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GIP(3–30)NH₂ is a potent competitive antagonist of the GIP receptor and effectively inhibits GIP-mediated insulin, glucagon, and somatostatin release

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Abstract

Alternative processing of the precursor protein pro-GIP results in endogenously produced GIP(1–30)NH₂, that by DPP-4 cleavage in vivo results in the metabolite GIP(3–30)NH₂. We showed previously that GIP(3–30)NH₂ is a high affinity antagonist of the human GIPR in vitro. Here we determine whether it is suitable for studies of GIP physiology in rats since effects of GIP agonists and antagonists are strictly species-dependent. Transiently transfected COS-7 cells were assayed for cAMP accumulation upon ligand stimulation or assayed in competition binding using human¹²⁵I-GIP(1–42) as radioligand. In isolated perfused rat pancreata, insulin, glucagon, and somatostatin-releasing properties were evaluated. Competition binding demonstrated that on the rat GIP receptor (GIPR), rat GIP(3–30)NH₂ bound with high affinity (K_i of 17 nM), in contrast to human GIP(3–30)NH₂ (K_i of 250 nM). In cAMP studies, rat GIP(3–30)NH₂ inhibited GIP(1–42)-induced rat GIPR activation and schild-plot analysis showed competitive antagonism with a pA₂ of 13 nM and a slope of 0.9 ± 0.09. Alone, rat GIP(3–30)NH₂ displayed weak, low-potent partial agonistic properties (EC₅₀ >1 µM) with an efficacy of 9.4% at 0.32 µM compared to GIP(1–42). In perfused rat pancreata, rat GIP(3–30)NH₂ efficiently antagonized rat GIP(1–42)-induced insulin, somatostatin, and glucagon secretion. In summary, rat GIP(3–30)NH₂ is a high affinity competitive GIPR antagonist and effectively antagonizes GIP-mediated G protein-signaling as well as pancreatic hormone release, while human GIP(3–30)NH₂, despite a difference of only one amino acid between the two (arginine in position 18 in rat GIP(3–30)NH₂; histidine in human), is unsuitable in the rat system. This underlines the importance of species differences in the GIP system, and the limitations of testing human peptides in rodent systems.

1. Introduction

GIP(1–42) is known as a postprandial gut hormone secreted from enteroendocrine K cells of the small intestine [1] together with other gut hormones [2,3]. Following a meal, GIP(1–42) enters the circulation and potentiates glucose-mediated insulin secretion from the pancreas [4]. Additional pancreatic effects may include stimulation of glucagon secretion from the α-cells [5,6] and somatostatin release from δ-cells [7,8]. The GIP receptor (GIPR) is widely expressed in various tissues besides the pancreas including adipose, bone, and lung tissue [9,10]. Particularly, the relationship between adipose tissue biology and the GIP system has received much interest. GIPR knock out mice are resistant to diet-induced obesity and crossing this mouse with the leptin mutant (ob/ob) mouse, which is an established mouse model for hyperphagic obesity, reduced weight gain by 23% [11], whereas transgenic GIPR expression in adipose tissue in global GIPR knock out mice restores diet-induced body weight gain [12]. Moreover, a recent study showed that heterogeneous abrogation of the GIP gene displays

Abbreviations: BSA, bovine serum albumin; DPP-4, dipeptidyl peptidase-4; GIP, glucose-dependent insulinotropic polypeptide; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP-1, glucagon-like peptide-1; HBS, HEPES-buffered saline.

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an intermediate phenotype in regard to high fat diet-induced insulin resistance and weight gain when compared to wild type and homogenous abrogation [13]. If GIP’s physiology in rodents is mirrored in humans, these results support the use of GIPR antagonists as potential therapeutics for the treatment of obesity.

Various strategies have been pursued in the search for GIPR antagonists. Antibodies raised against both GIP(1–42) [14,15] or the GIPR [16,17], a small molecule antagonist [18], amino acid substitutions of GIP(1–42) [19], and various GIP(1–42) truncations and modifications such as e.g. Pro3(GIP) [20–24] have all been reported to be effective, but none have been found suitable for human studies. In 2006, we showed that the dipeptidyl peptidase-4 (DPP-4)-mediated metabolite, porcine GIP(3–42), antagonized porcine GIP (1–42)-mediated cAMP accumulation, but had no antagonistic effects in anesthetized pigs at physiological concentrations [22]. Recently, an alternative processing of the precursor protein pro-GIP was shown to occur in the α-cells of the pancreas and in a subset of the K-cells of the small intestine, which potentially leads to the secretion of GIP(1–30)NH2 [25,26]. We combined the previously reported N-terminal truncation GIP(3–42) with this C-terminally truncated GIP(1–30)NH2 to design the GIP(3–30)NH2, which is a naturally occurring metabolite of the DPP-4 cleaved GIP(1–30)NH2, and demonstrated that GIP(3–30)NH2 is an effective competitive antagonist on the human GIPR [27]. In fact, it was superior to other truncations of the N-terminus (GIP(2–, 4–, 5–, 6–, 7–, 8–, and 9–30)NH2) and to GIP(1–42) in terms of basic binding affinity and antagonistic properties of the human GIPR in vitro. In the present study, we determine whether GIP(3–30)NH2 is sufficiently active in the rat model system to be used for studies elucidating the role of GIP in physiology and pathophysiology.

2. Materials and methods

2.1. Materials

Rat GIP(1–42) (cat. No. 027-12) was purchased from Phoenix Pharmaceuticals, Karlsruhe, Germany. Human GIP(1–42) (H5645) was purchased from Bachem, Bubendorf, Switzerland. Human and rat GIP(3–30)NH2 and GIP(1–30)NH2 were synthesized by CASLO ApS, Lyngby, Denmark. All peptides had a purity of more than 95% by HPLC analysis and had the correct mass spectrometry controlled molecular weight. cDNAs of the human and rat GIPR were purchased from Origene, Rockville, Maryland, USA (SC110906, RN212314, and MC216211, respectively) and cloned into the pCMV-Script vector. Human 125I-labeled GIP(1–42) and 125I-labeled Tyr11-somatostatin were purchased from PerkinElmer Life Sciences, Skovlunde, Denmark (NEX402 and NEX389, respectively). 125I-labeled glucagon and human 125I-insulin were kind gifts from Novo Nordisk A/S.

2.2. Animals

All animal care and experimental procedures were complied with institutional guidelines and approved by the Danish Animal Experiments Inspectorate (2013-15-2934-00833). Studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [28].

Male Wistar rats (220–250 g) were purchased from Janvier, Le Genest-Saint-Isle, France. The animals were housed in plastic-bottomed wire-lidded cages in air-conditioned (21 °C) and humidity controlled (55%) rooms with a 12:12 h light-dark cycle and free access to standard rat chow and water. Animals were acclimatized for at least one week before use.

2.3. Transfections and tissue culture

COS-7 cells were cultured at 10% CO2 and 37 °C in Dulbecco’s modified Eagle’s medium 1885 supplemented with 10% foetal bovine serum, 2 mM glutamine, 180 units/ml penicillin, and 45 g/ml streptomycin. Transient transfection of the COS-7 cells for cAMP accumulation and competition binding was performed using the calcium phosphate precipitation method with the addition of chloroquine [29].

2.4. cAMP assay

Transiently transfected COS-7 cells were seeded in white 96-well plates at a density of 3 × 103 cells/well. One day after, the cells were washed twice with Hepes-buffered saline (HBS) buffer and incubated with HBS and 1 mM 3-isobutyl-1-methylxanthine (IBMX) for 30 min at 37 °C. To test agonists, ligands were added and incubated for 30 min at 37 °C. In order to test for antagonistic properties, the cells were preincubated for 10 min with the antagonist with subsequent addition of the agonist and incubated for a further 20 min. The HitHunter™ cAMP XS assay (DiscoveRx, Herlev, Denmark) was carried out according to the manufacturer’s instructions.

2.5. Competition binding assay

COS-7 cells were seeded in clear 96-well plates the day after transient transfection. The number of cells added per well was governed by the apparent expression efficiency of the receptor, aiming for 5–10% specific binding of the radioactive ligand. The following day, cells were assayed by competition binding for 4 h at 4 °C using 15–40 PM of 125I-labeled GIP(1–42) as well as unlabeled ligand in a total volume of 100 μl per well in 50 mM Hepes buffer (pH 7.2) supplemented with 0.5% bovine serum albumin (BSA) (binding-buffer). After incubation, the cells were washed twice in 100 μl per well ice-cold binding buffer and lysed using 175 μl per well of 200 mM NaOH with 1% SDS for 30 min. Nonspecific binding was determined as the binding in the presence of 100 nM unlabeled ligand. The samples were analysed by the Wallac Wizard 1470 Gamma Counter.

2.6. Isolated perfused rat pancreas

Male Wistar rats (220–250 g) were anaesthetized (0.0158 mg fentanyl citrat + 0.5 mg fluanisone + 0.25 mg midazolam/100 g; Pharmacy Service, Denmark) and the pancreas was dissected and perfused in situ as described previously [22]. Briefly, the pancreas was perfused in a single-pass system through both the coeliac and the superior mesenteric artery via a catheter inserted into the aorta. All other aortic branches were ligated. The venous effluent was collected for 1 min intervals via a catheter in the portal vein, and stored at −20 °C until analysis. The pancreas was perfused with a modified Krebs Ringer bicarbonate buffer containing in addition of 5% dextran (Pharmacosmos, Holbaek, Denmark), 0.1% BSA, fumarate, glutamate, and pyruvate (5 mM of each), and 7 mM glucose. Flow rate was kept constant at 4 ml/min, perfusion buffer was heated and oxygenated (95% O2, 5% CO2), and pressure was continuously measured throughout the experiment. Rat GIP (3–30)NH2 and rat GIP(1–42) were infused as test substances through a sidearm infusion pump at a flow rate of 0.2 ml/min. Arginine (10 mM) was infused at the end of each experiment as a positive control.
2.7. Hormone analysis

Hormone concentrations in the perfusion effluent were measured using in-house radioimmunoassays. Glucagon was measured using a side viewing antiserum (code no 4304) recognizing a modified sequence of glucagon, using synthetic glucagon for standards and 125I-labeled glucagon as tracer [30]. Insulin was measured using an antibody cross-reacting strongly with rat insulin I and II (code no. 2006–3). As standard we used human insulin and the tracer was 125I-labeled human insulin [31]. Somatostatin concentrations were determined using a rabbit antiserum (code no. 1758) raised against synthetic cyclic somatostatin, recognizing both somatostatin 14 and -28 [32], somatostatin 14 as standard and 125I-labeled Tyr11-somatostatin as tracer.

2.8. Data- and statistical analysis

IC50, EC50, and Ki values were determined by nonlinear regression using GraphPad Prism 7 (San Diego, California, United States of America). Sigmoid curves were fitted logistically with a Hill slope of 1.0 for the activation curves and -1.0 for the inhibition of CAMP and binding. Ki values were calculated using the Cheng-Prusoff formula under the assumption of one class of binding sites. Dose ratios (DR) for the Schild analyses were based on the potency shift of rat GIP(1–42) in the presence of a given rat GIP(3–30)NH2 concentration, relative to the absence of GIP(3–30)NH2. Schild plots were performed with log(antagonist concentration, relative to the absence of GIP(1–42)) (ordinate) and log(antagonist concentration) (abscissa) to estimate the slopes and Ki values. For the rat pancreas perfusion data, baseline subtracted hormone output responses were evaluated using one-way ANOVA for repeated measurements. All calculations were performed using the software GraphPad Prism 7 with p-values <0.05 being considered significantly different.

2.9. Sequence alignments

The amino acid sequences of the rat/human GIP were acquired from GenBank of NCBI. The alignment was done in Geneious 6.0.5 using MAFFT v6.814b. The BLOSUM62 matrix was applied with gap open penalty and offset value of 1.53 and 0.123, respectively. The sequence logo was generated using the web-based program WebLogo (http://weblogo.berkeley.edu) and the various mammalian GIP sequences were acquired from ensembl.org and uniprot.org.

3. Results

3.1. Human GIP(3–30)NH2 displays a surprisingly low affinity on the rat GIPR compared to rat GIP(3–30)NH2

In order to determine whether the double truncation of GIP(1–42), which leads to GIP(3–30)NH2, is an effective antagonist in vivo using the rat as a model system, we initially evaluated the affinity of the ligand in vitro. Competition binding was conducted on transiently transfected COS-7 cells expressing the rat GIPR with 125I-labeled GIP(1–42) as the radioligand. GIP(1–30)NH2 was included to enable assessment of the significance of the C-terminus in terms of GIPR binding. In light of our recent study identifying major interspecies differences between rodents and humans within the GIP system [24], both rat and human GIP(1–42), GIP(1–30)NH2, and GIP(3–30)NH2 were included. Human and rat GIP(1–42) were found to bind to the rat GIPR with equally high affinities (Ki of 1.1 nM for human GIP(1–42) and Ki of 0.88 nM for rat GIP(1–42)) (Fig. 1A). In contrast, rat GIP(1–30)NH2 displayed a statistically significant improved affinity compared to human GIP(1–30)NH2 with Ki of 0.4 and 1.5, respectively (Fig. 1B). This species difference became even more pronounced for GIP(3–30)NH2 which showed a 15-fold shift in affinity. For rat GIP(3–30)NH2, the Ki was 17 nM, which is a 19-fold reduction compared to rat GIP(1–42). In contrast, human GIP(3–30)NH2 had a Ki of 250 nM and thus a 227-fold lower affinity for the rat GIPR compared to human GIP(1–42). When looking at the sequence differences between species (Fig. 1D), only one amino acid (position 18 with arginine in rat and histidine in human GIP) differs between rat and human GIP(1–30)NH2/GIP(3–30) NH2. In fact, among the 42 sequences of GIP identified so far, a histidine is found at this position in all human and non-human primates (10 sequences), whereas GIP in the remaining 32 species has an arginine (Fig. 2A, B). Importantly, this alteration of position 18 had a large effect on the binding properties of GIP(3–30)NH2, a minor effect on GIP(1–30)NH2 binding, whereas it did not affect the binding affinity of GIP(1–42).

3.2. GIP(3–30)NH2 is an antagonist of the rat GIPR

To investigate whether the high affinity of GIP(3–30)NH2 reflects high antagonistic potency, as observed in the human GIP system [27], we chose the GIPR-induced cAMP accumulation, a well-established signaling pathway for GIPR activation [33,34], in transiently transfected COS-7 cells. GIP(1–42) and GIP(1–30)NH2 were included to examine whether both forms activate the rat GIPR in a similar manner. For GIP(3–30)NH2, the evaluation was conducted both in the absence and presence of rat or human GIP (1–42) in amounts corresponding to ~60% Emax. As previously shown [24], rat GIP(1–42) was more potent and efficacious on the rat GIPR compared to human GIP(1–42) with EC50 values of 11 and 58 pM and Emax values of 100 and 76%, respectively (Fig. 3A). In contrast, rat and human GIP(1–30)NH2 were more similar to EC50 values of 18 and 38 pM and Emax values of 92 and 87%, respectively (Fig. 3B). Due to the higher potency and efficacy of rat GIP(1–42) compared to human GIP(1–42), 10 pM and 316 pM were chosen to achieve ~60% of Emax for the evaluation of GIP(3–30)NH2 antagonism of rat and human GIP(1–42), respectively. In the absence of GIP(1–42), rat GIP(3–30)NH2 displayed a low-potent partial agonistic profile with an efficacy of 9.4% at 0.32 μM (Fig. 3C), while no agonistic properties were observed for human GIP(3–30)NH2 even at the highest concentration (0.32 μM). In the presence of rat GIP(1–42), rat GIP(3–30)NH2 antagonized rat GIPR-induced cAMP accumulation dose-dependently with an estimated EC50-value of 118 nM. A similar pattern was observed for human GIP(3–30)NH2 which dose-dependently inhibited human GIP(1–42), however, with an estimated EC50-value of 380 nM (Fig. 3D). Thus, rat GIP(3–30)NH2 is more potent as an antagonist on the rat GIPR than human GIP(3–30)NH2, a pattern that mimicked the low affinity obtained for human GIP(3–30)NH2 as compared to the rat counterpart (Fig. 1C), and rat GIP(3–30)NH2 was therefore chosen for further investigation.

3.3. Rat GIP(3–30)NH2 is a high affinity competitive antagonist of the rat GIPR

To determine the nature of the antagonistic properties of rat GIP (3–30)NH2 on the rat GIPR, CAMP accumulation was measured as a function of increasing concentrations of rat GIP(1–42) in the absence or presence of fixed concentrations of rat GIP(3–30)NH2 (Fig. 4A). Rightward shifts in potency of rat GIP(1–42) were observed with increased rat GIP(3–30)NH2 concentration which is in line with the antagonistic properties (Fig. 3C). At concentrations from 17.8 to 316 nM of rat GIP(3–30)NH2, the potency (EC50) of rat GIP(1–42) decreased 2.6 to 28-fold compared to the absence of rat GIP(3–30)NH2. Based on these EC50 values for

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rat GIP(1–42), a Schild plot analysis was conducted (Fig. 4B). This analysis determines whether an antagonist acts competitively; if so, the equilibrium inhibitory constant (K_i) can be determined from the X-axis intercept (pA_2). A straight line relating the potency shifts to the antagonist concentration, which still elicited a prominent release of each of the three pancreatic hormones of interest, was determined in the perfusion model. Three different concentrations of rat GIP(1–42) were tested (10 pM, 100 pM and 1 nM). From these experiments, 1 nM rat GIP(1–42) was chosen due to a significant release of all three hormones at this concentration (data not shown). 1

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rat GIP(1–42) was chosen due to a significant release of all three hormones at this concentration (data not shown). 1 nM rat GIP(1–42) was quite potent and rat GIP(3–30)NH_2 was for any of the hormonal responses during the single perfusion with rat GIP(3–30)NH_2 (Fig. 5A–C). Thus, a significant GIPR antagonism by GIP(3–30)NH_2 on pancreatic insulin, glucagon, and somatostatin secretion was confirmed (Fig. 5A–C, total output shown as columns).

4. Discussion

Our study demonstrates that rat GIP(3–30)NH_2 is a high affinity competitive antagonist on the rat GIPR in vitro and in the surviving perfused rat pancreas, whereas human GIP(3–30)NH_2 displays much lower affinity and a consequent lower antagonistic potency on the rat GIPR. This indicates that human GIP(3–30)NH_2 is irrelevant in the rat GIP system, whereas rat GIP(3–30)NH_2 can be used as a tool to study the GIP physiology when using the rat as a model system. To substantiate this, we show that rat GIP(3–30)NH_2 inhibits GIP(1–42)-mediated hormone release from the intact pancreas as evident from the strong inhibition of GIP(1–42)-mediated insulin, glucagon, and somatostatin release from β-, α-, and δ-cells of the pancreas (Fig. 5). This establishes GIP(3–30)NH_2 as an effective antagonist in a physiological system.

4.1. The C-terminal truncation improves antagonistic properties of GIP (3–30)NH_2 versus GIP(3–42), but not agonism of GIP(1–30)NH_2 versus GIP(1–42)

The function of the 12 amino acids of the C-terminus of GIP(1–42) remains elusive. We previously showed that human GIP(3–30) NH_2 inhibited the human GIPR more potently compared to human GIP(3–42) [27]. Moreover, a recent study reported that palmitoylated human GIP(3–30)NH_2Cex (where Cex is a C-terminal extension of exendin) was able to antagonize GIP(1–42)-mediated insulin release in vitro from a rat β-cell line (BRIN-BD11 cells) [35]. However, when comparing the corresponding agonists (GIP(1–42) and GIP(1–30)NH_2) the differences are indistinguishable in terms of cAMP accumulation [27,36–38]. From a physiological perspective, both molecular forms have been shown to stimulate insulin secretion [26,39] and β-cell survival equipotently [40]; however, GIP(1–30)NH_2 has a reduced effect on lipoprotein lipase...
activity [40] and a reduced inhibitory effect on gastric acid secretion in rats [41]. In addition, a controversy exists in terms of gastric somatostatin release as GIP(1–30)NH₂ has been shown to be equipotent in mice [25] and less potent in rats [42], compared to GIP(1–42). In structure-activity studies, all the pivotal amino acids involved in the GIPR interaction are found within the first 30 amino acids [38,43], with the C-terminal part initiating binding with the extracellular receptor domains (ECD) while the N-
of this, the truncations GIP(15–30)NH₂ and GIP(15–30) still retain receptor segments [43,44]. Particularly important GIP(1–42) terminal part is thought to interact with the transmembrane receptor segments [43,44]. Particularly important GIP(1–42) residues involved in the binding to the ECD include Phe22, Val23, and Tyr1/C24. Following binding of GIP(1–42)’s C-terminal to the ECD, Tyr1 appears to interact with multiple amino acids of the transmembrane receptor segments of the GIPR [45,46] and is pivotal for receptor activation [47], which is in line with the inactivation of GIP by DPP-4 and with the present study showing GIP(3–30)NH₂ as an effective antagonist [22,48]. Furthermore, alanine screening of the N-terminus of human GIP(1–42) has identified Glu3, Gly4, Thr5, and Ile7 as highly important for GIP(1–42)-induced insulin secretion in a rat cell line [47]. This so-called two step receptor activation not only describes GIPR activation, but is thought as a general activation mechanism for secretin-like receptors [44,49–52], and even for receptors outside the secretin-like receptors, such as the chemokine receptors, which belong to the class of rhodopsin-like receptors [44,53]. Moreover, when determining the degree of conservation of GIP between mammalian species, most of the variation is found among residues 30–42 (Fig. 2B). In the present study, we found that human and rat GIP(1–42) bound with equal affinity to the rat GIPR, and only minor differences between human and rat GIP(1–30)NH₂ were observed. As previously shown, rat GIP(1–42) had greater agonistic potency and efficacy with respect to cAMP accumulation compared to human GIP(1–42) on the rat GIPR [24]. Surprisingly, a similar species difference was not observed between human and rat GIP(1–30)NH₂ indicating that the activity via G₄₃₅₃ is independent of whether an arginine (rat) or a histidine (human) is found in position 18. When looking at the rat ligands only, GIP(1–42) and GIP(1–30)NH₂ activated the rat GIPR in a completely identical manner confirming that the C-terminus does not have an impact on the agonistic properties. This is in agreement with previous work showing that human GIP(1–42) and GIP(1–30)NH₂ activate the human GIPR in an identical manner [27].

4.2. Position 18 impacts the antagonistic potential of GIP(3–30)NH₂ on the rat GIPR

In contrast to the single C-terminally truncated GIP(1–30)NH₂, the double truncated GIP(3–30)NH₂ showed species-dependent variation as the rat GIP(3–30)NH₂ displayed both higher affinity and antagonistic potency of GIP-induced cAMP accumulation for the rat GIPR compared to human GIP(3–30)NH₂. Since position 18 is the only difference between human and rat GIP(3–30)NH₂, the N-terminal truncation very likely exposes position 18 differently to the extracellular GIPR binding domain and thereby explains the shift in affinity. When taking a closer look at the GIP sequence among 41 species, most variations are observed in the C-terminal region (Fig. 2) and in comparison to the other incretin hormone, Glucagon-Like Peptide-1 (GLP-1), GIP is much less conserved between species [54]. However, position 18 remains conserved showing only a conservative substitution between histidine (in primates) and arginine (in rodents) - providing stronger antagonism with an arginine at this position. Confirming this, we observed a better antagonistic potency with porcine GIP(3–42), (arginine at position 18) compared to human GIP(3–42) (histidine at position 18) on the human GIPR [27]. Thus despite another variation in position 34 (serine vs. asparagine in pig vs. human, respectively), it is likely that this will also apply for the C-terminal truncated forms, such as GIP(3–30)NH₂.

4.3. Caution should be exercised when testing human GIP analogues in rodents

Researchers striving for the elucidation of human GIP physiology have used rodent in vivo models extensively. Much attention has been given to the presumed GIPR antagonist, (Pro3)GIP, in rodent models [19,55–57], however, we recently discovered that human (Pro3)GIP is a partial agonist on the rodent GIPRs but a full agonist on the human GIPR [24]. Thus, interspecies differences at both the receptor and ligand level are important to consider when evaluating the potential of a new compound, a fact that has been neglected in previous studies (Tables 1 and 2). In addition, there are many differences related to the organ of interest between researchers striving for the elucidation of human GIP physiology have used rodent in vivo models extensively. Much attention has been given to the presumed GIPR antagonist, (Pro3)GIP, in rodent models [19,55–57], however, we recently discovered that human (Pro3)GIP is a partial agonist on the rodent GIPRs but a full agonist on the human GIPR [24]. Thus, interspecies differences at both the receptor and ligand level are important to consider when evaluating the potential of a new compound, a fact that has been neglected in previous studies (Tables 1 and 2). In addition, there are many differences related to the organ of interest between.
rodents and humans, as for instance within the endocrine pancreas. This includes the topographical organization the rodent islets with the β-cells concentrated in the core surrounded by a mantle of α-cells and δ-cells. In contrast, the different cells of the human islet are highly dispersed [58–61], which may promote different potential paracrine effects and different intra-islet communication. On a structural level, our study emphasizes differences between GIPR, GIP(1–42), and GIP(3–30)NH₂ of rodent or human sequences (Figs. 1 and 3) and establishes that application of the DPP-4 metabolite of human GIP(1–30)NH₂, 1 nM rat GIP(1–42), 1 µM rat GIP(3–30)NH₂ or 10 mM arginine, n = 6 independent experiments. The glucose concentration was 7 mM and data are mean ± SEM. Baseline subtracted total outputs of insulin (A), glucagon (B), and somatostatin (C) during the 10 min infusion of rat GIP(1–42)/rat GIP(3–30)NH₂ are compared using a one-way ANOVA with multiple comparisons; mean ± S.E.M., ***P < 0.0001, **P < 0.01, and *P < 0.05.

Fig. 5. Antagonistic effects of rat GIP(3–30)NH₂ on insulin, glucagon, and somatostatin secretion are observed in perfused rat pancreata. Insulin (A), glucagon (B), and somatostatin (C) secretion following stimulation of perfused rat pancreata by either 1 nM rat GIP(1–42) and 1 µM rat GIP(3–30)NH₂ with a 5 min preincubation of 1 µM rat GIP(3–30)NH₂, 1 nM rat GIP(1–42), 1 µM rat GIP(3–30)NH₂ or 10 mM arginine, n = 6 independent experiments. The glucose concentration was 7 mM and data are mean ± SEM. Baseline subtracted total outputs of insulin (A), glucagon (B), and somatostatin (C) during the 10 min infusion of rat GIP(1–42)/rat GIP(3–30)NH₂ are compared using a one-way ANOVA with multiple comparisons; mean ± S.E.M., ***P < 0.0001, **P < 0.01, and *P < 0.05.

Conflicts of interest

A.H.S.U., M.N.G., L.S.G., C.B.C., B.S., B.H., and M.M.R. declare that they have no conflict of interest. J.J.H. has served as a consultant or advisor to Novartis Pharmaceuticals, Novo Nordisk, Merck Sharp & Dohme, and Roche and has received fees for lectures from Novo Nordisk, Merck Sharp & Dohme, and GlaxoSmithKline.
Overview of truncated GIP variants tested on the rat GIPR. The table displays an overview of truncated GIP variants of various different species tested on the rat GIPR both in vitro and in vivo. Percentages compared to full agonist GIP(1–42) or GIP(1–30) results. Results showing no binding, agonism or antagonism are in italic. GIP = species sequence of ligand, B = bovine, P = porcine, Hu = human, US = unknown species, UC = unknown concentration.

### Table 1: Overview of truncated GIP variants tested on the rat GIPR

<table>
<thead>
<tr>
<th>Truncation</th>
<th>GIP Species</th>
<th>Function</th>
<th>Binding</th>
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<tbody>
<tr>
<td>(3–30)NH2</td>
<td>R</td>
<td>COS cells: cAMP, antagonism (IC50 120 nM), low partial agonist, Emax 9.4% activity (0.32 μM) vs. GIP(1–42)</td>
<td>COS cells: IC50 17nM</td>
</tr>
<tr>
<td>(6–30)NH2</td>
<td>P</td>
<td>CHO cells: cAMP: 58% inhibition (0.1 μM)</td>
<td>CHO cells: Equal IC50 to GIP(1–42)</td>
</tr>
<tr>
<td>(7–30)NH2</td>
<td>P</td>
<td>CHO cells: 25% cAMP inhibition (0.1 μM)</td>
<td>CHO cells: IC50 24 nM</td>
</tr>
<tr>
<td>(10–30)</td>
<td>P</td>
<td>CHO cells: 10 μM decreases GIP(1–42): cAMP by 34%</td>
<td>CHO cells: IC50 177 nM</td>
</tr>
<tr>
<td>(15–30)NH2</td>
<td>P</td>
<td>CHO cells: 50% inhibition in cAMP (10 μM)</td>
<td>CHO cells: 187 fold decreased IC50</td>
</tr>
<tr>
<td>(16–30)NH2</td>
<td>P</td>
<td>CHO cells: cAMP, antagonism (10 μM): 30% of GIP(7–30)</td>
<td>CHO cells: IC50 1400 nM</td>
</tr>
<tr>
<td>(17–30)NH2</td>
<td>P</td>
<td>CHO cells: cAMP, antagonism (10 μM): 20% of GIP(7–30)</td>
<td>CHO cells: IC50 2530 nM</td>
</tr>
<tr>
<td>(19–30)NH2</td>
<td>P</td>
<td>CHO cells: 40% cAMP production (20 μM)</td>
<td>CHO cells: 52% binding (10 μM)</td>
</tr>
<tr>
<td>(21–30)NH2</td>
<td>P</td>
<td>CHO cells: No cAMP (1 μM)</td>
<td>CHO cells: No binding (1 μM)</td>
</tr>
<tr>
<td>(3–42)</td>
<td>US</td>
<td>BRIN-BD11: 30% cAMP (EC50 ~1 nM) and 1 μM inhibits 45%. 70% inhibition of insulin release by 1 μM</td>
<td>CHO cells: IC50 1270 nM</td>
</tr>
<tr>
<td>(4–42)</td>
<td>US</td>
<td>BRIN-BD11: 50% cAMP, 30% inhibition of insulin (1 μM)</td>
<td>CHO cells: IC50 138nM</td>
</tr>
<tr>
<td>(6–42)</td>
<td>US</td>
<td>BRIN-BD11: 35% cAMP (1 μM), insulin inhibition 70%</td>
<td>CHO cells: IC50 200 nM</td>
</tr>
<tr>
<td>(7–42)</td>
<td>US</td>
<td>BRIN-BD11: 80% cAMP (1 μM), insulin inhibition 40%</td>
<td>CHO cells: IC50 2.0 nM</td>
</tr>
<tr>
<td>(8–42)</td>
<td>US</td>
<td>RINm5F: 30-fold decrease of GIP(1–42) potency (5 μM)</td>
<td>CHO cells: IC50 24 nM</td>
</tr>
<tr>
<td>(9–42)</td>
<td>US</td>
<td>BRIN-BD11: 16% cAMP (1 μM), insulin inhibition 65%</td>
<td>CHO cells: IC50 1540 nM</td>
</tr>
<tr>
<td>(15–42)</td>
<td>P</td>
<td>CHO cells: cAMP, antagonism (10 μM): 40% of GIP(7–30)</td>
<td>CHO cells: IC50 177 nM</td>
</tr>
<tr>
<td>(17–42)</td>
<td>B</td>
<td>Pancreas perfusion: 32% insulin response (5 ng/ml)</td>
<td>CHO cells: IC50 1270 nM</td>
</tr>
<tr>
<td>(31–44)</td>
<td>US</td>
<td>CHO cells and L293 cells: No cAMP or GIP inhibition (10 μM)</td>
<td>CHO cells: IC50 0.4 μM</td>
</tr>
<tr>
<td>(3–39)</td>
<td>B</td>
<td>Pancreas: Full insulin response (1 nmol/kg/h)</td>
<td>CHO cells: IC50 20.0 nM</td>
</tr>
<tr>
<td>(1–39)</td>
<td>B</td>
<td>Pancreas: Full insulin response (1 nmol/kg/h)</td>
<td>RINm5F: IC50 10-fold decreased IC50</td>
</tr>
<tr>
<td>(1–30)OH</td>
<td>Hu</td>
<td>Pancreas: Full insulin response (1 nmol/kg/h)</td>
<td>CHO cells: IC50 4.0 μM</td>
</tr>
<tr>
<td>(1–39)</td>
<td>P</td>
<td>Pancreas perfusion: Full insulin response (1 μM)</td>
<td>CHO cells: IC50 2.0 nM</td>
</tr>
</tbody>
</table>

### Species Function Binding References

- **BRIN-BD11**: Inhibition of insulin, IC50 138nM
- **RINm5F**: 40% insulin inhibition (10 ng/ml) IC50 1270 nM
- **CHO cells**: 187 fold decreased IC50
- **CHO cells**: IC50 1400 nM
- **CHO cells**: IC50 24 nM
- **CHO cells**: IC50 200 nM
- **CHO cells**: IC50 1540 nM
- **CHO cells**: IC50 177 nM
- **BRIN-BD11**: 30% cAMP (EC50 ~1 nM)
- **BRIN-BD11**: 50% cAMP, 30% inhibition of insulin (1 μM)
- **BRIN-BD11**: 35% cAMP (1 μM), insulin inhibition 70%
- **BRIN-BD11**: 80% cAMP (1 μM), insulin inhibition 40%
- **BRIN-BD11**: 65% cAMP (1 μM), insulin inhibition 55%
- **BRIN-BD11**: 60% insulin release (1 ng/ml) IC50 1270 nM
- **CHO cells**: IC50 1270 nM
- **CHO cells**: IC50 177 nM
- **CHO cells**: IC50 1400 nM
- **CHO cells**: IC50 24 nM
- **CHO cells**: IC50 200 nM
- **CHO cells**: IC50 1540 nM
- **CHO cells**: IC50 177 nM
- **CHO cells**: IC50 177 nM
**Table 2**
Overview of truncated GIP variants tested on various GIPR species. The table displays an overview of truncated GIP variants of various different species tested on different species of GIPR both in *vitro* and *in vivo*. Percentages comparing full agonist GIP(1-42) or GIP(1-30) results. Results showing no binding, agonism or antagonism are in italic. GIP = species sequence of ligand, Hu = human, P = porcine, B = bovine, M = mouse, Ha = hamster, US = unknown species.

<table>
<thead>
<tr>
<th>Truncation</th>
<th>GIP</th>
<th>GIPR from other species (human, mouse, porcine, bovine, hamster)</th>
<th>Function</th>
<th>Binding</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>N- and C-terminal (mainly antagonists)</td>
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<tr>
<td>(2–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: 20% efficacy in cAMP, EC₅₀ of 3.7 nM, Antagonism (1 μM): 50 % inhibition of GIP(1–42) with EC₅₀ of 22 nM</td>
<td>COS cells: IC₅₀ of 14 nM</td>
<td>[27]</td>
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<tr>
<td>(3–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Antagonism (0.1 μM): 100 % inhibition of GIP(1–42) with EC₅₀ of 12 nM</td>
<td>COS cells: IC₅₀ of 2.3 nM</td>
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<tr>
<td>(4–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Antagonism (1 μM): 50 % inhibition of GIP(1–42) with EC₅₀ of 108 nM</td>
<td>COS cells: IC₅₀ of 22 nM</td>
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<tr>
<td>(5–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Antagonism (0.1 μM): 85 % inhibition of GIP(1–42) with EC₅₀ of 12 nM</td>
<td>COS cells: IC₅₀ of 5.9 nM</td>
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<td>(6–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Antagonism (0.1 μM): 75 % inhibition of GIP(1–42) with EC₅₀ of 342 nM</td>
<td>COS cells: IC₅₀ of 347 nM</td>
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<tr>
<td>(7–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Antagonism (0.1 μM): 75 % inhibition of GIP(1–42) with EC₅₀ of 137 nM</td>
<td>COS cells: IC₅₀ of 26 nM</td>
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<tr>
<td>(8–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: No intrinsic cAMP, Antagonism (0.1 μM): 50 % inhibition of GIP(1–42) with EC₅₀ of 133 nM</td>
<td>COS cells: IC₅₀ of 79 nM</td>
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<tr>
<td>(9–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: No intrinsic cAMP, Antagonism (0.1 μM): 25 % inhibition of GIP(1–42) with EC₅₀ of 450 nM</td>
<td>COS cells: IC₅₀ of 307 nM</td>
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<tr>
<td>(17–30)NH₂</td>
<td>US</td>
<td>US</td>
<td>CHO cells: 1 μM inhibits cAMP (0.1 μM GIP) 15 % BRIN-BD11: 1 μM inhibits insulin (0.1 μM GIP) 11 %</td>
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<tr>
<td>(18–28)</td>
<td>Hu</td>
<td>P</td>
<td>CHO cells: No binding (1 μM)</td>
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<td>[65]</td>
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<tr>
<td>(19–30)NH₂</td>
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<td>Ha</td>
<td>Pancreas membranes: No binding</td>
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<tr>
<td>(21–26)</td>
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<td>P</td>
<td>CHO cells: No binding (1 μM)</td>
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<td>[65]</td>
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<td>N-terminal (mainly antagonists)</td>
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<tr>
<td>(3–42)</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: cAMP, antagonism (1μM): 60 % inhibition of GIP(1–42) with EC₅₀ of 671 nM</td>
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<td></td>
<td>P</td>
<td>Hu</td>
<td>COS cells: cAMP, antagonism (1μM): 75 % inhibition of GIP(1–42) with EC₅₀ of 32 nM</td>
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<td>Anaesthetized pig: No inhibitory effect on insulinoetropic effects of GIP</td>
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<td>(4–42)</td>
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<td>Ha</td>
<td>Pancreas membranes: IC₅₀ of 5 nM</td>
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<tr>
<td></td>
<td>US</td>
<td>US</td>
<td>CHO cells: 1 μM inhibits cAMP (0.1 μM GIP) 40 % BRIN-BD11: 1 μM inhibits insulin (0.1 μM GIP) 23 %</td>
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<td>Ob/ob: Glucose increase (25 nmol/kg)</td>
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<td>US</td>
<td>M</td>
<td>Ob/ob: Glucose increase (25 nmol/kg)</td>
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<td>Ob/ob: Glucose increase (25 nmol/kg)</td>
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<td>Ob/ob: Glucose increase (25 nmol/kg)</td>
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<td></td>
<td>(7–42)</td>
<td>US</td>
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<td>Ob/ob: Glucose increase (25 nmol/kg)</td>
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<td>Ob/ob: Glucose increase, sig. insulin decrease (25 nmol/kg)</td>
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<td>(13–42)</td>
<td>Hu</td>
<td>Hu</td>
<td>Titratin calorimetry: 6-fold lower affinity</td>
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<td>(15–42)</td>
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<td>Titratin calorimetry: 10-fold lower affinity</td>
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<td>Pancreas membranes: IC₅₀ 0.5 μM</td>
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<td>Hu</td>
<td>Titratin calorimetry: 15-fold lower affinity</td>
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<td>Titratin calorimetry: 17-fold lower affinity</td>
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<td>Titratin calorimetry: 100-fold lower affinity</td>
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<td>Titratin calorimetry: No binding</td>
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<td>Ha</td>
<td>Pancreas membranes: No binding (10 μM)</td>
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<td>[69]</td>
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<td>(1–16)</td>
<td>B</td>
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<td>Pancreas membranes: No binding (10 μM)</td>
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<td>M</td>
<td>Ob/ob: No effect on glucose/insulin (25 nmol/kg)</td>
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<td></td>
<td>(1–30)NH₂</td>
<td>Hu</td>
<td>Hu</td>
<td>COS cells: Full agonist (cAMP), EC₅₀ equal to GIP(1–42)</td>
<td>COS cells: IC₅₀ equal to GIP(1–42)</td>
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<td>(1–30)OH</td>
<td>Hu</td>
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<td>CHO cells: 11-fold lower affinity than GIP(1–42)</td>
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</table>

**Acknowledgements**

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**References**


antagonist (Pro3)GIP prevents the development of diabetes and related metabolic abnormalities associated with genetically inherited obesity in ob/ob mice, Diabetologia 50 (7) (2007) 1532–1540.


