GLP-1 and GIP receptor signaling in beta cells – A review of receptor interactions and co-stimulation

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A B S T R A C T

Glucagon-like peptide 1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR) are two class B1 G protein-coupled receptors, which are stimulated by the gastrointestinal hormones GLP-1 and GIP, respectively. In the pancreatic beta cells, activation of both receptors lead to increased cAMP and glucose-dependent insulin secretion. Marketed GLP-1R agonists such as dulaglutide, lixibulin, etanotecil, and semaglutide constitute an expanding drug class with beneficial effects for persons suffering from type 2 diabetes and/or obesity. In recent years another drug class, the GLP-1R/GIPR co-agonists, has emerged. Especially the peptide-based, co-agonist tirzepatide is a promising candidate for a better treatment of type 2 diabetes by improving glycemic control and weight reduction. The mechanism of action for the co-agonists include biased signaling of the GLP-1R as well as potent GIPR signaling. Since the implications of co-targeting these closely related receptors concomitantly are challenging to study in vivo, the pharmacodynamic mechanisms and downstream signaling pathways of the GLP-1R/GIPR co-agonists in general, are not fully elucidated. In this review, we present the individual signaling pathways for GLP-1R and GIPR in the pancreatic beta cell with a focus on the shared signaling pathways of the two receptors and interpret the implications of GLP-1R/GIPR co-activation in the light of recent co-activating therapeutic compounds.

1. Introduction

Insulin is an essential hormone that facilitates glucose uptake in peripheral tissues, promoting blood glucose control, and basic cellular functions. Following nutrient ingestion, the postprandial insulin secretion is mainly caused by a rise in plasma glucose and a nutrient-induced secretion of the gastrointestinal hormones, glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [1,2]. GLP-1 and GIP are responsible for the incretin effect, which is the potentiation of glucose-induced insulin secretion seen when nutrients pass the gastrointestinal tract (compared to intravenous nutrient administration) [3,4]. GLP-1 and GIP exert their physiological actions via stimulation of the two G protein-coupled receptors (GPCRs): the GLP-1 receptor (GLP-1R) and the GIP receptor (GIPR), respectively. Besides being located in the pancreatic endocrine islets, the GLP-1R is expressed in the gastrointestinal tract, the cardiovascular system, brain, kidneys, and immune cells [6,7]. It consists of three domains, the extracellular N-terminus, the transmembrane core domain, and an intracellular C-terminal domain. The large N-terminus binds the C-terminus of the GLP-1 peptide, while the transmembrane domain binds the N-terminus of the GLP-1 peptide [8] (Fig. 1). The GLP-1R belongs to the class B1 GPCRs, which – in broad terms - are activated by a two-step

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docked into the transmembrane domain TMD of the receptor. This C-terminal alpha helical part of the ligand. Then the ligand N-terminus is interaction of the extra-cellular domain ECD of the receptor with the to this model, the ligand binding to the receptor is initiated by an testinal tract and the cardiovascular system), but is also distinctly satiety from receptor expressed in the central nervous system [9]. Many receptors expressed at beta cells and alpha cells, respectively, reduced result in receptor activation and downward signaling e.g. through G -enzyme dipeptidylpeptidase 4 (DPP-4).

Fig. 1. Glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), exenatide, liraglutide, semaglutide, and tirzepatide. Aminoacidic sequences with areas important for receptor activation and binding, the alpha helical segments as well as the cleavage site of the inactivating enzyme dipeptidylpeptidase 4 (DPP-4). “X” marks an α-aminoisobutyryl (Aib) linker.

 binding mechanism upon binding of their endogenous ligands. According to this model, the ligand binding to the receptor is initiated by an interaction of the extra-cellular domain ECD of the receptor with the C-terminal alpha helical part of the ligand. Then the ligand N-terminus is docked into the transmembrane domain TMD of the receptor. This results in receptor activation and downward signaling e.g. through G proteins.

The corresponding ligand, GLP-1, is secreted from enteroendocrine L cells in response to nutrient stimulation, and its activation of GLP-1Rs result in insulin secretion and inhibited glucagon secretion from receptors expressed at beta cells and alpha cells, respectively, reduced gastric emptying rate from GLP-1Rs in the ventricle and stimulation of satiety from receptor expressed in the central nervous system [9]. Many drugs targeting the GLP-1R have been developed for the treatment of type 2 diabetes and obesity such as liraglutide, semaglutide, exenatide, and dulaglutide (Table 1). Compared to endogenous GLP-1, these all have prolonged elimination half-lives and most often longer receptor residence times [10–12].

The GIPR is also expressed at the pancreatic beta cells and other overlapping tissues with the GLP-1R (the nervous system, the gastrointestinal tract and the cardiovascular system), but is also distinctly expressed in adipocytes and bone cells [13–15]. As for the interaction of GLP-1 with its receptor, GIP also use its C-terminal alpha-helix (position 6–30) to interact the N-terminal extracellular domain of the GIPR; an interaction which leads to the engagement of the N-terminus of GIP with the receptor core (Fig. 1). The GIPR then undergoes conformational changes and activation of downstream intracellular signaling [16–18]. GIP is secreted from endocrine K cells primarily located in the proximal part of the small intestine. Upon stimulation by nutrients, GIP is secreted to the blood stream and stimulates insulin secretion, lipid deposition in the adipose tissue, reduces bone resorption, increases bone formation and increases gastrointestinal blood flow [19–21].

Endogenous GIPR ligands include GIP(1-42), GIP(1-30)NH₂, GIP(3-42), and GIP(3-30)NH₂ [22] (Table 1) and currently, the only drugs marketed that affects the GIPR signaling, are the dipeptidylpeptidase-4 (DPP-4) inhibitors which inhibit the degradation of DPP-4 substrates (Fig. 1), among these GIP and GLP-1, and therefore (indirectly) result in increased GIPR activation [23,24].

Activation of both the GLP-1R and GIPR result in insulin secretion from the pancreatic beta cells, and presently, the concept of co-targeting the GLP-1R and GIPR is explored [18,25–29] and utilized in the development of improved compounds to treat type 2 diabetes [25]. The aims of this review are to describe and elaborate on the individual signaling pathways for these two receptors, evidence of signaling interactions and shared mechanisms, as well as mechanisms and implications of GLP-1R-GIPR co-activation.

2. GPCRs of the human pancreatic beta cells

GPCRs are transmembrane proteins with seven alpha-helical segments separated by alternating intracellular and extracellular loop regions. The GPCRs are allocated in five families where the GLP-1R and GIPR are found within the secretin family, also classified as class B [31,32]. Upon stimulation by an extracellular stimuli (ligand), GPCRs undergo conformational changes, and triggers downstream intracellular signals by coupling with G proteins (or other intracellular proteins such as arrestins), causing a wide range of both physiological and pathological processes [33–35]. A ligand can activate or block an agonist-induced GPCR resulting in agonism or antagonism, respectively, of the receptor signaling. A ligand can also be biased towards one signaling pathway over another relative to a reference ligand. This means that receptor activation induced by a biased ligand e.g. can result in selective G protein recruitment and reduced arrestin recruitment compared to the endogenous ligand causing a diverse cellular response [36]. This concept has been shown for several agonists targeting the GLP-1R, such as the Val8-GLP-1 [37] and several variants of exendin 4 [11,38,39]. The GPCRs are highly attractive drug targets for many human diseases [40], and drugs targeting the GLP-1R and GIPR are in clinical development or already marketed as treatments of type 2 diabetes or obesity (Table 1).

The beta cell is one of four cell types present in the islets of Langerhans, which are distributed throughout the endocrine pancreas. In response to hormones such as GLP-1 and GIP, nutrients, and neuronal stimuli the beta cells secrete insulin to maintain the plasma glucose levels in a small physiological range for optimal function of all tissues in the body [41,42]. The pancreatic beta cell is central for the physiological roles of GLP-1 and GIP and, thus, GLP-1R and GIPR signaling pathways. Glucose is the trigger of insulin secretion in beta cells, and GLP-1 and GIP both potentiate the glucose-stimulated insulin secretion via an increased cAMP concentration intracellularly resulting in an amplified insulin secretion [43]. Thus, the two agonists have small or no insulinotropic effect when blood glucose reach fasting concentrations, while they strongly potentiates insulin secretion during elevated (e.g. postprandial) blood glucose concentrations [44–46].

Several GPCRs have been identified on the surface of the human beta cell surface (Fig. 2) including GPR56, calcitonin receptor-like receptor (CALCRL), glucagon receptor, secretin receptor, GIPR, GLP-1R, alpha 2 adrenergic receptor, cholecystokinin receptor 1 (CCKR1), C-X-C motif chemokine receptor 4 (CXCR4), endothelin receptor type A, GPR4, GPR40, GPR41, GPR43, GPR55, GPR119, GPR120, growth hormone secretagogue receptor, oxytocin receptor, somatostatin receptor 1-3 [47–50]. Drugs in development that target some of these GPCRs on the beta cell are Fasiglifam/TAK-875 (GPR40 agonist), JTT-851 (GPR40 agonist), LY2881835 (GPR40 agonist), PSN821 (GPR119 agonist), MBX-2982 (GPR119 agonist), GSK1292263 (GPR119 agonist) and DS-8500a (GPR119 agonist) [51].

ADRA2A/C, alpha 2 adrenergic receptor A/C; CALCRL, calcitonin receptor-like receptor; CCKR1, cholecystokinin receptor 1; CXCR4, C-X-C motif chemokine receptor 4; ETA, endothelin receptor type A; GCCR, glucagon receptor; GHSR, growth hormone secretagogue receptor; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP-1R, glucagon-like peptide 1 receptor; GPR, G protein-coupled receptor;OXTR, oxytocin receptor; SCTR, secretin receptor; SSTR 1-3, somatostatin receptor 1-3.

3. GLP-1 receptor signaling in the human beta cell

To stimulate insulin secretion, and in the presence of elevated blood glucose concentrations, GLP-1R activation in pancreatic beta cells promote recruitment and activation of Gs protein leading to adenylyl cyclase-mediated cAMP production, elevation of Ca²⁺, and ERK1/2 phosphorylation (Fig. 3) [6,62,76–78]. cAMP production lead to activation of protein kinase A (PKA) as well as exchange protein directly activated by cAMP (EPAC) and is directly involved in increasing
Table 1
Established and future (potential) therapeutic compounds and widely used GLP-1R and GIPR ligands. Potencies and efficacies of ligands activating the glucagon-like peptide 1 receptor (GLP-1R) and/or glucose-dependent insulinotropic polypeptide receptor (GIPR) (in vitro studies). See references in the table.

| Therapeutic compounds | GLP-1R | GIPR | Status | Reference |
|-----------------------|--------|------|--------|-----------|
| Dulaglutide            | Full agonist EC₅₀ = 0.0125 nM; Emax = 100% | No activity | On the market (worldwide) | [52] |
| Lixisenatide (Lixumia, Adlyxin) | Full agonist EC₅₀ = 0.076 nM; Emax = 100% | No activity | On the market in Europe and the USA | [53] |
| Exenatide             | Full agonist EC₅₀ = 0.11 nM; Emax = 75% | No activity | On the market (worldwide) | [54] |
| Semaglutide (Ozempic, Rybelsus) | Full agonist EC₅₀ = 0.15 nM; Emax = 100% | No activity | On the market (worldwide) | [55] |
| Albiglutide           | Full agonist EC₅₀ = 0.24 nM; Emax = 100% | No activity | On the market in Europe and the USA | [56] |
| Liraglutide           | Full agonist EC₅₀ = 0.95 nM; Emax = 100% | No activity | On the market (worldwide) | [54] |
| Tirzepatide (LY3298176) | Full agonist EC₅₀ = 71.5 nM; Emax = 97% | Full agonist EC₅₀ = 0.18 nM; Emax = 101% | Ongoing clinical trials | [58] |
| Avasuclide (Exenin-9-39) | Antagonist EC₅₀ = 200 nM; Emax = 100% | No activity | Drug under development (type 2 diabetes) | [60] |
| HISHS-2001 | Partial agonist EC₅₀ = 0.1 nM; Emax = 58% | Full agonist EC₅₀ = 1.58 nM; Emax = 106% | Drug under development (type 2 diabetes) | [61] |
| MAR709 | Full agonist EC₅₀ = 0.36 nM; Emax = 100% | Full agonist EC₅₀ = 0.899 nM; Emax = 100% | Drug under development (type 2 diabetes) | [61] |

| Other ligands | GLP-1R | GIPR | Status | Reference |
|---------------|--------|------|--------|-----------|
| GLP-1(7-36)NH₂ | Full agonist EC₅₀ = 0.11 nM; Emax = 100% | No activity | Human circulating peptide | [62] |
| GLP-1(1-36) | Full agonist EC₅₀ = 0.019 nM; Emax = 100% | No activity | Human circulating peptide | [63] |
| Exendin-4 | Full agonist EC₅₀ = 0.02 nM; Emax = 87% | No activity | Ligand | [64] |
| GLP-1(7-37) | Full agonist EC₅₀ = 0.25 nM; Emax = 100% | No activity | Human circulating peptide | [65] |
| GLP-1(Gly8) | Full agonist EC₅₀ = 0.36 nM; Emax = 100% | No activity | Ligand | [65] |
| GLP-1(1-36)NH₂ | Partial agonist EC₅₀ = 158 nM; Emax = 79% | No activity | Ligand | [64] |
| GLP-1(9-36)NH₂ | Partial agonist EC₅₀ = 310 nM; Emax = 18% | No activity | Ligand | [68] |
| GIP(1-42) | No activity | Full agonist EC₅₀ = 0.006 nM; Emax = 100% | Human circulating peptide | [62,22] |
| GIP(1-30)NH₂ | No activity | Full agonist EC₅₀ = 0.01 nM; Emax = 102% | Human circulating peptide | [62,22] |
| GIP(2-30)NH₂ | No activity | Partial agonist EC₅₀ = 3.7 nM; Emax = 20% | Ligand | [22] |
| Pro3(GIP) | No activity | Partial agonist EC₅₀ = 4.7 nM; Emax = 90% | Ligand | [71] |
| GIP(3-30)NH₂ | No activity | Antagonist IC₅₀ = 10.2 nM; Emax = 100% | Human circulating peptide | [14,72] |
| GIP(3-42) | No activity | Antagonist IC₅₀ = 1000 nM; Emax = 100% | Human circulating peptide | [14] |
| Truncated GIP peptides | No activity | Agonists and antagonists | Ligands | [22] |
| CY-5 | Agonist EC₅₀ = 0.57 nM; Emax = not reported | Agonist EC₅₀ = 0.75 nM; Emax = not reported | Ligand | [73] |
| Peptide 19 | Full agonist EC₅₀ = 0.039 nM; Emax = 95.4% | Partial agonist EC₅₀ = 0.0074 nM; Emax = 66.7% | Ligand | [74] |
| DA-JC4 | Full agonist EC₅₀ = 0.07 nM; Emax = 100% | Full agonist EC₅₀ = 0.013 nM; Emax = 100% | Ligand | [75] |
| GLP-1R/GIPR/ GCGRtriagonist | Partial agonist EC₅₀ = 2.13 nM; Emax = 80% | Partial agonist EC₅₀ = 1.96 nM; Emax = 66% | Ligand | [62] |

proinsulin gene transcription and subsequent insulin secretion [79–81]. Activated PKA and EPAC enhance insulin granular exocytosis by insulin granular priming and phosphorylates sulfonylurea receptor (SUR1) K<sub>ATP</sub> channel subunit and thereby closes K<sub>ATP</sub>-channels in the plasma membrane [13,43,82], leading to membrane depolarization, and opening of the voltage gated Ca<sup>2+</sup>-channels. This leads to an increased influx of extracellular Ca<sup>2+</sup>, which triggers fusion of intracellular insulin-containing granules with the plasma membrane and thereby insulin secretion. Additionally, PKA activates transducer of regulated CREB (TORC2) and cAMP response element-binding protein (CREB), which leads to beta cell proliferation through CREB- and TORC2-mediated IRS2 gene expression [83,84]. In both rodent and human beta cells, EPAC2 also inhibits the function of the K<sub>ATP</sub>-Channels through activation of SUR1 [83,85]. Furthermore, CRE activation leads to stimulation of beta cell proliferation through CREB-mediated IRS-2 gene expression [80,86]. The activated CREB also results in higher Bcl-2 activity, and inhibition of the pro-apoptotic bax, which is involved in beta cell survival processes and thereby maintain and promote beta cell functions [9,80]. GLP-1 also stimulate expression of GLUT2 transporters and glucokinase leading to an increased intracellular glucose
Fig. 2. G protein-coupled receptors (GPCRs) on the human beta cell. Class B1, B2, and A GPCRs of the beta cell as well as therapeutic compounds on the market (black) and in development (grey).

For the non-G protein-dependent pathways, GLP-1R activation leads to c-SRC kinase-mediated transactivation of the epidermal growth factor tyrosine kinase receptor (EGFR) [13,84,87,88] (Fig. 3). This leads to activation of PI3K, which activates AKT/PKB, leading to phosphorylation of the nuclear transcription factor Foxo1 [9,89]. Phosphorylated Foxo1 can inactivate PDX-1, which in murine beta cells, has been demonstrated to lead to anti-apoptotic effects [9,13,90,91]. Moreover, beta arrestin 1 binds to the GLP-1R upon activation by GLP-1 where the conformational receptor changes initiate phosphorylation of transmembrane helix 7 by G protein-coupled receptor kinases (GRKs) resulting in recruitment of beta arrestins. The beta arrestin recruitment results in ERK1/2 activation, beta cell proliferative and insulin secretion [6,80,92]. Additionally, beta arrestin 1 also plays a role in receptor desensitization by at least two mechanisms: binding to the receptor and, thus, preventing G protein recruitment and by promoting receptor trafficking by internalization and recycling [14,92–94] (Fig. 3), although arrestin-independent internalization of the GLP-1R has been reported [95,96]. Furthermore, GLP-1R internalization is mediated by agonist-induced stimulation of the G_{ai} pathway, which leads to receptor recycling to the plasma membrane or transport to lysosomes resulting in proteolysis. Beside this, G_{ai} coupling leads to hydrolysis of PIP_{2} via phospholipase C activation, an increased Ca^{2+} accumulation, and phosphorylation of ERK 1/2 and beta cell proliferative processes [96]. Thus, multiple pathways are activated in the beta cell down stream of the GLP-1R.

4. GIP receptor signaling in the human beta cells

To stimulate insulin secretion after nutrient ingestion [16], GIP binds to and activates the GIPR on the beta cell surface. As for the GLP-1R, the activation of the GIPR promotes recruitment and activation of the G_{as} protein, which in turn activates the adenylate cyclase to stimulate CAMP production (Fig. 4). Again, the increased cAMP [97] activates PKA and EPAC [98]. PKA and cAMP activate a series of proteins including the mitogen-activated protein kinase (MAPK) cascades, and phosphorylates ERK1/2, which regulates genes involved in proliferative and anti-apoptotic processes [99,100]. The GIPR activation of PKA leads to insulin secretion by the same mechanisms as described for the GLP-1R [13,101,102]. GIPR activation can also promote non-insulinotropic actions, such as controlling pancreatic beta cell proliferation and survival (Fig. 4) [100,103]. Furthermore, PKA inhibits AMPK, which results in dephosphorylation and nuclear import of TORC2 [104]. CREB and TORC2 form a complex promoting the transcription of the anti-apoptotic gene bcl2 [104]. Activation of Akt/PKB/Pi3K promotes phosphorylation of the nuclear transcription factor Foxo1, which in turn inactivates bax and other apoptosis-related factors, resulting in downregulation of the pro-apoptotic signaling pathway [105]. The upregulation of bcl2 and the

**Fig. 3. Overview of glucagon-like peptide (GLP-1) receptor activation in the beta cell.** AMPK, AMP-activated protein kinase; AC, Adenylate cyclase; AKT/PKB, Protein kinase B; cAMP, Cyclic adenosine monophosphate; Ca^{2+}, Calcium; CREB, cAMP response element-binding protein; EGFR, Epidermal growth factor receptor; EPAC2, Exchange protein activated by cAMP2/cAMP-guanine nucleotide exchange factor (GEF) II; ERK 1/2, Extracellular signal-regulated kinase; G_{as}, G alpha subunit; GLP-1, Glucagon like peptide; IRS2, Insulin receptor substrate 2; K^{+}, Kaliumpotassium; MAPK, Mitogen-activated protein kinase; Na^{+}, Natrium; Na^{+}/Ca^{2+} exchange; PDK1, Phosphoinositide 3-kinase; c-Src, protooncogene tyrosine-protein kinase Src; SURL1, Sulfonilurea receptor; TORC2, Transducer of regulated CREB activity 2; VDCC, Voltage-dependent Ca^{2+} channel.

**Fig. 4. Glucose-dependent insulinotropic polypeptide (GIP) receptor activation in the beta cell.** AMPK, AMP-activated protein kinase; AC, Adenylate cyclase; AKT/PKB, Protein kinase B; Bcl2, B-cell CLL/Lymphoma; CAMP, Cyclic adenosine monophosphate; Ca^{2+}, Calcium; CREB, cAMP response element-binding protein; EPAC2, Exchange protein activated by cAMP2/cAMP-guanine nucleotide exchange factor (GEF) II; ERK 1/2, Extracellular signal-regulated kinase; G_{as}, G alpha subunit; GIP, Glucose-dependent insulinotropic polypeptide; K^{+}, Kaliumpotassium; MAPK, Mitogen-activated protein kinase; Na^{+}, Natrium; Na^{+}/K^{+} exchange; PKA, Protein Kinase A; RISK, RAAS inhibitor-sensitive kinase; SURL1, Sulfonilurea receptor; TORC2, Transducer of regulated CREB activity 2; VDCC, Voltage-dependent Ca^{2+} channel.
downregulation of bax leads to increased beta cell survival. Additionally, activation of Akt/PKB will result in suppressing the anti-proliferative and anti-survival mechanism of p38 MAPK and c-Jun N-terminal kinase (JNK), leading to suppressed mitochondrial translocation of BAD and BimEL and thereby the subsequent activation of caspase-3 promoting proliferation [100,103,104,106]. An important difference between GIPR and GLP-1R is that the GLP-1R activates EGFR leading to proliferation and anti-apoptosis, which is not the case for the GIPR [13].

When GIP is bound to its receptor, the receptor undergoes conformational changes that also initiate the phosphorylation of helix 7 by G protein-coupled receptor kinases (GRK). This leads to the recruitment of beta-arrestin 1 and 2 (Fig. 4). The beta-arrestins play a key role in receptor desensitization by blocking the $G_{\alpha\text{i}}$ proteins and in receptor trafficking by internalization and recycling [14,62,107]. In contrast to the GLP-1R, where the internalization is both beta arrestin dependent and independent (e.g. $G_{\alpha\text{i}}$ mediated pathway), the arrestins are necessary for GIPR internalization [107]. The coated pits that contain the GIPRs are separated from the plasma membrane by the membrane-remodeling GTPase dynamin leading to the construction of early endosomes, in which the GIPR is suggested to trigger production of cAMP and PKA activation [108] before recycling back to the beta cell surface.

5. Shared signaling pathways of the GIP and GLP-1 receptors in beta cells

Sharing the essential physiological task of stimulating postprandial insulin secretion, GLP-1R and GIPR in beta cells have many common intracellular pathways (Fig. 5A). Both receptors signal through activation of adenylyl cyclase resulting in increased intracellular cAMP, activating PKA and EPAC2 pathways which result in increased secretion of insulin, beta-cell proliferation, and survival/anti-apoptosis. Both GPCRs are desensitized by recruitment of beta-arrestins, followed by receptor internalization (though the GLP-1R internalization does not rely on arrestins for internalization), recycling, and blockage of the $G_{\alpha\text{i}}$ recruitment.

However, as for the differences, the activation of the EGFR pathway, leading to activation of PI3K and increased beta cell survival, is only activated by the GLP-1R and not the GIPR. Furthermore, the GLP-1R can be internalized by recruitment of beta arrestins and/or $G_{\alpha\text{i}}$ [96] (Fig. 3) and the internalization process can be affected by several ligands [38,94] where the internalization process of the GIPR is not that easily affected by novel ligands but completely rely on arrestins [61,74]. Moreover, activation of the GIPR in beta cells has been shown to result in MAPK-induced signaling pathways, which is not seen for in the GLP-1R.

6. The rationale for GLP-1R-GIPR co-agonists

Due to the therapeutic potential of increased insulin secretion and improved beta cell health in patients with type 2 diabetes, GLP-1R-GIPR co-agonists are now in drug development programs [110]. Impressively, synergistic effects have been reported for a GLP-1R-GIPR co-agonist, tirzepatide, promoting higher insulin responses than separate administration of each hormone. Tirzepatide has potent glucose lowering and weight loss effects compared to the established GLP-1R agonists and demonstrates dose-dependent reductions in HbA1c in patients with type 2 diabetes [25,61,111-115]. At the pharmacodynamics level, tirzepatide has been identified as an "imbalanced GLP-1R-GIPR co-agonist" implying a favor for GIPR over GLP-1R with equal affinity for the GIPR compared to endogenous GIP and slightly lower affinity for the GLP-1R than endogenous GLP-1. Also implied in the imbalanced profile of tirzepatide is a favor of GIPR over GLP-1 in receptor desensitization at the GLP-1R which leads to weaker ability to drive receptor internalization compared with endogenous GLP-1 (Fig. 5B) [111]. This bias, relative to endogenous GLP-1, results in an increased amount of GLP-1R on the cell surface thereby increasing the intracellular signaling induced by the drug and stronger insulinoceptive properties [11]. Based on in vitro receptor signaling studies, these tizepatide actions happen with concentrations of ~1–10 nM reaching the maximal activity of up to 100 nM [111] which is the same concentration ranges as the native ligands (GLP-1 and GIP, respectively). Other GLP-1R-GIPR co-agonists are in preclinical/clinical development (Table 1) [60,61,73], and compared to tirzepatide, the GLP-1R-GIPR co-agonist HISHS-2001 has slightly higher GLP-1R and GIPR potencies (cAMP production) and lower beta arrestin-2 recruitment to the GLP-1R [60]. However, it is not yet known

![Shared GLP-1 and GIP receptor signaling in the beta cell](image)

**Fig. 5.** A and B: Shared glucagon-like peptide 1 receptor (GLP-1R) and glucose-dependent insulinoceptive polypeptide receptor (GIPR) signaling in the beta cell (A) and tirzepatide signaling in the beta cell (B). AMPK, AMP-activated protein kinase; AC, Adenylate cyclase; AKT/PKB, Protein kinase B; Bcl2, B-cell CLL/Lymphoma; cAMP, Cyclic adenosine monophosphate; Ca$^{2+}$, Calcium; CREB, cAMP response element-binding protein; EPAC2, Exchange protein activated by cAMP2/cAMP-guanine nucleotide exchange factor (GEF) II; ERK 1/2, Extracellular signal-regulated kinase; $G_{\alpha\text{i}}$, G alpha subunit; GIP, Glucose-dependent insulinoceptive polypeptide; GLP-1, Glucagon-like peptide 1; IRS2, Insulin receptor substrate 2; K$^{+}$, Kalium; MAPK, Mitogen-activated protein kinase; Na$^{+}$, Natrium; KATP, Kalium-Natrium ATPase; PKA, Protein Kinase A; PI3K, phosphoinositide 3-kinase; c-Src, protooncogene tyrosine-protein kinase Src; SUR1, Sulfonylurea receptor; TORC2, Transducer of regulated CREB activity 2; VDCC, Voltage-dependent Ca$^{2+}$ channel.
if the biased mode of action seen for tirzepatide and HISHS-2001, will be a defining drug class effect.

7. Perspectives on mono and co-targeting GLP-1R and GIPR

7.1. Receptor expression relationship

The therapeutic implications of GLP-1R-GIPR co-agonism not only rely of the impact of the individual receptor activation, but undoubtedly also on the interplay between the two receptors and their signaling pathways. As an example, the signaling profiles of cells co-expressing GLP-1R and GIPR (in a fixed receptor expression ratio) suggest that GIPR negatively impact GLP-1R expression and GLP-1R agonist actions [74]. In the same system, GLP-1-GIPR co-agonists primarily interfere with the beta arrestin recruitment of the GLP-1R and the $G_{\text{o}}$ recruitment of the GIPR, inducing biased signaling as well as affecting the cell surface expressions. Moreover, co-expressed GIPR also severely reduce GLP-1R internalization, beta arrestin recruitment, and cAMP accumulation [116]. With this in mind, the classical pharmacodynamics term of agonism and antagonism, might not be sufficient to describe and determine the GLP-1R-GIPR targeting drugs needed e.g. to improve insulin secretion and beta cell function. For the GLP-1R-GIPR co-agonists tirzepatide and HISHS-2001 (Table 1, Fig. 5B), the biased activation of the GLP-1R results in increased amount of this receptor on the cell surfaces, while GIPR agonism result in proportionally higher GIPR internalization and thereby reduced receptor expression compared to the GLP-1R. In other terms, the receptor expression pattern and relationship could be an important factor for the success of GLP-1R-GIPR targeting type 2 diabetes treatment.

Another factor for addressing the studies of GLP-1R-GIPR co-activation and development of co-targeting drugs is the ratio of receptor activation e.g. the ratio of GLP-1 and GIP or the pharmacodynamics properties of a co-targeting compound towards each receptor. Taking pharmacodynamic properties of each receptor-ligand relationship, pharmacokinetic properties of the ligands as well as the factors of receptor expression highlighted above, and the presented shared signaling pathways into account, we leave this question unanswered. As described, numerous factors affect the implications of GLP-1R-GIPR co-agonism and if the receptors are activated more than 50 % (above ligand $E_{50}$), the ratio of receptor activation could likely be a minor factor.

7.2. Clinical GLP-1R-GIPR co-activation

As illustrated in Fig. 5A, the GLP-1R and GIPR share several intracellular signaling properties and in case of glucose-dependent insulin secretion in humans, the two hormones have been shown to act additively [2,3,21]. Presuming the mechanisms of action in the beta cells are common [119], the additive responses indicate a wide dynamic range of action in the beta cells of healthy individuals instead of parallel/distinct mechanisms. On top of that, the separate, unshared pathways (Figs. 3 and 4) may provide further basis for additive effects.

In a pathological state, such as type 2 diabetes, the beta cell responses to GLP-1 and GIP are however reduced, or in some cases, absent [120]. However, after a period of blood glucose normalization in patients with type 2 diabetes, the sensitivity and insulinotropic responses to GLP-1 and GIP are restored, indicating that the glucotoxicity affecting the beta cell function in general, are at least partly, reversible and that the intracellular machinery downstream of the GLP-1R and GIPR is intact [121,122]. Supporting the statement of intact GLP-1R, GIPR, and signaling properties in the beta cells in spite of the diabetic state, patients with the genetic diabetes variant (MODY3), where the insulin secretion is reduced, both GIP and GLP-1 administration results in increased insulin secretion [123].
increased lipid deposition in the adipose tissue [143,144] beyond its action on beta cells. Both GLP-1 and GIP administration also result in increased in heart rate [142,145], and could affect cardiac and vascular musculature [142,145]. GLP-1R-GIPR co-agonism will therefore not only affect the beta cells but several organs related to nutrient metabolism and glucose control. For most of the mentioned parameters, GLP-1R-GIPR co-agonism seem to have anti-diabetes and anti-obesity properties, however, especially glucagon secretion and appetite have, in acute studies, shown non-beneficial effects: In patients with type 2 diabetes, already treated with GLP-1R agonists, a GIP infusion resulted in higher glucagon secretion [145,146]. The presence of the GLP-1R on the human alpha cells has not been confirmed [147] but the GLP-1Rs are present on the delta cells and therefore glucagon-suppressing actions of GLP-1 can be via somatostatin [148]. The interplay between the GLP-1R and GIPR, which is present on the alpha cell [145], could therefore likely be affected by several extracellular factors as well as distinct receptor pharmacodynamics relations. For the other parameter ‘appetite’, persons with obesity had a higher food intake during infusion of the combination of GLP-1 and GIP than GLP-1 alone [150]. Fortunately, the clinical trials of the GLP-1R-GIPR co-agonist tirzepatide do not support the findings of higher glucagon levels and food intake [25] but whether this is due to the more complex islet interplay of GLP-1-induced glucagon suppression, the imbalanced and biased signaling profile of the specific compound or the physiological response to the long-term exposure of GLP-1R-GIPR co-agonism remains to be clarified.

7.6. Conclusions

Via common signaling pathways in the beta cells, GLP-1R and GIPR activation result in insulin secretion and novel drug classes such as GLP-1R-GIPR co-agonists in drug development result in efficient anti-diabetes treatment. The combination of GLP-1 agonism and GIP antagonism does, however, also improve glucose tolerance and induce weight loss in preclinical and non-human primate studies. Based on in vitro pharmacological studies, the interplay of the two receptors and their intracellular signaling reveal that receptor internalization and selective signaling pathway activation form the basis of a complex but close relationship between the GLP-1R and GIPR. The synergistic actions seen using the biased and imbalanced GLP-1R and GIPR co-agonist tirzepatide, is an example of promising new drug classes that selectively modify the receptor expression and signaling pathways resulting in improved treatment.

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A. Mayendorf et al.

9

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