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An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans

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ABSTRACT

G-protein coupled receptors (GPCRs) regulate hormone secretion from islets of Langerhans, and recently developed therapies for type-2 diabetes target islet GLP-1 receptors. However, the total number of GPCRs expressed by human islets, as well as their function and interactions with drugs, is poorly understood. In this review we have constructed an atlas of all GPCRs expressed by human islets: the 'islet GPCRome'. We have used this atlas to describe how islet GPCRs interact with their endogenous ligands, regulate islet hormone secretion, and interact with drugs known to target GPCRs, with a focus on drug/receptor interactions that may affect insulin secretion. The islet GPCRome consists of 293 GPCRs, a majority of which have unknown effects on insulin, glucagon and somatostatin secretion. The islet GPCRs are activated by 271 different endogenous ligands, at least 131 of which are present in islet cells. A large signalling redundancy was also found, with 119 ligands activating more than one islet receptor. Islet GPCRs are also the targets of a large number of clinically used drugs, and based on their coupling characteristics and effects on receptor signalling we identified 107 drugs predicted to stimulate and 184 drugs predicted to inhibit insulin secretion. The islet GPCRome highlights knowledge gaps in the current understanding of islet GPCR function, and identifies GPCR/ligand/drug interactions that might affect insulin secretion, which are important for understanding the metabolic side effects of drugs. This approach may aid in the design of new safer therapeutic agents with fewer detrimental effects on islet hormone secretion.

Contents

1. Introduction

1.1. Role of islet hormones in maintaining fuel homeostasis

Islets of Langerhans are clusters of endocrine cells distributed throughout the pancreas that account for 1–2% of the pancreas mass. Islets from all mammalian species are similar in size and contain approximately 1000 endocrine cells. The insulin-secreting β-cell comprises 80–90% of all cells in rodent islets but only 50–60% of the human islet (Cabrera et al., 2006; Kim et al., 2009). Islets also contain glucagon-secreting α-cells and somatostatin-secreting δ-cells, and the balance of islet hormone secretion is critically important in the maintenance of fuel homeostasis. Thus, elevations in circulating glucose concentrations are sensed by β-cells through GLUT1/2 transporters that allow glucose to enter β-cells, where it is metabolised to stimulate insulin secretion when the glucose concentration exceeds 3 mM (in man) and 5 mM (mice). Insulin circulates in the blood and acts at skeletal

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myocytes and adipocytes to facilitate glucose uptake via membrane insertion of the insulin-sensitive glucose transporter GLUT4, and it also stimulates fuel storage in liver, fat and skeletal muscle. When glucose levels fall in the post-absorptive state glucagon is secreted from islet α-cells and this counter-regulatory hormone restores blood glucose concentrations by stimulating glycogenolysis, gluconeogenesis and lipolysis. Like insulin, somatostatin secretion also increases in response to elevations in glucose and this islet hormone can act in a paracrine manner at β-cells to inhibit insulin release, thus adding a level of fine tuning to minimise the possibility of hypoglycaemia. Collectively, the reciprocal stimulation and inhibition of insulin/somatostatin and glucagon maintains plasma glucose levels within the healthy range of 3.5 mM (during fasting) and 7 mM (post-prandially).

Islets are highly innervated, obtaining input from parasympathetic and sympathetic neurotransmitters and biologically active neuropeptides, and they also have a robust vascular supply that allows circulating agents to regulate islet secretory function (Cabrera et al., 2006; Kim et al., 2009). This permits close regulation of islet hormone secretion, with neurotransmitters such as noradrenaline inhibiting insulin secretion and stimulating glucagon release to increase glucose availability during times of stress. In addition, the incretin GLP-1, released from gastrointestinal L-cells in response to food intake, reaches islet capillary beds via the circulation and reduces blood glucose concentrations through stimulation of insulin secretion and inhibition of glucagon release.

1.2. Human islet G-protein coupled receptors

A diverse range of neurotransmitters, neuropeptides and blood-borne regulatory agents exert their effects on islet cells by binding to specific cell surface receptors, the majority of which are seven transmembrane spanning G-protein coupled receptors (GPCRs) that sense changes in the local cellular environment. The roles of some islet GPCRs, such as those responding to the neurotransmitters acetylcholine and noradrenaline with stimulation and inhibition of insulin secretion, respectively, or to the incretin hormones GLP-1 and GIP with potentiation of glucose-stimulated insulin secretion, are well known (Cataland et al., 1974; Kreymann et al., 1987; Ahren, 2000). However, the roles of many other islet GPCRs are poorly understood and very little is known about islet GPCR expression or how the integrated signalling via these GPCRs contributes to deliver the final fine tuning of islet hormone secretion. Moreover, a majority of studies are focused on a limited population of well-known GPCRs, with functional studies carried out predominantly using rodent islets or rodent islet cell lines, which may not always be translatable to human physiology. This review focuses on the expression and function of all known non-olfactory GPCRs in human islets of Langerhans.

1.3. Human islet G-protein coupled receptor signalling pathways

The human GPCR superfamily is the largest membrane receptor family in the human genome (Fredriksson et al., 2003). GPCRs regulate a large number of functions in the human body, and they are also important targets of modern medicines. Signalling downstream of GPCR activation following ligand binding is complex, and the net signalling output from individual GPCRs is determined by several different factors such as presence and nature of agonists, constitutive signalling and receptor internalisation, receptor heteromerisation and availability of guanine nucleotide-binding proteins (G-proteins) (Baker & Hill, 2007). GPCR signalling pathways in islet cells, as in all other cell types, involve the interaction of ligand-bound GPCRs with G-proteins, which results in the enzymatic regulation of second messenger generation. The nature of the second messenger signalling depends on which type of G-protein is activated by the GPCR. The major G-protein-mediated signalling pathways are transduced through GPCR interactions with the α subunits of Gs (activates adenylate cyclase to stimulate cAMP production), Gi/Go (inhibits adenylate cyclase to decrease cAMP production), Gq/G11 (activates phospholipase C to generate diacylglycerol and inositol 1,4,5 trisphosphate, which mobilises intracellular Ca^{2+}) and G12/13 (activates the small GTPase Rho to regulate actin cytoskeleton remodelling). GPCRs can also signal via the modulation of ion channel activity, and many Gi/Go coupled GPCRs elevate Ca^{2+} by activating Ca^{2+} flux channels (Billington & Penn, 2003), or by direct modulation of exocytosis (Rosengren et al., 2010). GPCR signalling is very complex, and often involves the simultaneous activation of several second messenger systems (Gs and Gq/G11, Gq/G11 and G12/13 or Gi/Go and Gq/G11), with the net effect of activation or inhibition of cAMP production and simultaneous mobilisation of Ca^{2+} and/or effects on the actin cytoskeleton. In addition, some GPCRs signal via other pathways, such as Wnt signalling by Frizzled receptors (Komiya & Habas, 2008).

1.4. Definition of the human G-protein coupled receptor repertoire and quantification of human islet G-protein coupled receptor expression

In this review data from the IUPHAR GPCR database (Sharman et al., 2011), GeneCards.org, ingenuity.com and PubMed.gov have been used to define an up-to-date functional human GPCRome. The large olfactory receptor subfamily of GPCRs, as well as vomeronasal and pseudogene GPCRs, were not included in this study. Although we could detect human islet mRNAs encoding the receptors BAI1, GPR137, P2RY11 and SSTR5 using non-quantitative PCR, we were not able to identify any qPCR primers suitable for the relative quantification of expression of these receptors (as indicated in Fig. 3). In addition, mRNAs encoding the hydroxycarboxylic acid GPCRs HCAR2 and HCAR3 were not quantified due to the extreme sequence homology between the two genes, which makes it impossible to determine the expression of each of the individual receptors.

Most GPCRs are expressed at relatively low levels, making accurate expression profiling using microarrays challenging (Nagalakshmi et al., 2008), so alternative technologies including quantitative RT-PCR (qPCR) and RNA-sequencing are often used. The relative expression of GPCR mRNAs in mouse islets has been profiled previously by qPCR (Regard et al., 2007, 2008) and we have now used a similar approach to create this atlas of GPCR mRNA expression by human islets. Quantification of the 384 non-odorant human GPCRs currently known was performed using Qiagen's QuantiTect qPCR primers and QuantiFast kits as described elsewhere (Amisten, 2012) with cDNA templates obtained from human islets isolated at the Islet Transplantation Units at King's College London and at the Oxford Centre for Diabetes, Endocrinology and Metabolism. All islet donors were non-diabetic (50% male, 50% female), with an age range of 43–59 years (mean 49.5 ± 3.5) and BMI of 22–33 kg/m^2 (mean 28.2 ± 2.3). Human islet GPCR mRNA expression data were normalised against GAPDH mRNA expression in the same samples using the ΔΔCt method (Pfaffl, 2001). All GPCR primers were also used to quantify the GPCRomes in eight unrelated human tissues and cell lines, and the obtained expression profiles from all tissues (data not shown) were matched with published expression and functional data to minimise false negative results due to non-functional qPCR primers.

1.5. The human islet G-protein coupled receptor atlas

Of the 384 GPCRs screened, we detected and quantified 293 GPCR mRNAs in human islets by qPCR, which accounts for 76.3% of all known functional, non-odorant GPCRs. Data were manually extracted from PubMed, Ingenuity Pathways Analysis (www.ingenuity.com), the IUPHAR GPCR database (Sharman et al., 2011) and Drug-Bank (Knox et al., 2011) to identify endogenous ligands with established agonist or antagonist effects at these GPCRs. As described in Section 2, at least one endogenous ligand is known for 210 (71.7%) of the GPCRs that we detected in human islets, while 83 (28%) are classified as