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# GIP(3–30)$NH_2$ 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)$NH_2$, that by DPP-4 cleavage *in vivo* results in the metabolite GIP(3–30)$NH_2$. We showed previously that GIP(3–30)$NH_2$ 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 assessed for cAMP accumulation upon ligand stimulation or assayed in competition binding using human $^{125}$I-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)$NH_2$ bound with high affinity ($K_i$ of 17 nM), in contrast to human GIP(3–30)$NH_2$ ($K_i$ of 250 nM). In cAMP studies, rat GIP(3–30)$NH_2$ inhibited GIP(1–42)-induced rat GIPR activation and schild-plot analysis showed competitive antagonism with a $pA_2$ of 13 nM and a slope of 0.9 ± 0.09. Alone, rat GIP(3–30)$NH_2$ displayed weak, low-potent partial agonistic properties ($EC_{50}$ > 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)$NH_2$ efficiently antagonized rat GIP(1–42)-induced insulin, somatostatin, and glucagon secretion. In summary, rat GIP(3–30)$NH_2$ 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)$NH_2$, despite a difference of only one amino acid between the two (arginine in position 18 in rat GIP(3–30)$NH_2$; 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)$NH_2$ [25,26]. We combined the previously reported N-terminal truncation GIP(3–42) with this C-terminally truncated GIP(1–30)$NH_2$ to design the GIP(3–30)$NH_2$ (which is a naturally occurring metabolite of the DPP-4 cleaved GIP(1–30)$NH_2$), and demonstrated that GIP(3–30)$NH_2$ 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)$NH_2$) and to GIP(3–42) 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)$NH_2$ 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)$NH_2$ and GIP(1–30)$NH_2$ 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 $^{125}$I-labeled GIP(1–42) and $^{125}$I-labeled Tyr$^{11}$-somatostatin were purchased from PerkinElmer Life Sciences, Skovlunde, Denmark (NEX402 and NEX389, respectively). $^{125}$I-labeled glucagon and human $^{125}$I-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 acclimatised for at least one week before use.

### 2.3. Transfections and tissue culture

COS-7 cells were cultured at 10% $CO_2$ 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 * $10^4$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 $^{125}$I-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% $O_2$, 5% $CO_2$), and pressure was continuously measured throughout the experiment. Rat GIP (3–30)$NH_2$ 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.