Targeting islet G-protein-coupled receptors for type 2 diabetes therapy

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The majority of people with diabetes have type 2 diabetes (T2D), where hyperglycaemia occurs because the islet β-cells are unable to secrete enough insulin, usually in the context of insulin resistance that arises because of fat mass expansion. There are a range of pharmacotherapies in current use to treat T2D and pharmaceutical companies are actively engaged in the development of novel therapies for better glucose control. Ligands that target G-protein-coupled receptors (GPCRs) are obvious candidates because they are used successfully for a wide range of disorders and GLP-1 receptor agonists, which are a relatively recent class of diabetes therapy, have proved to be very effective in treating T2D. We provide here an overview of current successes, some drawbacks and future possibilities for GPCR-based T2D therapies.

Diabetes: where we were in 1921

The heartache that parents faced over a century ago when a child was diagnosed with diabetes and knowing that there was nothing they could do to halt the inexorable march towards starvation and death is almost unimaginable. However, the relief at the discovery of insulin in 1921 and its almost miraculous ability to normalize blood glucose levels following its rapid clinical introduction in 1922 is easy to appreciate. The first recipient of insulin in January 1922 was 14-year-old Leonard Thompson, who weighed only 29 kg and was close to death. This was an astonishing breakthrough that allowed him to live for another 13 years, when he succumbed to pneumonia. Leonard and his contemporaries were very lucky that they had not been born a few years earlier and they soon adjusted to injecting this life-saving hormone that allowed them to utilize and store the fuel contained within their food.

Stratification of diabetes

Diabetes was initially considered to be a single disorder where there was insufficient insulin secretion, which could be effectively treated by provision of exogenous insulin. In this respect, the very high sequence homology between human, bovine and porcine insulins meant that extracting this vital hormone from cow or pig pancreases was an efficient and effective way of commercially producing insulin for therapeutic use in diabetes. However, by the 1930s, it was apparent that some people who had overt hyperglycaemia associated with diabetes did not respond to injected insulin with the expected normalization of blood glucose levels, and the English physician Harold Himsworth was the first to stratify diabetes into subtypes that were insulin-sensitive and insulin-insensitive. His statement that "It therefore appears that in insulin-sensitive diabetics the disease is due to deficiency of insulin, whilst in insulin-insensitive patients diabetes mellitus results, not from lack of insulin, but from lack of an unknown factor which renders the body sensitive to insulin" was the first recognition that diabetes wasn't simply a consequence of insulin deficiency but that it could result from insufficient sensitivity to insulin.

We now know that the subjects in Himsworth's study who were unable to lower their glucose levels following insulin delivery were not missing a factor responsible for allowing insulin sensitivity, but that they were insulin resistant, most likely as a consequence of impaired insulin receptor signalling. In due course, the term insulin-dependent diabetes mellitus was coined for those individuals who were classified as 'insulin-sensitive' and non-insulin-dependent diabetes mellitus was used for those who were 'insulin insensitive'. It later became apparent that these terms were not sufficiently descriptive, and that β-cell autoimmune association was the defining feature of insulin-dependent diabetes. The currently used nomenclatures of type 1 (T1D) and type 2 (T2D) diabetes were therefore introduced by Andrew Cudworth in 1976, who proposed that T1D was characterized by "juvenile onset, ketosis prone, insulin-dependent diabetes, irrespective of age of onset, and

showing a significant association with the HLA system", and that T2D was "maturity onset, insulin-independent diabetes which has no association with the HLA system".

Therapies for diabetes

Once it had been established by Himsworth that diabetes was not a single entity, it became clear that what would be termed T2D required different therapeutic intervention to the insulin administration approach used to control glycaemia in T1D. Two very effective classes of oral diabetes medications were introduced in the 1950s: sulphonylureas, which directly stimulate insulin secretion, and biguanides, which enhance insulin action. These were the only therapies in clinical use until the 1990s, when α-glucosidase inhibitors, thiazolidinediones and glinides were made available and this was followed by introduction of GLP-1 receptor agonists, DPP4 inhibitors and, most recently, SGLT2 inhibitors. Table 1 summarizes the classes of medications used to treat T2D, their modes of action and disadvantages associated with their use.

G-protein-coupled receptors (GPCRs) as targets for novel diabetes therapies

GPCRs are a large family of cell surface proteins that have a common structure, consisting of seven transmembrane domains, with an extracellular N-terminal domain and intracellular C-terminal domain. Binding of an agonist to its cognate GPCR results in a conformational change that allows the activated receptor to facilitate exchange of the GDP bound to the associated heterotrimeric G-protein for GTP. This leads to dissociation of the G-protein into a separate GTP-bound Gα subunit and a Gβγ dimer. Interaction of Gα subunits with two main effectors, adenylate cyclase (AC) and phospholipase C (PLC), regulates second messenger generation, downstream signalling and cellular function (Figure 1). Thus, $G\alpha_s$ and $G\alpha_i$ activate and inhibit AC, respectively, resulting in $G\alpha_s$-mediated elevation in intracellular cyclic AMP levels and $G\alpha_i$-induced reduction in cyclic AMP. $G\alpha_q$ activation of PLC results in hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. GPCR signalling is terminated by Gα subunit GTPase activity, which hydrolyses GTP to GDP and the GDP-bound Gα subunit recombines with the Gβγ dimer.

Humans have approximately 800 GPCRs, but although GPCR ligands account for at least a third of all drugs in clinical use the number of GPCRs targeted clinically is actually rather low. Part of the reason for this is that around half of the identified GPCRs are involved in olfaction and many are orphans, for which an activating endogenous ligand has not been identified so development of therapeutic ligands for those receptors relies on protein crystallography, *in silico* modelling and ligand screening approaches. Nonetheless, the accessible extracellular ligand binding sites of GPCRs make them suitable for therapeutic exploitation and GPCR ligands are used to treat numerous conditions such as asthma, schizophrenia, hypertension and migraine. Targeting GPCRs is a relatively recent approach for T2D treatment, but the effectiveness of GLP-1 receptor agonists in maintaining normoglycaemia has led to increased interest in identifying novel GPCR candidates for T2D therapy.

GLP-1 is a peptide hormone secreted by enteroendocrine L-cells in the distal gastrointestinal tract in response to food intake. It acts at GLP-1 receptors, which are widely expressed, to exert a range of beneficial actions that reduce blood glucose levels in people with T2D (Figure 2). Its main effect is to activate islet β-cell GLP-1 receptors to potentiate glucose-stimulated insulin secretion, and this elevation in circulating insulin has glucose lowering effects through promotion of glucose uptake and storage. GLP-1 also acts at islet α-cells to inhibit glucagon secretion and thus decrease hepatic glucose output, and it also delays gastric emptying so that the rate of increase of blood glucose levels is reduced. An additional major benefit of GLP-1 is its action at GLP-1 receptors on POMC/CART neurons of the arcuate nucleus to reduce food intake. Decreased GLP-1 levels have been reported in people with T2D but its half-life of a few minutes means that the native peptide cannot be used therapeutically. Nonetheless, multiple GLP-1 receptor analogues with considerably longer half-lives have been introduced to clinical use over the past 15 years and they are effective at normalizing blood glucose levels, with the added benefit of promoting weight loss. In fact, the anorectic actions of the GLP-1 receptor agonist liraglutide have led to it being approved in 2020 by the UK as a stand-alone anti-obesity pharmacotherapy. Furthermore, the weight loss effects of liraglutide have recently led to its use in people with T1D, allowing a reduction in daily insulin requirements. The major drawback with GLP-1 receptor agonists is that since they are peptides they must be delivered by subcutaneous injection, just as insulin is. However, a version of semaglutide (Rybelsus®) has been developed containing sodium *N*-(8-[2-hydroxylbenzoyl] amino) caprylate, which enhances absorption and protects the peptide from proteolytic degradation in the stomach, allowing it to be administered orally. Rybelsus use in multiple clinical trials led to reductions in HbA1c levels and weight, with minimal side effects, and it was approved in the UK in 2020.

Diabetes

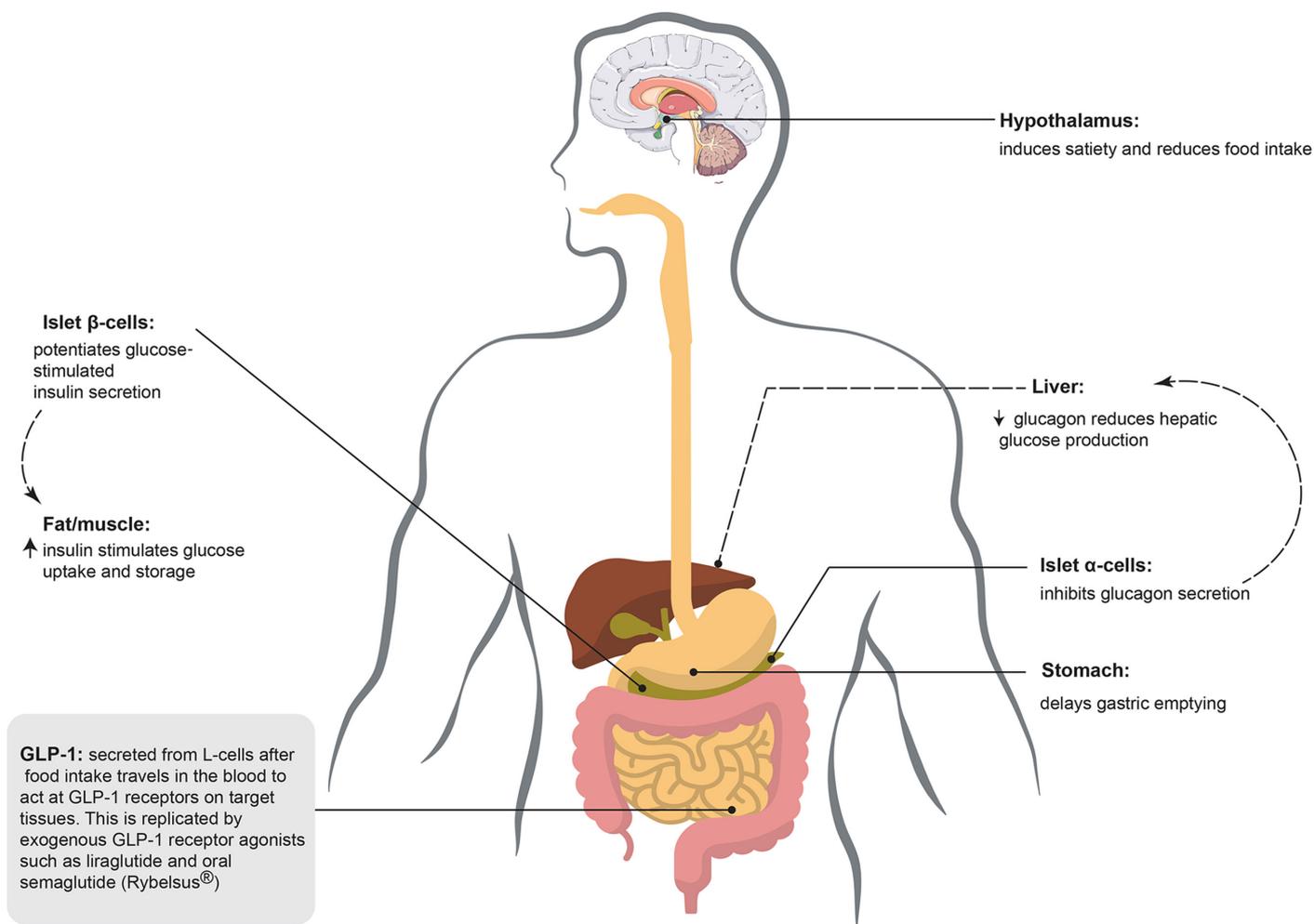**Table 1.** Drugs currently used to treat type 2 diabetes.

First clinical use	Drug family	Glucose-lowering mechanism of action	Disadvantages
1950s	Biguanides e.g., metformin (Glucophage)	Activation of liver AMP kinase to reduce expression of genes required for gluconeogenesis	Very few disadvantages but there are rare cases of lactic acidosis. Metformin is usually the initial therapy of choice
1950s	Sulphonylureas e.g., glibenclamide (Daonil)	Direct closure of β-cell ATP-sensitive potassium channels to stimulate insulin secretion	Hypoglycaemia and weight gain; the 2019 American Diabetes Association guidelines suggest that sulphonylureas should be used as a last-line T2D therapy
1990s	Glinides e.g., repaglinide (Prandin)	Direct closure of β-cell ATP-sensitive potassium channels to stimulate insulin secretion	Similar to sulphonylureas but less problematic as glinides have shorter half-lives
1990s	Thiazolidinediones e.g., pioglitazone (Actos)	Activation of nuclear PPARγ receptors, mainly in adipocytes, to stimulate anabolic gene transcription	Increased bladder cancer risk; higher risk of cardiovascular disease; weight gain
1990s	α-Glucosidase inhibitors e.g., acarbose (Glucobay)	Inhibition of enterocyte α-glucosidase to decrease oligosaccharide cleavage and thus reduce the rate of post-prandial glucose absorption	Flatulence and diarrhoea as undigested carbohydrates pass into the large intestine
2000s	GLP-1 receptor agonists e.g., liraglutide (Victoza)	Activation of β-cell GLP-1 receptors to increase cyclic AMP, activate protein kinase A and thus potentiate glucose-induced insulin secretion. Also inhibition of glucagon secretion, delayed gastric emptying and decreased appetite	Administration by subcutaneous injection (but Rybelsus® is taken orally); increased risk of gallbladder and biliary diseases
2000s	DPP4 inhibitors e.g., sitagliptin (Januvia)	Inhibition of dipeptidyl peptidase 4 inactivation of endogenous GLP-1, leading to increased insulin secretion through elevations in cAMP (as for GLP-1 receptor agonists)	Increased risk of inflammatory bowel disease and bile duct cancer; joint pain
2010s	SGLT2 inhibitors e.g., canagliflozin (Invokana)	Inhibition of kidney tubule SGLT2 transporters to block renal glucose reabsorption, leading to excess glucose excretion in the urine	Urinary tract infections; increased risk of gangrene and lower limb amputations; increased risk of ketoacidosis
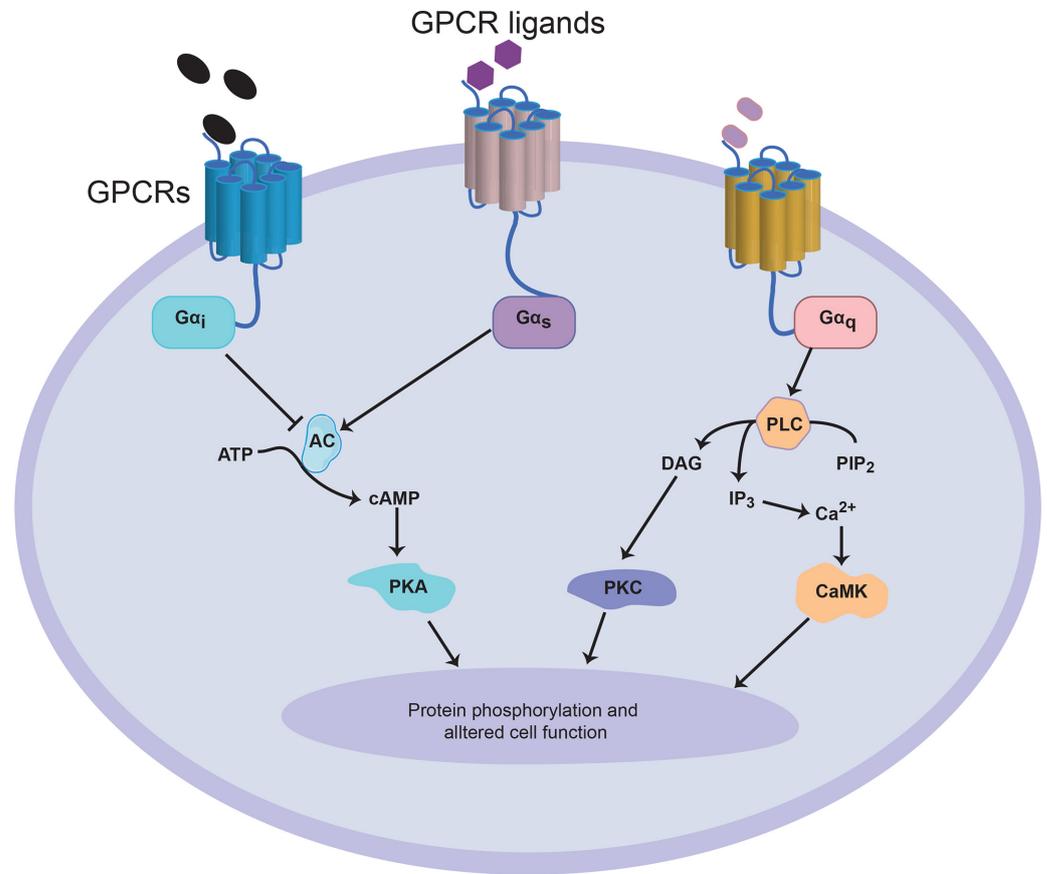
Figure 1. GPCR coupling to adenylate cyclase and phospholipase C.

There is scope for more widespread targeting of GPCRs and the success of GLP-1 receptor agonists as therapies for T2D, and the clinical introduction of an orally acting member of this class, opens up opportunities for development of novel GPCR-based diabetes therapies. A useful starting point is the islet as maintenance of β-cell mass and potentiation of glucose-induced insulin secretion are of key importance in normalizing hyperglycaemia. We have identified that human islets express approximately 300 non-olfactory GPCRs and in the past decade numerous studies have investigated islet GPCRs with therapeutic potential, with a particular focus on those coupled to $G\alpha_s$ and $G\alpha_q$. One candidate of initial interest was $G\alpha_s$-coupled GPR119, which is expressed by β-cells and GLP-1 secreting L-cells, but GPR119-selective agonists were of low efficacy in clinical trials. More recent research indicating that GPR119 agonism can preserve β-cell mass in diabetic mice and the generation of additional selective GPR119 agonists means that there may still be opportunities for using GPR119 ligands to treat T2D. The $G\alpha_q$-coupled long-chain fatty acid receptor FFAR1 is also present on β-cells and L-cells, and a range of FFAR1 ligands have been evaluated in T2D clinical trials. Thus far, adverse effects and toxicity have limited progress,

with phase III clinical trials of the potent and selective FFAR1 agonist TAK-875 curtailed because of liver safety concerns. However, modified versions of some of these ligands have been designed and their effects in diabetic mice are promising. The clear benefits of GLP-1 receptor agonists have been harnessed by combining them with other GPCR ligands in unimolecular co-agonist and tri-agonist therapies. For example, drugs that activate receptors for the other incretin, GIP, in addition to GLP-1 receptors improve glucose homeostasis and reduce weight in clinical trials, and weight loss and glucose-reducing effects of tri-agonists for GLP-1, GIP and glucagon receptors have also been identified. It may seem counter-intuitive to activate glucagon receptors to treat T2D, given glucagon's glucose-liberating action at the liver. However, this polyagonist approach harnesses the lipolytic and anorexigenic actions of glucagon receptor activation in combination with the insulin secretagogue effects of GLP-1 and GIP receptor activation, thus reducing the risk of hyperglycaemia. Simultaneous activation of distinct GPCRs by co- and tri-agonists may have additive or synergistic effects, and their evaluation in T2D animal models and clinical trials is likely to be become more prevalent in the future than use of single agonist drugs.

Hypothalamus: induces satiety and reduces food intake

Islet β-cells: potentiates glucose-stimulated insulin secretion

Fat/muscle: ↑ insulin stimulates glucose uptake and storage

Liver: ↓ glucagon reduces hepatic glucose production

Islet α-cells: inhibits glucagon secretion

Stomach: delays gastric emptying

GLP-1: secreted from L-cells after food intake travels in the blood to act at GLP-1 receptors on target tissues. This is replicated by exogenous GLP-1 receptor agonists such as liraglutide and oral semaglutide (Rybelsus®)

Figure 2. Multiple beneficial effects of GLP-1 receptor agonists on glucose homeostasis.

Diabetes: where we are in 2021

In 1921 the world population was approximately 1.9 billion and although the incidence of diabetes at that time is unknown it can be estimated at less than 20 million given that it was mainly a T1D phenotype in the largely normal weight populace of that era. Fast forward to 2021 and there are currently 7.8 billion people worldwide, of whom over 460 million have diabetes, 90% with T2D. The huge increase in T2D incidence is largely a consequence of the dramatic increase in obesity in recent decades and, given that people with T2D also develop hyperglycaemia-driven micro- and macrovascular complications, it is essential that effective T2D therapies are available to properly regulate blood glucose levels. Of particular interest are those drugs, such as liraglutide, that can induce weight loss as well as promoting β-cell function and it will be important that future GPCR therapies have multiple beneficial modes of action. For example, the use of polyagonist approaches may lead to development of unimolecular GPCR agonists that potentiate insulin secretion, increase β-cell mass, induce satiety, stimulate lipolysis and inhibit gluconeogenesis. In addition, further understanding of metabolic signalling downstream of GPCRs, using technological advances such as 'designer receptors activated by a designer drug' technology, will be important in opening up new GPCR-targeted drugs for T2D. In the meantime, the recent introduction of oral Rybelsus® is a very welcome addition to the T2D GPCR therapeutic armoury. ■

Further reading

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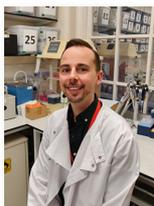
Patricio Atanes is a Diabetes UK-funded Post-Doctoral Research Associate working in the Department of Diabetes at King's College London. His research aims to elucidate novel cross-talk between metabolic tissues with islet GPCRs during obesity that may lead to development of new therapeutics for type 2 diabetes. Email: patricio.atanes_juiz@kcl.ac.uk