Molecular interactions of full-length and truncated GIP peptides with the GIP receptor – A comprehensive review

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A R T I C L E  I N F O

Keywords: GIP(1–42) GIP receptor Agonists Antagonists

A B S T R A C T

Enzymatic cleavage of endogenous peptides is a commonly used principle to initiate, modulate and terminate action for instance among cytokines and peptide hormones. The incretin hormones, glucose-dependent insulino-tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), and the related hormone glucagon-like peptide-2 (GLP-2) are all rapidly N-terminally truncated with severe loss of intrinsic activity. The most abundant circulating form of full length GIP(1–42) is GIP(3–42) (a dipeptidyl peptidase-4 (DPP-4) product). GIP(130)NH2 is another active form resulting from prohormone convertase 2 (PC2) cleavage of proGIP. Like GIP(1–42), GIP (1–30)NH2 is a substrate for DPP-4 generating GIP(3–30)NH2 which, compared to GIP(3–42), binds with higher affinity and very efficiently inhibits GIP receptor (GIPR) activity with no intrinsic activity. Here, we review the action of these four and multiple other N- and C-terminally truncated forms of GIP with an emphasis on molecular pharmacology, i.e. ligand binding, subsequent receptor activation and desensitization. Our overall conclusion is that the N-terminus is essential for receptor activation as GIP N-terminal truncation leads to decreased/lst intrinsic activity and antagonism (similar to GLP-1 and GLP-2), whereas the C-terminal extension of GIP(1–42), as compared to GLP-1, GLP-2 and glucagon (29–33 amino acids), has no apparent impact on the GIPR in vitro, but may play a role for other properties such as stability and tissue distribution. A deeper understanding of the molecular interaction of naturally occurring and designed GIP-based peptides, and their impact in vivo, may contribute to a future therapeutic targeting of the GIP system – either with agonists or with antagonists, or both.

1. The GIP system and GIP ligands used for human studies

Glucose-dependent insulino-tropic polypeptide (GIP) is a 42 amino acid peptide hormone secreted from enteroendocrine K cells in response to nutrient intake [1]. Like its sister incretin hormone, glucagon-like peptide-1 (GLP-1), GIP potentiates glucose-induced insulin secretion in the postprandial state [2,3]. The GIP receptor (GIPR) is expressed in a variety of organs where it elicits multiple functions. Its expression and function in the endocrine pancreas, adipocytes, cardiovascular system and in bone cells are well established, whereas the functional consequences of its presence in the gastrointestinal tract, lungs, leukocytes, spleen, and in the central nervous system remain to be elucidated [4–6]. Moreover, ectopic GIPR expression has been identified in endocrine organs with hyperactivity, such as in pituitary adenomas in patients with acromegaly and in the adrenal glands in patients with meal-related Cushing’s disease, where it has been suggested to mediate a food-induced burst in hormone release initiated by GIP released from the GI tract [7].

The therapeutic potential of GLP-1 was recognized when it was demonstrated to be capable of stimulating insulin secretion in patients with type 2 diabetes (T2D) [8]. Nowadays, GLP-1 forms the basis of several marketed treatments of type 2 diabetes and obesity exploiting its preserved insulino-tropic properties and the inhibition of glucagon secretion, gastric emptying and appetite and food intake [9]. Unlike GLP-1, the insulino-tropic action of GIP is severely impaired in patients with T2D, and a defective GIPR activity has been suspected to be a contributing factor, as supported by animal studies where a hyperglycemia-mediated downregulation of GIPR expression was observed in diabetic rodents [10,11]. However, a causal link between identified GIPR SNPs and the GIP insensitivity in patients with T2D or obesity [12–18] has not been found. Possibly, the function of the GIPR is not impaired in human diabetes, as also suggested from recent human

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https://doi.org/10.1016/j.peptides.2019.170224
Received 7 October 2019; Received in revised form 25 November 2019; Accepted 27 November 2019
Available online 03 December 2019
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studies, where GLP-1/GIP co-agonists were claimed to activate the GIPR (in addition to the GLP-1 receptor (GLP-1R)) in patients with T2D [7,19,20].

Both full length GIP(1–42) and the naturally occurring truncated GIP peptides GIP(1–30)NH₂ and GIP(3–30)NH₂ have been administered to humans. Infusions of GIP(1–42) (for two to six hours) in healthy individuals potentiate glucose-stimulated insulin secretion, augment glucagon secretion during hypoglycaemia, decrease bone resorption, increase blood flow and deposition of triacylglycerol in adipose tissue, mediate intestinal vasodilation, decrease blood pressure and increase heart rate [21–27] (see also Holst et al., this issue). In contrast, GIP (1–42) infusions in patients with T2D result in very low insulin secretion [28–30]. In a single study, GIP(1–30)NH₂ has been confirmed as a potent GIPR agonist when measuring insulin, glucagon, and markers of bone metabolism during various plasma glucose levels [31]. The GIPR antagonist, GIP(3–30)NH₂, has been used as a tool to elucidate GIP physiology, and in these studies endogenous GIP has now been confirmed as an important incretin hormone in healthy men, but also confirmed its lack of effect in patients with T2D [32–35] (see also Gasbjerg et al., this issue). In contrast to the many studies in humans, where GIP agonists (GIP(1–42) and GIP(1–30)NH₂) and the antagonist (GIP(3–30)NH₂) have been used to describe the acute physiological and pathophysiological roles of the GIP system, no long-term studies have been performed in humans with GIPR ligands as monotherapy. Multiple animal studies have evaluated GIPR functions and ligands, however, the results are often confounded by the marked species differences [36]. Furthermore, the conflicting results between rodent and human GIP pharmacology is contributed not only by potential differences in endogenous GIPR signaling but also by the assumption of similar agonist efficacy among species when performing pharmacological studies. Moreover, in rodents the GIP physiology is complicated and unclear as the endogenous GIPR signaling but also by the assumption of similar agonist pharmacology is contributed not only by potential differences in endogenous GIPR signaling but also by the assumption of similar agonist efficacy among species when performing pharmacological studies. Moreover, in rodents the GIP physiology is complicated and unclear as illustrated by reports that both GIP overexpression and GIPR gene deletion in mice resulted in improved glycaemic control and in a phenotype in which the N-terminal part of the peptide moves to and docks into the TMD. This mean that, in terms of structure, the receptor has a transmembrane domain (TMD) consisting of seven transmembrane α-helices (helix I-VII), a single intracellular α-helix (helix VIII) and a well-structured, large extra-cellular N-terminal domain (EC). The GIPR receptor family recognizes a series of remarkably homogenous ligands, i.e. peptides of > 27 amino acid residues and of mostly α-helical nature [41,42]. The exact activation mechanism of class B1 GPCRs, including the GIPR, is largely unknown. Educated guesses are based on data from X-ray crystallography, cryogenic electron microscopy (cryo-EM) or extrapolation of knowledge from other classes of GPCRs. Based on crystal and cryo-EM structures of the single GLP-1R and the glucagon receptor (GCGR) (Fig. 1), the active and inactive states have been compared and reinforced by mutation studies. These studies have revealed the particular residues of importance in ligand binding and/or receptor activation within this receptor family [43–46].

However, the intricate cascade of changes in residue interactions and receptor conformations upon ligand binding, which moves the receptor from an inactive to active state, remains unknown. Currently, the elopment upon ligand binding, which moves the receptor from an inactive to active state, remains unknown. Currently, the (Fig.2) is the leading paradigm in the field of class B1 GPCRs. two-step binding and activation model (Fig.2) is the leading paradigm in the field of class B1 GPCRs. This model suggests that the peptide ligand diffuses through the interstitial fluid shifting between having α-helical fragments or a disordered secondary structure. The ECD of the receptor recognizes and “catches” the middle or C-terminal part (residues 15–30) of the ligand [48] after which the N-terminal part of the peptide moves to and docks into the TMD. During this process or as a result of this process, the well-conserved PLLG (proline-lysine-lysine-glycine) motif of the receptor in the middle of helix VI gets destabilized and changes conformation. The bottom of helix VI (as also seen in Fig. 1) moves 10–20 Å outwards, making an angle of 40–60° with helix VII and creates space in the intracellular part of the receptor for cytosolic protein complexes to bind [49]. However, evidence of the exact transformation from active to inactive (or vice versa) is still lacking (Fig. 2).

The signaling of the GIPR occurs mainly through binding of Gαs proteins which invoke multiple signaling cascades including increased levels of the downstream second messenger CAMP (Fig. 3). Additionally, the conformational changes of the receptor also enhance the phosphorylation of helix VIII by G protein-coupled receptor kinases (GRKs), which subsequently recruit β-arrestins to this part of the receptor. β-arrestins desensitize the receptor by blocking the binding of Gαs proteins and by initiation of receptor trafficking, i.e. internalization and recycling [50–54]. Moreover, the GIPR has also been reported to signal from intracellular vesicles, i.e. endosomes [55]. A naturally occurring variant of the GIPR, GIPR-[E354Q], has recently been shown to enhance the internalization rate through enhanced GIP residence time [56]; in agreement with the previous demonstration of decreased receptor recycling to the cell surface after internalization [54].
3. GIP peptides - agonists and antagonists obtained by truncations of GIP

Various GIP agonists and antagonists have been characterized in vitro and in vivo in rodents and some even in humans and have contributed to the understanding of the physiological actions of GIP [57] (summarized in Fig. 4). GIP(1–42) is a substrate for the enzyme DPP-4 and has a short half-life in plasma [58]. DPP-4 cleaves peptide bonds following proline or alanine residues when positioned as the second amino acid counted from the N-terminus [59]. Therefore, the main circulating GIP(1–42) variant is the N-terminally truncated DPP-4 metabolite GIP(3–42) [58,60] (Fig. 4 and 5A). GIP(1–42) is processed from the precursor protein proGIP by the prohormone convertase (PC) 1/3 [61], but also contains a PC2 cleavage motif, i.e. Gly31;Lys32;Lys33, which after cleavage yields GIP(1–30)NH2 and GIP(34–42). PC2 is expressed in pancreatic α-cells [62] and a subpopulation of enteroendocrine K cells [63] and the C-terminal truncated GIP(1–30)NH2 seems to have similar agonistic properties as full length GIP(1–42) [63,64]. Like GIP(1–42), GIP(1–30)NH2 is also a substrate for the enzyme DPP-4 producing the N-terminally truncated GIP(3–30)NH2 (Fig. 5A).

3.1. GIPR agonists

As mentioned above, humans have (at least) two active circulating GIP variants – GIP(1–42) and the C-terminally truncated version, GIP (1–30)NH2 [63,64] (Fig. 5A). Since GIP(1–30)NH2 is only present at very low plasma concentrations (low picomolar range), its physiological role is expected to be minimal or even absent [65]. From a molecular pharmacologically point of view, the two peptides bind to the human GIPR with similar affinities and furthermore activate the receptor with identical potencies and efficacies in terms of Gαi3 signaling [66] (Fig. 5B) and β-arrestin 2 recruitment (unpublished, Gabe et al). Several N-terminal variants of these two peptides have been studied and shown to have profound impact on receptor activity, which is reviewed in the following sections.

3.1.1. Functional consequences of N-terminal truncations of GIP(1–42)

The N-terminus of GIP is essential for receptor activation as previously displayed by NMR, in silico molecular modeling, in vitro and in vivo experiments [66–73]. Specifically, the N-terminal Tyr1 of GIP has been described to interact with residues of three different transmembrane helixes of the GIPR, namely Glu224 (helix III), Arg300 (helix V), and Phe357 (helix VI) [70,73]. In addition, Ala2 is believed to play an important role as it is thought to interact with residues of helix III and form a hydrogen bond with Thr5 of GIP which seems to be important for receptor activation [70]. This is confirmed by in vitro studies in which N-terminal truncation of either Tyr1 (GIP(2–42)) or both Tyr1 and Ala2 (GIP(3–42)) result in low efficacy or no activity [66–68]. In addition, GIP(3–42) does not induce any insulin secretion from rat islets or pig pancreas [68]. Further N-terminal truncations of GIP(1–42) result in partial agonism (GIP(4–42), GIP(5–42), GIP(6–42), GIP(7–42) and GIP(8–42)) or full activity (GIP(9–42)) [67]. In contrast, N-terminal truncations of GIP(1–30)NH2 result in no intrinsic activity [66] except for GIP(19–30)NH2 which acted as a partial agonist [74] (Fig. 4). Thus, N-terminal truncations of GIP(1–30)NH2 show less intrinsic activity compared to N-terminal truncations of GIP(1–42). Since the naturally occurring GLP-1(7–36)NH2 and GLP-2(1–33) also lose activity and become partial agonists, when truncated N-terminally to GLP-1(9–36)NH2 and GLP-2(3–33), respectively, an intact N-terminus seems necessary to gain full receptor activation within the class B1 GPCR family [75–78] (Fig. 6A and B, respectively). As confirmed by the overall receptor structures and proposed activation mechanism, described in section 2, the GIP system resembles the GLP-1 and GLP-2 systems in this way.

Fig. 2. The general two-step binding and activation model of class B1 GPCRs. The peptide ligand is in equilibrium between being disordered and α-helical. Ligand binding to the receptor is initiated when the ECD of the receptor tethers the C-terminal part of the peptide ligand after which the N-terminal of the ligand is docked into the TMD. This activates the receptor and mediates G protein-dependent signaling. Modified from [67]. GPCR: G protein-coupled receptor, ECD: extracellular domain, TMD: transmembrane domain.
3.1.2. The C-terminus of GIP peptides and GIPR agonism

GIP, when comparing itself to being C-terminally extended (amino acid 31–42), distinguishes itself by its naturally occurring class B1 peptides (Fig. 4A). From NMR data, GIP(1–42) appears to have a well-defined backbone (between residues Phe6–Gln29) and a less well-defined helix (between residues Lys30–Trp36) [41]. Detailed analysis of the chemical shifts suggests that within the stable helical part of the peptide, one can distinguish two helical segments, i.e. between residues Ser8–Lys16 and Gln19–Lys30 [69]. A crystal structure published in 2007, proposes that the binding interface of GIP(1–42) to the ECD spans from Asp15 to Lys30 and that since residues Lys32 to Gln42 could not be resolved, it is expected that the C-terminus shows a high degree of disorder [48]. The disorder observed in the last 12 residues of GIP (1–42), along with the fact that it does not bind to the ECD, nor to residues close to the TMD, indicate that these residues are of little importance for GIPR binding and receptor activation. As previously mentioned, GIP(1–42) and GIP(1–30)NH₂ bind with identical affinity to the GIPR confirming the (in vitro) redundancy of residues Gly31–Gln42 [63,66]. Until very recently, no in vivodifferences have been described regarding the intrinsic activity between GIP(1–42) and GIP(1–30)NH₂. However, a recent human study indicated that GIP(1–30)NH₂ increases the glucagon levels more than GIP(1–42), where the presence of the C-terminus improves the agonistic action of the N-terminally truncated GIP peptides, as compared to the same N-terminal truncations in the absence of the C terminus [67]. Furthermore, due to the difference in structure, it could be speculated that GIP(1–42) and GIP(1–30)NH₂ may have different pharmacokinetic profiles, such as tissue distribution and clearance, however, this still remains to be verified.

3.2. GIPR antagonists

Many approaches have been applied to develop potent and efficacious GIPR antagonists to study GIP physiology such as GIP or GIPR antibodies and even a vaccination against GIP in animal studies has been described [80–84]. In addition, several peptide-based antagonistic variants have been identified involving N- and C-terminal modifications of GIP(1–42) and GIP(1–30)NH₂ (Fig. 4) [66,67,85,86].

3.2.1. The N-terminus of GIP peptides and GIPR antagonism

As previously described, class B1 GPCRs are believed to be activated in a two-step ligand binding-activation mechanism where the binding of the peptide N-terminus to the transmembrane domain of the receptor results in receptor activation [47–49] (Fig. 2). It is therefore to be expected that N-terminal truncations or modifications of the ligand will result in decreased efficacy. One of the first GIP peptides to be described as an antagonist was (Pro3)GIP [85]. This peptide was used in a
number of rodent studies to inhibit GIPR activity and this resulted in improved diabetic parameters and weight loss [85,87–90]. (Pro3)GIP was long believed to be an antagonist of the GIPR system, however, a thorough in vitro study revealed that human (Pro3)GIP is a full agonist of human GIPR and that the rodent variants are partial agonists of the rodent and human GIPRs [36]. Other amino acid substitutions at position 3 were found to be partial agonists of human GIPR [86]. This underlines the impact of the N-terminus in receptor activation, as also emphasized by the observation that the most potent GIPR peptide antagonists are N-terminal truncations of GIP(1–30)NH₂ (Fig.4). Truncation of the first two amino acids and up to 16 amino acids of GIP(1–30)NH₂ leads to GIPR antagonists [66,74,91–94], among which GIP(3–30)NH₂ and GIP(5–30)NH₂ are most potent. Since GIP(3–30)NH₂ is found naturally within the human circulation (being the DPP-4 product of GIP(1–30)NH₂) this peptide has already been used in several clinical studies [24,32–35,95,96]. It has, however, to be taken into account that GIP(3–30)NH₂ shows species-dependent variation [97], once again emphasizing that the GIP system is much less conserved between species than for example the GLP-1 system [98].

### 3.2.2. The C-terminus of GIP peptides and GIPR antagonism

In comparison to GIPR agonism, C-terminally truncated GIP peptides have improved antagonistic properties. This is clearly exemplified by the two naturally occurring GIPR antagonists, GIP(3–42) and GIP(3–30)NH₂. In vitro, both peptides antagonize the human GIPR, however, GIP(3–30)NH₂ is 26-fold more potent in doing so than GIP(3–42) [66]. In addition, porcine GIP(3–42) was not able to inhibit GIP(3–42) mediated insulin secretion in pigs at physiological concentrations [68], whereas human GIP(3–30)NH₂ was able to reduce exogenous GIP-induced insulin secretion by at least 82% in humans [95]. This emphasizes that the C-terminus of GIP plays a role in GIPR antagonism as its absence improves the antagonistic action of the N-terminally truncated GIP peptides [66]. The lack of impact for the C-terminus tail is also corroborated by GIP variants in which the C-terminus of exendin(1–39)
were inserted in exchange for the GIP C-terminus with lipitations to improve protein binding and thereby half-life extension [99].

4. Conclusion

Based on the current literature it is clear that endogenous GIP peptides are suitable tools in the study of human GIP physiology/pathophysiology. Furthermore, due to the lack of success in designing small molecules targeting class B1 receptors, as opposed to the huge number of small molecule drugs developed for class A GPCRs, peptide-based GIPR ligands could be future therapeutics. The N-terminus of the GIP peptide is essential for receptor activation and N-terminal truncation leads to decreased or even lost intrinsic activity (similar to GLP-1 and GLP-2). The C-terminal extension of GIP, as compared to GLP-1, GLP-2 and glucagon, has no apparent interaction with the GIPR and may be important for properties such as stability, tissue accessibility and tissue specific signaling. Modifications of the C-terminus minimally affect receptor activation, while its absence improves the antagonistic action for the GIPR. As such, nature has created a tool box of peptide agonists and antagonists that can be exploited from a pharmaceutical point of view, as was the case for the discovery of the successful agonsists based on GLP-1 [100,101]. In addition, a large number of chimeric dual or even triple acting compounds have already been designed on the basis of the native peptides and their receptor interactions [19,20,102–105].

Declaration of Competing Interest

MBNG, W.CvdV, FXS, LSG, and MMR declare that they have no conflict of interest.

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