

# Molecular Pharmacology of the Incretin Receptors

Suleiman Al-Sabah

Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait

## Key Words

Incretin · Glucagon-like peptide-1 · Glucose-dependent insulinotropic polypeptide · Internalization · Dimerization · G protein-coupled receptor

## Abstract

The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are important regulators of insulin and glucagon secretion as well as lipid metabolism and appetite. These biological functions make their respective receptors (GIPR and GLP-1R) attractive targets in the treatment of both type 2 diabetes mellitus (T2DM) and obesity. The use of these native peptides in the treatment of these conditions is limited by their short half-lives. However, long-acting GLP-1R agonists and inhibitors of the enzyme that rapidly inactivates GIP and GLP-1 (dipeptidyl peptidase IV) are in clinical use. Although there is a loss of response to both hormones in T2DM, this effect appears to be more pronounced for GIP. This has made targeting GIPR less successful than GLP-1R. Furthermore, results demonstrating that GIPR knockout mice were resistant to diet-induced obesity suggested that GIPR antagonists may prove to be useful therapeutics. More recently, molecules that activate both receptors have shown promise in terms of glycemic and body weight control. This review focused on recent advances in the understanding of the signaling mechanisms and regulation of these two clinically important receptors.

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## Introduction

Oral glucose load is known to elicit a much larger insulin response than glucose administered intravenously [1]. This phenomenon, termed the ‘incretin effect’, can account for more than half of the insulin secreted in response to a meal [2]. This effect is mediated by incretin hormones secreted from the gut in response to nutrient ingestion, which act to potentiate insulin secretion in a glucose-dependent manner. To date, only two incretin hormones have been identified: glucose-dependent insulinotropic polypeptide (GIP, formerly known as gastric inhibitory polypeptide) and glucagon-like peptide-1 (GLP-1) [3]. GIP is synthesized in K cells, which are found predominantly in the duodenum and jejunum. The active 42-amino acid peptide is derived from a 153-amino acid precursor by posttranslational processing by prohormone convertase 1/3 [4]. GLP-1, on the other hand, is a posttranslational product of the proglucagon gene. Tissue-specific expression and posttranslational processing of this gene result in several peptides with important physiological functions other than GLP-1 (such as glucagon and oxyntomodulin) [5]. Full-length GLP-1(1–37) is cleaved from the proglucagon precursor in intestinal L cells (found predominantly in the ileum) but must be further processed to produce GLP-1(7–37) and GLP-1(7–36) amide before the peptide becomes biologically active. GLP-1(7–37) and GLP-1(7–36) amide are equipotent; however, the majority of the circulating active peptide is

GLP-1(7–36) amide [6]. For simplicity, the term GLP-1 will be used to describe GLP-1(7–36) amide through the rest of this review.

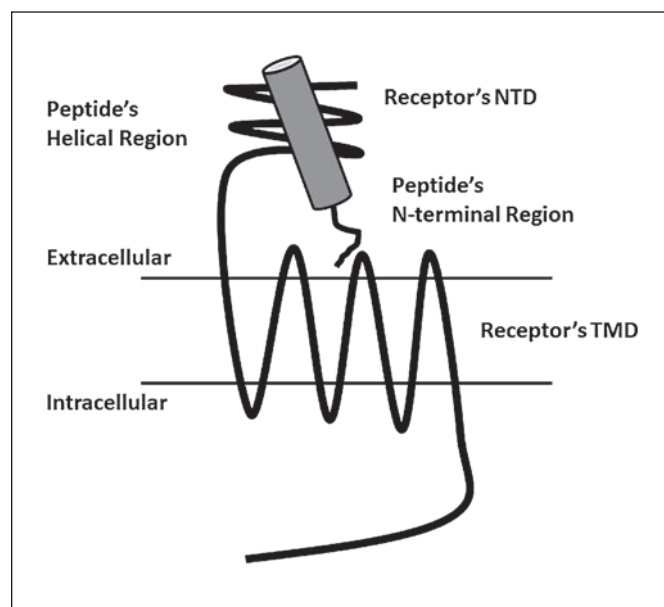
The drivers of incretin hormone secretion are complex and may include input from the nervous and endocrine systems. However, the primary stimulus for the secretion of both GIP and GLP-1 is the ingestion of glucose, although other nutrients such as lipids and amino acids also stimulate their secretion [7]. From a pharmacological perspective it is interesting to note that the commonly used antidiabetic medication metformin increases the secretion of GLP-1 when taken orally [8] and this may contribute to the antidiabetic effect of this drug.

Loss of the incretin effect is an early characteristic of type 2 diabetes mellitus (T2DM) and while large doses of GLP-1 can overcome this impairment, it is unclear whether the same is true for GIP [9, 10]. The reasons for this are unclear but several studies have demonstrated that hyperglycemia negatively affects GIP receptor (GIPR) signaling to a greater extent than the GLP-1 receptor (GLP-1R) [11, 12].

GIP and GLP-1 are rapidly inactivated by the enzyme dipeptidyl peptidase IV (DPP-IV), also known as CD-26, which severely limits the use of native GIP and GLP-1 in the treatment of T2DM [13, 14]. To overcome this, both long-acting GLP-1R agonists and DPP-IV inhibitors have been developed and are currently used clinically to treat T2DM [15]. GIPR has received less attention than GLP-1R as a drug target. However, as GIPR knockout mice were shown to be resistant to diet-induced obesity [16], several studies suggest that the use of GIPR antagonists may be a suitable approach to treat both T2DM and obesity [17, 18]. More recently, single molecules that activate both GIPR and GLP-1R have shown promise as effective antidiabetic and antiobesity drugs [19]. The biology of the incretin hormones has been extensively reviewed elsewhere [3, 7, 20]. Therefore, the focus of this review was on recent advances in the understanding of the signaling mechanisms and regulation of the two incretin hormone receptors.

### The Incretin Receptors

Both the receptors for GIP and GLP-1 are members of the secretin family or class B G protein-coupled receptors (GPCRs) [21]. Although GLP-1R and GIPR share considerable sequence homology (approximately 40%), they display extremely high selectivity for their respective ligands [22]. Family B GPCRs have a large extracellular N-



**Fig. 1.** A model for peptide ligand binding to incretin receptors. The helical region of the peptide ligand binds to the NTD of the receptor, allowing the N-terminal region of the peptide to interact with the receptor's TMD.

terminal domain (NTD) linked to the 7-transmembrane helical domain that is characteristic of all GPCRs. The C-terminal region of the peptide ligand binds the NTD of the receptor, facilitating a secondary interaction between the N-terminal region of the peptide and the 'core' or transmembrane domain (TMD) of the receptor (fig. 1). This secondary interaction is thought to lead to activation of the receptor, allowing the TMD to interact with and activate heterotrimeric G proteins [23–25]. However, recent data for both GIPR and GLP-1R have shown that peptides that comprise the central region of GIP and GLP-1 are also able to activate their respective receptors, albeit with reduced potency [26, 27]. Therefore, this situation requires a rethinking of the current model of ligand binding and activation for this class of receptors.

Structures of both the NTD of GIPR and GLP-1R in complex with their respective ligands had been solved and has confirmed that the C-terminal region of the peptide binds to this domain [28–30]. The structure of the TMD of either GIPR or GLP-1R is yet to be determined. To date, the only members of the class B family of GPCRs which have crystal structures of their TMD published are the corticotropin-releasing factor receptor 1 and the glucagon receptor [31, 32]. These structures provide valuable insight into the structure and function of this family

of receptors and can be used as a template to model the structure of GIPR and GLP-1R. However, as they do not include the NTD, the orientation of this region in relation to the TMD and how peptide ligands alter this structure remain to be solved.

### Receptor Expression

GIPR and GLP-1R are both expressed in pancreatic islet  $\beta$ -cells where they mediate the incretins' amplification of glucose-induced insulin secretion, insulin biosynthesis and inhibition of  $\beta$ -cell apoptosis. GIPR is also expressed in pancreatic  $\alpha$ -cells but it is still unclear whether GLP-1R is expressed in other islet cell types [20]. Whereas GLP-1 inhibits glucagon, the actions of GIP on this counterregulatory hormone appear to be glucose dependent [33]. Based on studies using quantitative polymerase chain reaction, GLP-1R may be expressed at 10 times that of GIPR in pancreatic islets [34]. GLP-1R has been reported to be expressed in pancreatic ducts, which may contribute to the reports of pancreatitis in patients treated with GLP-1R agonists. It should be noted, however, that diabetic patients are at greater risk of developing acute pancreatitis regardless of the medication they are taking and that more recent studies did not find an association between the use of incretin-based therapies and increased risk of acute pancreatitis [35, 36]. GIPR is also expressed in the adrenal cortex, adipose tissue, bone, some areas of the brain, and endothelium amongst others. Interestingly, the GIPR gene includes a PPAR- $\gamma$  response element in the promoter region, and treatment with troglitazone increases GIPR expression in isolated mouse islets [37]. GLP-1R is also expressed in the central and peripheral nervous system and other tissues, including the heart, lungs, kidneys, stomach, and intestines. The broad tissue distribution of GIPR and GLP-1R accounts for the pleiotropic effects of the incretin hormones [20], which include neuro- and cardioprotective effects, regulation of adipose tissue and bone for GIP and regulation of food intake for GLP-1.

### Splice Variants

GIPR exists as two isoforms: one 466 amino acids in length and the other 493 amino acids long. The additional 27 amino acids in the long form are located at the junction between transmembrane helix 7 and the C-terminal tail. Both isoforms bind GIP with similar high affinity, resulting in dose-dependent increases in intracellular cAMP

[38, 39]. The functional significance of the long form is currently unknown and it is unclear whether it is actually expressed in human pancreatic cells. Interestingly, a novel splice variant of GIPR which acts as dominant negative has been identified in murine pancreatic  $\beta$ -cells [40]. This splice variant is severely truncated (263 amino acids) due to a frame shift that introduces a stop codon to transmembrane helix 4 [40]. To date, an equivalent splice variant has not been identified in human pancreatic  $\beta$ -cells.

### Cell Signaling

Although they have been reported to couple to other G proteins, GIPR and GLP-1R signal primarily through  $G_s$ , leading to the activation of adenylate cyclase and an increase in intracellular cAMP. In pancreatic  $\beta$ -cells this leads to the activation of protein kinase A, which inhibits  $K^+$  channels, resulting in membrane depolarization and the opening of voltage-gated  $Ca^{2+}$  channels. The increase in  $Ca^{2+}$  influx triggers the release of  $Ca^{2+}$  from intracellular stores (calcium-induced calcium release) and the subsequent fusion of insulin-containing vesicles with the plasma membrane and, ultimately, insulin release [41]. The increase in cAMP also leads to the activation of exchange protein activated by cAMP-2 (Epac2), which enhances transcription of the proinsulin gene [42].

Activation of GLP-1R has been shown to lead to transactivation of the epidermal growth factor receptor [43]. A signaling complex of arrestin2 and c-Src is thought to facilitate this transactivation [44]. The subsequent activation of phosphatidylinositol-3 kinase and Akt is thought to mediate the proliferative and prosurvival effects of GLP-1 on the pancreatic  $\beta$ -cell.

### Constitutive Activity

GIPR has been shown to display considerable ligand-independent or basal activity, whereas GLP-1R is relatively silent in the absence of an agonist [45]. The physiological relevance of this constitutive activity is not entirely clear but a mutation resulting in a glutamic acid-to-glutamine substitution in the 6th transmembrane of the GIPR results in a receptor with lower basal activity [46]. Subjects who are homozygous for this polymorphism have been shown to have lower fasting and post-oral glucose tolerance test serum C-peptide, which suggests that the high basal activity of GIPR may play an important role in normal glycemic regulation [47].

## Homologous Desensitization and Internalization

Homologous desensitization refers to the loss of response to subsequent stimulation following agonist stimulation of a particular receptor [48]. For many GPCRs, such as the prototypical  $\beta_2$ -adrenergic receptor ( $\beta_2$ -AR), it had been established that this process involves phosphorylation of the agonist-activated receptor at serine and threonine residues in the receptor's C-terminal tail and intracellular loop regions by the family of kinases known as GPCR kinases [49]. The phosphorylated receptor can still signal through G proteins at this stage but phosphorylation also facilitates the binding of another family of proteins known as arrestins to the agonist-activated receptor. Arrestin binding prevents the receptor from interacting with G proteins, desensitizing the G protein-dependent component [50]. Arrestin also mediates internalization of the receptor by targeting the receptor to clathrin-coated pits as well as activation of G protein-independent signaling pathways such as the mitogen-activated protein kinase cascade. Once internalized, the receptor may be dephosphorylated and recycled to the plasma membrane (resensitization) or targeted to lysosomes for degradation [51].

Homologous desensitization and internalization are, however, two distinct phenomena. It is also clear that not all GPCRs are desensitized or internalized by the same mechanisms [52]. Some receptors such as the  $\beta_3$ -AR (which lacks the same phosphorylation sites in its C-terminal region as the  $\beta_2$ -AR) may not undergo homologous desensitization, and when desensitization has been observed for this receptor it appears to be due to the downregulation of receptor mRNA or reduced  $G_s$  expression [53].

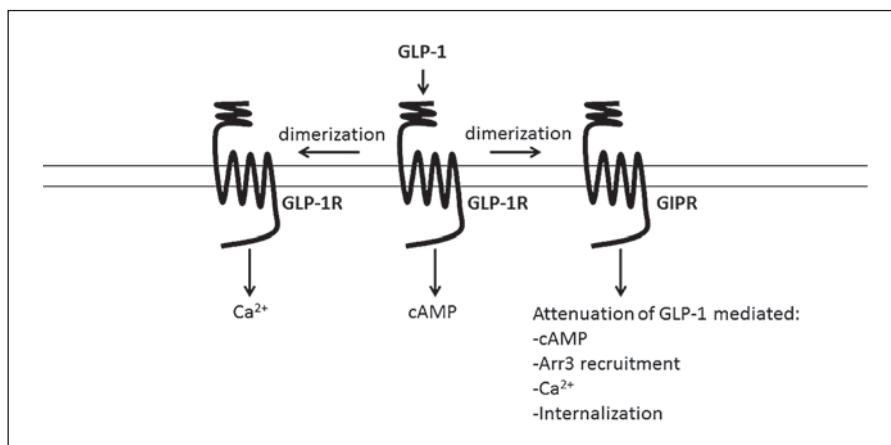
Following agonist stimulation, GLP-1R is rapidly internalized [54–56]. An earlier study has suggested that this was via a clathrin-coated pit-dependent process [57] but more recent work has shown that agonist-induced internalization of GLP-1R is caviolin-1 and dynamin dependent and arrestin independent [58]. Interestingly, internalization of GLP-1R is mediated by the  $G_{\alpha_q}$  pathway [59]. It has been known for some time that GLP-1R is capable of coupling to multiple G proteins [60]. However, the significance of this promiscuity was not completely appreciated at the time. Although the internalization of GLP-1R may contribute to the desensitization to subsequent stimulation observed *in vitro*, its physiological significance is unclear, as chronic exposure to the GLP-1R agonist exendin-4 does not result in an attenuation of GLP-1R-mediated glucose homeostasis or receptor downregulation *in vivo* [61].

Intriguingly, the inhibition of GLP-1R internalization has been shown to attenuate both cAMP production in BRIN-BD11 cells and insulin secretion in pancreatic  $\beta$ -cells [55, 62]. This finding suggests that internalization of GLP-1R is an integral part of the signaling process. Using fluorescently labelled ligands and receptors, Kuna et al. [55] have shown that agonist-bound GLP-1R colocalizes with adenylate cyclase in endosomes. Taken together, these findings suggest that GLP-1R continues to signal following internalization. Endocytotic generation of cAMP has also been observed for other members of the secretin family of GPCRs such as the glucagon and parathyroid hormone receptors (PTHr) [63, 64]. For the PTHr this phenomenon has been reported to be mediated by arrestin2 [65]. Although various groups, using a variety of techniques, have demonstrated that stimulation of GLP-1R results in the recruitment of both arrestin2 and arrestin3, both internalization and desensitization appear to be arrestin-independent processes for this particular receptor [66, 67]. Furthermore, knockdown of arrestin2 in cultured pancreatic  $\beta$ -cells results in a reduction of both GLP-1-stimulated cAMP and insulin production [62].

Based on their interaction with arrestin, GPCRs have been classified as either class A or class B receptors. Class A receptors such as  $\beta_2$ -AR only interact transiently with arrestin at the plasma membrane, whereas class B receptors such as PTHr cointernalize with arrestin following agonist stimulation [68]. In this regard, GLP-1R appears to be a class A receptor as, unlike PTHr, it does not cointernalize with arrestin [43]. The exact mechanism by which arrestin contributes to GLP-1R signaling still remains to be fully elucidated.

In contrast to GLP-1R, the role of internalization in signaling through GIPR has not been studied in as much detail and appears to be a somewhat controversial subject. When expressed in 3T3-L1 adipocytes, GIPR was shown to be constitutively internalized and recycled to the plasma membrane, resulting in no net change in cell surface expression [69]. This finding is compatible with the fact that GIPR displays a high level of constitutive activity in terms of cAMP production. Stimulation of this cell line with GIP causes a decrease in the amount of GIPR at the cell surface and desensitization to further stimulation with GIP. This process has been shown to be driven by a reduction in the rate of recycling of the receptor to the plasma membrane rather than an increase in internalization kinetics. However, the mechanistic details by which GIPR is sequestered are currently unknown. Furthermore, when expressed in either HEK-293 or COS-7 cells, even micro-

**Fig. 2.** Impact of homo- and heterodimerization in GLP-1R signaling. Arr3 = Arrestin3. Disruption of GLP-1R homodimer formation severely inhibits GLP-1-mediated  $\text{Ca}^{2+}$  mobilization, suggesting that homodimerization is involved in this signaling pathway. GLP-1 has also been shown to induce heterodimerization of GLP-1R and GIPR. Coexpression of GIPR with GLP-1R attenuates GLP-1-mediated cAMP production, arrestin3 recruitment,  $\text{Ca}^{2+}$  mobilization, and GLP-1R internalization. This suggests that GIPR acts as a negative regulator of GLP-1R signaling.



molar concentrations of GIP were insufficient to cause internalization of GIPR [70]. These findings demonstrate that GIPR behavior is cell context dependent.

### Dimerization

GPCRs have been shown to function as monomers, homo- and heterodimers and also higher-order oligomers [71, 72]. Resonance energy transfer studies have demonstrated that both GLP-1R and GIPR can form homodimers [73]. For GLP-1R the primary dimerization interface has been shown to be located in transmembrane helix 4. Disruption of GLP-1R dimer formation by various means abolished high-affinity GLP-1 binding and differentially affected signaling. GLP-1-mediated cAMP production and ERK1/2 phosphorylation were moderately inhibited, whereas intracellular calcium mobilization was markedly attenuated [72].

GLP-1R and GIPR also form heterodimers. The surface expression of an N-glycosylation-deficient GIPR mutant was rescued when coexpressed with GLP-1R, suggesting that dimer formation may occur during the maturation process. In the same study, GLP-1R and GIPR were shown to be heterodimerized by the use of BRET (bioluminescence resonance energy transfer) [74]. BRET studies have also shown that GLP-1 promotes GLP-1R/GIPR dimer formation and that this can be reversed by treatment with GIP [67]. When coexpressed with GLP-1R in HEK-293 cells, GIPR impaired GLP-1-mediated calcium signaling and recruitment of arrestin to GLP-1R. Reversing dimer formation by treatment with GIP restored arrestin recruitment. In a more recent study coexpression of GIPR with GLP-1R in HEK-293 cells not only impaired

arrestin recruitment to GLP-1R but also GLP-1-stimulated cAMP production, ERK phosphorylation, calcium signaling, and internalization of GLP-1R [70] (fig. 2). In this study, however, costimulation with GIP did not rescue GLP-1-mediated recruitment of arrestin to GLP-1R. These recent studies highlight the functional consequences of GLP-1R/GIPR dimerization and may have relevance in the development of T2DM.

### Conclusion

GIP and GLP-1 are important regulators of metabolism, including glycemia and pancreatic  $\beta$ -cell function. GLP-1R agonists such as exendin-4 (Byetta) and liraglutide (Victoza) are currently used clinically to treat T2DM and potentially have utility in the treatment of obesity. These two drugs are soon to be joined by other GLP-1R agonists with longer durations of action. Therefore, a detailed understanding of the desensitization, internalization and subsequent signaling of GLP-1R is warranted. Recent work in this area has shown that internalization appears to be an important part of the signaling process for GLP-1R, and inhibiting this process attenuates insulin release. DPP-IV inhibitors such as sitagliptin prolong the circulating half-life of both GIP and GLP-1 and are also used to treat T2DM. While GIPR agonists are not used clinically, recent reports of GIPR/GLP-1R coagonists have shown promise in terms of glucose control and weight loss. As GIPR/GLP-1R heterodimerization has been shown to regulate GLP-1R signaling, it will be important to understand how targeting both receptors with a single molecule affects this process and may highlight new opportunities to treat T2DM and obesity.

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