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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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Abstract——**Type 2 diabetes (T2D) mellitus 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. Although 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maintain normal blood glucose levels in individuals with chronic disease, many of these medicines are outdated, have severe side effects, and often become less efficacious over time, necessitating the need for insulin therapy.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 physiologic 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 insights 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 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 insights into GPCR signaling and the importance of GRK function in modulating β-cell physiology.

Significance Statement——Pancreatic β cells contain a diverse array of G protein–coupled receptors (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 GPCR kinases while also highlighting the necessity of investigating islet-enriched GPCRs that have largely been unexplored to unveil novel treatment strategies.

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 diabetes (T1D); type 2 diabetes (T2D); rare monogenic forms of diabetes, such as maturity onset diabetes of the young, 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 (Flannick et al., 2016; Riddle et al., 2020; International Diabetes Federation, 2021; Reed et al., 2021).

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 (Kahn et al., 2014; Reed et al., 2021). 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 (Johnson and Kushner, 2018; Page and Johnson, 2018; Bar-Tana, 2020). This disease is preventable in most cases, so early detection is paramount to ensure that 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 (nonalcoholic fatty liver disease, steatosis), that can result in death if left untreated (Ritz et al., 1999; Goldberg, 2001; Gross et al., 2005; Mazzone et al., 2008; Alonso-Magdalena et al., 2011; Corkey, 2012a,b; Wilding,

2014; Tilg et al., 2017; Cole and Florez, 2020; Reed et al., 2021).

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 five clusters of diabetes: severe autoimmune diabetes, severe insulin-deficient diabetes, severe insulin-resistant diabetes, mild obesity-related diabetes, and mild age-related diabetes (MARD), where severe autoimmune diabetes 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 regimens 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 precede β-cell dysfunction and T2D or β-cell hyperstimulation and hyperinsulinemia causes obesity and insulin resistance, leading to T2D. As is becoming apparent, both are likely 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 (Kahn, 2003; Weir and Bonner-Weir, 2004; Shanik et al., 2008; Corkey, 2012a,b; Esser et al., 2020; Johnson, 2021). Nevertheless, in each case, the hallmark of the disease is hyperglycemia accompanied with insulin resistance, and often obesity that is left untreated can progress into life-threatening complications.

The primary treatment of 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 (Kahn et al., 2014; Reed et al., 2021). However, there is a significant population of individuals with T2D in whom 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 Food and Drug Administration (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 noninsulin 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 (GLP-1) receptor (GLP-1R) have been shown to increase functional β-cell mass (Tamura et al., 2015; Fusco et al., 2017; 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 (Del Guerra et al., 2005; Maedler et al., 2005; Kahn et al., 2006; 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 (Drucker, 2018; Sriram and Insel, 2018). In 2022, another incretin-activated GPCR, the gastric inhibitory peptide (GIP) receptor (GIPR), was approved by the FDA for the treatment of T2D with tirzepatide, a GLP-1R/GIPR dual agonist (Chavda et al., 2022; El et al., 2023). 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 (Husted et al., 2017; Perreault et al., 2021).

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

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 (Bratanova-Tochkova et al., 2002; Henquin et al., 2002, 2006). 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 gradually rises over time in humans and rats (Curry et al., 1968; Berglund, 1980; Henquin et al., 2006). 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 physiologic range. The glucose then binds glucokinase (hexokinase IV) to generate glucose-6-phosphate (G6P) and initiates 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 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 with 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 (Wilson, 2003; Matschinsky and Wilson, 2019). 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 (Bratanova-Tochkova et al., 2002; Henquin et al., 2002, 2006).

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 (Bratanova-Tochkova et al., 2002; Henquin et al., 2002, 2006; 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 glucose-stimulated insulin secretion (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 small interfering RNA 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 (Prentki et al., 2013; Campbell and Newgard, 2021).

Multiple studies utilizing knockout of different TCA cycle, NADH shuttle, and pentose shunt pathway enzymes impair GSIS, helping to demonstrate the importance of glucose metabolism and its connection to insulin secretion independent of the K_{ATP} channel (Goehring et al., 2011; Spégel et al., 2013; Zhang et al., 2021a). 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 (Zhao et al., 2014; Ferdaoussi et al., 2015; 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
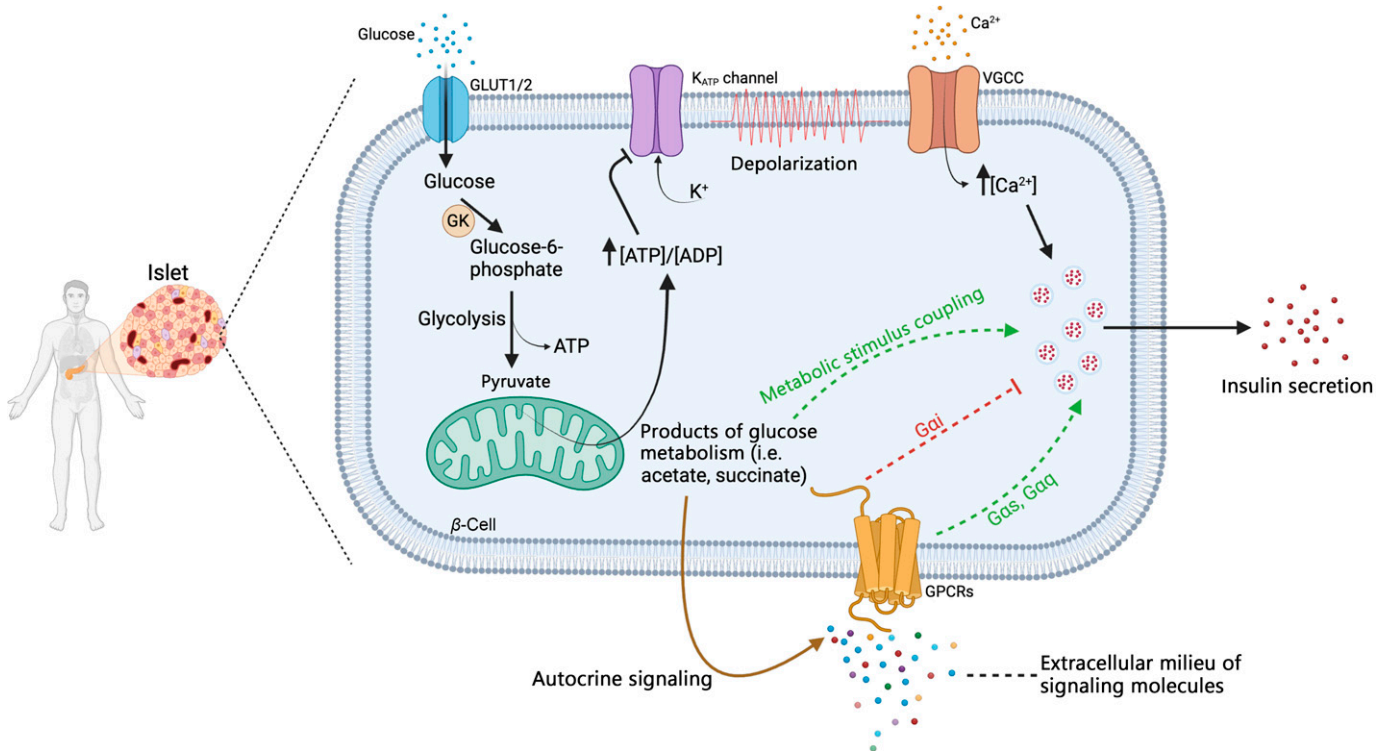
Fig. 1. Insulin secretion in the β cell. The pancreas is an organ in the abdomen, located behind the stomach in humans. It is composed of two major parts: the exocrine and endocrine pancreas. The exocrine pancreas releases enzymes that aid in the digestion of food and makes up most of the pancreas. The 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–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, long-chain fatty acids, 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 represent the amplifying pathway of insulin secretion. Created with BioRender.com

β-cell–expressed GPCRs that transmit the extracellular milieu of small molecules and peptide hormones to an intracellular secretory response (Oh and Olefsky, 2016; Husted et al., 2017; Riddy et al., 2018; Oliveira de Souza et al., 2021).

II. G Protein–Coupled Receptor Signaling

A. Overview of G Protein–Coupled Receptors

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 mediated in part by the large N-terminus of these GPCRs (Hollenstein et al., 2014; Congreve et al., 2020). Class C GPCRs (glutamate receptor–like) include 22 members and have the unique requirement for dimerization to initiate signaling (El Moustaine et al., 2012; Levitz et al., 2016). 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 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. G Protein–Coupled Receptor 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 (TMs; 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 (Siu et al., 2013; Manglik et al., 2015; Wingler and Lefkowitz, 2020; 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 (Lagerström and Schiöth, 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 Gβγ subunits then dissociate from the activated receptor and interact with effector molecules to mediate changes in intracellular signaling. Gα subunits regulate adenylyl cyclase, phospholipase C (PLC)-β, and RhoGEFs, whereas Gβγ subunits can regulate various effectors, including G protein–gated inwardly rectifying potassium channels (GIRKs) and phosphoinositide 3-kinase (PI3K)-γ.

The heterotrimeric G proteins consist of Gα, Gβ, and Gγ subunits. There are 16 Gα, five Gβ, and 14 Gγ subunits. In the cell, Gβ and Gγ subunits are constitutively associated as a Gβγ 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 protein kinase A (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 $G\alpha$ subunits have an intrinsic GTPase activity, allowing them to hydrolyze GTP to GDP inactivating themselves. For some $G\alpha$ subunits, the hydrolysis of GTP is accelerated by regulator of G protein signaling (RGS) proteins that can bind $G\alpha$ proteins and stimulate GTPase activity (Lambert et al., 2010). Additionally, mutant forms of $G\alpha$ 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 G Protein–Coupled Receptor Kinases 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 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 (also known as arrestin-1 and arrestin-4, respectively) are found exclusively in the visual system within the retina, whereas the two nonvisual 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 toward degradation or recycling pathways. Degradation mechanisms include β-arrestin–mediated ubiquitination of the receptor designated for lysosomal degradation, whereas 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; Reiter and Lefkowitz, 2006; Gurevich and Gurevich, 2019).

III. G Protein–Coupled Receptors Regulating β-Cell Insulin Dynamics

A. The Incretin Effect: GLP-1 Receptor and Gastric Inhibitory Peptide Receptor

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, GLP-1 and 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) (Drucker, 2006; Nauck and Meier, 2018; Boer and Holst, 2020).

B. Glucagon-Like Peptide-1 Receptor

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 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 (Light et al., 2002; Tang et al., 2017). 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 endoplasmic reticulum 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 et al., 1999; Tsuboi et al., 2003; Holz, 2004). 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 (Deriy et al., 2009; Leech et al., 2010). 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 that the islet has sufficient β-cell mass and insulin secretory capacity, both of which are lost in T2D.

C. Gastric Inhibitory Peptide Receptor

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 triagonist 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., 2019a). 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 (Nauck and Meier, 2018; Boer and Holst, 2020).

The signaling mechanisms by which GIPR enhances insulin secretion, other than increased cAMP, are poorly understood. Some studies have shown that 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 (WT) 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 antiapoptotic 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. Glucagon Receptor

The proglucagon proprotein is well conserved across species and gives rise to various metabolic hormones often referred to as proglucagon-derived peptides. In the pancreatic α cells, proglucagon processing by prohormone-converting enzyme (PC)-2 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, which, 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 (Vergari et al., 2019; El et al., 2021).

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 (Xu and Xie, 2009; Janah et al., 2019).

The prevailing dogma is that insulin decreases glucose levels in a fed state and that glucagon increases glucose levels in a fasted state. Although 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 antihypoglycemic effects mediated by the liver. Mice overexpressing the GCGR on β cells treated with glucagon exhibited a fourfold 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., 2019b). 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 proglucagon-derived peptides (Campbell and Drucker, 2015; Capozzi et al., 2019a). 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 triagonists/antagonist treatment regimens 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 due in part 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, whereas antisense oligonucleotides against the FFA1 reduced linoleic acid induced insulin secretion by 50% (Salehi et al., 2005; Briscoe et al., 2006). Additionally, FFA1 knockout mice displayed fasting hyperglycemia, glucose intolerance, and insulin resistance compared with 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 increases in GSIS (Steneberg et al., 2005). Furthermore, pharmacologic and genetic inhibition of FFA1 in vitro abrogates FFA-mediated potentiation of GSIS (Itoh et al., 2003; Salehi et al., 2005; Briscoe et al., 2006). 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 endoplasmic reticulum 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, whereas 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). Although TAK-875 progressed to phase 3 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. 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). Tang et al. (2015) 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 that 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 that 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 versus FFA3 regulate this coupling in human versus mouse islets remains obscure. Based on the available data, FFA2 antagonism in humans is likely the best approach to treating T2D.

G. Muscarinic Receptor Subtype 3

It has been known that parasympathetic cholinergic input can increase insulin secretion. This effect is mediated by the muscarinic receptor agonist acetylcholine, which activates all muscarinic receptor subtypes (Gilon and Henquin, 2001). Of the five 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).

Although 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, whereas 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 (ARs). Although 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 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; $α_1$ ARs include $α_{1A}$, $α_{1B}$, and $α_{1C}$ subtypes that activate G_q and PLCβ to regulate intracellular Ca^{2+}; and $α_2$ARs 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 β2-adrenergic receptors ($β_2$ARs).

Early studies implicating a role for βARs in regulating blood glucose found that $β_2$AR-selective agonists were able to stimulate insulin secretion in isolated human pancreatic islets, although not in rat islets (Lacey et al., 1990). The $β_2$AR 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 $β_2$ AR was expressed in islets and that the expression decreased with age, resulting in impaired insulin secretion, whereas studies in $β_2AR^{-/-}$ mice found impaired glucose-promoted insulin secretion (Santulli et al., 2012). Interestingly, pancreas-specific deletion of the $β_2$AR 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). Although 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 G Protein–Coupled Receptors 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. Although 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, whereas G_i signaling serves to inhibit these processes.

IV. Orphan Receptors and Other G Protein–Coupled Receptors

There are over 300 known GPCRs that are expressed in mouse and human pancreatic islets (Amisten et al., 2017a). Although 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 (Soga et al., 2005; Ning et al., 2008; Godlewski et al., 2009). One of the first studies showed that the GPR119 agonist AR231453 was able to increase insulin secretion in rodent islets, an effect 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, Calcium-Sensing Receptor, 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 (Oh and Olefsky, 2016; Husted et al., 2017; Riddy

et al., 2018). 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 as 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 (Li et al., 2020; Trauelsen et al., 2021). 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 (Squires et al., 2014; Oh et al., 2016; Wang et al., 2016; Husted et al., 2017).

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 and 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.

V. G Protein–Coupled Receptor Kinase Function in the β Cell

A. G Protein–Coupled Receptor Kinase Family

The GRKs are a family of seven serine/threonine protein kinases that phosphorylate agonist-activated GPCRs (Gainetdinov et al., 2004; Gurevich et al., 2012; Komolov and Benovic, 2018). 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 (Kühn, 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 $β_2AR$ (Benovic et al., 1987, 1986). 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 RGS 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
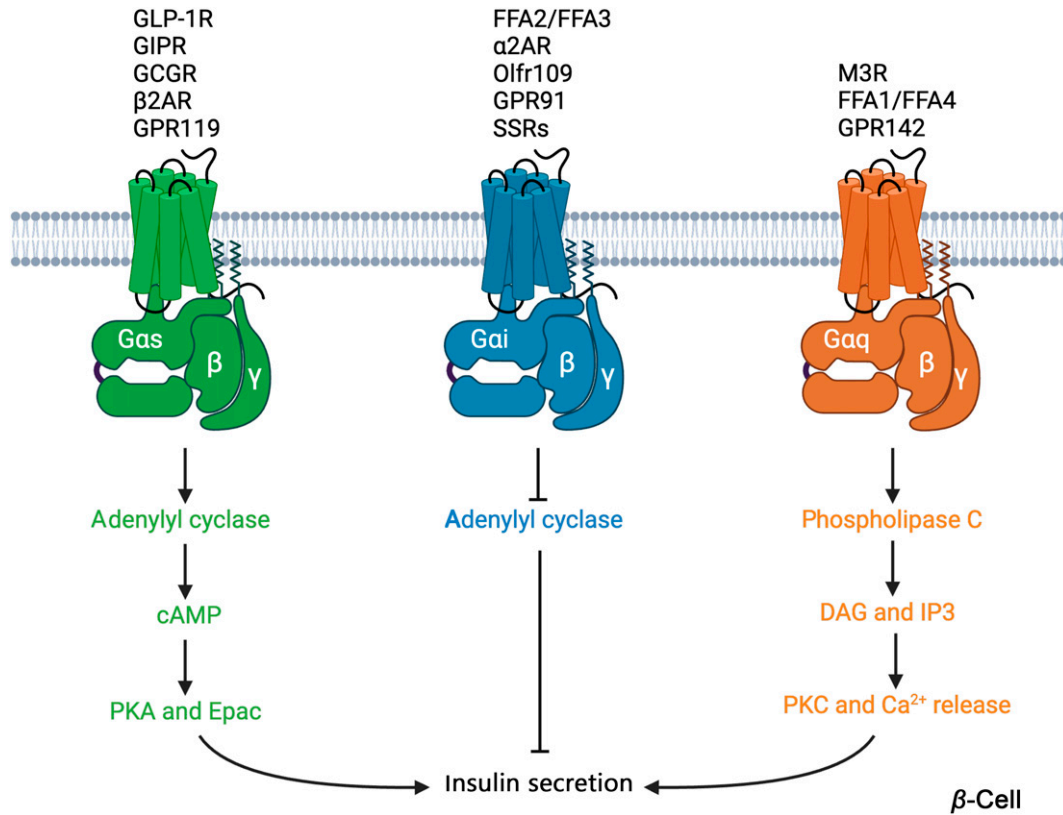
Fig. 2. GPCR-mediated activation and inhibition of insulin secretion in pancreatic β cells. There are many GPCRs resident on β cells. Typically, GPCRs that couple to G_s and G_q stimulate insulin secretion through adenylyl cyclase and phospholipase Cβ, respectively. GPCRs that are G_i coupled inhibit 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

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). Although 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 (Liggett, 2011; Drube et al., 2022; Kawakami et al., 2022).

B. G Protein–Coupled Receptor Kinases Implicated in Diabetes

GRKs are known for their role in regulating GPCR signaling throughout the body, thereby modulating cellular changes in virtually all physiologic systems. GRK2, GRK3, GRK5, and GRK6 are expressed in numerous tissues and have been implicated in several diseases, including neurologic disorders (Alzheimer and Parkinson), cardiovascular disease (hypertrophy and heart failure), and metabolic diseases, including insulin resistance and T2D (Suo et al., 2004, 2007;

Ahmed et al., 2015; Murga et al., 2019; Pfleger et al., 2019). For example, insulin-induced GLUT4 translocation is dependent upon insulin receptor signaling that leads to the activation of 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 like the insulin receptor to use heterotrimeric G proteins typically involved in GPCR signaling (Imamura et al., 1999a,b; 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 small interfering RNA 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 were 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 used a genetic approach where a genome-wide association studies analysis revealed that the single nucleotide polymorphism 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 do not 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 nondiabetic (ND) individuals. The mRNA expression of GRK5 was higher in all patients with the allele and 40% more in T2D cases compared with nondiabetic 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, Homeostatic Model Assessment for 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 is also interesting that alleles of the rs10886471 SNP were associated with increased T2D risk (Xia et al., 2014), whereas 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). Although 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. G Protein–Coupled Receptor Kinase Regulation of β-Cell G Protein–Coupled Receptors

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 (Sonoda et al., 2008; Kong et al., 2010; 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 use 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.

Although 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 the increased size of the readily releasable pool of insulin granules in these studies. Further analysis showed that this effect was mediated in part 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, reinforcing 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. (2021) and Lee et al. (2021) supported whole-animal data with insulin secretion studies in isolated islets, whereas

Snyder et al. (2021) did not use isolated islets but did have targeted deletion of GRK2 in the pancreas. Furthermore, Snyder et al. (2021) used only glucose stimulation, whereas Arcones et al. (2021) and Lee et al. (2021) used glucose with GLP-1R activation. 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 versus 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 (Hamamdzic et al., 1995; Diviani et al., 1996; Eckhart et al., 2000). 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 provides evidence for an important role of GRK5 in regulating insulin secretion.

D. Novel Roles of G Protein–Coupled Receptor Kinases 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 that 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). Although 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 (Willets et al., 2002; Nobles et al., 2011; Burns et al., 2014). Moreover, GRK6 regulation of oxidative stress in hematopoietic stem cells 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. (2022) identified a heterozygous missense mutation in *GRK6* using whole-exome sequencing in two related patients with early onset T2D. 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. 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, whereas 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 with 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 (Nagamatsu et al., 1987; Schuppin and Rhodes, 1996; Skelly et al., 1996). Some studies have shown that the rate of proinsulin-to-insulin conversion is also increased by glucose (Nagamatsu and Grodsky., 1987, 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 (Davidson et al., 1988; Zhou and Lindberg, 1993; Martin et al., 1994; Alarcon et al., 2006; Stiernet et al., 2006; Wang et al., 2014). 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 (Oliveira 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 physiologic and pathophysiological conditions (Zhu et al., 2017a), whereas 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.

VI. The Human Islet G Protein–Coupled Receptor Transcriptome

A. Islet Enriched G Protein–Coupled Receptor 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
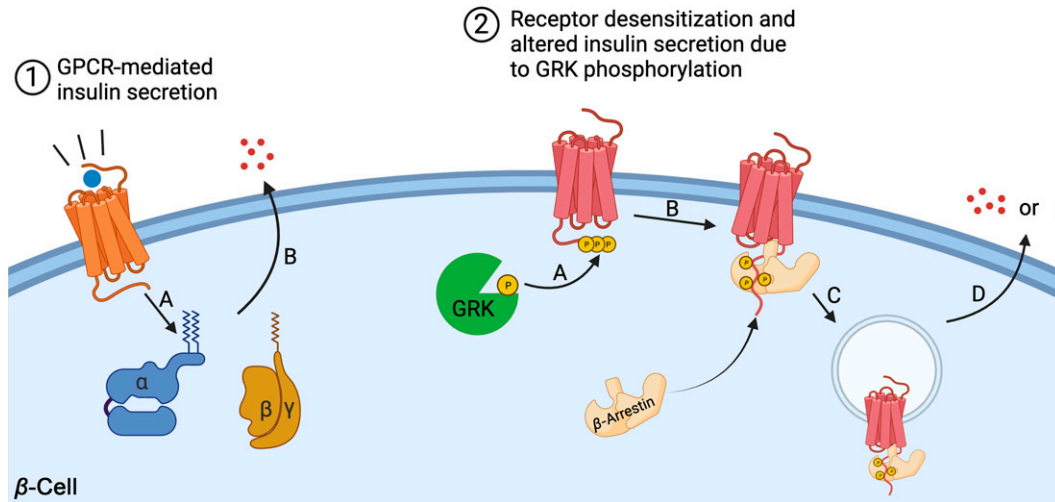
Fig. 3. Changes in GPCR-mediated insulin secretion regulated by GRKs. The β cell contains multiple GPCRs capable of increasing or decreasing insulin secretion based on their G protein coupling. In part 1, a ligand-bound receptor is activated and stimulates the dissociation and activity of the heterotrimeric G proteins (A). The Gα subunits then control the release of insulin through various downstream pathways described previously, where they either increase (Gα_s, Gα_q) or decrease (Gα_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

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 use 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 et al. (2013) performed a transcriptomic analysis on human islets from nondiabetic, middle-aged, nonobese donors. To accomplish this, they isolated the islets from human pancreatic samples, extracted the RNA, and performed quantitative polymerase chain reaction 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 nonodorant 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*, which 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 five, emphasizing the fact that higher mRNA expression does not necessarily correlate to more prominent function within the islets (Amisten 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 toward 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 is 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 α-latrotoxin from the black widow spider (Krasnoperov et al., 1997). These receptors are involved in brain function and embryonic development (Müller et al., 2015; Scholz 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 (Lelianova et al., 1997; Müller et al., 2015; Nazarko et al., 2018). However, some studies showed that α-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 plays 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 nonadhesion 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 coreleased 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 physiologic 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 transretinoic 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 (Storto et al., 2006; Soni et al., 2013). 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 in insulin secretion. Moreover, glutamate

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[a]	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 embryonic development	Unknown	Muller et al., 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[b]	GPRC5B	C	G_i?	Glutamate; involved in cytokine-induced apoptosis	Decrease	Soni et al., 2013
GPRC5C[a]	GPRC5C	C	G_s?	Transretinoic acid (vitamin C); prevents cytokine-induced apoptosis	Increase	Amisten et al., 2017b
HT1RF	5-HT1F	A	Unknown	Serotonin; inhibits glucagon release in α cells	Decrease (paracrine-signaling mechanism)	Almaca et al., 2016
CCR9[c]	CCR9	A	G_i	Chemokine receptor; involved in inflammation	Decrease	Atanes et al., 2020
HTR2B[c]	5-HT2B	A	G_q	Serotonin; increased α cell proliferation	Increase	Bennet et al., 2016
GPR156[c]	GPR156	C	G_i	Hair cell orientation, cell polarity	Unknown	Kindt et al., 2021
GPR39[c]	GPR39	A	G_s, G_q	Zinc-sensing receptor	Increase	Holst et al., 2009; Laitakari et al., 2021

[a]Downregulated in T2D.
[b]Upregulated in T2D.
[c]Upregulated in obesity.

treatment of GPRC5B knockdown islets secreted more insulin and were protected against cytokine-induced apoptosis compared with 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 (Storto et al., 2006; Soni et al., 2013).

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 with 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 transretinoic acid as both insulin secretion and cAMP levels were reduced in transretinoic 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. Although 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 a high-fat diet (Ohara-Imaizumi et al., 2013; Kim et al., 2015; Bennet et al., 2016). More recently, a study revealed that increased serotonin released under high-glucose conditions acts in a paracrine manner to inhibit glucagon releasee from α cells in isolated human islets (Almaca et al., 2016). Additionally, activation of 5-HT1F in mouse models after intravenous 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 contributing to hyperglucagonemia in T2D.

B. Changes in Human Islet G Protein–Coupled Receptor Gene Expression Due to Type 2 Diabetes 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 RNA-sequencing (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. (2016) performed whole-islet RNAseq and single cell RNA sequencing (scRNAseq) on islets isolated from nondiabetic and diabetic donors to determine changes in gene expression in α, β, δ, and pancreatic polypeptide cells. 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 that 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 nonobese 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 (Ohara-Imaizumi et al., 2013; Kim et al., 2015; Bennet et al., 2016). 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 of the top 10 most upregulated GPCR genes 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 (Soni et al., 2013; Kindt et al., 2021).

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. (2022) performed an analysis where they separated differentially expressed GPCR genes by their known G protein coupling, not including receptors that bind multiple G proteins. They found that genes for G_i-coupled receptors were the most enhanced in obese subjects compared with 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. (2022) 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*. 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 et al., 2009). Together, these data suggest that GPR39 could be activated in an autocrine manner to reinforce insulin secretion since zinc will be coreleased 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 Versus Human Islet G Protein–Coupled Receptor 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; Xin et al., 2016; Atanes et al., 2021; Lyu et al., 2022). Although informative, changes in gene expression do not always correlate with analogous changes in protein expression, and it is 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. (2017a) performed a comparative analysis looking at

GPCR gene expression in islets isolated from commonly used mouse strains as well as human islets.

They found that only three 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 United States. 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. (2017a) revealed that for the SST1 receptor, only human islets were sensitive to inhibition of insulin secretion by SST1 agonists. 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 with 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 used 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 shows that 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, scRNA-seq 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 with 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. Single Cell RNA Sequencing 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 quantitative polymerase chain reaction and next-generation sequencing 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 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 (Dorrell et al., 2016; Aguayo-Mazzucato et al., 2017; Benninger and Kravets, 2022; Chen et al., 2022; Gottmann et al., 2022; Shrestha et al., 2022; Vivoli et al., 2023). 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 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 (Haque et al., 2017; Baysoy et al., 2023). For example, insulin mRNA expression is used to identify β cells, whereas 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 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, 2017a; Atanes et al., 2021; Lyu et al., 2022). To date, these focused analyses do not 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 versus T2D) or enriched specifically in β cells (Elgamal et al., 2023; Hrovatin et al., 2023; Yang et al., 2023).

A recent report by Elgamal et al. (2023) took advantage of the Human Pancreas Analysis Consortium. The Human Pancreas Analysis Consortium 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. These data are publicly available via the web portal PANC-DB (Kaestner et al., 2019; Shapira et al., 2022). Elgamal et al. (2023) 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 and 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 differential gene expressions under certain conditions as well as identity marker genes that are specific to a cell type.

Gene set enrichment analysis 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 (TSH) receptor (TSHR) that was increased approximately fourfold (Elgamal et al., 2023). This gene was also upregulated fourfold in an scRNAseq study done in T2D mouse models (Hrovatin et al., 2023). The TSHR is a class A GPCR that is activated by the glycoprotein 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 central nervous system and metabolism (Kleinau et al., 2017; Vieira et al., 2022). 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 et al. (2023) 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). 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, stimulate 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 (Gerdes et al., 2014;

Cho et al., 2022; Li et al., 2022). 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 Patched (PTCH), located at the base of cilia. The PTCH ligand, sonic hedgehog (SHH), binds to PTCH, repressing inhibition of Smoothened (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 that this pathway may have an important role in β cells (Gerdes et al., 2014; Cho et al., 2022; Li et al., 2022; 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 activates β-catenin and transcriptional programs that control similar processes as hedgehog signaling, including embryonic development, cell polarity, and cell proliferation (May-Simera and Kelley, 2012; Wheway et al., 2018). There is evidence that the WNT and hedgehog signaling pathways undergo crosstalk 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 plays 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 central nervous, endocrine, and cardiovascular systems and can stimulate adenylyl cyclase activity 1000 times greater than VIP through its ability to activate the PACAP GPCR, PAC1 (Miyata et al., 1990; Koves et al., 2020). In mouse β cells, PACAP overexpression enhances insulin release (Yamamoto et al., 2003). Additionally, PACAP-38, the mature form of the PACAP proprotein, 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 (Leech et al., 1996; Liu et al., 2019). 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 (Sekar et al., 2017; Sureshkumar et al., 2021). It is worth noting that there was a discrepancy in the mRNA and protein expression, with *ADCYAP1* mRNA specifically enriched in β cells, whereas PACAP protein was detected in δ cells via immunohistochemistry (Elgamal et al., 2023; Yang et al., 2023). It is therefore essential that transcriptomic data are validated when feasible at the protein level.

One of the major pitfalls of scRNAseq is that although there is now a wealth of scRNAseq datasets, they often do not overlap and have different gene expression profiles complicating data interpretation and conclusions (Chen et al., 2019; Elgamal et al., 2023; Hrovatin et al., 2023; Yang et al., 2023). 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 are not detected at all (Fredriksson and Schiöth, 2005; Qiu, 2020). Information is lost in a computational analysis if transcription of a gene is low or is not 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 (Elgamal et al., 2023). For example, transcriptomic profiles will differ in islets from a cadaveric donor versus 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 that 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, mild age-related diabetes, and severe insulin-deficient diabetes (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 versus T2D transcriptomic profiles (Elgamal 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. (2023) 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, to help compartmentalize scRNAseq data. 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. Although we focused on GPCR genes that were highly expressed in islets, differentially expressed during metabolic stress, and conserved among humans and mice, functional data are 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, are 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.

VII. 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 classic 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

This is a review and does not contain any data.

Authorship Contributions

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

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