knockout of GCGR/GLP-1R (Capozzi et al., 2019). These insulinotropic effects of glucagon are promulgated by the increased cAMP levels due to GCGR and GLP-1R activation that set β-cell tone. The GCGR and GLP-1R share significant homology and functional overlap and can both be activated by various PGDPs (Campbell and Drucker, 2015; Capozzi et al., 2019). Therefore, it has been shown that much of the α-cell released glucagon activates the GLP-1R thus equipping the β-cell with not only a means to enhance insulin secretion through GCGR and GLP-1R but also its growth and survival through GLP-1R signaling (Svendsen et al., 2018). Consequently, different combinations of dual- and tri-agonists/antagonist treatment regiments targeting GLP-1R/GIPR/GCGR are in clinical trials to harness the complex pharmacology of these metabolic hormones in the treatment of not only T2D, but also T1D and obesity.

E. FFA1 and FFA4

β-cell lipid content influences the secretory ability to release insulin. This is in part due to the inhibition of carnitine palmitoyltransferase 1 (CPT-1) and reduction in fatty

acid oxidation that provides substrates for various aspects of the amplifying phase of insulin secretion (Campbell and Newgard, 2021). Interestingly, some of preserved free fatty acids signal in a paracrine and autocrine manner to stimulate islet β-cell increasing insulin secretion. These cellular free fatty acids bind and activate free fatty acid receptors on the β-cell membrane, namely FFA1 (GPR40), a GPCR that is activated by long chain fatty acids such as linoleic acid and palmitic acid (Ferdaoussi et al., 2012). Linoleic acid activation of FFA1 in mouse MIN6 cells increased insulin secretion where antisense oligonucleotides against the FFA1 reduced linoleic acid induced insulin secretion by 50% (Briscoe et al., 2006; Salehi et al., 2005). Additionally, FFA1 knockout mice displayed fasting hyperglycemia, glucose intolerance, and insulin resistance compared to their WT littermates when on a high fat diet (HFD). The striking increases in insulin secretion following intravenous glucose and lipid administration in WT mice was lost in the FFA1 knockout mice (Kebede et al., 2008). Similarly, whole animal FFA1 knockout mice given just glucose had normal glucose tolerance but islets isolated from these mice had impaired FFA mediated increase in GSIS (Steneberg et al., 2005). Furthermore, pharmacologic and genetic inhibition of FFA1 *in vitro* abrogates FFA mediated potentiation of GSIS (Briscoe et al., 2006; Itoh et al., 2003; Salehi et al., 2005). Together, these studies provide support for FFA1 as a small molecule target to increase insulin secretion in patients with T2D.

The discovery that FFA1 activation augmented insulin secretion in a glucose-dependent manner led to the development of the FFA1 agonist TAK-875 (Negoro et al., 2010). FFA1 is a G_q-coupled receptor that is highly expressed in the pancreas. Stimulation of FFA1 by long chain FFAs or TAK-875 mediates insulin release through

G_q-PLCβ activation and increased intracellular calcium by activation of ER IP3 receptors and enhanced influx of calcium through L-type calcium channels (Usui et al., 2019). Additionally, both β-arrestin1 and 2 can bind and internalize FFA1 while knockdown of β-arrestin2 attenuated the insulinotropic effect of TAK-875 *in vitro* and in mouse islets suggesting that there are β-arrestin mediated mechanisms of insulin secretion following FFA1 agonism in addition to G_q-dependent signaling (Mancini et al., 2015). TAK-875 and AMG-837 were two of the major drugs developed that were partial agonists for FFA1 thus potentiating GSIS. AMG-1638, however, was a full agonist for FFA1 and in addition to augmenting GSIS, it could also stimulate the release of incretins from the enteroendocrine cells (Luo et al., 2012). While TAK-875 progressed to phase III clinical trials due to its superior efficacy, the trials were abruptly terminated due to`` liver toxicity associated with the metabolism of the drug.

The most prominent receptor similarity that exists between FFA1 and FFA4 is that they both bind long chain fatty acids (LCFAs). Otherwise, FFA4 does not share significant homology with FFA1 but is more similar to the spingosine-1 phosphate receptor (S1PR). Nonetheless, FFA4 has been implicated in the release of incretin hormones similar to FFA1 as well as having β-cell protective properties and the ability to increase peripheral insulin sensitivity (Milligan et al., 2015). FFA4 is also a G_q-coupled receptor as shown by the absence of calcium and IP3 increases in G_q knockout HEK293 cells after FFA4 stimulation (Alvarez-Curto et al., 2016). However, G_i coupling has also been proposed since pertussis toxin (PTX) treatment reduces the ability of FFA4 to release the satiety hormone ghrelin in addition to somatostatin from pancreatic δ-cells (Engelstoft et al., 2013; Stone et al., 2014). Therefore, G_q coupling may be the

principal mode of G-protein coupling to FFA4 but cell type specific differences may exist where G_i is the preferred G-protein. Knockout of FFA4 in mice leads to glucose intolerance and hyperglycemia (Suckow et al., 2014). Furthermore, FFA4 mRNA expression in patients with T2D or high blood sugar is reduced in islets and was associated with an attenuated response of FFA4 activated by omega 3 fatty acids to prevent apoptosis (Taneera et al., 2012).

F. FFA2 and FFA3

The short chain fatty acid (SCFA) receptors FFA2 and FFA3 are expressed on human and mouse islets where FFA2 seems to be the predominant SCFA receptor in human islets. Multiple studies have shown that FFA2/FFA3 knockout in whole animals and mouse pancreatic islets or FFA2/FFA3 antagonism improved insulin secretion and glucose tolerance. However, some publications challenge the metabolic enhancement in these studies highlighting the necessity to better understand these receptors in β-cell biology (Priyadarshini et al., 2016). In 2015, Tang *et al.* published a pivotal study that described metabolic phenotypes of FFA2/FFA3 signaling in mice and human models of insulin secretion where decreased FFA2/FFA3 signaling promoted insulin secretion. Dual knockout of these receptors in the intestinal cells had no effect on glucose levels indicating the glucoregulatory changes in FFA2/FFA3 are due to their pancreatic location. Moreover, knockout of each SCFA receptor alone had glucoregulatory metrics that were either unchanged from WT or smaller to that observed for the dual FFA2/FFA3 knockout. Interestingly, the SCFA, acetate, is locally produced in β-cells due to the metabolism of glucose through glycolysis. Inhibition of β-cell glycolysis by 2-

deoxyglucose or pyruvate decarboxylase (pyruvate conversion to acetate) by moniliformin reduces the production of acetate. FFA2/FFA3 knockout MIN6 cells that were kept in a small volume to allow for accumulation of acetate through β-cell glycolysis had considerably increased insulin secretion in the absence and presence of GLP-1 (Tang et al., 2015). These data suggest an autoregulatory mechanism by the β-cell to ensure it does not release excessive quantities of insulin by signaling to itself and other β-cells in an autocrine/paracrine manner.

Importantly, many discrepancies between mouse and human FFA2/FFA3 have been described largely due to differential receptor pharmacology between the species thus highlighting the importance of well-designed experiments and controls (Bolognini et al., 2016). Generally, it is accepted that these receptors are G_i coupled and inhibit the release of insulin following stimulation with SCFAs such as acetate, butyrate, or propionate. This is supported by the attenuation of GLP-1 enhanced insulin secretion after treatment with acetate that was lost following PTX pretreatment in MIN6 cells and endocBH1, a human β-cell model (Tang et al., 2015). Additionally, FFA2 agonism by the FFA2 specific agonist, 4-CMTB, in human pseudo-islets inhibited GSIS. These findings are confounded by the opposing responses of mouse islets where FFA2 stimulation elicits increased or decreased GSIS in a concentration dependent manner. Here, low FFA2 activation induced by lower concentrations of 4-CMTB increased GSIS in a G_q dependent manner as inhibition by the specific G_q inhibitor FR900359 ablated this response. Conversely, at high concentrations of 4-CMTB, GSIS was reduced which could be partly reversed by PTX treatment indicating G_i function (Lorza-Gil et al., 2020). From these and other studies, it is accepted that FFA2 can couple to G_q and G_i but

whether ligand concentrations, ligand structure, or variable expression of FFA2 vs FFA3 regulate this coupling in human vs mouse islets remains obscure. Based on the available data, FFA2 antagonism in humans is likely the best approach to treating T2D.

G. M3R

It has been known that parasympathetic cholinergic input can increase insulin secretion. This effect is mediated by the muscarinic receptor agonist acetylcholine that activates all muscarinic receptor subtypes (Gilon and Henquin, 2001). Of the 5 muscarinic receptors, the muscarinic receptor subtype 3 (M3R) is expressed on the β-cells and is responsible for increased insulin secretion (Duttaroy et al., 2004). This was supported by studies demonstrating that expression of constitutively active forms of M3R in mouse β-cells, to mimic continued agonist presence, markedly improved GSIS and glucose tolerance as well as reduced hyperglycemia (Gautam et al., 2006). In early studies, the insulin secretory response to acetylcholine was attributed to G_q-dependent pathways that led to the activation of PLCβ and increased intracellular calcium concentrations (Ruiz de Azua et al., 2011). However, a β-arrestin dependent pathway was also discovered in which M3R phosphorylation in rodent β-cells and islets was necessary for the recruitment of β-arrestin and subsequent activation of PKD1 to enhance sustained second phase insulin release. In this report, phosphorylation deficient M3R mice exhibited G-protein bias but displayed worse insulin secretion following M3R activation (Kong et al., 2010). Additional studies support an important role for the Gβ5-R7 protein complex in M3R signaling in mouse MIN6 cells (Wang et al., 2017).

While the studies discussed above were primarily focused on rodent β-cells, the Caicedo laboratory has provided insight on cholinergic signaling in human islets. Initial studies showed that cholinergic innervation of human islets is minimal and that it is the α-cells that release acetylcholine and sensitize β-cells to optimally respond to changes in glucose concentration (Rodriguez-Diaz et al., 2011). These investigators also found that endogenous acetylcholine stimulates β-cells through both M3 and M5 receptor subtypes while somatostatin-secreting δ-cells respond to acetylcholine through M1 muscarinic receptors (Molina at al., 2014). Thus, cholinergic signaling regulates insulin secretion through both direct and indirect input to β-cells in the human islet.

GPCRs that have many subtypes often have endogenous agonists that are not selective among the subtypes. This is the case for the muscarinic receptors where the endogenous agonist acetylcholine can activate all five subtypes. Additionally, they are peripherally distributed and serve critical functions such as regulating smooth muscle contraction and cardiac tone making it difficult to selectively target the M3R for T2D (Abrams et al., 2006). Structure based small molecule discovery campaigns have established the small molecule positive allosteric modulator VU0119498 as a potentiator of M3R signaling since mice lacking M3R in β-cells do not respond to VU0119498. These mice also had mild side effects since concentrations that elicited insulin secretion also affected peripheral muscarinic receptor activity (Zhu et al., 2019). These and other studies have garnered excitement as improvement of small molecule drugs that specifically target β-cell M3Rs could have significant therapeutic benefit in the treatment of T2D (Ito et al., 2019; Zhu et al., 2020).

H. Adrenergic Receptors

Blood glucose levels are also regulated by the sympathetic nervous system which releases norepinephrine and epinephrine to activate adrenergic receptors. While much of this activation occurs in peripheral tissues, adrenergic receptors are also expressed in β-cells and have been implicated in β-cell function (Riddy et al., 2018). The adrenergic receptors (ARs) fall into three major classes: βARs include β1, β2 and β3 subtypes that primarily activate G_s and adenylyl cyclase to promote cAMP production; α_1ARs include α_{1A}, α_{1B} and α_{1C} subtypes that activate G_q and PLCβ to regulate intracellular Ca^{2+}, and α_2ARs include α_{2A}, α_{2B} and α_{2C} subtypes that activate G_i family members to inhibit cAMP production (Bylund et al., 1994). The primary adrenergic receptor subtypes that have been implicated in β-cell function include the α_{2A}ARs and β_2ARs.

Early studies implicating a role for βARs in regulating blood glucose found that β_2AR-selective agonists were able to stimulate insulin secretion in isolated human pancreatic islets, although not in rat islets (Lacey et al., 1990). The β_2AR is a G_s-coupled receptor in β-cells that stimulates insulin secretion following catecholamine binding (Haffner and Kendall, 1992). Studies in mouse pancreatic islets showed that the β_2AR was expressed in islets and that the expression decreased with age resulting in impaired insulin secretion, while studies in $\beta_2AR^{-/-}$ mice found impaired glucose-promoted insulin secretion (Santulli et al., 2012). Interestingly, pancreas-specific deletion of the β_2AR gene in mice resulted in glucose intolerance and impaired insulin secretion but only in females (Ceasrine et al., 2018). This effect appears to be due to increased production of Vascular Endothelial Growth Factor-A in female neonatal β-

cells that results in hyper-vascularized islets and disruption of insulin production and exocytosis. Thus, the β_2AR is expressed on human and some rodent pancreatic β-cells and plays a stimulatory role in insulin secretion.

A link between α_2ARs and inhibition of insulin secretion was first observed in normal individuals (Porte, 1967) and then by the demonstration that α_2AR antagonists could enhance glucose-promoted insulin secretion in diabetic patients (Robertson et al., 1976). While the α_{2A}AR appears to be the primary subtype expressed in pancreatic β-cells, there may also be some expression of the α_{2C}AR subtype (Amisten et al., 2013). Interestingly, studies identified an *ADRA2A* polymorphism in some individuals that resulted in overexpression of the α_{2A}AR and impaired glucose-promoted insulin secretion (Rosengren et al., 2010, 2012). The reduced glucose responsiveness of isolated islets from these individuals could be corrected with an α_2AR antagonist (Rosengren et al., 2010). Moreover, α_2AR antagonist treatment of patients with the *ADRA2A* variant helped to correct the insulin response (Tang et al., 2014). Polymorphisms in *ADRA2A* were also found to correlate with the risk of gestational diabetes in Caucasian women (Kawai et al., 2017). Taken together, these studies highlight an important role for the α_{2A}AR in regulating inhibition of insulin secretion.

I. Use of designer GPCRs in β-cells

It is worth noting that the use of cell-specific designer GPCRs and conditional G-protein knockout mice has also proven useful in dissecting the role of G protein-dependent pathways in β-cells. While this topic has been covered in recent reviews

(Wang et al., 2021; Wess, 2022), we would like to highlight a few publications that have helped to better define the role of specific G protein pathways in β-cell function. These include the demonstration that G_q and G_s signaling increase first- and second-phase insulin release and β-cell mass (Guettier et al., 2009), that G_q/G_{11}-mediated signaling potentiates insulin secretion (Sassmann et al., 2010), and that selective activation of β-cell G_q improves β-cell function and glucose homeostasis (Jain et al., 2013). In contrast, G_i-mediated signaling leads to decreased β-cell proliferation and impaired glucose homeostasis (Berger et al., 2015). Taken together, these studies provide additional support for G_s and G_q signaling in insulin release and β-cell proliferation while G_i signaling serves to inhibit these processes.

I. Orphan Receptors and other GPCRs

There are over 300 known GPCRs that are expressed in mouse and human pancreatic islets (Amisten et al., 2017a). While most studies investigating GPCR physiology are performed in rodent models due to the poor availability of human islets, many GPCRs have become attractive therapeutic targets as a result of preclinical studies identifying the potential clinical implications of these receptors. A small subset of these receptors and their role on the β-cells of the islets are discussed below.

A. GPR119

Many orphan GPCRs are often referred to as their orphan connotation even after their endogenous ligand has been identified (i.e., FFARs). This is sometimes due to a lack of agreeable consensus as to what the major endogenous agonists are. GPR119 is

a class A GPCR that increases cAMP after activation by various lipids including 2-monoacylglycerol and anandamide as well as oleoylethanolamide (OEA). It is highly expressed on β-cells and has been shown in numerous studies to increase glucose induced insulin secretion in various rodent models (Godlewski et al., 2009; Soga et al., 2005; Ning et al., 2008). One of the first studies showed that the GPR119 agonist AR231453 was able to increase insulin secretion in rodent islets that was abrogated when GPR119 was genetically removed (Chu et al., 2007). GPR119 is also present on enteroendocrine cells where it enhances insulin secretion by its ability to stimulate GLP-1 and GIP release from the gut (Chu et al., 2008).

The plant *Hoodia Gordinii* is a succulent that is native to Africa that has been used for centuries by the Xhomani bushmen as an anorectic and thirst suppressant during hunting trips. One group identified that plant extracts of this succulent had strong activation of GPR119 and could increase insulin secretion from β-cells. This group identified the steroid glycoside Gordonoside F isolated from *H. gordonii* as a potent activator of GPR119 (Zhang et al., 2014). However, subsequent studies questioned the validity of GPR119 in rodent models as human clinical trials employing GPR119 agonists were not as efficacious as expected from the preclinical data (Ritter et al., 2016). In one study, mice with GPR119 knockout in β-cells had virtually no change in GSIS or glucose tolerance suggesting that GPR119 was dispensable for insulin secretion (Panaro et al., 2017). These discrepant findings suggest major species differences in GPR119 pharmacology and activation that may be improved by increasing the bioavailability, pharmacokinetics, and specificity of GPR119 agonists to activate GPR119 in humans.

B. Olfr109, GPR91, GPR99, CaSR, and GPR142

The β-cell is well equipped as a sensor of various metabolites translating extracellular nutrient information to an intracellular response to fine tune insulin secretion. These abilities are largely due to the extracellular sensing of metabolites, glucose, and hormones by β-cell resident channels and GPCRs. However, the β-cell also autoregulates itself by responding to extracellular metabolites and small peptides that it generates (Riddy et al., 2018; Oh and Olefsky, 2016; Husted et al., 2017). These β-cell intrinsic metabolic products add further complexity to the regulation of insulin dynamics. For example, acetate produced from glycolysis within the β-cells can induce G_i signaling inhibiting insulin secretion as previously described (Tang et al., 2015). The β-cells harbor additional GPCRs that serve a similar autoregulatory role in regulating insulin synthesis and secretion in a glucose-dependent manner.

The Olfr109 is a β-cell expressed olfactory receptor that can modulate insulin secretion in response to β-cell generated byproducts. Olfr109 is activated by the endogenous insulin peptide insB:9-23 that is released in conjunction with insulin. This receptor is also activated by denatured insulin. Olfr109 is a G_i-coupled receptor that prevents cAMP accumulation and inhibits insulin secretion. Furthermore, its activation recruits β-arrestin1 which leads to transcriptional programs that induce islet autoimmunity and macrophage infiltration (Cheng et al., 2022). Therefore, antagonizing this receptor could serve a therapeutic benefit in T2D.

Similarly, the TCA cycle intermediates, succinate and α-ketoglutarate, activate GPR91 (SUCNR1) and GPR99, respectively (He et al., 2004). Previous studies have

shown that succinate acts as a metabolic stimulus coupling factor in β-cells where it enhances proinsulin biosynthesis and insulin secretion in a glucose-dependent manner (Alarcon et al., 2002; Attali et al., 2006). Whether this is dictated by GPR91 activation has not been investigated. However, mice with GPR91 knockout fed a high fat diet had hyperglycemia and reduced insulin secretion (McCreath et al., 2015). Whether this is incumbent upon β-cell generated or circulating succinate from other sources remains to be determined but supports a role for GPR91 in regulating insulin secretion. Interestingly, this receptor has promiscuous G-protein coupling as it has been reported to activate G_s, G_i, and G_q signaling, although most studies indicate G_i coupling (Trauelsen et al., 2021; Li et al., 2020). Therefore, this receptor can modulate both cAMP and calcium levels which could have important implications in its regulation of insulin release. The other TCA cycle intermediate mentioned, α-ketoglutarate, activates GPR99, a G_q-coupled receptor. Much less is known about GPR99 but high levels of α-ketoglutarate are associated with cardiovascular disease (An et al., 2021). More studies are needed to elucidate the role of GPR99 and whether β-cell GPR99 plays a role in insulin secretion. There is also evidence that the class C calcium sensing receptor (CasR) and the aromatic amino acid sensing receptor GPR142 respond to products of autophagy in an autocrine manner stimulating insulin secretion in states of fasting in a G_q-dependent manner. Tryptophan activation of GPR142 induced GSIS in various rodent animal models (Husted et al., 2017; Oh et al., 2016; Squires et al., 2014; Wang et al., 2016).

Pancreatic β-cells harness a robust capability to respond to various stimuli from a diverse range of ingested food products, hormones, and even neurotransmitters to

modulate the release of the critical hormone insulin. Combined with glucose, many of these stimuli activate β-cell resident GPCRs where G_s- and G_q-coupled receptors stimulate insulin secretion while G_i-coupled receptors inhibit insulin secretion (Fig. 2). However, these mechanisms are complicated by GRK-mediated phosphorylation of these receptors which leads to β-arrestin recruitment, desensitization and often GPCR internalization. The section below primarily describes what we know about GRK function in β-cells, as there is little known about the roles of GRKs in α- and δ-cells. Since GRK phosphorylation of GPCRs promotes arrestin binding, we also briefly highlight some of the literature on the role of β-arrestins in β-cells.

II. GRK function in the β-cell

A. GRK family

The GRKs are a family of 7 serine/threonine protein kinases that phosphorylate agonist-activated GPCRs (Gainetdinov et al., 2004; Komolov and Benovic, 2018; Gurevich et al., 2012). They are classified based on sequence homology and functional similarities into the GRK1/7, GRK2/3, and GRK4-6 subfamilies. The GRK1/7 subfamily includes GRK1, which is expressed in retinal rods and cones, and GRK7, found only in retinal cones. GRK1 is also referred to as rhodopsin kinase as it was discovered as the kinase that phosphorylates light-activated rhodopsin (Kuhn, 1978; Lorenz et al., 1991). The GRK2/3 subfamily is ubiquitously expressed and originated from the identification of a protein kinase originally called the β-adrenergic receptor kinase that could phosphorylate the agonist-activated β_2-adrenergic receptor (β_2AR) (Benovic et al., 1986;

Benovic et al., 1987). The GRK4-6 subfamily includes GRK5 and GRK6 which are ubiquitously expressed and GRK4, which is restricted to the testes, brain, and kidney.

Although the GRKs diverge on their lipid modifications, expression, and subcellular localization, they share a similar domain architecture and activation mechanism (Komolov and Benovic, 2018). GRKs have a bilobal Regulator of G protein signaling homology (RH) domain consisting of RH terminal and bundle subdomains. The RH domain flanks the catalytic domain which is comprised of the kinase small lobe and large lobe separated by a catalytic cleft that coordinates ATP binding. Of the many regulatory features intrinsic to the GRKs, a prominent one is an ionic lock that involves intramolecular contacts between the RH bundle subdomain and kinase large lobe. In the inactive conformation, the ionic lock stabilizes the kinase in an inactive open conformation. Upon GPCR binding, the ionic lock is broken causing the RH bundle subdomain to swing away from the catalytic domain and allowing the kinase small and large lobes to move closer together (Komolov et al., 2017; Chen et al., 2021). While there is some functional redundancy, many GPCRs are selectively phosphorylated by a specific GRK or group of GRKs. The receptor-GRK preference is influenced by the type of ligand bound to the receptor, GPCR subtype, cell type, G-protein interactions, GRK expression, and even hierarchal phosphorylation where the phosphorylation by one GRK is facilitated by previous phosphorylation by other kinases. Therefore, determining the GRK specificity for various receptors is poorly understood for many GPCRs and the subject of many recent reports (Drube et al., 2022; Kawakami et al., 2022; Liggett, 2011).

B. GRKs implicated in diabetes

GRKs are known for their role in regulating GPCR signaling throughout the body thereby modulating cellular changes in virtually all physiological systems. GRK2, GRK3, GRK5, and GRK6 are expressed in numerous tissues and have been implicated in several diseases including neurological disorders (Alzheimer's and Parkinson's), cardiovascular disease (hypertrophy and heart failure), and metabolic diseases including insulin resistance and T2D (Suo et al., 2007; Suo et al., 2004; Pfleger et al., 2019; Murga et al., 2019; Ahmed et al., 2015). For example, insulin induced GLUT4 translocation is dependent upon insulin receptor signaling that leads to the activation of phosphatidyl inositol 3-kinase (PI3K) and Akt to initiate the trafficking of GLUT4 containing vesicles to the plasma membrane to mobilize glucose from the blood. In cultured adipocytes, this is influenced by G_q as constitutively active G_q increased GLUT4 translocation up to 70% in a PI3K-dependent manner. Overexpression of G_q had a similar effect whereas G_q inhibition using a G_q antibody or RGS2 (which increases GTP hydrolysis by G_q) reduced GLUT4 translocation after insulin receptor activation. These studies revealed the ability of a receptor tyrosine kinase (RTK) like the insulin receptor to utilize heterotrimeric G-proteins typically involved in GPCR signaling (Imamura et al., 1999a; Imamura et al., 1999b; Kanzaki et al., 2000). Since it was also shown that the RH domain of GRK2 binds G_q, it was postulated that GRK2 would be able to interfere with insulin receptor-G_q signaling (Carman et al., 1999; Usui et al., 2004). Indeed, studies using GRK2 overexpression, GRK2 antibodies, and GRK2 siRNA support a role for GRK2 in inhibiting insulin induced GLUT4 translocation by inhibiting G_q mediated activation of PI3K. This effect was independent of GRK2 kinase

activity but relied on GRK2 binding to G_q. Thus, this study demonstrates that GRK2 inhibition can ameliorate insulin resistance, potentially having clinical relevance in patients with T2D.

More recent studies employed inducible GRK2 ablation in various metabolic animal models to ascertain the potential of GRK2 suppression to reduce insulin resistance. Since GRK2 is upregulated in metabolic disease, it was postulated that reducing GRK2 levels could improve metabolic phenotypes. Mice given a high fat diet to induce a model of insulin resistance and metabolic disease exhibited improved metabolic markers following GRK2 suppression. This included reversal of insulin resistance, normalized blood sugar levels, and improved glucose tolerance. Additionally, the mice ceased to gain weight, fat mass was reduced, and proinflammatory cytokines in the liver lessened (Vila-Bedmar et al., 2015). Other mouse studies revealed that GRK2 suppression reduced endothelial dysfunction in a mouse model of diabetes by improving glucose homeostasis in the liver (Taguchi et al., 2017). Furthermore, pharmacological inhibition of GRK2 in diabetic mice improved glucose tolerance and insulin sensitivity, and reduced markers of impaired cardiac function such as oxidative stress and proinflammatory cytokines (Cipolletta et al., 2019). Collectively, these studies highlight the potential clinical utility of GRK2 inhibition in the treatment of T2D and the complications secondary to T2D as GRK2 reduction could improve both outcomes in animal models.

The first studies implicating GRK5 in T2D utilized a genetic approach where a GWAS analysis revealed that the SNP rs10886471 in intron 3 in the *GRK5* gene was associated with higher fasting plasma insulin and T2D but not with fasting plasma

glucose in Chinese Han patients. The association with T2D had genome wide significance (Li et al., 2013; Xia et al., 2014). East Asians are more susceptible to T2D from genetic predispositions as this population is not typically obese and thus don't normally develop T2D from weight/diet induced etiologies. These studies reported that the T2D risk increasing allele of rs10886471 was associated with increased GRK5 mRNA levels in a group containing both diabetic and non-diabetic individuals. The mRNA expression of GRK5 was higher in all patients with the allele and 40% more in T2D cases compared to non-diabetic controls. Although these results were from a cell type (blood cells) typically not a major factor in the progression of diabetes, these data suggest that increased GRK5 expression might contribute to increased T2D risk.

The increased fasting insulin levels associated with higher GRK5 expression could be a sign of GRK5 mediated defects in insulin sensitivity or β-cell function. To address this, the response of Chinese Hans with T2D to repaglinide, a meglitinide drug that increases insulin secretion, especially postprandial insulin secretion, through its action on the K_{ATP} channel, was investigated (Shang et al., 2018). In this study, the C containing alleles of rs10886471 had an improved response to repaglinide in the postprandial release of insulin accompanied by improvements in T2D metrics such as fasting plasma glucose, HOMA-IR (insulin resistance), and HbA1c. The pronounced response to repaglinide in these patients suggests that GRK5 is regulating K_{ATP} dynamics and subsequent insulin secretion. It's also interesting that alleles of the rs10886471 SNP were associated with increased T2D risk (Xia et al., 2014), while the C containing alleles improve insulin secretion following repaglinide treatment (Shang et al., 2018). This suggests that multiple mechanisms of GRK5 action may be involved.

Future studies will need to assess the role of GRK5 in the insulin secreting β-cells as well as the insulin sensitive peripheral tissues such as the liver, muscle, and fat. These studies also highlight the importance of population specific approaches to the treatment of T2D, as the GRK5 risk allele was only observed in this east Asian population and not detected in European populations.

Animal studies also support a role for GRK5 in diabetes as GRK5 knockout mice have impaired glucose tolerance and insulin sensitivity, perturbed Akt signaling, and develop hepatic steatosis (Wang et al., 2012). While the tissues involved in mediating these effects of GRK5 were not determined, mRNA expression of genes involved in hepatic glucose and lipid homeostasis were involved. Tissue specific knockouts of GRK5 will help to decipher the cell type specific role of GRK5 and its contribution to insulin resistance and insulin secretion.

C. GRK regulation of β-cell GPCRs

Numerous GPCRs resident on β-cells modulate insulin processing and secretion including the FFA receptors (FFA1-FFA4), incretin receptors (GLP-1 and GIPR), GCGR, and somatostatin receptors (SSR2 and SSR5) as well as many orphan and olfactory receptors. However, surprisingly little is known about how β-cell GRKs regulate G-protein and β-arrestin-dependent mechanisms of insulin secretion through their ability to regulate GPCRs. Many reports have described the phosphorylation-dependent recruitment of β-arrestin that in turn elicits β-cell responses to modify insulin secretion (Kong et al., 2010; Sonoda et al., 2008; Zhu et al., 2017a). Since the kinase specificity of these interactions is often lacking, it seems likely that GRKs will play a central role in

this process. Historically, determining GRK substrates has been difficult due to the large number of potential GPCR and non-GPCR substrates and low expression of most GPCRs. Consequently, the GRK specificity of most GPCRs has not been elucidated. Recent advances in our ability to study GRK specific phosphorylation of receptors has been gained in cell models that utilize various combinations of GRK knockouts (Drube et al., 2022; Kawakami et al., 2022). These platforms in combination with different tissue specific GRK knockout or knock-in animals will enable a better understanding of GRK selectivity at different receptors and their role in physiology including β-cell expressed GPCRs that regulate insulin secretion.

While knockout of GRK2 is embryonic lethal, hemizygous GRK2 mice are viable (Matkovich et al., 2006), and incretin mediated release of insulin from pancreatic β-cells was improved (Arcones et al., 2021). Interestingly, this effect was only seen in the first phase of insulin secretion where GRK2 suppression followed by oral feeding to ensure incretin release enhanced insulin secretion. This correlated with increased size of the readily releasable pool of insulin granules in these studies. Further analysis showed that this effect was in part mediated by the GLP-1R signaling axis as GLP-1R agonists augmented early phase insulin release *in vivo* and in isolated islets from hemizygous GRK2 mice. *In vitro* studies in HEK293 cells revealed that GRK2 was recruited to the activated GLP-1R and that its kinase activity was required for receptor phosphorylation and β-arrestin recruitment. This suggests that the improved secretory response to GLP-1 in GRK2 hemizygous mice may be due to decreased desensitization of GLP-1R (Arcones et al., 2021). A parallel report that used pharmacological inhibitors of GRK2 confirmed these findings (Lee et al., 2021). These studies found that inhibition of GRK2

reduced GLP-1 mediated β-arrestin recruitment to GLP-1R and enhanced insulin secretion in isolated islets and rodent β-cell lines reenforcing GRK2 as a negative regulator of GLP-1R signaling.

Interestingly, a subsequent study reported that silencing GRK2 in MIN6 cells led to diminution of GSIS that was recovered by PTX treatment (Snyder et al., 2021). This suggested that loss of GRK2-mediated regulation/phosphorylation of G_i-coupled receptors allowed these receptors to influence β-cell secretory tone by maintaining G_i signaling and inhibiting GSIS. Furthermore, in mice with pancreatic specific GRK2 knockout, a similar effect was seen where insulin secretion was reduced and cardiac function attenuated with both exacerbated by a high fat diet. Essentially, GRK2 had a positive effect on insulin secretion by keeping G_i signaling in check (Snyder et al., 2021). This contrasts with previous reports that show GRK2 inhibition as having beneficial outcomes in metabolic parameters such as glucose tolerance (Usui et al., 2004; Vila-Bedmar et al., 2015; Taguchi et al., 2017; Cipolletta et al., 2019). However, in these studies, GRK2 reduction included whole animal genetic suppression and pharmacological inhibition making it difficult to ascertain what GRK2 expressing tissues were responsible for the metabolic outcomes. Arcones *et al*. and Lee *et al*. supported whole animal data with insulin secretion studies in isolated islets whereas Snyder *et al.* did not use isolated islets but did have targeted deletion of GRK2 in the pancreas. Furthermore, Snyder *et al.* used only glucose stimulation whereas Arcones *et al.* and *Lee at al.* used glucose with GLP-1R activation (Arcones et al., 2021; Lee et al., 2021; Snyder et al., 2021). Taken together, these studies emphasize the importance of not only understanding global reduction in GRK2 levels but also tissue specific suppression

of GRK2. Additionally, GRK2 mediated effects on the β-cell are variable in response to the stimulatory environment (i.e., glucose alone vs glucose plus GLP-1) highlighting the necessity to distinguish secretory outcomes under different conditions.

The other incretin receptor, GIPR, was also shown to be regulated by a GRK and influence insulin secretion. In this study, GIPR phosphorylation was GRK2-dependent leading to the recruitment of β-arrestin1 and a reduction in GIP induced cAMP production. Moreover, GRK2 overexpression in the rodent β-cell line BT3C attenuated GIP triggered insulin release. The effects of GRK2 overexpression were only seen in incretin mediated insulin release as it had no effect on GSIS in BT3C cells (Tseng and Zhang, 2000).

Much less has been reported on the role of GRK5 in the regulation of β-cell GPCRs. However, many GPCRs resident on the β-cell are substrates for GRK5 in other cell types and *in vitro* systems. For example, the β_2AR can be phosphorylated by GRK5 (Komolov et al., 2017). As previously discussed, the β_2AR is a G_s-coupled receptor that stimulates insulin secretion in β-cells following catecholamine binding (Haffner and Kendall, 1992). GRK5 also phosphorylates α2-adrenergic receptors which are G_i coupled and inhibit insulin secretion (Eckhart et al., 2000; Diviani et al., 1996; Hamamdzic et al., 1995). The internalization of expressed GCGR in HEK293 cells increased with GRK5 overexpression as well as GRK2 and GRK3 overexpression. This resulted in increased β-arrestin1 and 2 recruitment and association with clathrin and caveolae involved in receptor endocytosis (Krilov et al., 2011). Future studies will clarify the role of GRK5 in the pancreatic β-cell. The seemingly GPCR-independent

potentiation of GSIS induced by repaglinide in patients with a genetic GRK5 abnormality provide evidence for an important role of GRK5 in regulating insulin secretion.

D. Novel roles of GRKs in β-cells

Although there is considerable evidence for a role of GRK2 and GRK5 in insulin resistance and insulin secretion leading to T2D, much less is known about the contribution made by the other broadly expressed GRKs, GRK3 and GRK6. Nonspecific inhibitors of GRK2/GRK3 exhibited a potentiating effect on GLP-1 induced insulin secretion but genetic approaches suggest this is primarily because of GRK2. Similarly, most studies for GRK6 involve investigation into the biology of various blood cell types and GRK6 knockout mice have reduced hematopoietic stem cells and progenitor populations that fail to differentiate. This is partly due to increased oxidative stress and increased ROS found in GRK6 knockout mice (Le et al., 2016). GRK6 knockout mice also have a reduced ability to clear apoptotic red blood cells which leads to autoimmune disease (Nakaya et al., 2013). While less is known about GRK6 in metabolic disease, it is worth noting that GRK6 substrates include the β_2AR, FFA4 and M3R, all critical mediators of insulin secretion and β-cell function (Nobles et al., 2011; Burns et al., 2014; Willets et al., 2002). Moreover, GRK6 regulation of oxidative stress in HSCs and lymphoid progenitors suggests that GRK6 could serve a similar role in β-cells, which are extremely sensitive to oxidative stress and ROS (Mukai et al., 2022).

Recently, Steyaert *et al.* identified a heterozygous missense mutation in *GRK6* using whole exome sequencing in two related patients with early onset T2D (Steyaert et al., 2022). This mutation resulted in a proline to serine change at residue 384 within the

large lobe of the catalytic domain and segregated with the disease with a LOD score of 2. These individuals presented with immunoreactive gastrin that was elevated significantly above reference values. Interestingly, the gastrin immunoreactivity was primarily due to the precursor gastrin peptides, preprogastrin (11 kDa) and progastrin (8.9 kDa). Similar findings were reported for proinsulin with levels several orders of magnitude higher than reference values suggesting the presence of a processing and/or secretory defect in β-cells. Taken together, the unique clinical phenotype triggered by GRK6 mutation has interesting implications in the role of this kinase in prohormone processing.

Based on the above findings, the potential role of GRK6 in insulin processing and secretion was studied in MIN6 cells. Initial studies demonstrated that a high affinity selective inhibitor of GRK5 and GRK6 enhanced GSIS in MIN6 cells while proinsulin processing was blunted resulting in significantly lower cellular insulin levels (Uehling et al., 2021; Varney et al., 2022). A similar effect was seen in GRK6 knockdown cells where cellular levels of insulin decreased dramatically accompanied by attenuated insulin secretion levels. Proinsulin secretion, however, was increased in GRK6 knockdown cells consistent with the insulin processing defect and the phenotype of the GRK6-P384S patients described above. Interestingly, this study also found that GRK6-P384S was mislocalized being found in the cytoplasm and nucleus as compared to the plasma membrane localization of wild type GRK6 (Varney et al., 2022).

Another striking finding from this study was the reduced expression and activity of the prohormone converting enzymes, PC1, PC2, and CPE (Varney et al., 2022). These enzymes have a critical role in converting proinsulin to insulin within secretory

granules (Smeekens et al., 1992; Creemers et al., 1998; Meier et al., 2022), that was defective in β-cells devoid of GRK6 as apparent by their reduced activity. The prohormone converting enzymes are induced under stimulatory conditions such as acute stimulation with glucose where the translation of preexisting mRNAs encoding these proteins is enhanced to increase protein levels and increase insulin output (Skelly et al., 1996; Schuppin and Rhodes, 1996; Nagamatsu et al., 1987). Some studies have shown the rate of proinsulin to insulin conversion is also increased by glucose (Nagamatsu and Grodsky., 1987; Nagamatsu and Grodsky, 1988). Additionally, PC1 and PC2 have optimal activity that is dependent upon the calcium concentration and pH level within the secretory granule, both of which are altered upon various stimulatory conditions including glucose or GLP-1R activation (Zhou and Lindberg, 1993; Davidson et al., 1988; Martin et al., 1994; Alarcon et al., 2006; Wang et al., 2014; Stiernet et al., 2006). Therefore, many potential pathways exist to modulate secretory granule dynamics. These include the plethora of GPCR signaling described above that is largely unexplored. Based on experiments using GRK6 mutants, reduced insulin processing is likely due to GRK6 interaction with a GPCR and the subsequent changes in downstream G protein and β-arrestin signaling pathways. Therefore, like GRK2 and GRK5, GRK6 also contributes to the regulation of insulin output in β-cells. It is evident that more investigation into the GRK regulation of β-cell GPCRs is necessary to better understand GPCR control of insulin secretion (Fig. 3).

E. Role of arrestins in β-cells.

Since an important function of GRKs is to phosphorylate GPCRs and promote the binding of arrestins, we also briefly discuss the role of arrestins in β-cell function. This is an area that has been thoroughly investigated using subtype-selective knockout mice and is the subject of several excellent recent reviews (de Souza et al., 2021; Pydi et al., 2022; Guven and Onay-Besikci, 2023). Briefly, studies from the Wess laboratory using β-arrestin1 and 2 knockout mice demonstrated that β-arrestin2 is an essential regulator of pancreatic β-cell function under both physiological and pathophysiological conditions (Zhu et al., 2017a), while hepatic β-arrestin2 was also found to have an essential role in maintaining euglycemia (Zhu et al., 2017b). β-arrestin1 was found to have a role in regulating insulin secretion (Barella et al., 2019) as well as a role in adaptive β-cell mass expansion during obesity (Barella et al., 2021). These studies have identified important roles for β-arrestins in β-cell function and suggest that these proteins may represent promising targets for the treatment of T2D.

III. The Human Islet GPCR transcriptome

A. Islet enriched GPCR genes with limited functional data

As previously discussed, GPCRs play a critical role in the regulation and maintenance of hormone secretion from islets with most studies investigating the role of various GPCRs in β-cell insulin secretion. We have discussed in depth the role of the well-known receptors including GLP-1R, GIPR, and M3R. However, most studies utilize the use of rodent islets which raises the concern for translatability to human islets as there are clear differences among the species (Eizirik et al., 1994; MacDonald et al., 2011; Alcazar and Buchwald, 2019). Moreover, although GPCR signaling clearly

modulates islet hormone secretion in a multitude of ways, only a small subset of GPCRs have received substantial investigation with only two receptors successfully targeted by FDA approved drugs (GLP-1R in 2007 and GIP in 2022). Therefore, there is an urgent need to better understand GPCR biology in islets given their proven utility as drug targets, especially for receptors that have been vastly understudied.

To address this shortcoming, Amisten and co-workers performed a transcriptomic analysis on human islets from non-diabetic, middle-aged, non-obese donors (Amisten et al., 2013). To accomplish this, they isolated the islets from human pancreatic samples, extracted the RNA, and performed qPCR on cDNA libraries from the human islets using multiple primer sets for each GPCR being analyzed. The gene expression for each GPCR was normalized to GAPDH as a control. To validate their primers, they did the same analysis in other tissues and cell lines and matched their expression profiles against known expression profiles and functional data to minimize false negatives. This group excluded odorant receptors and identified 293 GPCR mRNAs after screening for all 384 non-odorant receptors. Interestingly, they found that 210 of the 293 receptors had a known ligand whereas the rest were classified as orphan receptors. Moreover, ligand analysis revealed that 110 of the GPCRs detected are activated by 178 peptides/proteins, 99 are activated by 87 small molecule compounds, and the remainder are activated by monoatomic ions (e.g., Ca^{2+}) or large macromolecules. A large fraction of each of these classes of ligands are known to activate more than one receptor in addition to being present/expressed in islets or surrounding neurons and cells. The sheer diversity in GPCR expression as well as the multitude of ligands that can activate these receptors often originating from the islets

portrays the capacity for autocrine and paracrine signaling mechanisms within the islet that can be controlled by islet GPCRs to fine tune hormone secretion to the extracellular milieu of nutrients and food byproducts.

As expected, one of the more highly expressed genes was *GLP-1R* where it is known to increase β-cell insulin secretion and inhibit α-cell glucagon secretion. However, it is not the highest expressed gene or even in the top 5, emphasizing the fact that higher mRNA expression doesn't necessarily correlate to more prominent function within the islets (Amisen et al., 2013). The opposite is also true where although both *GCGR* and *SSTR*s are expressed above trace levels, their relative mRNA abundance is much lower than several of the other GPCRs and both receptors are known to have important functions within the β-cell (Amisten et al., 2013). Another caveat from this analysis is that the mRNA abundance detected is from whole islets which are composed of multiple cell types. Therefore, the less prominent cell types like δ-cells could be skewed towards what is happening in the β-cells. Additionally, changes within α- or δ-cells could be masked by the much more abundant β-cells so without separating the cell types, these data are likely most representative of gene expression levels in β-cells. Nonetheless, combining these gene expression profiles in islets with known functional data for the corresponding receptors such as G protein coupling can lead to exciting new areas of investigation.

The transcriptomic analysis revealed that the most highly expressed GPCR mRNAs in islets included the adhesion receptors *GPR56*, *LPHN1*, and *ELTD1* with *GPR56* being the most highly expressed of all GPCR genes (Amisten et al., 2013). These adhesion GPCRs are involved in coordinating contacts between cells and the

extracellular matrix as well as having signaling capabilities, although this is not well characterized (Vizurraga et al., 2020). GPR56 is activated by collagen type III and leads to the activation of $G_{12/13}$ and the RhoA signaling cascade that in muscle cells, potentiates muscle hypertrophy following mechanical resistance (White et al., 2014). In islets, not much is known about $G_{12/13}$ function so it's hard to know how GPR56-mediated signaling would influence hormone secretion. However, some studies have shown that GPR56 can increase insulin secretion since downregulation of this receptor in mouse islets reduced cAMP and subsequently insulin secretion (Duner et al., 2016). Additionally, collagen III treatment of mouse islets protected against cytokine induced apoptosis and augmented GSIS. These effects were not seen in GPR56 knockout islets suggesting that GPR56 is also G_s-coupled (Olaniru et al., 2018). Another study showed a similar effect where collagen III was protective against apoptosis in mouse MIN6 cells and human islets (Olaniru et al., 2021). Interestingly, in this study, GPR56 was constitutively internalized and trafficked with or without exogenous agonist and its internalization was increased after treatment with collagen III. Therefore, GPR56 is not only the most abundant GPCR gene expressed in islets but has been shown to regulate insulin secretion and could potentially be a new target for T2D.

The latrophillin-1 receptor, LPHN1, is part of a group of adhesion GPCRs that were originally discovered as receptors activated by alpha-latrotoxin from the black widow spider (Krasnoperov et al., 1997). These receptors are involved in brain function and embryonic development (Scholz et al., 2015; Muller et al., 2015). Studies have shown it to be coupled to all G proteins except $G_{12/13}$, so its role in islets is unclear (Muller et al., 2015; Lelianova et al., 1997; Nazarko et al., 2018). However, some

studies showed that alpha-latrotoxin could induce vesicle release from MIN6 cells (Lang et al., 1998; Lajus et al., 2006). Interestingly, a more recent report showed that its family member, LPHN3, is coupled to G_i and decreases insulin secretion suggesting that the latrophillin family of GPCRs play a role in insulin secretion from β-cells (Rothe et al., 2019). The latrophillin like orphan adhesion GPCR, ELTD1, is involved in tumor angiogenesis and is hypothesized to be a target for treating retinoblastoma migration and invasion (Guihurt Santiago et al., 2021). To date, nothing is known about how ELTD1 influences islet biology except that its mRNA levels are high in human islets.

Other non-adhesion GPCRs that were highly expressed include *CasR*, *GPR119*, and *FFAR1* whereas *HTR1F*, *GPRC5B*, and *GPCR5C* were moderately expressed (Amisten et al., 2013). GPR119 and FFA1 were discussed previously, and both induce insulin secretion. The gene for CasR was the second most abundant GPCR gene expressed in human islets (Amisten et al., 2013) (Table 1). This receptor is a class C GPCR activated primarily by the monoatomic ion calcium but also by magnesium. Calcium is present at high levels within insulin granules and is co-released with insulin in pancreatic β-cells (Jones et al., 2007). One study showed that activating CasR with the calcimimetic R-568 in MIN6 cells stimulated insulin secretion at physiological calcium concentrations but the potentiation of GSIS by CasR activation was blunted by PLCβ inhibition suggesting that CasR is signaling through G_q in β-cells (Gray et al., 2006). Another study showed that aged mice had increased levels of *CasR* mRNA and this correlated with compensatory insulin secretion in insulin resistant mice (Oh et al., 2016). In α-cells, CasR regulated α-cell proliferation as CasR inactivation prevented growth and this was dependent on G_q-signaling (Gong et al., 2023). Interestingly,

another report that used gain of function CasR mutations showed that these mice were hyperglycemic and hypoinsulinemic. Interestingly, they had reduced β-cell mass but enhanced α-cell mass and glucagon secretion consistent with the previous study showing increased α-cell growth with CasR activation. Antagonizing this mutant CasR restored glucose tolerance and insulin levels in the heterozygotes and glucose tolerance in homozygotes (Babinsky et al., 2017). Therefore, there seems to be a fine balance between CasR stimulation and overstimulation that can be detrimental to the islets, both in α- and β-cells, that can influence the paracrine communication between them.

Other interesting receptor genes that were abundantly expressed in islets include the class C orphan receptors *GPCR5B* and *GPRC5C* (Amisten et al, 2013). The expression of these receptors was found to be induced by trans-retinoic acid, which is produced from vitamin A (Robbins et al., 2000). GPRC5B shares high sequence similarity to another class C GPCR, the metabotropic glutamate receptor which stimulates insulin secretion from β-cells (Soni et al, 2013; Storto et al., 2006). Interestingly, *GPRC5B* mRNA and protein is upregulated in T2D and was the most abundant orphan receptor of the GPRC5 subgroup in human islets (Soni et al., 2013) (Table 1). Knockdown of *GPRC5B* in isolated mouse islets enhances both basal and glucose GSIS suggesting that GPRC5B plays a negative role on insulin secretion. Moreover, glutamate treatment of GPRC5B knockdown islets secreted more insulin and were protected against cytokine induced apoptosis compared to controls. Increased insulin secretion was not observed with retinoic acid treatment between control and GPRC5B islets suggesting that glutamate may be acting on this receptor and initiating

G_i signaling mechanisms since downregulation of GPRC5B leads to improved β-cell function. This is consistent with the increased expression of GPRC5B protein in T2D (Soni et al., 2013). Interestingly, it was also shown that the increased insulin response to glutamate mediated by the metabotropic glutamate receptor 5 (mGluR5) was due to receptors on the insulin granules rather than at the β-cell surface suggesting that receptors on insulin granules play an important role in insulin secretion and that the closely related GPRC5B that is activated by glutamate may also contribute to this signaling (Soni et al., 2013; Storto et al., 2006).

Similar to *GPRC5B*, *GPRC5C* is highly expressed in human pancreatic islets (Amisten et al., 2013). However, its mRNA and protein expression are downregulated in islets from T2D donors and islets from old mice had downregulated gene and protein expression of GPRC5C compared to newborns, both trends that were the opposite for GPRC5B (Soni et al., 2013; Amisten et al., 2017b) (Table 1). As might be expected from these trends, knockdown of GPRC5C led to reduced GSIS in mouse islets as well as decreased cAMP levels suggesting a G_s-coupled mechanism for this receptor. Intriguingly, unlike GPRC5B, GPRC5C seems to be activated by trans-retinoic acid as both insulin secretion and cAMP levels were reduced in trans-retinoic acid treated mouse islets with GPRC5C knockdown. This was not observed in GPCR5C knockdown islets treated with glutamate. Furthermore, GPRC5C knockdown islets were more susceptible to cytokine induced cell death and proliferation (Amisten et al., 2017b). Taken together, these data suggest that GPRC5B and GPRC5C have opposing roles in regulating β-cell mass and function with GPRC5B likely signaling through G_i and GPRC5C through G_s. While more investigation is needed, the differential protein

expression of GPRC5B and GPRC5C in islets from T2D donors combined with transcriptomic analysis showing high expression in human islets suggest that these GPCRs could serve as valuable targets in the treatment of T2D and that food high in vitamin A (carrots, sweet potatoes) might be a valuable addition to T2D diets.

As was described for the CasR, the insulin granules of the β-cell contain numerous molecules, proteins, and monoatomic ions that are often GPCR ligands. Another example of this on pancreatic β-cells is the presence of serotonin within the granules, a prototypical neurotransmitter that activates serotonin GPCRs (Rorsman and Renstrom, 2003; McCorvy and Roth, 2015). One of these GPCR genes, *HT1RF* which encodes the 5-HT1F receptor, was found at high levels within human islets (Amisten et al., 2013) (Table 1). In β-cells, serotonin is produced and secreted where it acts in an autocrine manner to increase β-cell mass, proliferation, and insulin secretion though 5-HT2B (a GPCR) and 5-HT3 (an ion channel) when under metabolic stress such as pregnancy or high fat diet (Bennet et al., 2016; Kim et al., 2015; Ohara-Imaizumi et al., 2013). More recently, a study revealed that increased serotonin released under high glucose conditions acts in a paracrine manner to inhibit glucagon release from α-cells in isolated human islets (Almaca et al., 2016). Additionally, activation of 5-HT1F in mouse models after IV administration of the 5-HT1F agonist LY344864, reduced serum glucagon levels consistent with what was observed *ex vivo* in human islets (Almaca et al., 2016). The characteristic loss of β-cell insulin granule exocytosis in T2D would likely reduce paracrine signaling of β-cell released serotonin activation of α-cell 5-HT1RF resulting in hypersecretion of glucagon from α-cells and potentially contribute to hyperglucagonemia in T2D.

B. Changes in human islet GPCR gene expression due to T2D and obesity

The transcriptomic analysis of GPCRs on human islets has revealed significant insight into the potential regulation of islet biology based on the high expression of various GPCRs that has led to studies investigating the role of these receptors in hormone secretion and islet function. However, the sheer complexity of the potential signaling mechanisms that could arise from the 293 currently known GPCRs expressed on islets and identifying which ones to investigate underscore the difficulty in assessing islet biology, especially as it relates to GPCR signaling. This is especially true since many of these receptors are orphan receptors and therefore do not have readily available pharmacological tools to assess their function. To help circumvent this problem, transcriptomic analyses from RNAseq data in human populations of metabolic duress such as T2D and obesity could help reveal critical proteins in islets, especially β-cells, that have differential expression under these conditions. In this way, the vast number of receptors in islets could be reduced to a smaller number of interesting targets due to the changes in their expression in subjects with T2D or obesity.

Recently, Xin *et al.* performed whole islet RNAseq and scRNAseq on islets isolated from non-diabetic and diabetic donors to determine changes in gene expression in α-, β-, δ-, and pancreatic polypeptide (PP) cells (Xin et al., 2016). As expected, many genes were differentially expressed between donors with or without T2D. A major portion of these were in pathways that are known to modulate the concentration of cAMP which is not surprising given the critical role of cAMP in regulating hormone secretion and cell growth in islet cells. Not surprisingly, many of

these differentially expressed genes were for GPCRs. Notably in β-cells, these included decreased expression of *GLP-1R*, *CasR*, and *FFA4* where *FFA4* was the most downregulated, even being significant in scRNAseq data in addition to whole islet RNAseq data. As previously discussed, these receptors typically have a positive role on insulin secretion which is consistent with their downregulation in individuals with T2D suggesting they may have reduced function in diabetic patients. Obviously, much is known about the GLP-1R but further study into β-cell CasR and FFA4 might be revealing since they show gene downregulation in T2D. This data also raises concerns as to the effectiveness of GLP-1R agonists in T2D subjects with severe GLP-1R downregulation and could help in selecting more effective treatments for patients based on their specific islet gene and protein expression.

A similar analysis was done for individuals with or without obesity (Atanes et al., 2021). In this study, which focused on human islet GPCR expression as opposed to all genes, islets were isolated from non-obese and obese donors and analyzed for GPCR gene expression. They again found that *GPR56* was the most expressed GPCR gene among both groups. Interestingly, there was not an obesity dependent change in the abundance of this gene even though *GPR56* has been shown to be downregulated in individuals with T2D. However, many other adhesion receptors were upregulated in obese subjects including *ADGRG6* (GPR126), which is activated by type 4 collagen, indicating that these adhesion receptors may have a critical role in islet function in obesity (Paavola et al., 2014).

The most upregulated GPCR mRNA in obesity was for the chemokine receptor CCR9 (Atanes et al., 2021) (Table 1). However, the authors note that this may be the

result of infiltrating inflammatory cells such as macrophages due to the increased presence of fat depots in obese islets. Evidence for this comes from studies where CCR9 activation by its agonist CCL25 had similar effects on insulin secretion in islets from lean and obese donors (Atanes et al., 2020). They did show however that CCL25 activation of the G_i-coupled CCR9 in isolated islets inhibits GSIS and enhanced cytokine induced apoptosis suggesting that this receptor could be explored as a therapeutic target (Atanes et al., 2020).

One of the more interesting receptors that was upregulated 10-fold in islets and was the 10th most upregulated GPCR gene from obese subjects was *HTR2B*, which encodes the 5-HT2B serotonin receptor (Amisten et al., 2013; Atanes et al., 2021) (Table 1). As noted, multiple studies have shown that β-cells can synthesize serotonin and that 5-HT2B activation enhances β-cell proliferation and insulin secretion (Bennet et al., 2016; Kim et al., 2015; Ohara-Imaizumi et al., 2013). Together, this suggests that upregulation of this receptor may be an adaptive response in obese islets to compensate for the obesogenic environment that can be deleterious to β-cell function in susceptible individuals. Another top 10 most upregulated GPCR gene was *GPR156*, a class C GPCR (Atanes et al., 2021) (Table 1). Very little is known about this receptor, although one study showed that it is G_i-coupled suggesting that upregulation of this gene could exacerbate the metabolic phenotype in islets of obese subjects like that seen for the class C GPRC5B in rodent models (Kindt et al., 2021; Soni et al., 2013).

Interestingly, various bitter taste receptors were also among the top 10 most upregulated (*TAS2R16*) or most downregulated (*TAS2R1* and *TAS2R38*) genes (Atanes et al., 2021). Relatively little is known about bitter taste receptors, especially in

islets, as most are orphan receptors. There is evidence to suggest, however, that bitter taste GPCRs are activated by exogenous compounds including artificial sweeteners like acesulfame K and saccharin potentially disrupting hormone secretion (Kuhn et al., 2004). This is an intriguing finding given that obese individuals ingest a multitude of nutrients and food additives, many of which we know little about regarding metabolic health. Moreover, several bitter taste receptors were also among the 10 most expressed GPCRs in islets including TAS2R45 in lean subjects and TAS2R41, TAS1R3, and TAS2R45 in obese subjects (Atanes et al., 2021). TAS2R16 was also among the top 10 most upregulated genes in obese subjects (Atanes et al., 2021). These data suggest that the bitter taste receptors may play a critical role in the biology of islets and could represent potential targets in the treatment of T2D and obesity, especially TAS2R16. One could also postulate that these receptors are activated by the numerous additives found in many food products, particularly artificial flavors that could be damaging islet health.

Lyu *et al.* performed an analysis where they separated differentially expressed GPCR genes by their known G-protein coupling, not including receptors that bind multiple G proteins (Lyu et al., 2022). They found that genes for G_i-coupled receptors were the most enhanced in obese subjects compared to lean controls. This is congruent with the notion that an upregulation of G_i-coupled receptors, especially in β-cells, would render this cell type less adept at responding to the metabolically demanding environment of obesity leading to increased fat deposition and weight gain exacerbating the disease. Multiple studies have shown that G_i-coupled receptors can reduce β-cell mass and decrease insulin secretion so antagonism at these receptors could prove

therapeutically useful in metabolic diseases, especially T2D (Berger et al., 2015; Dickerson et al., 2022). The G_q-coupled GPCRs also showed significant upregulation but not to the extent seen for G_i (Lyu et al., 2022). G_q-coupled receptors typically potentiate insulin secretion by activating PLCβ and stimulating increases in cellular calcium so an upregulation in these receptors could indicate compensatory mechanisms for the adverse metabolic environment in obesity (Husted et al., 2017). Surprisingly, this analysis revealed that the majority of both G_s- and $G_{12/13}$-coupled receptor genes were unaltered in obese subjects. Signaling through G_s is known to increase β-cell function and insulin secretion and a handful of G_s-coupled receptor genes were upregulated in obese subjects such as the *MC2R* which was increased more than 30-fold and was the second most upregulated gene. This analysis also revealed that genes for receptors with unknown G protein coupling were significantly upregulated which could partially explain why G_s-coupled receptors did not show robust upregulation in obese subjects since many of them may have been unaccounted for. This also unveils an underexploited area that there are still many orphan GPCR genes and GPCRs with unknown G protein coupling whose expression is significantly upregulated in obese individuals highlighting a major gap in our knowledge of GPCR signaling in islet biology.

Lyu *et al.* followed their G protein coupling data by showing alterations in GPCR mRNAs from human islets relative to the fold change in expression and showed that many of the top gene hits were orphan GPCRs including *GPR146*, *GPR39*, *GPR110*, and *GPR171* (Lyu et al., 2022). GPR39 has been shown to be a zinc sensing receptor, an intriguing finding since zinc plays a vital role within β-cells (Holst et al., 2009). Zinc is transported inside secretory granules to aid in the packaging of insulin hexamers during

granule maturation where downregulation and mutations in ZnT8, the major zinc transporter in β-cells, leads to impaired insulin secretion and diabetes in mouse models. Many mutant variants of this gene are associated with T2D in humans as well (Nicolson et al., 2009; Huang et al., 2019). Both insulin secretion *in vivo* after oral glucose administration and *ex vivo* from isolated islets treated with glucose had reduced insulin secretion in GPR39 null mice (Laitakari et al., 2021). GPR39 expression is also specific to the insulin containing β-cells in islets (Holst, 2009). Together, these data suggest that GPR39 could be activated in an autocrine manner to reenforce insulin secretion since zinc will be co-released with insulin from β-cell granules. Therefore, the investigation of these orphan receptors could prove useful in uncovering new pathways and treatment options for metabolic disease since they are highly represented in human islet transcriptomes and the few functional studies for these receptors have suggested important roles in islet biology.

C. Mouse vs human islet GPCR transcriptomes

The transcriptomic analyses described above were all done using isolated islets from human donors highlighting the relevance to human physiology (Amisten et al., 2013; Atanes et al., 2021; Xin et al., 2016; Lyu et al., 2022). Although informative, changes in gene expression do not always correlate with analogous changes in protein expression and it's possible that changes in protein expression may not have a functional outcome. Therefore, functional studies in cells and animal models are imperative to deciphering islet biology and hormone secretion. Unfortunately, most of the functional data investigating islet biology, especially for β-cells have been garnered

from studies using rodent models and rodent β-cell lines like MIN6 and INS-1. These studies have proven to be valuable as in the case of GLP-1R agonists but the biology of islets in rodents is not always conserved in humans. To address this issue, Amisten *et al.* performed a comparative analysis looking at GPCR gene expression in islets isolated from commonly used mouse strains as well as human islets (Amisten et al., 2017a).

They found that only 3 of the most abundant human GPCR genes were shared with the top 10 mouse GPCR genes. These included *GPR56* which was the highest in each species followed by *GLP-1R* and *FFAR1*. *GPR56* has been the highest expressed GPCR in human islets in all the described RNAseq analyses further supporting its potential importance in islets. Both GLP-1R and FFAR1 have received rigorous investigation as both can robustly stimulate β-cell insulin secretion with GLP-1R agonists widely used clinically in the US. FFAR1 had shown great potential as a drug target in β-cells but small molecules targeting this receptor were discontinued in clinical trials due to liver toxicity (Negoro et al, 2010; Luo et al., 2012; Milligan et al., 2015). Nonetheless, this analysis reveals that some of the most highly expressed GPCRs that are conserved between mouse and human islets have translated well to the clinic validating the utility of this approach. This is important because 71 GPCR genes were exclusively found in humans including *HT1RF*, *ADRA2C*, and *SST1*. Functional studies by Amisten *et al.* revealed that for the SST1 receptor, only human islets were sensitive to inhibition of insulin secretion by SST1 agonists (Amisten et al., 2017a). Even among the most expressed GPCR mRNAs in each species, the relative abundance of these could be quite different between mice and humans. For example, human GLP-1

expression was approximately 10-fold less in human islets compared to mouse islets although this could reflect differences in the housekeeping genes they were referenced against (Amisten et al., 2017a). Regardless, this approach and similar approaches can be utilized to confirm that the GPCR or protein of interest is expressed in both humans and mice so that rodents can be used as more translatable models until human resources become more readily available.

The most conserved abundantly expressed GPCR genes among mice and humans aside from *GPR56*, *GLP-1R*, and *FFA1R* include *GPRC5B*, *GPRC5C*, *CasR*, *GPR119*, and *GIPR* which have all been discussed in this review and have been reported to have similar characteristics between human and mouse models (Amisten et al., 2017a). This is critical as researchers can determine if using rodent islets to assess the function of GPCRs expressed at extremely low levels in these animals is worthwhile or whether using approaches to introduce these receptors in rodent models is warranted. Another important missing factor is the need to identify the expression of these receptors in specific cell types within islets. This is especially critical in mouse islets where β-cells can be >80% of the cells within an islet and could mask mRNA expression of the other cell types in RNAseq data from whole islets. For example, islet RNAseq data from mice and humans shows that *HTR1F* may be exclusive to human islets. However, protein staining in islets show this receptor is present in α-cells from human and mouse islets but only minimally expressed in β-cells. In this case, studying the *HT1RF* in α-cells of mouse islets can serve as a good proxy to the human situation as was shown (Almaca et al., 2016). This is also consistent with the much higher proportion of α-cells in human islets where RNAseq data from whole human islets will

be less obtrusive to α-cell mRNA expression in humans than in mice. To avoid masking gene expression profiles of rarer islet cell types, scRNAseq is necessary but these methods are more cumbersome and expensive limiting their use. This is especially true for α- and δ-cells which are present at much lower levels compared to β-cells in mice making it difficult to sort them for RNA analysis. Identifying protein expression in all of these cell types is also imperative but antibodies to detect the proteins are not always available or reliable so RNA expression data becomes critical in helping to determine the interest to initiate a study.

D. scRNAseq in human and mouse β-cells

In the above transcriptomic section describing GPCR gene expression in islets, bulk RNAseq was primarily done where the primary islet tissue was isolated from human or mouse donors, lysed, and reverse transcribed to generate cDNA libraries that were then amplified, quantitated, and identified using qPCR and Next Generation Sequencing (NGS) to determine changes in gene expression. The caveat of this method is that tissues, in this case the islets, contain several cell types and therefore gene expression signals are a representation of the average expression of that gene in the entire tissue. This can be insightful data for comparing the islet transcriptomes of non-diabetic (ND) and diabetic individuals to assess changes in islet gene expression mediated by the disease. Often in these studies the changes in gene expression are assumed to be primarily because of β-cells as they represent the majority of endocrine cells in both mouse and human islets. However, numerous recent studies have indicated a profound heterogeneity within endocrine cells that can vary across mouse

and human donors based on donor characteristics (BMI, age, experimental condition, disease state) as well as changes in cell type numbers that can confound the assumption that β-cells represent the majority of the gene expression signal (Benninger and Kravets, 2022; Dorrell et al., 2016; Vivoli et al., 2023; Gottmann et al., 2022; Aguayo-Mazzucato et al., 2017; Shrestha et al., 2022; Chen et al., 2022). Therefore, it is critical to ascertain gene expression profiles of individual endocrine cell types to really understand their contribution to the gene expression signature in T2D to help supplement bulk RNAseq data.

To circumvent this problem, investigators have begun utilizing scRNAseq methodologies that have become much more robust in recent years. Using this method, the isolated islets can be dissociated into individual cells, separated using microfluidics and other separation methods into labeled single cell droplets, and be subjected to the aforementioned protocol to lyse the cells and determine mRNA expression in individual cells. Bioinformatic tools are then used to determine which cell type was identified based on marker genes for each cell type (Baysoy et al., 2023; Haque et al., 2017). For example, insulin mRNA expression is used to identify β-cells while glucagon mRNA expression identifies α-cells. The cell number of each cell type can be quantified generating an islet atlas of the different endocrine cells. These bioinformatic pipelines can also determine the differential gene expression (DEG) under different conditions, such as in T2D islets (Elgamal et al., 2023; Yang et al., 2023). For this review, we have focused on bulk RNAseq datasets that were specifically focused on GPCR gene expression changes in pancreatic islets under various conditions (Amisten et al., 2013; Amisten et al., 2017; Atanes et al., 2021., Lyu et al., 2022). To date, these focused

analyses don't exist for GPCR gene expression from scRNAseq studies derived from islets. However, many groups have performed scRNAseq analysis in pancreatic islets that allowed us to manually examine their differentially expressed gene datasets to probe which GPCRs and GPCR regulatory proteins in β-cells may have important functions based on those that are highly altered under different conditions (ND vs T2D) or enriched specifically in β-cells (Elgamal et al., 2023; Yang et al., 2023; Hrovatin et al., 2023).

A recent report by Elgamal and coworkers took advantage of the Human Pancreas Analysis Consortium (HPAP) (Elgamal et al., 2023). HPAP was developed to collect and characterize islets from healthy and diseased human donors to gain insight into the pathogenesis of T1D and T2D. This includes a full panel of islet profiling including RNA sequencing, functional studies including insulin secretion, and histology to name a few. This data is publicly available via the web portal PANC-DB (Kaestner et al., 2019; Shapira et al., 2022). Elgamal et al. analyzed the scRNAseq data for 65 donors that included a mix of individuals that were healthy or had T1D or T2D. They then applied their bioinformatic pipeline to determine and quantitate endocrine cell types, subpopulations of endocrine cells, as well as changes in the islet atlas in disease states that can also be stratified based on donor characteristics such as age and gender. These bioinformatic pipelines also included mechanisms to determine DEGs under certain conditions as well as identity marker genes that are specific to a cell type.

Gene set enrichment analysis (GSEA) revealed that pathways involved in 'protein hormone receptor activity' have some of the most upregulated genes in T2D donors. One example is the thyroid stimulating hormone receptor (TSHR) that was

increased approximately four-fold (Elgamal et al., 2023). This gene was also upregulated four-fold in a scRNAseq study done in T2D mouse models (Hrovatin et al., 2023). The TSHR is a class A GPCR that is activated by the glycoprotein, thyroid stimulating hormone (TSH) and is known to be coupled to G_s and G_q. This hormone is critical for the growth and function of the thyroid gland and the release of thyroid hormones that are essential in numerous systems including the CNS and metabolism (Vieira et al., 2022., Kleinau et al., 2017). The TSHR has been reported to be expressed in rat islets at the protein level. In INS-1 cells, activation of the TSHR by TSH increased the expression of the GLUT2 transporter and glucose uptake. Additionally, INS-1 cells treated with TSH had increased glucokinase expression and better GSIS consistent with increased GLUT2 expression (Lyu et al., 2018). Another report used TSHR knockout mice and found that these mice have atrophied islets, glucose intolerance, and impaired insulin secretion (Yang et al., 2019). These studies suggest a protective mechanism in β-cells from donors with T2D where TSHR signaling increases the expression of insulin secretion genes to enhance insulin release.

Elgamal and coworkers also found that differentially expressed genes that modulate GPCR signaling were significantly altered in β-cells from donors with T2D. These included protein phosphatase 1 regulatory subunit 1A (PPP1R1A) and acetylcholinesterase (ACHE) (Elgamal et al., 2023). PPP1R1A is a target gene of MafA, a critical β-cell marker gene needed for proper GSIS. In INS-1 cells with PPP1R1A silenced, GSIS is impaired and contributes to β-cell dedifferentiation. Part of this effect is shown to be mediated by its ability to regulate GLP-1 amplification of GSIS in β-cells (Cataldo et al., 2021). In β-cells from T2D donors, PPP1R1A is downregulated

suggesting that incretin receptor signaling mediated by GLP-1 or GIP is potentially attenuated (Elgamal et al., 2023). Interestingly, β-cell *Ache* is upregulated in T2D donors (Elgamal et al., 2023). ACHE breaks down and inactivates acetylcholine which would modulate muscarinic receptor activity on β-cells (Soreq and Seidman, 2001). As previously described, muscarinic receptors are activated by acetylcholine and, for the M3R, stimulates insulin release (Kong et al., 2010; Zhu et al., 2019). With more pancreatic ACHE, the muscarinic receptor acetylcholine is broken down and presumably unable to stimulate insulin release. There is not extensive literature for ACHE in β-cells but one report shows *Ache* expression in β-cells and islets and that its expression is associated with apoptotic β-cells highlighting the potential importance of muscarinic receptor activation in treating T2D (Zhang et al., 2012).

It has become more appreciated in recent years that primary cilia in β-cells play important roles in β-cell function including insulin secretion (Li et al., 2022; Cho et al., 2022; Gerdes et al., 2014). The cilia are long slender cellular organelles that project into the intercellular space acting as a cellular antenna to detect and integrate signals from the surrounding environment to mediate cellular responses that for a long period were thought of as vestigial cellular organelles (Wheway et al., 2018). Of the many functions mediated by cilia, the hedgehog signaling pathway is a critical modality in regulating embryonic development and organogenesis and has been implicated in aberrant cell division and tumorigenesis in cancer. It also has the important role of regulating progenitor and stem cell populations as well as adult cell growth and maintenance including in β-cells (Petrova and Joyner, 2014; Yung et al., 2019).

The hedgehog signaling pathway in vertebrates is complex and centers around the activity of the smoothened receptor (Smo), part of the frizzled class of GPCRs. In brief, Smo is constitutively inhibited by the transmembrane Patched receptor (PTCH) located at the base of cilia. The PTCH ligand, sonic hedgehog (SHH), binds to PTCH repressing inhibition of Smo and allowing Smo translocation to the cilia base where it is phosphorylated by GRK2. This phosphorylation event initiates Smo movement up the cilia protrusion where it eventually activates the Gli transcription factors that then translocate to the nucleus inducing activation of target genes involved in development patterning and tissue growth (Briscoe and Therond, 2013). Smo may also have a canonical GPCR signaling mechanism but this is poorly understood and therefore Smo is often referred to as an atypical GPCR. PTCH appears to regulate Smo via its ability to transport plasma membrane cholesterol to bind to and activate Smo (Kowatsch et al., 2019; Radhakrishnan et al., 2020).

The combination of functional data showing cilia and hedgehog signaling in β-cells controlling insulin secretion with scRNAseq data showing differentially expressed genes in β-cells suggests this pathway may have an important role in β-cells (Li et al., 2022; Cho et al., 2022; Gerdes et al., 2014; Elgamal et al., 2023). For example, scRNAseq data revealed that PTCH2 was upregulated approximately 2.5-fold in T2D donors (Elgamal et al., 2023). This would likely have important ramifications in regulating Smo activity and Gli transcription in β-cells. Additionally, although not upregulated in T2D donors, hedgehog acetyl transferase like (HHATL) was identified as a novel β-cell marker gene. The hedgehog acetyl transferase, skinny hedgehog (SKI), coordinates lipid modification of the SHH ligand which is critical for its function to bind

PTCH and induce derepression of Smo signaling (Briscoe and Therond, 2013). One might suspect that HHATL will be involved in lipid modification of SHH ligands but this has not been determined. Together, these gene profiles in β-cells and T2D donors highlight the potential importance of cilia and hedgehog signaling in β-cell function.

In a related cilia pathway, the WNT/β-catenin pathway had significantly upregulated levels of WNT3 in β-cells from human T2D donors (Elgamal et al., 2023). WNT3 is a ligand for the class of frizzled GPCRs that activate β-catenin and transcriptional programs that control similar processes as hedgehog signaling including embryonic development, cell polarity, and cell proliferation (Wheway et al., 2018; May-Simera and Kelley, 2012). There is evidence that the WNT and hedgehog signaling pathways undergo cross talk and regulate each other with both playing critical roles in cell proliferation and growth (Ding and Wang, 2017). Since both pathways have upregulated components in donors with T2D and many reports have highlighted the importance of primary cilia in β-cell function, investigations into these GPCR mediated mechanisms of transcriptional regulation should reveal important insight into how the frizzled class of receptors play important roles in β-cell function.

In two independent scRNAseq studies, the gene for pituitary adenylate cyclase activating peptide (PACAP), *ADCYAP1*, was identified as a novel β-cell specific marker gene in addition to the canonical markers such as *Ins* and *MafA* (Elgamal et al., 2023; Yang et al., 2023). PACAP is a protein hormone that shares significant homology with the vasoactive intestinal polypeptide (VIP) and is part of the VIP/glucagon/secretin class of protein hormones that also includes GLP-1 (Sherwood et al., 2000). PACAP is the most ancestral and conserved of these hormones suggesting that it may be the original

ancestral molecule in which gene duplication events led to the divergence of the other protein hormones. Unsurprisingly, PACAP has numerous critical functions that are involved in cell proliferation and apoptosis as well as regulation of metabolism. It has roles in the CNS, endocrine, and cardiovascular systems and can stimulate adenylyl cyclase activity 1000x greater than VIP through its ability to activate the PACAP GPCR, PAC1 (Koves et al. 2020; Miyata et al., 1990). In mouse β-cells, PACAP overexpression enhances insulin release (Yamamoto et al., 2003). Additionally, PACAP-38, the mature form of the PACAP pro-protein, stimulates insulin release by activating the PAC1R in isolated rat islets. It accomplishes this via adenylyl cyclase, inhibition of potassium channels and membrane depolarization, and augmentation of calcium influx via voltage gated calcium channels (Liu et al., 2019; Leech et al.,1996). PACAP has also been shown to regulate glucagon release from islets and may be involved in the regulation of β-cell mass (Filipsson et al.,1997; Inoue et al., 2013). Together, these findings raise the intriguing possibility that PACAP and PAC1R signaling may have critical roles in β-cell function. Moreover, β-cell production and release of proteolytically mature PACAP-38 in response to glucose may represent a mechanism to ensure its own insulin release but also to communicate the feeding state to PAC1 receptors in the hypothalamus and other brain regions that control feeding behavior (Sureshkumar et al., 2022; Sekar et al., 2017). It's worth noting that there was a discrepancy in the mRNA and protein expression with *ADCYAP1* mRNA specifically enriched in β-cells while PACAP protein was detected in δ-cells via IHC (Elgawal et al., 2023; Yang et al., 2023). It is therefore essential that transcriptomic data is validated when feasible at the protein level.

One of the major pitfalls of scRNAseq is that while there is now a wealth of scRNAseq datasets, they often don't overlap and have different gene expression profiles complicating data interpretation and conclusions (Elgawal et al., 2023; Yang et al., 2023; Hrovatin et al., 2023; Chen et al., 2019). This method can also miss valuable information from genes that are not highly expressed including GPCRs which are known to be expressed at low levels. These missing data are known as 'dropouts' where bioinformatic pipelines either remove lowly expressed transcripts from the analysis or transcripts just aren't detected at all (Fredriksson and Schioth, 2005; Qui, 2020). Information is lost in a computational analysis if transcription of a gene is low or isn't captured in the cell isolation procedure and extraction of mRNA from cell to cell. Therefore, lowly expressed mRNAs, although potentially changing significantly in response to different conditions, are not captured and therefore not analyzed in bioinformatic pipelines. This is not as much of an issue with bulk RNAseq as individual cells are not separated giving larger sample sizes to extract mRNA although this sacrifices cell type specific resolution that can be achieved with scRNAseq. This amplifies an already complicated biology of comparing the transcriptomes of thousands of cells across heterogenous datasets that are not consistent, hence leading to poor overlap of scRNAseq datasets (Elgawal et al., 2023). For example, transcriptomic profiles will differ in islets from a cadaveric donor vs islets isolated from an individual who underwent a partial pancreatomy (Solimena et al., 2018; Wigger et al., 2021). Therefore, until these methodologies and bioinformatic pipelines are more normalized across independent groups and datasets, researchers should address potential gene hits with diligence and validate at the protein level when possible.

In the case of T2D, normalizing and compartmentalizing scRNAseq datasets is paramount to ensure reasonable hypotheses are made regarding gene profiles under various conditions. T2D is a highly heterogenous disease as many separate and independent paths exist for someone to develop glucose intolerance and hyperglycemia (Ha and Sherman, 2020). For example, T2D has recently been classified into subtypes such as mild obesity related diabetes (MOD), mild age-related diabetes (MARD), and severe insulin deficient diabetes (SIDD) (Ahlqvist et al, 2018). Organizing datasets based on donor characteristics such as age, gender, and subtype may help to compartmentalize and generate focused hypotheses. Also, pseudo bulk scRNAseq methods, where scRNAseq is performed but data from individual cells are pooled together for each donor sample rather than each cell serving as its own 'N' are likely better representations of populations and more accurate for comparing ND vs T2D transcriptomic profiles (Elgawal et al., 2023). The challenge is going to be normalizing methodology, data retrieval and analysis to refine these large datasets from well controlled and minimally manipulated in silico gene profiles to select robust candidate genes in diabetes. Hrovatin et al. have started to address this problem where they integrated the scRNAseq datasets from 56 mouse samples that have different characteristics including age, gender, and the diabetic mouse model used to create the mouse islet atlas (MIA), to help compartmentalize scRNAseq data (Hrovatin et al., 2023). For example, db/db mice and mice treated with low dose streptozotocin, both T2D models, had transcriptomes that mapped together but were different than that of a NOD mouse, a T1D model. An analogous approach should be taken in humans to make sure that large transcriptomic datasets are deposited with single cell isolation

methodologies, donor characteristics, and type of diabetes. This should help to resolve important diabetic genes under various conditions with endocrine cell type specific resolution. Ideally, this evolves into transcriptomes from different stages of the disease to identify β-cell markers foreshadowing β-cell dysfunction.

The above mentioned GPCRs represent only a small number of the 293 GPCRs expressed in human islets at the mRNA level. While we focused on GPCR genes that were highly expressed in islets, differentially expressed during metabolic stress, and conserved among humans and mice, functional data is often lacking for some of these GPCRs. Nevertheless, the importance of GPCR signaling in pancreatic islets and specifically in β-cells from RNAseq and functional data is abundantly clear. These transcriptomic analyses highlight the need to further investigate these receptors and their regulatory proteins as they could reveal new insights in our ability to treat metabolic diseases.

IV. Conclusions

The islets of Langerhans within the pancreas regulate metabolism throughout the body via the ability to detect and respond to nutrient and small molecule stimuli. This is accomplished through the spatial and temporal release of various hormones including glucagon and insulin that ultimately control the extent of nutrient breakdown and storage. When dysfunctional, this can have profound effects on metabolic regulation that can result in diseases like obesity and diabetes. Hormone release from the pancreatic islets, especially the β-cells has historically been understood to be mediated primarily by glucose. This is evident by the early drugs to treat insulin insufficiency and

diabetes including sulfonylureas and meglitinides which enhance pancreatic insulin output by augmenting the classical glucose pathway in β-cells. However, subsequent studies highlighted the complexity within the individual islet cell types and the ability of these cells to communicate with each other to modulate hormone release that in addition to glucose, is mediated by GPCRs. The islets express a multitude of GPCRs that can respond to hundreds of different ligands derived from food byproducts, gut microbiota, and intestinally released hormones that act in a coordinated manner to fine tune the islet hormonal response. These receptors not only have important roles in controlling the release of insulin from β-cells but also glucagon from α-cells and likely somatostatin release from δ-cells. Additionally, the human islet GPCR transcriptome reveals many potential receptors whose roles have not been investigated beyond their mRNA expression profile. To further this complexity, the regulation of these receptors by GRKs and β-arrestins is vastly understudied. Therefore, there is significant untapped potential in our understanding of islet biology that is controlled by GPCRs. Closing this gap could ultimately aid in the treatment of the many millions of people impacted by obesity and diabetes and lead to additional drugs that target GPCR signaling pathways with reduced side effects and better long-term outcomes.

Data Availability Statement

This is a review and does not contain any data.

Authorship contributions

Wrote or contributed to the writing of the manuscript: Varney, Benovic

References

Abrams P, Andersson KE, Buccafusco JJ, Chapple C, de Groat WC, Fryer AD, Kay G, Laties A, Nathanson NM, Pasricha PJ, Wein AJ (2006) Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *Br J Pharmacol* **148**:565-578.

Aguayo-Mazzucato C, van Haaren M, Mruk M, Lee TB Jr, Crawford C, Hollister-Lock J, Sullivan BA, Johnson JW, Ebrahimi A, Dreyfuss JM, Van Deursen J, Weir GC, Bonner-Weir S (2017) β Cell Aging Markers Have Heterogeneous Distribution and Are Induced by Insulin Resistance. *Cell Metab*. **25**:898-910.

Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spégel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark Å, Lahti K, Forsén T, Tuomi T, Rosengren AH, Groop L (2018) Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol* **6**:361-369.

Ahmed MR, Bychkov E, Li L, Gurevich VV, Gurevich EV (2015) GRK3 suppresses L-DOPA-induced dyskinesia in the rat model of Parkinson's disease via its RGS homology domain. *Sci Rep* **5**:10920.

Alarcon C, Wicksteed B, Prentki M, Corkey BE, Rhodes CJ (2002) Succinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation. *Diabetes* **51**:2496-2504.

Alarcon C, Wicksteed B, Rhodes CJ (2006) Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. *Diabetologia* **49**:2920-2929.

Alcazar O, Buchwald P (2019) Concentration-Dependency and Time Profile of Insulin Secretion: Dynamic Perifusion Studies with Human and Murine Islets. *Front Endocrinol (Lausanne)* **10**:680.

Almaça J, Molina J, Menegaz D, Pronin AN, Tamayo A, Slepak V, Berggren PO, Caicedo A (2016) Human Beta Cells Produce and Release Serotonin to Inhibit Glucagon Secretion from Alpha Cells. *Cell Rep* **17**:3281-3291.

Alonso-Magdalena P, Quesada I, Nadal A (2011) Endocrine disruptors in the etiology of type 2 diabetes mellitus. *Nat Rev Endocrinol* **7**:346-353.

Alsalim W, Tura A, Pacini G, Omar B, Bizzotto R, Mari A, Ahrén B (2016) Mixed meal ingestion diminishes glucose excursion in comparison with glucose ingestion via several adaptive mechanisms in people with and without type 2 diabetes. *Diabetes Obes Metab* **18**:24-33.

Alvarez-Curto E, Inoue A, Jenkins L, Raihan SZ, Prihandoko R, Tobin AB, Milligan G (2016) Targeted Elimination of G Proteins and Arrestins Defines Their Specific Contributions to Both Intensity and Duration of G Protein-coupled Receptor Signaling. *J Biol Chem* **291**:27147-27159.

Amisten S, Atanes P, Hawkes R, Ruz-Maldonado I, Liu B, Parandeh F, Zhao M, Huang GC, Salehi A, Persaud SJ (2017a) A comparative analysis of human and mouse islet G-protein coupled receptor expression. *Sci Rep* **7**:46600.

Amisten S, Mohammad Al-Amily I, Soni A, Hawkes R, Atanes P, Persaud SJ, Rorsman P, Salehi A (2017b) Anti-diabetic action of all-trans retinoic acid and the orphan G protein coupled receptor GPRC5C in pancreatic β-cells. *Endocr J* **64**:325-338.

Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ (2013) An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. *Pharmacol Ther* **139**:359-391.

An D, Zeng Q, Zhang P, Ma Z, Zhang H, Liu Z, Li J, Ren H, Xu D (2021) Alpha-ketoglutarate ameliorates pressure overload-induced chronic cardiac dysfunction in mice. *Redox Biol* **46**:102088.

Apovian CM, Okemah J, O'Neil PM (2019) Body Weight Considerations in the Management of Type 2 Diabetes. *Adv Ther*. **36**:44-58.

Arcones AC, Vila-Bedmar R, Mirasierra M, Cruces-Sande M, Vallejo M, Jones B, Tomas A, Mayor F Jr, Murga C (2021) GRK2 regulates GLP-1R-mediated early phase insulin secretion in vivo. *BMC Biol* **19**:40.

Artasensi A, Pedretti A, Vistoli G, Fumagalli L (2020) Type 2 Diabetes Mellitus: A Review of Multi-Target Drugs. *Molecules* **25**:1987.

Atanes P, Ashik T, Persaud SJ (2021) Obesity-induced changes in human islet G protein-coupled receptor expression: Implications for metabolic regulation. *Pharmacol Ther* **228**:107928.

Atanes P, Lee V, Huang GC, Persaud SJ (2020) The role of the CCL25-CCR9 axis in beta-cell function: potential for therapeutic intervention in type 2 diabetes. *Metabolism* **113**:154394.

Attali V, Parnes M, Ariav Y, Cerasi E, Kaiser N, Leibowitz G (2006) Regulation of insulin secretion and proinsulin biosynthesis by succinate. *Endocrinology* **147**:5110-5118.

Babinsky VN, Hannan FM, Ramracheya RD, Zhang Q, Nesbit MA, Hugill A, Bentley L, Hough TA, Joynson E, Stewart M, Aggarwal A, Prinz-Wohlgenannt M, Gorvin CM, Kallay E, Wells S, Cox RD, Richards D, Rorsman P, Thakker RV (2017) Mutant Mice With Calcium-Sensing Receptor Activation Have Hyperglycemia That Is Rectified by Calcilytic Therapy. *Endocrinology* **158**:2486-2502.

Barella LF, Rossi M, Zhu L, Cui Y, Mei FC, Cheng X, Chen W, Gurevich VV, Wess J (2019) β-Cell-intrinsic β-arrestin 1 signaling enhances sulfonylurea-induced insulin secretion. *J Clin Invest* **130**:3732-3737.

Barella LF, Rossi M, Pydi SP, Meister J, Jain S, Cui Y, Gavrilova O, Fulgenzi G, Tessarollo L, Wess J (2021) β-Arrestin-1 is required for adaptive β-cell mass expansion during obesity. *Nat Commun* **12**:3385.

Bar-Tana J (2020) Type 2 diabetes - unmet need, unresolved pathogenesis, mTORC1-centric paradigm. *Rev Endocr Metab Disord*. **21**:613-629.

Baysoy A, Bai Z, Satija R, Fan R (2023) The technological landscape and applications of single-cell multi-omics. *Nat Rev Mol Cell Biol*. **24**:695-713.

Bennet H, Mollet IG, Balhuizen A, Medina A, Nagorny C, Bagge A, Fadista J, Ottosson-Laakso E, Vikman P, Dekker-Nitert M, Eliasson L, Wierup N, Artner I, Fex M (2016) Serotonin (5-HT) receptor 2b activation augments glucose-stimulated insulin secretion in human and mouse islets of Langerhans. *Diabetologia* **59**:744-754.

Benninger RKP, Kravets V (2022) The physiological role of β-cell heterogeneity in pancreatic islet function. *Nat Rev Endocrinol*. **18**:9-22.

Benovic JL, Kühn H, Weyand I, Codina J, Caron MG, Lefkowitz RJ (1987) Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proc Natl Acad Sci U S A* **84**:8879-8882.

Benovic JL, Strasser RH, Caron MG, Lefkowitz RJ (1986) Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc Natl Acad Sci U S A* **83**:2797-2801.

Berger M, Scheel DW, Macias H, Miyatsuka T, Kim H, Hoang P, Ku GM, Honig G, Liou A, Tang Y, Regard JB, Sharifnia P, Yu L, Wang J, Coughlin SR, Conklin BR, Deneris ES, Tecott LH, German MS (2015) Gαi/o-coupled receptor signaling restricts pancreatic β-cell expansion. *Proc Natl Acad Sci U S A* **112**:2888-2893.

Berglund O (1980) Different dynamics of insulin secretion in the perfused pancreas of mouse and rat. *Acta Endocrinol* **93**:54-60.

Boer GA, Holst JJ (2020) Incretin Hormones and Type 2 Diabetes-Mechanistic Insights and Therapeutic Approaches. *Biology (Basel)* **9**:473.

Bolognini D, Tobin AB, Milligan G, Moss CE (2016) The Pharmacology and Function of Receptors for Short-Chain Fatty Acids. *Mol Pharmacol* **89**:388-398.

Bratanova-Tochkova TK, Cheng H, Daniel S, Gunawardana S, Liu YJ, Mulvaney-Musa J, Schermerhorn T, Straub SG, Yajima H, Sharp GW (2002) Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion. *Diabetes* **51**:S83-S90.

Bräuner-Osborne H, Wellendorph P, Jensen AA (2007) Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Curr Drug Targets* **8**:169-184.

Briscoe CP, Peat AJ, McKeown SC, Corbett DF, Goetz AS, Littleton TR, McCoy DC, Kenakin TP, Andrews JL, Ammala C, Fornwald JA, Ignar DM, Jenkinson S (2006) Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. *Br J Pharmacol* **148**:619-628.

Briscoe J, Thérond PP (2013) The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat Rev Mol Cell Biol*. **14**:416-29.

Burns RN, Singh M, Senatorov IS, Moniri NH (2014) Mechanisms of homologous and heterologous phosphorylation of FFA receptor 4 (GPR120): GRK6 and PKC mediate phosphorylation of Thr^{347}, Ser^{350}, and Ser^{357} in the C-terminal tail. *Biochem Pharmacol* **87**:650-659.

Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenburg U (1994) International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* **46**:121-136.

Campbell JE, Drucker DJ (2015) Islet α cells and glucagon--critical regulators of energy homeostasis. *Nat Rev Endocrinol* **11**:329-338.

Campbell JE, Newgard CB (2021) Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nat Rev Mol Cell Biol* **22**:142-158.

Campbell JE, Ussher JR, Mulvihill EE, Kolic J, Baggio LL, Cao X, Liu Y, Lamont BJ, Morii T, Streutker CJ, Tamarina N, Philipson LH, Wrana JL, MacDonald PE,

Drucker DJ (2016) TCF1 links GIPR signaling to the control of beta cell function and survival. *Nat Med* **22**:84-90.

Capozzi ME, Svendsen B, Encisco SE, Lewandowski SL, Martin MD, Lin H, Jaffe JL, Coch RW, Haldeman JM, MacDonald PE, Merrins MJ, D'Alessio DA, Campbell JE (2019) β Cell tone is defined by proglucagon peptides through cAMP signaling. *JCI Insight* **4**:e126742.

Capozzi ME, Wait JB, Koech J, Gordon AN, Coch RW, Svendsen B, Finan B, D'Alessio DA, Campbell JE (2019) Glucagon lowers glycemia when β-cells are active. *JCI Insight* **5**:e129954.

Carman CV, Parent JL, Day PW, Pronin AN, Sternweis PM, Wedegaertner PB, Gilman AG, Benovic JL, Kozasa T (1999) Selective regulation of Galpha(q/11) by an RGS domain in the G protein-coupled receptor kinase, GRK2. *J Biol Chem* **274**:34483-34492.

Carr RD, Larsen MO, Jelic K, Lindgren O, Vikman J, Holst JJ, Deacon CF, Ahrén B (2010) Secretion and dipeptidyl peptidase-4-mediated metabolism of incretin hormones after a mixed meal or glucose ingestion in obese compared to lean, nondiabetic men. *J Clin Endocrinol Metab* **95**:872-878.

Cataldo LR, Vishnu N, Singh T, Bertonnier-Brouty L, Bsharat S, Luan C, Renström E, Prasad RB, Fex M, Mulder H, Artner I (2021) The MafA-target gene PPP1R1A regulates GLP1R-mediated amplification of glucose-stimulated insulin secretion in β-cells. *Metabolism*. **118**:154734.

Ceasrine AM, Lin EE, Lumelsky DN, Iyer R, Kuruvilla R (2018) Adrb2 controls glucose homeostasis by developmental regulation of pancreatic islet vasculature. *eLife* **7**:e39689.

Chavda VP, Ajabiya J, Teli D, Bojarska J, Apostolopoulos V (2022) Tirzepatide, a New Era of Dual-Targeted Treatment for Diabetes and Obesity: A Mini-Review. *Molecules* **27**:4315.

Chen G, Ning B, Shi T (2019) Single-Cell RNA-Seq Technologies and Related Computational Data Analysis. *Front Genet*. **10**:317.

Chen K, Zhang J, Huang Y, Tian X, Yang Y, Dong A (2022) Single-cell RNA-seq transcriptomic landscape of human and mouse islets and pathological alterations of diabetes. *iScience*. **25**:105366.

Chen Q, Plasencia M, Li Z, Mukherjee S, Patra D, Chen CL, Klose T, Yao XQ, Kossiakoff AA, Chang L, Andrews PC, Tesmer JJG (2021) Structures of rhodopsin in complex with G-protein-coupled receptor kinase 1. *Nature* **595**:600-605.

Cheng J, Yang Z, Ge XY, Gao MX, Meng R, Xu X, Zhang YQ, Li RZ, Lin JY, Tian ZM, Wang J, Ning SL, Xu YF, Yang F, Gu JK, Sun JP, Yu X (2022) Autonomous sensing of the insulin peptide by an olfactory G protein-coupled receptor modulates glucose metabolism. *Cell Metab* **34**:240-255.

Cho JH, Li ZA, Zhu L, Muegge BD, Roseman HF, Lee EY, Utterback T, Woodhams LG, Bayly PV, Hughes JW (2022) Islet primary cilia motility controls insulin secretion. *Sci Adv*. **8**:8486.

Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, Chen R, Jones RM, Behan DP, Leonard J (2008) A role for

intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release. *Endocrinology* **149**:2038-2047.

Chu ZL, Jones RM, He H, Carroll C, Gutierrez V, Lucman A, Moloney M, Gao H, Mondala H, Bagnol D, Unett D, Liang Y, Demarest K, Semple G, Behan DP, Leonard J (2007) A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology* **148**:2601-2609. Erratum in: (2007) *Endocrinology* **148**:4753.

Cipolletta E, Gambardella J, Fiordelisi A, Del Giudice C, Di Vaia E, Ciccarelli M, Sala M, Campiglia P, Coscioni E, Trimarco B, Sorriento D, Iaccarino G (2019) Antidiabetic and Cardioprotective Effects of Pharmacological Inhibition of GRK2 in db/db Mice. *Int J Mol Sci* **20**:1492.

Cole JB, Florez JC (2020) Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol* **16**:377-390.

Congreve M, de Graaf C, Swain NA, Tate CG (2020) Impact of GPCR Structures on Drug Discovery. *Cell* **181**:81-91.

Corkey BE (2012a) Banting lecture 2011: hyperinsulinemia: cause or consequence? *Diabetes*. **61**:4-13.

Corkey BE (2012b) Diabetes: have we got it all wrong? Insulin hypersecretion and food additives: cause of obesity and diabetes? *Diabetes Care* **35**:2432-2437.

Creemers JW, Jackson RS, Hutton JC (1998) Molecular and cellular regulation of prohormone processing. *Semin Cell Dev Biol* **9**:3-10.

Curry DL, Bennett LL, Grodsky GM (1968) Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* **83**:572-584.

Davidson HW, Rhodes CJ, Hutton JC (1988) Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic beta cell via two distinct site-specific endopeptidases. *Nature* **333**:93-96.

de Souza CO, Sun X, Oh D (2021) Metabolic functions of G protein-coupled receptors and β-arrestin-mediated signaling pathways in the pathophysiology of type 2 diabetes and obesity. *Front Endocrinol (Lausanne)* **12**:715877.

Del Guerra S, Marselli L, Lupi R, Boggi U, Mosca F, Benzi L, Del Prato S, Marchetti P (2005) Effects of prolonged in vitro exposure to sulphonylureas on the function and survival of human islets. *J Diabetes Complications* **19**:60-64.

Deriy LV, Gomez EA, Jacobson DA, Wang X, Hopson JA, Liu XY, Zhang G, Bindokas VP, Philipson LH, Nelson DJ (2009) The granular chloride channel ClC-3 is permissive for insulin secretion. *Cell Metab* **10**:316-323.

Dickerson MT, Dadi PK, Zaborska KE, Nakhe AY, Schaub CM, Dobson JR, Wright NM, Lynch JC, Scott CF, Robinson LD, Jacobson DA (2022) $G_{i/o}$ protein-coupled receptor inhibition of beta-cell electrical excitability and insulin secretion depends on Na^+/K^+ ATPase activation. *Nat Commun* **13**:6461.

Ding M, Wang X (2017) Antagonism between Hedgehog and Wnt signaling pathways regulates tumorigenicity. *Oncol Lett*. **14**:6327-6333.

Diviani D, Lattion AL, Larbi N, Kunapuli P, Pronin A, Benovic JL, Cotecchia S (1996) Effect of different G protein-coupled receptor kinases on phosphorylation and desensitization of the alpha1B-adrenergic receptor. *J Biol Chem* **271**:5049-5058.

Dorrell C, Schug J, Canaday PS, Russ HA, Tarlow BD, Grompe MT, Horton T, Hebrok M, Streeter PR, Kaestner KH, Grompe M (2016) Human islets contain four distinct subtypes of β cells. *Nat Commun*. **7**:11756.

Drube J, Haider RS, Matthees ESF, Reichel M, Zeiner J, Fritzwanker S, Ziegler C, Barz S, Klement L, Filor J, Weitzel V, Kliewer A, Miess-Tanneberg E, Kostenis E, Schulz S, Hoffmann C (2022) GPCR kinase knockout cells reveal the impact of individual GRKs on arrestin binding and GPCR regulation. *Nat Commun* **13**:540.

Drucker DJ (2006) The biology of incretin hormones. *Cell Metab* **3**:153-165.

Drucker DJ (2018) Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. *Cell Metab* **27**:740-756.

Dunér P, Al-Amily IM, Soni A, Asplund O, Safi F, Storm P, Groop L, Amisten S, Salehi A (2016) Adhesion G Protein-Coupled Receptor G1 (ADGRG1/GPR56) and Pancreatic β-Cell Function. *J Clin Endocrinol Metab* **101**:4637-4645.

Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J (2004) Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. *Diabetes* **53**:1714-1720.

Eckhart AD, Duncan SJ, Penn RB, Benovic JL, Lefkowitz RJ, Koch WJ (2000) Hybrid transgenic mice reveal in vivo specificity of G protein-coupled receptor kinases in the heart. *Circ Res* **86**:43-50.

Eizirik DL, Pipeleers DG, Ling Z, Welsh N, Hellerström C, Andersson A (1994) Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. *Proc Natl Acad Sci U S A* **91**:9253-9256.

El K, Douros JD, Willard FS, Novikoff A, Sargsyan A, Perez-Tilve D, Wainscott DB, Yang B, Chen A, Wothe D, Coupland C, Tschöp MH, Finan B, D'Alessio DA, Sloop KW, Müller TD, Campbell JE (2023) The incretin co-agonist tirzepatide requires GIPR for hormone secretion from human islets. *Nat Metab* **5**:945-954.

El K, Gray SM, Capozzi ME, Knuth ER, Jin E, Svendsen B, Clifford A, Brown JL, Encisco SE, Chazotte BM, Sloop KW, Nunez DJ, Merrins MJ, D'Alessio DA, Campbell JE (2021) GIP mediates the incretin effect and glucose tolerance by dual actions on α cells and β cells. *Sci Adv* **7**:eabf1948.

El Moustaine D, Granier S, Doumazane E, Scholler P, Rahmeh R, Bron P, Mouillac B, Banères JL, Rondard P, Pin JP (2012) Distinct roles of metabotropic glutamate receptor dimerization in agonist activation and G-protein coupling. *Proc Natl Acad Sci U S A* **109**:16342-16347.

Elgamal RM, Kudtarkar P, Melton RL, Mummey HM, Benaglio P, Okino ML, Gaulton KJ (2023) An Integrated Map of Cell Type-Specific Gene Expression in Pancreatic Islets. *Diabetes*. **72**:1719-1728.

Engelstoft MS, Park WM, Sakata I, Kristensen LV, Husted AS, Osborne-Lawrence S, Piper PK, Walker AK, Pedersen MH, Nøhr MK, Pan J, Sinz CJ, Carrington PE, Akiyama TE, Jones RM, Tang C, Ahmed K, Offermanns S, Egerod KL, Zigman JM, Schwartz TW (2013) Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol Metab* **2**:376-392.

Esser N, Utzschneider KM, Kahn SE (2020) Early beta cell dysfunction vs insulin hypersecretion as the primary event in the pathogenesis of dysglycaemia. *Diabetologia*. **63**:2007-2021.

Ferdaoussi M, Bergeron V, Zarrouki B, Kolic J, Cantley J, Fielitz J, Olson EN, Prentki M, Biden T, MacDonald PE, Poitout V (2012) G protein-coupled receptor (GPR)40-dependent potentiation of insulin secretion in mouse islets is mediated by protein kinase D1. *Diabetologia* **55**:2682-2692.

Ferdaoussi M, Dai X, Jensen MV, Wang R, Peterson BS, Huang C, Ilkayeva O, Smith N, Miller N, Hajmrle C, Spigelman AF, Wright RC, Plummer G, Suzuki K, Mackay JP, van de Bunt M, Gloyn AL, Ryan TE, Norquay LD, Brosnan MJ, Trimmer JK, Rolph TP, Kibbey RG, Manning Fox JE, Colmers WF, Shirihai OS, Neufer PD, Yeh ET, Newgard CB, MacDonald PE (2015) Isocitrate-to-SENP1 signaling amplifies insulin secretion and rescues dysfunctional β cells. *J Clin Invest* **125**:3847-3860.

Filipsson K, Tornøe K, Holst J, Ahrén B (1997) Pituitary adenylate cyclase-activating polypeptide stimulates insulin and glucagon secretion in humans. *J Clin Endocrinol Metab*. **82**:3093-8.

Flannick J, Johansson S, Njølstad PR (2016) Common and rare forms of diabetes mellitus: towards a continuum of diabetes subtypes. *Nat Rev Endocrinol* **12**:394-406.

Flock G, Holland D, Seino Y, Drucker DJ (2011) GPR119 regulates murine glucose homeostasis through incretin receptor-dependent and independent mechanisms. *Endocrinology* **152**:374-383.

Foord SM, Bonner TI, Neubig RR, Rosser EM, Pin JP, Davenport AP, Spedding M, Harmar AJ (2005) International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharmacol Rev* **57**:279-288.

Fredriksson R, Schiöth HB (2005) The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharmacol*. **67**:1414-25.

Fusco J, Xiao X, Prasadan K, Sheng Q, Chen C, Ming YC, Gittes G (2017) GLP-1/Exendin-4 induces β-cell proliferation via the epidermal growth factor receptor. *Sci Rep* **7**:9100.

Gabe MBN, Sparre-Ulrich AH, Pedersen MF, Gasbjerg LS, Inoue A, Bräuner-Osborne H, Hartmann B, Rosenkilde MM (2018) Human GIP(3-30)NH_2 inhibits G protein-dependent as well as G protein-independent signaling and is selective for the GIP receptor with high-affinity binding to primate but not rodent GIP receptors. *Biochem Pharmacol* **150**:97-107.

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG (2004) Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* **27**:107-144.

Gaisano HY (2017) Recent new insights into the role of SNARE and associated proteins in insulin granule exocytosis. *Diabetes Obes Metab* **19**:115-123.

Gasbjerg LS, Helsted MM, Hartmann B, Jensen MH, Gabe MBN, Sparre-Ulrich AH, Veedfald S, Stensen S, Lanng AR, Bergmann NC, Christensen MB, Vilsbøll T, Holst JJ, Rosenkilde MM, Knop FK (2019) Separate and Combined Glucometabolic Effects of Endogenous Glucose-Dependent Insulinotropic Polypeptide and Glucagon-like Peptide 1 in Healthy Individuals. *Diabetes* **68**:906-917.

Gautam D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH, Cui Y, Mears D, Lu H, Deng C, Heard T, Wess J (2006) A critical role for beta cell M3 muscarinic acetylcholine

receptors in regulating insulin release and blood glucose homeostasis in vivo. *Cell Metab* **3**:449-461.

Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ (2003) Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A* **100**:1438-1443.

Gelling RW, Vuguin PM, Du XQ, Cui L, Rømer J, Pederson RA, Leiser M, Sørensen H, Holst JJ, Fledelius C, Johansen PB, Fleischer N, McIntosh CH, Nishimura E, Charron MJ (2009) Pancreatic beta-cell overexpression of the glucagon receptor gene results in enhanced beta-cell function and mass. *Am J Physiol Endocrinol Metab* **297**:E695-E707.

Gerdes JM, Christou-Savina S, Xiong Y, Moede T, Moruzzi N, Karlsson-Edlund P, Leibiger B, Leibiger IB, Östenson CG, Beales PL, Berggren PO (2014) Ciliary dysfunction impairs beta-cell insulin secretion and promotes development of type 2 diabetes in rodents. *Nat Commun*. **5**:5308.

Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* **22**:565-604.

Godlewski G, Offertáler L, Wagner JA, Kunos G (2009) Receptors for acylethanolamides-GPR55 and GPR119. *Prostaglandins Other Lipid Mediat* **89**:105-111.

Goehring I, Sauter NS, Catchpole G, Assmann A, Shu L, Zien KS, Moehlig M, Pfeiffer AF, Oberholzer J, Willmitzer L, Spranger J, Maedler K (2011) Identification of an

intracellular metabolic signature impairing beta cell function in the rat beta cell line INS-1E and human islets. *Diabetologia* **54**:2584-2594. Erratum in: (2019) *Diabetologia*

Goldberg IJ (2001) Clinical review 124: Diabetic dyslipidemia: causes and consequences. *J Clin Endocrinol Metab* **86**:965-971.

Gong Y, Yang B, Zhang D, Zhang Y, Tang Z, Yang L, Coate KC, Yin L, Covington BA, Patel RS, Siv WA, Sellick K, Shou M, Chang W, Danielle Dean E, Powers AC, Chen W (2023) Hyperaminoacidemia induces pancreatic α cell proliferation via synergism between the mTORC1 and CaSR-Gq signaling pathways. *Nat Commun* **14**:235.

Gottmann P, Speckmann T, Stadion M, Zuljan E, Aga H, Sterr M, Büttner M, Santos PM, Jähnert M, Bornstein SR, Theis FJ, Lickert H, Schürmann A (2022) Heterogeneous Development of β-Cell Populations in Diabetes-Resistant and -Susceptible Mice. *Diabetes*. **71**:1962-1978.

Gray E, Muller D, Squires PE, Asare-Anane H, Huang GC, Amiel S, Persaud SJ, Jones PM (2006) Activation of the extracellular calcium-sensing receptor initiates insulin secretion from human islets of Langerhans: involvement of protein kinases. *J Endocrinol* **190**:703-710.

Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T (2005) Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care* **28**:164-176.

Guettier JM, Gautam D, Scarselli M, Ruiz de Azua I, Li JH, Rosemond E, Ma X, Gonzalez FJ, Armbruster BN, Lu H, Roth BL, Wess J (2009) A chemical-genetic

approach to study G protein regulation of beta cell function in vivo. *Proc Natl Acad Sci U S A* **106**:19197-19202.

Guihurt Santiago J, Burgos-Tirado N, Lafontaine DD, Mendoza Sierra JC, Camacho RH, Vecchini Rodríguez CM, Morales-Tirado V, Flores-Otero J (2021) Adhesion G protein-coupled receptor, ELTD1, is a potential therapeutic target for retinoblastoma migration and invasion. *BMC Cancer* **21**:53.

Gurevich EV, Tesmer JJ, Mushegian A, Gurevich VV (2012) G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. *Pharmacol Ther* **133**:40-69.

Gurevich VV, Gurevich EV (2019) GPCR Signaling Regulation: The Role of GRKs and Arrestins. *Front Pharmacol* **10**:125.

Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, Fischer M, Chaudhari A, Ji M, Kapoun AM, Lam A, Lazetic S, Ma S, Mitra S, Park IK, Pickell K, Sato A, Satyal S, Stroud M, Tran H, Yen WC, Lewicki J, Hoey T (2012) Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci U S A* **109**:11717-11722.

Guven B, Onay-Besikci A (2023) Past and present of beta arrestins: A new perspective on insulin secretion and effect**.** *Eur J Pharmacol* **956**:175952.

Ha J, Sherman A (2020) Type 2 diabetes: one disease, many pathways. *Am J Physiol Endocrinol Metab*. **319**:E410-E426.

Haffner CA, Kendall MJ (1992) Metabolic effects of beta 2-agonists. *J Clin Pharm Ther* **17**:155-164.

Hamamdzic D, Duzic E, Sherlock JD, Lanier SM (1995) Regulation of alpha 2-adrenergic receptor expression and signaling in pancreatic beta-cells. *Am J Physiol* **269**:E162-E171.

Haque A, Engel J, Teichmann SA, Lönnberg T (2017) A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med*. **9**:75.

He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H, Ling L (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429**:188-193.

Heller S (2004) Weight gain during insulin therapy in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. **65**:S23-7.

Henquin JC, Ishiyama N, Nenquin M, Ravier MA, Jonas JC (2002) Signals and pools underlying biphasic insulin secretion. *Diabetes* **51**:S60-S67.

Henquin JC, Nenquin M, Stiernet P, Ahren B (2006) In vivo and in vitro glucose-induced biphasic insulin secretion in the mouse: pattern and role of cytoplasmic Ca2+ and amplification signals in beta-cells. *Diabetes* **55**:441-451.

Hollenstein K, de Graaf C, Bortolato A, Wang MW, Marshall FH, Stevens RC (2014) Insights into the structure of class B GPCRs. *Trends Pharmacol Sci* **35**:12-22.

Holst B, Egerod KL, Jin C, Petersen PS, Østergaard MV, Hald J, Sprinkel AM, Størling J, Mandrup-Poulsen T, Holst JJ, Thams P, Orskov C, Wierup N, Sundler F, Madsen OD, Schwartz TW (2009) G protein-coupled receptor 39 deficiency is associated with pancreatic islet dysfunction. *Endocrinology* **150**:2577-2585.

Holz GG (2004) Epac: A new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic beta-cell. *Diabetes* **53**:5-13.

Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF (1999) cAMP-dependent mobilization of intracellular Ca2+ stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca2+ signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* **274**:14147-14156.

Hrovatin K, Bastidas-Ponce A, Bakhti M, Zappia L, Büttner M, Salinno C, Sterr M, Böttcher A, Migliorini A, Lickert H, Theis FJ (2023) Delineating mouse β-cell identity during lifetime and in diabetes with a single cell atlas. *Nat Metab*. **5**:1615-1637.

Huang Q, Du J, Merriman C, Gong Z (2019) Genetic, Functional, and Immunological Study of ZnT8 in Diabetes. *Int J Endocrinol* **2019**:1524905.

Husted AS, Trauelsen M, Rudenko O, Hjorth SA, Schwartz TW (2017) GPCR-Mediated Signaling of Metabolites. *Cell Metab* **25**:777-796.

Imamura T, Ishibashi K, Dalle S, Ugi S, Olefsky JM (1999a) Endothelin-1-induced GLUT4 translocation is mediated via Galpha(q/11) protein and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. *J Biol Chem* **274**:33691-33695.

Imamura T, Vollenweider P, Egawa K, Clodi M, Ishibashi K, Nakashima N, Ugi S, Adams JW, Brown JH, Olefsky JM (1999b) G alpha-q/11 protein plays a key role in insulin-induced glucose transport in 3T3-L1 adipocytes. *Mol Cell Biol* **19**:6765-6774.

Inoue H, Shintani N, Sakurai Y, Higashi S, Hayata-Takano A, Baba A, Hashimoto H (2013) PACAP Inhibits β-cell Mass Expansion in a Mouse Model of Type II Diabetes: Persistent Suppressive Effects on Islet Density. *Front Endocrinol (Lausanne)*. **4**:27.

International Diabetes Federation (2021) *IDF Diabetes Atlas*, 10th edn., Brussels, Belgium

Ismail S, Gherardi MJ, Froese A, Zanoun M, Gigoux V, Clerc P, Gaits-Iacovoni F, Steyaert J, Nikolaev VO, Fourmy D (2016) Internalized Receptor for Glucose-dependent Insulinotropic Peptide stimulates adenylyl cyclase on early endosomes. *Biochem Pharmacol* **120**:33-45.

Ito Y, Kaji M, Sakamoto E, Terauchi Y (2019) The beneficial effects of a muscarinic agonist on pancreatic β-cells. *Sci Rep* **9**:16180.

Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M (2003) Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* **422**:173-176.

Jain S, Ruiz de Azua I, Lu H, White MF, Guettier JM, Wess J (2013) Chronic activation of a designer G_q-coupled receptor improves β-cell function. *J Clin Invest* **123**:1750-1762.

Janah L, Kjeldsen S, Galsgaard KD, Winther-Sørensen M, Stojanovska E, Pedersen J, Knop FK, Holst JJ, Wewer Albrechtsen NJ (2019) Glucagon Receptor Signaling and Glucagon Resistance. *Int J Mol Sci* **20**:3314.

Johnson JD, Kushner JA (2018) Endogenous insulin: its role in the initiation, progression and management of diabetes. *The Endocrinologist* **129**.

Johnson JD (2021) On the causal relationships between hyperinsulinaemia, insulin resistance, obesity and dysglycaemia in type 2 diabetes. *Diabetologia*. **64**:2138-2146.

Jones PM, Kitsou-Mylona I, Gray E, Squires PE, Persaud SJ (2007) Expression and function of the extracellular calcium-sensing receptor in pancreatic beta-cells. *Arch Physiol Biochem* **113**:98-103.

Kaestner KH, Powers AC, Naji A; HPAP Consortium; Atkinson MA (2019) NIH Initiative to Improve Understanding of the Pancreas, Islet, and Autoimmunity in Type 1 Diabetes: The Human Pancreas Analysis Program (HPAP). *Diabetes*. **68**:1394-1402.

Kahn SE (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*. **46**:3-19.

Kahn SE, Cooper ME, Del Prato S (2014) Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* **383**:1068-1083.

Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G; ADOPT Study Group (2006) Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* **355**:2427-2443. Erratum in: (2007) *N Engl J Med* **356**:1387-1388.

Kalwat MA, Cobb MH (2017) Mechanisms of the amplifying pathway of insulin secretion in the β cell. *Pharmacol Ther* **179**:17-30.

Kanzaki M, Watson RT, Artemyev NO, Pessin JE (2000) The trimeric GTP-binding protein (G(q)/G(11)) alpha subunit is required for insulin-stimulated GLUT4 translocation in 3T3L1 adipocytes. *J Biol Chem* **275**:7167-7175.

Katoh H, Aoki J, Yamaguchi Y, Kitano Y, Ichikawa A, Negishi M (1998) Constitutively active Galpha12, Galpha13, and Galphaq induce Rho-dependent neurite retraction through different signaling pathways. *J Biol Chem* **273**:28700-28707.

Kawai VK, Levinson RT, Adefurin A, Kurnik D, Collier SP, Conway D, Stein CM (2017) Variation in the α_{2A}-adrenergic receptor gene and risk of gestational diabetes. *Pharmacogenomics* **18**:1381-1386.

Kawakami K, Yanagawa M, Hiratsuka S, Yoshida M, Ono Y, Hiroshima M, Ueda M, Aoki J, Sako Y, Inoue A (2022) Heterotrimeric Gq proteins act as a switch for GRK5/6 selectivity underlying β-arrestin transducer bias. *Nat Commun* **13**:487.

Kebede M, Alquier T, Latour MG, Semache M, Tremblay C, Poitout V (2008) The fatty acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. *Diabetes* **57**:2432-2437. Erratum in: (2008) *Diabetes* **57**:3166.

Kim K, Oh CM, Ohara-Imaizumi M, Park S, Namkung J, Yadav VK, Tamarina NA, Roe MW, Philipson LH, Karsenty G, Nagamatsu S, German MS, Kim H (2015) Functional role of serotonin in insulin secretion in a diet-induced insulin-resistant state. *Endocrinology* **156**:444-452.

Kim T, Holleman CL, Nason S, Arble DM, Ottaway N, Chabenne J, Loyd C, Kim JA, Sandoval D, Drucker DJ, DiMarchi R, Perez-Tilve D, Habegger KM (2018) Hepatic Glucagon Receptor Signaling Enhances Insulin-Stimulated Glucose Disposal in Rodents. *Diabetes* **67**:2157-2166.

Kindt KS, Akturk A, Jarysta A, Day M, Beirl A, Flonard M, Tarchini B (2021) EMX2-GPR156-Gαi reverses hair cell orientation in mechanosensory epithelia. *Nat Commun* **12**:2861.

Kleinau G, Worth CL, Kreuchwig A, Biebermann H, Marcinkowski P, Scheerer P, Krause G (2017) Structural-Functional Features of the Thyrotropin Receptor: A Class A G-Protein-Coupled Receptor at Work. *Front Endocrinol (Lausanne)*. **8**:86.

Kobilka BK (2007) G protein coupled receptor structure and activation. *Biochim Biophys Acta* **1768**:794-807.

Komolov KE, Benovic JL (2018) G protein-coupled receptor kinases: Past, present and future. *Cell Signal* **41**:17-24.

Komolov KE, Du Y, Duc NM, Betz RM, Rodrigues JPGLM, Leib RD, Patra D, Skiniotis G, Adams CM, Dror RO, Chung KY, Kobilka BK, Benovic JL (2017) Structural and Functional Analysis of a β_2-Adrenergic Receptor Complex with GRK5. *Cell* **169**:407-421.

Kong KC, Butcher AJ, McWilliams P, Jones D, Wess J, Hamdan FF, Werry T, Rosethorne EM, Charlton SJ, Munson SE, Cragg HA, Smart AD, Tobin AB (2010) M3-muscarinic receptor promotes insulin release via receptor phosphorylation/arrestin-dependent activation of protein kinase D1. *Proc Natl Acad Sci U S A* **107**:21181-21186.

Kowatsch C, Woolley RE, Kinnebrew M, Rohatgi R, Siebold C. (2019) Structures of vertebrate Patched and Smoothened reveal intimate links between cholesterol and Hedgehog signaling. *Curr Opin Struct Biol.* **57**:204-214.

Köves K, Szabó E, Kántor O, Heinzlmann A, Szabó F, Csáki Á (2020) Current State of Understanding of the Role of PACAP in the Hypothalamo-Hypophyseal Gonadotropin Functions of Mammals. *Front Endocrinol (Lausanne)*. **11**:88.

Krasnoperov VG, Bittner MA, Beavis R, Kuang Y, Salnikow KV, Chepurny OG, Little AR, Plotnikov AN, Wu D, Holz RW, Petrenko AG (1997) alpha-Latrotoxin stimulates exocytosis by the interaction with a neuronal G-protein-coupled receptor. *Neuron* **18**:925-937.

Krilov L, Nguyen A, Miyazaki T, Unson CG, Williams R, Lee NH, Ceryak S, Bouscarel B (2011) Dual mode of glucagon receptor internalization: role of PKCα, GRKs and β-arrestins. *Exp Cell Res* **317**:2981-2994.

Kuhn C, Bufe B, Winnig M, Hofmann T, Frank O, Behrens M, Lewtschenko T, Slack JP, Ward CD, Meyerhof W (2004) Bitter taste receptors for saccharin and acesulfame K. *J Neurosci* **24**:10260-10265.

Kühn H (1978) Light-regulated binding of rhodopsin kinase and other proteins to cattle photoreceptor membranes. *Biochemistry* **17**:4389-4395.

Lacey RJ, Berrow NS, London NJ, Lake SP, James RF, Scarpello JH, Morgan NG (1990) Differential effects of beta-adrenergic agonists on insulin secretion from Pancreatic islets isolated from. rat and man. *J Mol Endocrinol* **5**:49-54.

Lagerström MC, Schiöth HB (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* **7**:339-357. Erratum in: (2008) *Nat Rev Drug Discov* **7**:542.

Laitakari A, Liu L, Frimurer TM, Holst B (2021) The Zinc-Sensing Receptor GPR39 in Physiology and as a Pharmacological Target. *Int J Mol Sci* **22**:3872.

Lajus S, Vacher P, Huber D, Dubois M, Benassy MN, Ushkaryov Y, Lang J (2006) Alpha-latrotoxin induces exocytosis by inhibition of voltage-dependent K+ channels and by stimulation of L-type Ca2+ channels via latrophilin in beta-cells. *J Biol Chem* **281**:5522-5531.

Lambert NA, Johnston CA, Cappell SD, Kuravi S, Kimple AJ, Willard FS, Siderovski DP (2010) Regulators of G-protein signaling accelerate GPCR signaling kinetics and govern sensitivity solely by accelerating GTPase activity. *Proc Natl Acad Sci U S A* **107**:7066-7071. Erratum in: (2012) *Proc Natl Acad Sci U S A* **109**:2175.

Lang J, Ushkaryov Y, Grasso A, Wollheim CB (1998) Ca2+-independent insulin exocytosis induced by alpha-latrotoxin requires latrophilin, a G protein-coupled receptor. *EMBO J* **17**:648-657.

Langenhan T, Aust G, Hamann J (2013) Sticky signaling--adhesion class G protein-coupled receptors take the stage. *Sci Signal* **6**:re3.

Lapadula D, Benovic JL (2021) Targeting Oncogenic $G\alpha_{q/11}$ in Uveal Melanoma. *Cancers (Basel)* **13**:6195.

Lasher AT, Srivastava H, Sun LY (2022) Insights into the Role of Glucagon Receptor Signaling in Metabolic Regulation from Pharmacological Inhibition and Tissue-Specific Knockout Models. *Biomedicines* **10**:1907.

Le Q, Yao W, Chen Y, Yan B, Liu C, Yuan M, Zhou Y, Ma L (2016) GRK6 regulates ROS response and maintains hematopoietic stem cell self-renewal. *Cell Death Dis* **7**:e2478.

Lee SP, Qi J, Xu G, Rankin MM, Littrell J, Xu JZ, Bakaj I, Pocai A (2021) GRK Inhibition Potentiates Glucagon-Like Peptide-1 Action. *Front Endocrinol (Lausanne)* **12**:652628.

Leech CA, Holz GG, Habener JF (1996) Signal transduction of PACAP and GLP-1 in pancreatic beta cells. *Ann N Y Acad Sci*. **805**:81-93.

Leech CA, Chepurny OG, Holz GG (2010) Epac2-dependent rap1 activation and the control of islet insulin secretion by glucagon-like peptide-1. *Vitam Horm* **84**:279-302.

Lelianova VG, Davletov BA, Sterling A, Rahman MA, Grishin EV, Totty NF, Ushkaryov YA (1997) Alpha-latrotoxin receptor, latrophilin, is a novel member of the secretin family of G protein-coupled receptors. *J Biol Chem* **272**:21504-21508.

Levitz J, Habrian C, Bharill S, Fu Z, Vafabakhsh R, Isacoff EY (2016) Mechanism of Assembly and Cooperativity of Homomeric and Heteromeric Metabotropic Glutamate Receptors. *Neuron* **92**:143-159.

Li H, Gan W, Lu L, Dong X, Han X, Hu C, Yang Z, Sun L, Bao W, Li P, He M, Sun L, Wang Y, Zhu J, Ning Q, Tang Y, Zhang R, Wen J, Wang D, Zhu X, Guo K, Zuo X, Guo X, Yang H, Zhou X; DIAGRAM Consortium; AGEN-T2D Consortium, Zhang X, Qi L, Loos RJ, Hu FB, Wu T, Liu Y, Liu L, Yang Z, Hu R, Jia W, Ji L, Li Y, Lin X (2013) A genome-wide association study identifies GRK5 and RASGRP1 as type 2 diabetes loci in Chinese Hans. *Diabetes* **62**:291-298.

Li X, Xie L, Qu X, Zhao B, Fu W, Wu B, Wu J (2020) GPR91, a critical signaling mechanism in modulating pathophysiologic processes in chronic illnesses. *FASEB J* **34**:13091-13105.

Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ (2003) Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* **278**:471-478.

Li ZA, Cho JH, Woodhams LG, Hughes JW (2022) Fluorescence imaging of beta cell primary cilia. *Front Endocrinol (Lausanne)*. **13**:1004136.

Liggett SB (2011) Phosphorylation barcoding as a mechanism of directing GPCR signaling. *Sci Signal* **4**:pe36.

Light PE, Manning Fox JE, Riedel MJ, Wheeler MB (2002) Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. *Mol Endocrinol* **16**:2135-2144.

Lin S, Han S, Cai X, Tan Q, Zhou K, Wang D, Wang X, Du J, Yi C, Chu X, Dai A, Zhou Y, Chen Y, Zhou Y, Liu H, Liu J, Yang D, Wang MW, Zhao Q, Wu B (2021) Structures of G_i-bound metabotropic glutamate receptors mGlu2 and mGlu4. *Nature* **594**:583-588.

Liu M, Yang X, Bai T, Liu Z, Liu T, Wang Y, Cui L, Liu Y, Zhang Y (2019) PACAP stimulates insulin secretion by PAC_1 receptor and ion channels in β-cells. *Cell Signal*. **61**:48-56.

Longuet C, Robledo AM, Dean ED, Dai C, Ali S, McGuinness I, de Chavez V, Vuguin PM, Charron MJ, Powers AC, Drucker DJ (2013) Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. *Diabetes* **62**:1196-1205.

Lorenz W, Inglese J, Palczewski K, Onorato JJ, Caron MG, Lefkowitz RJ (1991) The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the beta-adrenergic receptor kinase. *Proc Natl Acad Sci U S A* **88**:8715-8719.

Lorza-Gil E, Kaiser G, Rexen Ulven E, König GM, Gerst F, Oquendo MB, Birkenfeld AL, Häring HU, Kostenis E, Ulven T, Ullrich S (2020) FFA2-, but not FFA3-agonists inhibit GSIS of human pseudoislets: a comparative study with mouse islets and rat INS-1E cells. *Sci Rep* **10**:16497.

Lu D, Mulder H, Zhao P, Burgess SC, Jensen MV, Kamzolova S, Newgard CB, Sherry AD (2002) 13C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). *Proc Natl Acad Sci U S A* **99**:2708-2713.

Luo J, Swaminath G, Brown SP, Zhang J, Guo Q, Chen M, Nguyen K, Tran T, Miao L, Dransfield PJ, Vimolratana M, Houze JB, Wong S, Toteva M, Shan B, Li F, Zhuang R, Lin DC (2012) A potent class of GPR40 full agonists engages the enteroinsular axis to promote glucose control in rodents. *PLoS One* **7**:e46300.

Lyu J, Imachi H, Yoshimoto T, Fukunaga K, Sato S, Ibata T, Kobayashi T, Dong T, Yonezaki K, Yamaji N, Kikuchi F, Iwama H, Ishikawa R, Haba R, Sugiyama Y, Zhang H, Murao K (2018) Thyroid stimulating hormone stimulates the expression of glucose transporter 2 via its receptor in pancreatic β cell line, INS-1 cells. *Sci Rep*. **8**:1986.

Lyu Z, Zhao M, Atanes P, Persaud SJ (2022) Quantification of changes in human islet G protein-coupled receptor mRNA expression in obesity. *Diabet Med* **39**:e14974.

MacDonald MJ, Longacre MJ, Stoker SW, Kendrick M, Thonpho A, Brown LJ, Hasan NM, Jitrapakdee S, Fukao T, Hanson MS, Fernandez LA, Odorico J (2011) Differences between human and rodent pancreatic islets: low pyruvate

carboxylase, atp citrate lyase, and pyruvate carboxylation and high glucose-stimulated acetoacetate in human pancreatic islets. *J Biol Chem* **286**:18383-18396.

MacDonald PE, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB (2002) The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes* **51** Suppl 3:S434-S442.

Maedler K, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY (2005) Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J Clin Endocrinol Metab* **90**:501-506.

Mancini AD, Bertrand G, Vivot K, Carpentier É, Tremblay C, Ghislain J, Bouvier M, Poitout V (2015) β-Arrestin Recruitment and Biased Agonism at Free Fatty Acid Receptor 1. *J Biol Chem* **290**:21131-21140.

Manglik A, Kim TH, Masureel M, Altenbach C, Yang Z, Hilger D, Lerch MT, Kobilka TS, Thian FS, Hubbell WL, Prosser RS, Kobilka BK (2015) Structural Insights into the Dynamic Process of β2-Adrenergic Receptor Signaling. *Cell* **161**:1101-1111. Erratum in: (2015) *Cell* **162**:1431.

Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Del Cañizo-Gómez FJ (2016) Update on the treatment of type 2 diabetes mellitus. *World J Diabetes* **7**:354-395.

Martin SK, Carroll R, Benig M, Steiner DF (1994) Regulation by glucose of the biosynthesis of PC2, PC3 and proinsulin in (ob/ob) mouse islets of Langerhans. *FEBS Lett* **356**:279-282.

Matkovich SJ, Diwan A, Klanke JL, Hammer DJ, Marreez Y, Odley AM, Brunskill EW, Koch WJ, Schwartz RJ, Dorn GW 2nd (2006) Cardiac-specific ablation of G-protein

receptor kinase 2 redefines its roles in heart development and beta-adrenergic signaling. *Circ Res* **99**:996-1003.

Matschinsky FM, Wilson DF (2019) The Central Role of Glucokinase in Glucose Homeostasis: A Perspective 50 Years After Demonstrating the Presence of the Enzyme in Islets of Langerhans. *Front Physiol* **10**:148.

May-Simera HL, Kelley MW (2012) Cilia, Wnt signaling, and the cytoskeleton. *Cilia*. **1**:7.

Mazzone T, Chait A, Plutzky J (2008) Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet* **371**:1800-1809.

McCorvy JD, Roth BL (2015) Structure and function of serotonin G protein-coupled receptors. *Pharmacol Ther* **150**:129-142.

McCreath KJ, Espada S, Gálvez BG, Benito M, de Molina A, Sepúlveda P, Cervera AM (2015) Targeted disruption of the SUCNR1 metabolic receptor leads to dichotomous effects on obesity. *Diabetes* **64**:1154-1167.

Meier DT, Rachid L, Wiedemann SJ, Traub S, Trimigliozzi K, Stawiski M, Sauteur L, Winter DV, Le Foll C, Brégère C, Guzman R, Odermatt A, Böni-Schnetzler M, Donath MY (2022) Prohormone convertase 1/3 deficiency causes obesity due to impaired proinsulin processing. *Nat Commun* **13**:4761.

Meier JJ, Bonadonna RC (2013) Role of reduced β-cell mass versus impaired β-cell function in the pathogenesis of type 2 diabetes. *Diabetes Care* **36**:S113-S119.

Milligan G, Alvarez-Curto E, Watterson KR, Ulven T, Hudson BD (2015) Characterizing pharmacological ligands to study the long-chain fatty acid receptors GPR40/FFA1 and GPR120/FFA4. *Br J Pharmacol* **172**:3254-3265.

Milligan G, Kostenis E (2006) Heterotrimeric G-proteins: a short history. *Br J Pharmacol* **147** Suppl 1:S46-S55.

Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N, Arimura A (1990) Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem Biophys Res Commun*. **170**:643-8.

Mohammad S, Patel RT, Bruno J, Panhwar MS, Wen J, McGraw TE (2014) A naturally occurring GIP receptor variant undergoes enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. *Mol Cell Biol* **34**:3618-3629.

Molina J, Rodriguez-Diaz R, Fachado A, Jacques-Silva MC, Berggren P-O, Caicedo A (2014) Control of insulin secretion by cholinergic signaling in the human pancreatic islet. *Diabetes* **63**:2714-2726.

Montrose-Rafizadeh C, Avdonin P, Garant MJ, Rodgers BD, Kole S, Yang H, Levine MA, Schwindinger W, Bernier M (1999) Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. *Endocrinology* **140**:1132-1140.

Moore CA, Milano SK, Benovic JL (2007) Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol* **69**:451-482.

Mukai E, Fujimoto S, Inagaki N (2022) Role of reactive oxygen species in glucose metabolism disorder in diabetic pancreatic β-cells. *Biomolecules* **12(9)**:1228.

Müller A, Winkler J, Fiedler F, Sastradihardja T, Binder C, Schnabel R, Kungel J, Rothemund S, Hennig C, Schöneberg T, Prömel S (2015) Oriented Cell Division in

the C. elegans Embryo Is Coordinated by G-Protein Signaling Dependent on the Adhesion GPCR LAT-1. *PLoS Genet* **11**:e1005624.

Müller WA, Faloona GR, Aguilar-Parada E, Unger RH (1970) Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. *N Engl J Med* **283**:109-115.

Murga C, Arcones AC, Cruces-Sande M, Briones AM, Salaices M, Mayor F Jr (2019) G Protein-Coupled Receptor Kinase 2 (GRK2) as a Potential Therapeutic Target in Cardiovascular and Metabolic Diseases. *Front Pharmacol* **10**:112.

Nagamatsu S, Bolaffi JL, Grodsky GM (1987) Direct effects of glucose on proinsulin synthesis and processing during desensitization. *Endocrinology* **120**:1225-1231.

Nagamatsu S, Grodsky GM (1987) Analysis of proinsulin conversion activated by prior glucose: evidence that glucose stimulates synthesis of the conversion enzyme. *Biochem Biophys Res Commun* **148**:1418-1424.

Nagamatsu S, Grodsky GM (1988) Glucose-regulated proinsulin processing in isolated islets from rat pancreas. *Diabetes* **37**:1426-1431.

Nakaya M, Tajima M, Kosako H, Nakaya T, Hashimoto A, Watari K, Nishihara H, Ohba M, Komiya S, Tani N, Nishida M, Taniguchi H, Sato Y, Matsumoto M, Tsuda M, Kuroda M, Inoue K, Kurose H (2013) GRK6 deficiency in mice causes autoimmune disease due to impaired apoptotic cell clearance. *Nat Commun* **4**:1532.

Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W (1993b) Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* **91**:301-307.

Nauck MA, Meier JJ (2018) Incretin hormones: Their role in health and disease. *Diabetes Obes Metab* **20** Suppl 1:5-21.

Nauck MA, Meier JJ (2019) GIP and GLP-1: Stepsiblings Rather Than Monozygotic Twins Within the Incretin Family. *Diabetes* **68**:897-900.

Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W (1993a) Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in Type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **36**:741–744.

Nazarko O, Kibrom A, Winkler J, Leon K, Stoveken H, Salzman G, Merdas K, Lu Y, Narkhede P, Tall G, Prömel S, Araç D (2018) A Comprehensive Mutagenesis Screen of the Adhesion GPCR Latrophilin-1/ADGRL1. *iScience* **3**:264-278.

Negoro N, Sasaki S, Mikami S, Ito M, Suzuki M, Tsujihata Y, Ito R, Harada A, Takeuchi K, Suzuki N, Miyazaki J, Santou T, Odani T, Kanzaki N, Funami M, Tanaka T, Kogame A, Matsunaga S, Yasuma T, Momose Y (2010) Discovery of TAK-875: A Potent, Selective, and Orally Bioavailable GPR40 Agonist. *ACS Med Chem Lett* **1**:290-294.

Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkhandanyan AV, Koshkin V, Tarasov AI, Carzaniga R, Kronenberger K, Taneja TK, da Silva Xavier G, Libert S, Froguel P, Scharfmann R, Stetsyuk V, Ravassard P, Parker H, Gribble FM, Reimann F, Sladek R, Hughes SJ, Johnson PR, Masseboeuf M, Burcelin R, Baldwin SA, Liu M, Lara-Lemus R, Arvan P, Schuit FC, Wheeler MB, Chimienti F, Rutter GA (2009) Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. *Diabetes* **58**:2070-2083.

Ning Y, O'Neill K, Lan H, Pang L, Shan LX, Hawes BE, Hedrick JA (2008) Endogenous and synthetic agonists of GPR119 differ in signalling pathways and their effects on insulin secretion in MIN6c4 insulinoma cells. *Br J Pharmacol* **155**:1056-1065.

Nobles KN, Xiao K, Ahn S, Shukla AK, Lam CM, Rajagopal S, Strachan RT, Huang TY, Bressler EA, Hara MR, Shenoy SK, Gygi SP, Lefkowitz RJ (2011) Distinct phosphorylation sites on the β(2)-adrenergic receptor establish a barcode that encodes differential functions of β-arrestin. *Sci Signal* **4**:ra51.

Oh DY, Olefsky JM (2016) G protein-coupled receptors as targets for anti-diabetic therapeutics. *Nat Rev Drug Discov* **15**:161-172.

Oh YS, Seo EH, Lee YS, Cho SC, Jung HS, Park SC, Jun HS (2016) Increase of Calcium Sensing Receptor Expression Is Related to Compensatory Insulin Secretion during Aging in Mice. *PLoS One* **11**:e0159689.

Ohara-Imaizumi M, Kim H, Yoshida M, Fujiwara T, Aoyagi K, Toyofuku Y, Nakamichi Y, Nishiwaki C, Okamura T, Uchida T, Fujitani Y, Akagawa K, Kakei M, Watada H, German MS, Nagamatsu S (2013) Serotonin regulates glucose-stimulated insulin secretion from pancreatic β cells during pregnancy. *Proc Natl Acad Sci U S A* **110**:19420-19425.

Olaniru OE, Cheng J, Ast J, Arvaniti A, Atanes P, Huang GC, King AJF, Jones PM, Broichhagen J, Hodson DJ, Persaud SJ (2021) SNAP-tag-enabled super-resolution imaging reveals constitutive and agonist-dependent trafficking of GPR56 in pancreatic β-cells. *Mol Metab* **53**:101285.

Olaniru OE, Pingitore A, Giera S, Piao X, Castañera González R, Jones PM, Persaud SJ (2018) The adhesion receptor GPR56 is activated by extracellular matrix collagen III to improve β-cell function. *Cell Mol Life Sci* **75**:4007-4019.

Oliveira de Souza C, Sun X, Oh D (2021) Metabolic Functions of G Protein-Coupled Receptors and β-Arrestin-Mediated Signaling Pathways in the Pathophysiology of Type 2 Diabetes and Obesity. *Front Endocrinol (Lausanne)* **12**:715877.

Paavola KJ, Sidik H, Zuchero JB, Eckart M, Talbot WS (2014) Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. *Sci Signal* **7**:ra76.

Page MM, Johnson JD (2018) Mild Suppression of Hyperinsulinemia to Treat Obesity and Insulin Resistance. *Trends Endocrinol Metab* **29**:389-399.

Pamir N, Lynn FC, Buchan AM, Ehses J, Hinke SA, Pospisilik JA, Miyawaki K, Yamada Y, Seino Y, McIntosh CH, Pederson RA (2003) Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis. *Am J Physiol Endocrinol Metab* **284**:E931-E939.

Panaro BL, Flock GB, Campbell JE, Beaudry JL, Cao X, Drucker DJ (2017) β-Cell Inactivation of *Gpr119* Unmasks Incretin Dependence of GPR119-Mediated Glucoregulation. *Diabetes* **66**:1626-1635.

Pao CS, Benovic JL (2005) Structure/function analysis of alpha2A-adrenergic receptor interaction with G protein-coupled receptor kinase 2. *J Biol Chem* **280**:11052-11058.

Parker JC, Andrews KM, Allen MR, Stock JL, McNeish JD (2002) Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochem Biophys Res Commun* **290**:839-843.

Perreault L, Skyler JS, Rosenstock (2021) Novel therapies with precision mechanisms for type 2 diabetes mellitus. *J Nat Rev Endocrinol* **17**:364-377.

Petrova R, Joyner AL (2014) Roles for Hedgehog signaling in adult organ homeostasis and repair. *Development*. **141**:3445-57.

Pfleger J, Gresham K, Koch WJ (2019) G protein-coupled receptor kinases as therapeutic targets in the heart. *Nat Rev Cardiol* **16**:612-622.

Pierce KL, Premont RT, Lefkowitz RJ (2002) Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* **3**:639-650.

Porte D Jr (1967) A receptor mechanism for the inhibition of insulin release by epinephrine in man. *J Clin Invest* **46**:86-94.

Prentki M, Matschinsky FM, Madiraju SR (2013) Metabolic signaling in fuel-induced insulin secretion. *Cell Metab* **18**:162-185.

Priyadarshini M, Wicksteed B, Schiltz GE, Gilchrist A, Layden BT (2016) SCFA Receptors in Pancreatic β Cells: Novel Diabetes Targets? *Trends Endocrinol Metab* **27**:653-664.

Pydi SP, Barella LF, Zhu L, Meister J, Rossi M, Wess J (2022) β-arrestins as important regulators of glucose and energy homeostasis. *Annu Rev Physiol* **84**:17-40.

Qiu P (2020) Embracing the dropouts in single-cell RNA-seq analysis. *Nat Commun*. **11**:1169.

Radhakrishnan A, Rohatgi R, Siebold C. (2020) Cholesterol access in cellular membranes controls Hedgehog signaling. *Nat Chem Biol.* **16**:1303-1313.

Raz, I (2013) Guideline approach to therapy in patients with newly diagnosed type 2 diabetes. *Diabetes Care* **36**:S139–S144.

Reed J, Bain S, Kanamarlapudi V (2021) A Review of Current Trends with Type 2 Diabetes Epidemiology, Aetiology, Pathogenesis, Treatments and Future Perspectives. *Diabetes Metab Syndr Obes* **14**:3567-3602.

Reiter E, Lefkowitz RJ (2006) GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab* **17**:159-165.

Riddle MC, Philipson LH, Rich SS, Carlsson A, Franks PW, Greeley SAW, Nolan JJ, Pearson ER, Zeitler PS, Hattersley AT (2020) Monogenic Diabetes: From Genetic Insights to Population-Based Precision in Care. Reflections From a *Diabetes Care* Editors' Expert Forum. *Diabetes Care* **43**:3117-3128.

Riddy DM, Delerive P, Summers RJ, Sexton PM, Langmead CJ (2018) G Protein-Coupled Receptors Targeting Insulin Resistance, Obesity, and Type 2 Diabetes Mellitus. *Pharmacol Rev* **70**:39-67.

Ritter K, Buning C, Halland N, Pöverlein C, Schwink L (2016) G Protein-Coupled Receptor 119 (GPR119) Agonists for the Treatment of Diabetes: Recent Progress and Prevailing Challenges. *J Med Chem* **59**:3579-3592.

Ritz E, Rychlík I, Locatelli F, Halimi S (1999) End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. *Am J Kidney Dis* **34**:795-808.

Robbins MJ, Michalovich D, Hill J, Calver AR, Medhurst AD, Gloger I, Sims M, Middlemiss DN, Pangalos MN (2000) Molecular cloning and characterization of two

novel retinoic acid-inducible orphan G-protein-coupled receptors (GPRC5B and GPRC5C). *Genomics* **67**:8-18.

Robertson RP, Halter JB, Porte D Jr (1976) A role for alpha-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. *J Clin Invest* **57**:791–795.

Rodriguez-Diaz R, Dando R, Jacques-Silva MC, Fachado A, Molina J, Abdulreda MH, Ricordi C, Roper SD, Berggren P-O, Caicedo A (2011) Alpha cells secrete acetylcholine as a non-neuronal paracrine signal priming beta cell function in humans. *Nat Med* **17**:888-892.

Ronnebaum SM, Ilkayeva O, Burgess SC, Joseph JW, Lu D, Stevens RD, Becker TC, Sherry AD, Newgard CB, Jensen MV (2006) A pyruvate cycling pathway involving cytosolic NADP-dependent isocitrate dehydrogenase regulates glucose-stimulated insulin secretion. *J Biol Chem* **281**:30593-30602.

Rorsman P, Renström E (2003) Insulin granule dynamics in pancreatic beta cells. *Diabetologia* **46**:1029-1045.

Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK (2007) GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* **318**:1266-1273.

Rosengren A, Jing X, Eliasson L, Renström E (2008) Why treatment fails in type 2 diabetes. *PLoS Med*. **5**:e215.

Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V,

Luthman H, Renström E (2010) Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science* **327**:217–220.

Rosengren AH, Braun M, Mahdi T, Andersson SA, Travers ME, Shigeto M, Zhang E, Almgren P, Ladenvall C, Axelsson AS, Edlund A, Pedersen MG, Jonsson A, Ramracheya R, Tang Y, Walker JN, Barrett A, Johnson PR, Lyssenko V, McCarthy MI, Groop L, Salehi A, Gloyn AL, Renström E, Rorsman P, Eliasson L (2012) Reduced insulin exocytosis in human pancreatic beta-cells with gene variants linked to type 2 diabetes. *Diabetes* **61**:1726–1733.

Röthe J, Thor D, Winkler J, Knierim AB, Binder C, Huth S, Kraft R, Rothemund S, Schöneberg T, Prömel S (2019) Involvement of the Adhesion GPCRs Latrophilins in the Regulation of Insulin Release. *Cell Rep* **26**:1573-1584.

Ruiz de Azua I, Gautam D, Guettier JM, Wess J (2011) Novel insights into the function of β-cell M3 muscarinic acetylcholine receptors: therapeutic implications. *Trends Endocrinol Metab* **22**:74-80.

Russell-Jones D, Khan R (2007) Insulin-associated weight gain in diabetes--causes, effects and coping strategies. *Diabetes Obes Metab*. **9**:799-812.

Salehi A, Flodgren E, Nilsson NE, Jimenez-Feltstrom J, Miyazaki J, Owman C, Olde B (2005) Free fatty acid receptor 1 (FFA(1)R/GPR40) and its involvement in fatty-acid-stimulated insulin secretion. *Cell Tissue Res* **322**:207-215.

Santulli G, Lombardi A, Sorriento D, Anastasio A, Del Giudice C, Formisano P, Béguinot F, Trimarco B, Miele C, Iaccarino G (2012) Age-related impairment in insulin release: The essential role of β_2-adrenergic receptor. *Diabetes* **61**:692-701.

Sassmann A, Gier B, Grone H-J, Drews G, Offermanns S, Wettschureck N (2010) The Gq/G11-mediated signaling pathway is critical for autocrine potentiation of insulin secretion in mice**.** *J Clin Invest* **120**:2184-2193.

Scholz N, Gehring J, Guan C, Ljaschenko D, Fischer R, Lakshmanan V, Kittel RJ, Langenhan T (2015) The adhesion GPCR latrophilin/CIRL shapes mechanosensation. *Cell Rep* **11**:866-874.

Schuit F, Van Lommel L, Granvik M, Goyvaerts L, de Faudeur G, Schraenen A, Lemaire K (2012) β-cell-specific gene repression: a mechanism to protect against inappropriate or maladjusted insulin secretion? *Diabetes* **61**:969-975.

Schuppin GT, Rhodes CJ (1996) Specific co-ordinated regulation of PC3 and PC2 gene expression with that of preproinsulin in insulin-producing beta TC3 cells. *Biochem J* **313**:259-268.

Sekar R, Wang L, Chow BK (2017) Central Control of Feeding Behavior by the Secretin, PACAP, and Glucagon Family of Peptides. *Front Endocrinol (Lausanne)*. **8**:18. Erratum in: *Front Endocrinol (Lausanne)*. (2018) **9**:395.

Shang Z, Han F, Zhou X, Bao Z, Zhu J, Wang T, Lu Q, Du L, Li W, Lv D, Yin X (2018) A variant of GRK5 is associated with the therapeutic efficacy of repaglinide in Chinese Han patients with type 2 diabetes mellitus. *Drug Dev Res* **79**:129-135.

Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J (2008) Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care*. **31**:S262-8.

Shapira SN, Naji A, Atkinson MA, Powers AC, Kaestner KH (2022) Understanding islet dysfunction in type 2 diabetes through multidimensional pancreatic phenotyping: The Human Pancreas Analysis Program. *Cell Metab*. **34**:1906-1913.

Sherwood NM, Krueckl SL, McRory JE (2000) The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. *Endocr Rev*. **21**:619-70.

Shrestha S, Erikson G, Lyon J, Spigelman AF, Bautista A, Manning Fox JE, Dos Santos C, Shokhirev M, Cartailler JP, Hetzer MW, MacDonald PE, Arrojo E Drigo R (2022) Aging compromises human islet beta cell function and identity by decreasing transcription factor activity and inducing ER stress. *Sci Adv*. **8**:3932.

Siu FY, He M, de Graaf C, Han GW, Yang D, Zhang Z, Zhou C, Xu Q, Wacker D, Joseph JS, Liu W, Lau J, Cherezov V, Katritch V, Wang MW, Stevens RC (2013) Structure of the human glucagon class B G-protein-coupled receptor. *Nature* **499**:444-449.

Skelin Klemen M, Dolenšek J, Slak Rupnik M, Stožer A (2017) The triggering pathway to insulin secretion: Functional similarities and differences between the human and the mouse β cells and their translational relevance. *Islets* **9**:109-139.

Skelly RH, Schuppin GT, Ishihara H, Oka Y, Rhodes CJ (1996) Glucose-regulated translational control of proinsulin biosynthesis with that of the proinsulin endopeptidases PC2 and PC3 in the insulin-producing MIN6 cell line. *Diabetes* **45**:37-43.

Smeekens SP, Montag AG, Thomas G, Albiges-Rizo C, Carroll R, Benig M, Phillips LA, Martin S, Ohagi S, Gardner P (1992) Proinsulin processing by the subtilisin-related

proprotein convertases furin, PC2, and PC3. *Proc Natl Acad Sci U S A* **89**:8822-8826.

Snyder J, Lackey AI, Brown GS, Diaz M, Yuzhen T, Sato PY (2021) GRK2 contributes to glucose mediated calcium responses and insulin secretion in pancreatic islet cells. *Sci Rep* **11**:11129.

Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, Matsumoto S, Kamohara M, Hiyama H, Yoshida S, Momose K, Ueda Y, Matsushime H, Kobori M, Furuichi K (2005) Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. *Biochem Biophys Res Commun* **326**:744-751. Erratum in: (2005) *Biochem Biophys Res Commun* **329**:417.

Solimena M, Schulte AM, Marselli L, Ehehalt F, Richter D, Kleeberg M, Mziaut H, Knoch KP, Parnis J, Bugliani M, Siddiq A, Jörns A, Burdet F, Liechti R, Suleiman M, Margerie D, Syed F, Distler M, Grützmann R, Petretto E, Moreno-Moral A, Wegbrod C, Sönmez A, Pfriem K, Friedrich A, Meinel J, Wollheim CB, Baretton GB, Scharfmann R, Nogoceke E, Bonifacio E, Sturm D, Meyer-Puttlitz B, Boggi U, Saeger HD, Filipponi F, Lesche M, Meda P, Dahl A, Wigger L, Xenarios I, Falchi M, Thorens B, Weitz J, Bokvist K, Lenzen S, Rutter GA, Froguel P, von Bülow M, Ibberson M, Marchetti P (2018) Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. *Diabetologia*. **61**:641-657.

Soni A, Amisten S, Rorsman P, Salehi A (2013) GPRC5B a putative glutamate-receptor candidate is negative modulator of insulin secretion. *Biochem Biophys Res Commun* **441**:643-648.

Sonoda N, Imamura T, Yoshizaki T, Babendure JL, Lu JC, Olefsky JM (2008) Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. *Proc Natl Acad Sci U S A* **105**:6614-6619.

Soreq H, Seidman S (2001) Acetylcholinesterase--new roles for an old actor. *Nat Rev Neurosci*. **2**:294-302.

Spégel P, Sharoyko VV, Goehring I, Danielsson AP, Malmgren S, Nagorny CL, Andersson LE, Koeck T, Sharp GW, Straub SG, Wollheim CB, Mulder H (2013) Time-resolved metabolomics analysis of β-cells implicates the pentose phosphate pathway in the control of insulin release. *Biochem J* **450**:595-605.

Squires PE, Jones PM, Younis MY, Hills CE (2014) The calcium-sensing receptor and β-cell function. *Vitam Horm* **95**:249-267.

Sriram K, Insel PA (2018) G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs? *Mol Pharmacol* **93**:251-258.

Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H (2005) The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab* **1**:245-258.

Steyaert W, Varney MJ, Benovic JL, Creemers J, Speeckaert MM, Coucke PJ, Delanghe JR (2022) Hypergastrinemia, a clue leading to the identification of an atypical form of diabetes mellitus type 2. *Clin Chim Acta* **532**:79-83.

Stiernet P, Guiot Y, Gilon P, Henquin JC (2006) Glucose acutely decreases pH of secretory granules in mouse pancreatic islets. Mechanisms and influence on insulin secretion. *J Biol Chem* **281**:22142-22151.

Stone VM, Dhayal S, Brocklehurst KJ, Lenaghan C, Sörhede Winzell M, Hammar M, Xu X, Smith DM, Morgan NG (2014) GPR120 (FFAR4) is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from murine islets of Langerhans. *Diabetologia* **57**:1182-1191.

Storto M, Capobianco L, Battaglia G, Molinaro G, Gradini R, Riozzi B, Di Mambro A, Mitchell KJ, Bruno V, Vairetti MP, Rutter GA, Nicoletti F (2006) Insulin secretion is controlled by mGlu5 metabotropic glutamate receptors. *Mol Pharmacol* **69**:1234-1241.

Suckow AT, Polidori D, Yan W, Chon S, Ma JY, Leonard J, Briscoe CP (2014) Alteration of the glucagon axis in GPR120 (FFAR4) knockout mice: a role for GPR120 in glucagon secretion. *J Biol Chem* **289**:15751-15763.

Suo Z, Cox AA, Bartelli N, Rasul I, Festoff BW, Premont RT, Arendash GW (2007) GRK5 deficiency leads to early Alzheimer-like pathology and working memory impairment. *Neurobiol Aging* **28**:1873-1888.

Suo Z, Wu M, Citron BA, Wong GT, Festoff BW (2004) Abnormality of G-protein-coupled receptor kinases at prodromal and early stages of Alzheimer's disease: an association with early beta-amyloid accumulation. *J Neurosci* **24**:3444-3452.

Sureshkumar K, Saenz A, Ahmad SM, Lutfy K (2021) The PACAP/PAC1 Receptor System and Feeding. *Brain Sci*. **12**:13.

Svendsen B, Larsen O, Gabe MBN, Christiansen CB, Rosenkilde MM, Drucker DJ, Holst JJ (2018) Insulin Secretion Depends on Intra-islet Glucagon Signaling. *Cell Rep* **25**:1127-1134.

Taguchi K, Hida M, Hasegawa M, Narimatsu H, Matsumoto T, Kobayashi T (2017) Suppression of GRK2 expression reduces endothelial dysfunction by restoring glucose homeostasis. *Sci Rep* **7**:8436.

Tamura K, Minami K, Kudo M, Iemoto K, Takahashi H, Seino S (2015) Liraglutide improves pancreatic Beta cell mass and function in alloxan-induced diabetic mice. *PLoS One* **10**: e0126003.

Taneera J, Lang S, Sharma A, Fadista J, Zhou Y, Ahlqvist E, Jonsson A, Lyssenko V, Vikman P, Hansson O, Parikh H, Korsgren O, Soni A, Krus U, Zhang E, Jing XJ, Esguerra JL, Wollheim CB, Salehi A, Rosengren A, Renström E, Groop L (2012) A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab* **16**:122-134.

Tang C, Ahmed K, Gille A, Lu S, Gröne HJ, Tunaru S, Offermanns S (2015) Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat Med* **21**:173-177.

Tang JS, Li QR, Li JM, Wu JR, Zeng R (2017) Systematic Synergy of Glucose and GLP-1 to Stimulate Insulin Secretion Revealed by Quantitative Phosphoproteomics. *Sci Rep* **7**:1018.

Tang Y, Axelsson AS, Spégel P, Andersson LE, Mulder H, Groop LC, Renström E, Rosengren AH (2014) Genotype-based treatment of type 2 diabetes with an α2A-adrenegic receptor antagonist. *Sci Transl Med.* **6**:257ra139.

Tilg H, Moschen AR, Roden M (2017) NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* **14**:32-42.

Trauelsen M, Hiron TK, Lin D, Petersen JE, Breton B, Husted AS, Hjorth SA, Inoue A, Frimurer TM, Bouvier M, O'Callaghan CA, Schwartz TW (2021) Extracellular succinate hyperpolarizes M2 macrophages through SUCNR1/GPR91-mediated Gq signaling. *Cell Rep* **35**:109246.

Tseng CC, Zhang XY (2000) Role of G protein-coupled receptor kinases in glucose-dependent insulinotropic polypeptide receptor signaling. *Endocrinology* **141**:947-952.

Tsuboi T, da Silva Xavier G, Holz GG, Jouaville LS, Thomas AP, Rutter GA (2003) Glucagon-like peptide-1 mobilizes intracellular Ca2+ and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. *Biochem J* **369**:287-299.

Uehling DE, Joseph B, Chung KC, Zhang AX, Ler S, Prakesch MA, Poda G, Grouleff J, Aman A, Kiyota T, Leung-Hagesteijn C, Konda JD, Marcellus R, Griffin C, Subramaniam R, Abibi A, Strathdee CA, Isaac MB, Al-Awar R, Tiedemann RE (2021) Design, Synthesis, and Characterization of 4-Aminoquinazolines as Potent Inhibitors of the G Protein-Coupled Receptor Kinase 6 (GRK6) for the Treatment of Multiple Myeloma. *J Med Chem* **64**:11129-11147. Erratum in: (2022) *J Med Chem* **65**:886-887.

Usui I, Imamura T, Satoh H, Huang J, Babendure JL, Hupfeld CJ, Olefsky JM (2004) GRK2 is an endogenous protein inhibitor of the insulin signaling pathway for glucose transport stimulation. *EMBO J* **23**:2821-2829.

Usui R, Yabe D, Fauzi M, Goto H, Botagarova A, Tokumoto S, Tatsuoka H, Tahara Y, Kobayashi S, Manabe T, Baba Y, Kurosaki T, Herrera PL, Ogura M, Nagashima K, Inagaki N (2019) GPR40 activation initiates store-operated Ca^{2+} entry and potentiates insulin secretion via the IP3R1/STIM1/Orai1 pathway in pancreatic β-cells. *Sci Rep* **9**:15562.

Varney MJ, Steyaert W, Coucke PJ, Delanghe JR, Uehling DE, Joseph B, Marcellus R, Al-Awar R, Benovic JL (2022) G protein-coupled receptor kinase 6 (GRK6) regulates insulin processing and secretion via effects on proinsulin conversion to insulin. *J Biol Chem* **298**:102421.

Vergari E, Knudsen JG, Ramracheya R, Salehi A, Zhang Q, Adam J, Asterholm IW, Benrick A, Briant LJB, Chibalina MV, Gribble FM, Hamilton A, Hastoy B, Reimann F, Rorsman NJG, Spiliotis II, Tarasov A, Wu Y, Ashcroft FM, Rorsman P (2019) Insulin inhibits glucagon release by SGLT2-induced stimulation of somatostatin secretion. *Nat Commun* **10**:139.

Vieira IH, Rodrigues D, Paiva I (2022) The Mysterious Universe of the TSH Receptor. Front Endocrinol (Lausanne). 13:944715.

Vila-Bedmar R, Cruces-Sande M, Lucas E, Willemen HL, Heijnen CJ, Kavelaars A, Mayor F Jr, Murga C (2015) Reversal of diet-induced obesity and insulin resistance by inducible genetic ablation of GRK2. *Sci Signal* **8**:ra73.

Vivoli A, Ghislain J, Filali-Mouhim A, Angeles ZE, Castell AL, Sladek R, Poitout V (2023) Single-Cell RNA Sequencing Reveals a Role for Reactive Oxygen Species and Peroxiredoxins in Fatty Acid-Induced Rat β-Cell Proliferation. *Diabetes*. **72**:45-58.

Vizurraga A, Adhikari R, Yeung J, Yu M, Tall GG (2020) Mechanisms of adhesion G protein-coupled receptor activation. *J Biol Chem* **295**:14065-14083.

Wang J, Carrillo JJ, Lin HV (2016) GPR142 Agonists Stimulate Glucose-Dependent Insulin Secretion via Gq-Dependent Signaling. *PLoS One* **11**:e0154452.

Wang L, Liu Y, Yang J, Zhao H, Ke J, Tian Q, Zhang L, Wen J, Wei R, Hong T (2014) GLP-1 analog liraglutide enhances proinsulin processing in pancreatic β-cells via a PKA-dependent pathway. *Endocrinology* **155**:3817-3828.

Wang L, Shen M, Wang F, Ma L (2012) GRK5 ablation contributes to insulin resistance. *Biochem Biophys Res Commun* **429**:99-104.

Wang L, Zhu L, Meister J, Bone DB, Pydi SP, Rossi, Wess J (2021) Use of DREADD technology to identify novel targets for anti-diabetic drugs. *Annu Rev Pharmacol Toxicol* **61**:421-440.

Wang Q, Pronin AN, Levay K, Almaca J, Fornoni A, Caicedo A, Slepak VZ (2017) Regulator of G-protein signaling Gβ5-R7 is a crucial activator of muscarinic M3 receptor-stimulated insulin secretion. FASEB J 31:4734-4744.

Weir GC, Bonner-Weir S (2004) Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes*. **53**:S16-21.

Wess J (2022) In vivo metabolic roles of G proteins of the G_i family studied with novel mouse models. *Endocrinology* **163**:bqab245.

Wheway G, Nazlamova L, Hancock JT (2018) Signaling through the Primary Cilium. *Front Cell Dev Biol*. **6**:8.

White JP, Wrann CD, Rao RR, Nair SK, Jedrychowski MP, You JS, Martínez-Redondo V, Gygi SP, Ruas JL, Hornberger TA, Wu Z, Glass DJ, Piao X, Spiegelman BM

(2014) G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy. *Proc Natl Acad Sci U S A* **111**:15756-15761.

Wigger L, Barovic M, Brunner AD, Marzetta F, Schöniger E, Mehl F, Kipke N, Friedland D, Burdet F, Kessler C, Lesche M, Thorens B, Bonifacio E, Legido-Quigley C, Barbier Saint Hilaire P, Delerive P, Dahl A, Klose C, Gerl MJ, Simons K, Aust D, Weitz J, Distler M, Schulte AM, Mann M, Ibberson M, Solimena M (2021) Multi-omics profiling of living human pancreatic islet donors reveals heterogeneous beta cell trajectories towards type 2 diabetes. *Nat Metab*. **3**:1017-1031.

Wilding JP (2014) The importance of weight management in type 2 diabetes mellitus. *Int J Clin Pract* **68**:682-691.

Willets JM, Challiss RA, Nahorski SR (2002) Endogenous G protein-coupled receptor kinase 6 Regulates M3 muscarinic acetylcholine receptor phosphorylation and desensitization in human SH-SY5Y neuroblastoma cells. *J Biol Chem* **277**:15523-15529.

Williams DM, Jones H, Stephens JW (2022) Personalized Type 2 Diabetes Management: An Update on Recent Advances and Recommendations. *Diabetes Metab Syndr Obes* **15**:281-295.

Wilson JE (2003) Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. *J Exp Biol* **206**:2049-2057.

Wingler LM, Lefkowitz RJ (2020) Conformational Basis of G Protein-Coupled Receptor Signaling Versatility. *Trends Cell Biol* **30**:736-747.

Xia Z, Yang T, Wang Z, Dong J, Liang C (2014) GRK5 intronic (CA)n polymorphisms associated with type 2 diabetes in Chinese Hainan Island. *PLoS One* **9**:e90597.

Xin Y, Kim J, Okamoto H, Ni M, Wei Y, Adler C, Murphy AJ, Yancopoulos GD, Lin C, Gromada J (2016) RNA Sequencing of Single Human Islet Cells Reveals Type 2 Diabetes Genes. *Cell Metab* **24**:608-615.

Xu G, Kaneto H, Lopez-Avalos MD, Weir GC, Bonner-Weir S (2006) GLP-1/exendin-4 facilitates beta-cell neogenesis in rat and human pancreatic ducts. *Diabetes Res Clin Pract* **73**:107-110.

Xu Y, Xie X (2009) Glucagon receptor mediates calcium signaling by coupling to G alpha q/11 and G alpha i/o in HEK293 cells. *J Recept Signal Transduct Res* **29**:318-325.

Yamamoto K, Hashimoto H, Tomimoto S, Shintani N, Miyazaki J, Tashiro F, Aihara H, Nammo T, Li M, Yamagata K, Miyagawa J, Matsuzawa Y, Kawabata Y, Fukuyama Y, Koga K, Mori W, Tanaka K, Matsuda T, Baba A (2003) Overexpression of PACAP in transgenic mouse pancreatic beta-cells enhances insulin secretion and ameliorates streptozotocin-induced diabetes. *Diabetes*. **52**:1155-62.

Yang T, Yan C, Yang L, Tan J, Jiang S, Hu J, Gao W, Wang Q, Li Y (2023) Identification and validation of core genes for type 2 diabetes mellitus by integrated analysis of single-cell and bulk RNA-sequencing. *Eur J Med Res*. **28**:340.

Yang Y, Chen Y, Chen J, Zhang D, Wang J, Mao X, Wei X, Li X, Ma X, Liu C, Wang K (2019) The Adverse Effects of Thyrotropin Absence on Pancreatic *β* Cell Function in Mice. *J Diabetes Res*. **2019**:9536032.

Yung T, Poon F, Liang M, Coquenlorge S, McGaugh EC, Hui CC, Wilson MD, Nostro MC, Kim TH (2019) Sufu- and Spop-mediated downregulation of Hedgehog

signaling promotes beta cell differentiation through organ-specific niche signals. *Nat Commun*. **10**:4647.

Zhang B, Yang L, Yu L, Lin B, Hou Y, Wu J, Huang Q, Han Y, Guo L, Ouyang Q, Zhang B, Lu L, Zhang X (2012) Acetylcholinesterase is associated with apoptosis in β cells and contributes to insulin-dependent diabetes mellitus pathogenesis. *Acta Biochim Biophys Sin (Shanghai)*. **44**:207-16.

Zhang GF, Jensen MV, Gray SM, El K, Wang Y, Lu D, Becker TC, Campbell JE, Newgard CB (2021a) Reductive TCA cycle metabolism fuels glutamine- and glucose-stimulated insulin secretion. *Cell Metab* **33**:804-817.

Zhang S, Ma Y, Li J, Ma J, Yu B, Xie X (2014) Molecular matchmaking between the popular weight-loss herb Hoodia gordonii and GPR119, a potential drug target for metabolic disorder. *Proc Natl Acad Sci U S A* **111**:14571-14576.

Zhang Y, Han C, Zhu W, Yang G, Peng X, Mehta S, Zhang J, Chen L, Liu Y (2021b) Glucagon Potentiates Insulin Secretion Via β-Cell GCGR at Physiological Concentrations of Glucose. *Cells* **10**:2495.

Zhao S, Mugabo Y, Iglesias J, Xie L, Delghingaro-Augusto V, Lussier R, Peyot ML, Joly E, Taïb B, Davis MA, Brown JM, Abousalham A, Gaisano H, Madiraju SR, Prentki M (2014) α/β-Hydrolase domain-6-accessible monoacylglycerol controls glucose-stimulated insulin secretion. *Cell Metab* **19**:993-1007.

Zhou Y, Lindberg I (1993) Purification and characterization of the prohormone convertase PC1(PC3). *J Biol Chem* **268**:5615-5623.

Zhu L, Almaça J, Dadi PK, Hong H, Sakamoto W, Rossi M, Lee RJ, Vierra NC, Lu H, Cui Y, McMillin SM, Perry NA, Gurevich VV, Lee A, Kuo B, Leapman RD,

Matschinsky FM, Doliba NM, Urs NM, Caron MG, Jacobson DA, Caicedo A, Wess J (2017a) β-arrestin-2 is an essential regulator of pancreatic β-cell function under physiological and pathophysiological conditions. *Nat Commun* **8**:14295.

Zhu L, Rossi M, Cui Y, Lee RJ, Sakamoto W, Perry NA, Urs NM, Caron MG, Gurevich VV, Godlewski G, Kunos G, Chen M, Chen W, Wess J (2017b) Hepatic β-arrestin-2 is essential for maintaining euglycemia. *J Clin Invest* **127**:2941-2945.

Zhu L, Rossi M, Cohen A, Pham J, Zheng H, Dattaroy D, Mukaibo T, Melvin JE, Langel JL, Hattar S, Matschinsky FM, Appella DH, Doliba NM, Wess J (2019) Allosteric modulation of β-cell M_3 muscarinic acetylcholine receptors greatly improves glucose homeostasis in lean and obese mice. *Proc Natl Acad Sci U S A* **116**:18684-18690.

Zhu L, Rossi M, Doliba NM, Wess J (2020) Beta-cell M_3 muscarinic acetylcholine receptors as potential targets for novel antidiabetic drugs. *Int Immunopharmacol* **81**:106267.

Footnotes

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Table 1. Highly expressed and understudied GPCRs in islets of normal and diseased individuals

Gene	GPCR	Class	G protein coupling	Known ligand/function	Effects on insulin secretion	References
ADGRG1[$]	GPR56	Adhesion	$G_{12/13}$, G_s?	Collagen type III; potentiates muscle hypertrophy	increase	White et al., 2014; Olaniru et al., 2018
ADGRL1	LPHN1	Adhesion	G_s, G_q, G_i	Brain function and	unknown	Muller et al.,

				embryonic development		2015; Nazarko et al., 2018
ADGRL4	ELTD1	Adhesion	unknown	Tumor angiogenesis	unknown	Guihurt et al., 2021
CasR	CasR	C	G_q	Calcium sensing receptor	increase	Gong et al., 2023
GPRC5B[#]	GPRC5B	C	G_i?	Glutamate; involved in cytokine induced apoptosis	decrease	Soni et al., 2013
GPRC5C[$]	GPRC5C	C	G_s?	trans-retinoic acid (vitamin C); prevents cytokine induced apoptosis	increase	Amisten et al., 2017b
HT1RF	5-HT1F	A	unknown	Serotonin; inhibits glucagon release in alpha cells	decrease (paracrine signaling mechanism)	Almaca et al., 2016
*CCR9**	CCR9	A	G_i	Chemokine receptor; involved in inflammation	decrease	Atanes et al., 2020
*HTR2B**	5-HT2B	A	G_q	Serotonin; increased beta cell proliferation	increase	Bennet et al., 2016
*GPR156**	GPR156	C	G_i	Hair cell orientation, cell polarity	unknown	Kindt et al., 2021
*GPR39**	GPR39	A	G_s, G_q	Zinc sensing receptor	increase	Holst et al., 2009; Laitakari et al., 2021

[#]upregulated in T2D
[$]downregulated in T2D
*upregulated in obesity

Figure Legends

Figure 1. Insulin secretion in the β-cell. The pancreas is an organ in the abdomen located behind the stomach in humans. It is composed of two major parts: the exocrine and endocrine pancreas. The exocrine pancreas releases enzymes that aid in the digestion of food and makes up most of the pancreas. The endocrine pancreas is

composed of the islets of Langerhans which are small clusters of cells (shown above) producing hormones critical for fuel storage and metabolic homeostasis. These include the glucagon secreting α-cells, the somatostatin secreting δ-cells, the pancreatic-polypeptide (PP) secreting cells and the insulin producing β-cells which are depicted in this schematic and make up the majority of the cells in islets in both rodents and humans. The β-cells are highly regulated to ensure insulin is produced and secreted properly to maintain normoglycemia. First, extracellular glucose enters the β-cell through GLUT1/2 transporters where it binds to glucokinase. Glucokinase phosphorylates glucose forming glucose-6-phosphate that undergoes glycolysis to form pyruvate and generate ATP. The pyruvate then enters the mitochondria where it supports the TCA cycle and oxidative phosphorylation to generate more ATP. This increase in the ATP/ADP ratio in the cell inhibits the ATP sensitive K_{ATP} channel. Channel closure ensues, potassium efflux is prevented, and the membrane depolarizes. This change in membrane potential activates the voltage gated calcium channels (VGCCs) allowing for rapid calcium influx and insulin exocytosis. GPCRs resident on the β-cell further influence insulin secretion through G-protein- and β-arrestin-dependent signaling pathways activated in several ways including through gut derived metabolites from food, gut derived hormones, and signaling molecules originating from neighboring islet cells as well as the β-cell itself. This creates an extracellular milieu of signals that include SCFAs, LCFAs, acetate, monoatomic ions, GLP-1, and GIP to name a few. Additionally, products of β-cell glucose metabolism modulate insulin secretion either through autocrine signaling mechanisms that can involve GPCRs or metabolic stimulus coupling pathways that alter the β-cell redox state and metabolic signaling pathways

that support exocytosis. Black arrows represent the triggering pathway and colored arrows the amplifying pathway of insulin secretion. Created with BioRender.com

Figure 2. GPCR mediated activation and inhibition of insulin secretion in pancreatic β-cells. There are many GPCRs resident on β-cells. Typically, GPCRs that couple to G_s and G_q stimulate insulin secretion through adenylyl cyclase and phospholipase Cβ, respectively. GPCRs that are G_i coupled inhibit insulin secretion by preventing the production of cAMP. Some GPCRs are listed for each GPCR-G-protein coupling emphasizing the ones discussed in this review. Signaling for $G_{12/13}$ is not included because very little data exists for $G_{12/13}$ mediated insulin secretion. Created with BioRender.com

Figure 3. Changes in GPCR mediated insulin secretion regulated by GRKs. The β-cell contains multiple GPCRs capable of increasing or decreasing insulin secretion based on their G protein coupling. In part 1, a ligand bound receptor is activated and stimulates the dissociation and activity of the heterotrimeric G proteins (A). The Gα subunits than control the release of insulin through various downstream pathways described previously where they either increase ($G\alpha_s$, $G\alpha_q$) or decrease ($G\alpha_i$) secretion (B). However, the control of insulin secretion can also be modulated by receptor desensitization mediated by GRK phosphorylation shown in part 2 which has largely been unexplored. Here, GRKs phosphorylate the activated receptor blunting G protein signaling (A). This triggers β-arrestin recruitment to the phosphorylated receptor (B) initiating receptor internalization and downregulation (C). Depending on the receptor

and the G protein coupling, this will either increase or decrease insulin secretion (D). Furthermore, β-arrestin signaling within the β-cell following receptor internalization has also been shown to modulate insulin secretion adding further complexity to the release of insulin following GPCR activation and GRK phosphorylation. Similar mechanisms are also likely present in the other hormone secreting cells of the islets including α-cells and δ-cells but have not been well studied. Created with Biorender.com

Figure 1

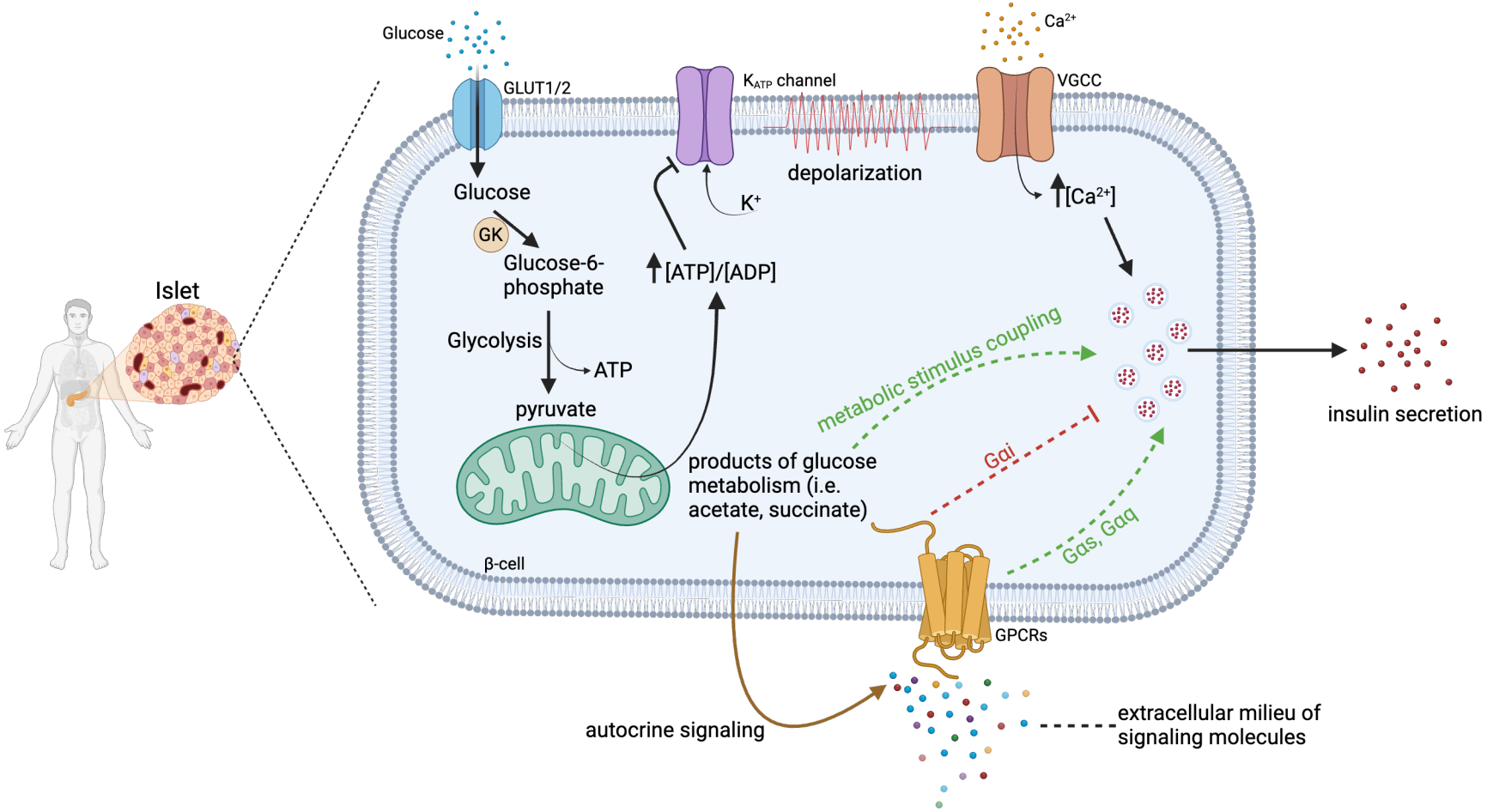

Figure 2

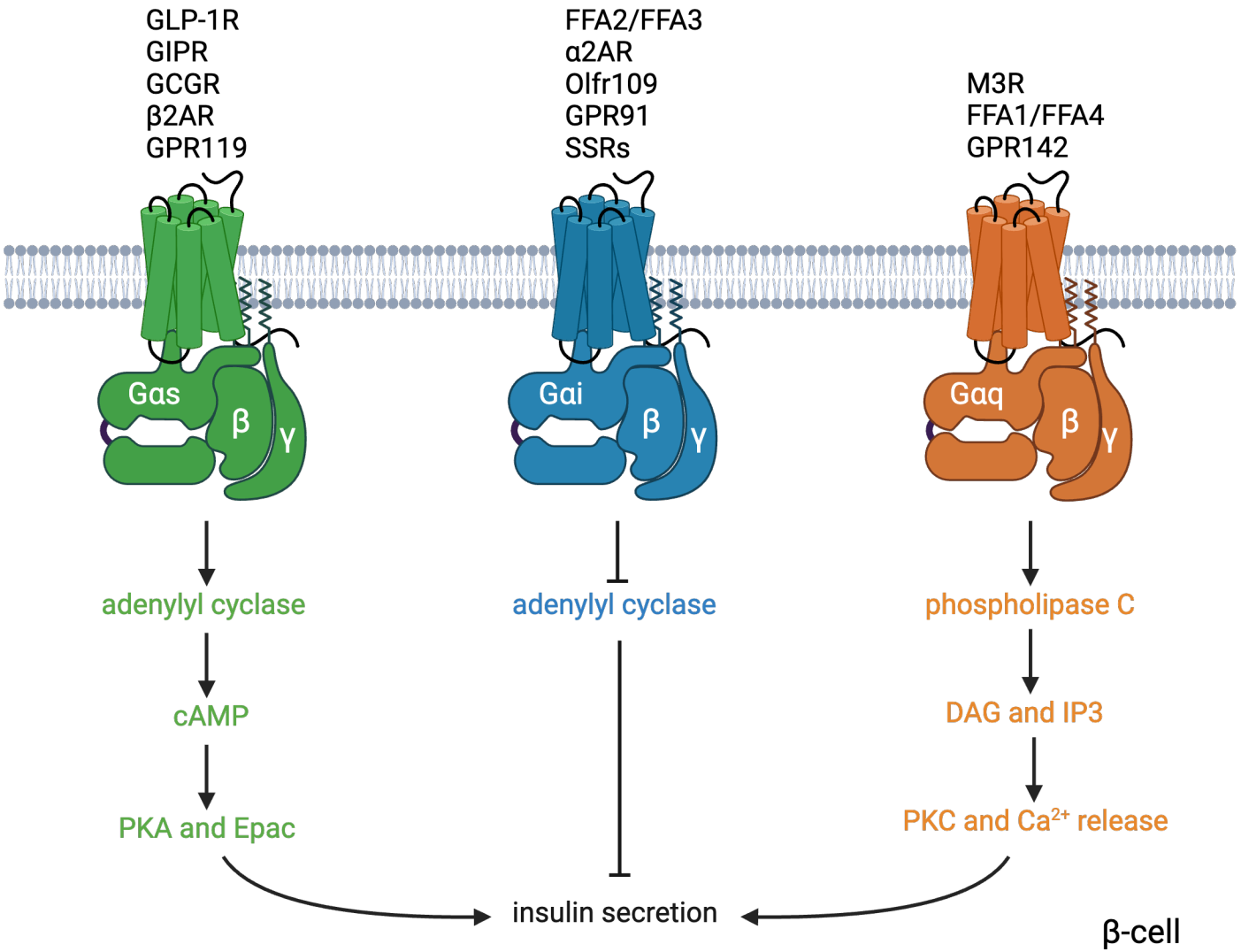

Figure 3