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Functional elements of the gastric inhibitory polypeptide receptor: Comparison between secretin- and rhodopsin-like G protein-coupled receptors

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ABSTRACT

Innovative crystallographic techniques have resulted in an exponential growth in the number of solved G-protein coupled receptor (GPCR) structures and a better understanding of the mechanisms of class A receptor activation and G protein binding. The recent release of the type 1 receptor for the corticotropin-releasing factor and the glucagon receptor structures, two members of the secretin-like family, gives the opportunity to understand these mechanisms of activation in this family of GPCRs. Here, we addressed the comparison of the functional elements of class A and secretin-like GPCRs, using the glucose-dependent insulinotropic polypeptide receptor (GIPR) as a model receptor. Inactive and active models of GIPR permitted to select, by structural homology with class A GPCRs, several residues that may form key interactions presumably involved in receptor activation and Gs coupling, for pharmacological evaluation. Mutants on these amino acids were expressed in HEKT 293 cells and characterized in terms of GIP-induced cAMP production. We identified various functional domains spanning from the peptide-binding to the G protein pockets: including: a network linking the extracellular part of transmembrane (TM) 6 with TMs 2 and 7; a polar lock that resembles the ionic-lock in class A GPCRs; an interaction between TMs 3 and 7 that favors activation; and two clusters of polar/charged and of hydrophobic residues that interact with the C-terminus of the Gα. The results show that despite the low degree of sequence similarity between rhodopsin- and secretin-like GPCRs, the two families share conserved elements in their mechanisms of activation and G protein binding.

1. Introduction

G protein-coupled receptors (GPCRs) mediate nearly all human cellular responses to hormones and neurotransmitters and are the target of about 30% of currently marketed therapeutic agents [1]. GPCRs are grouped into six main classes (named A to F) according to sequence homology and functional similarity, with only four of them (A, B, C and F) present in vertebrates [2,3]. The class A or rhodopsin-like family is by far the largest and has received most of the attention. Class B GPCRs are a smaller group of receptors that consist of subfamilies B1 or secretin-like (the classical hormone receptors), B2 or adhesion receptors and B3 or Methuselah-like proteins [4]. Secretin-like receptors are promising targets in a large number of diseases such as chronic inflammation, neurodegeneration, diabetes, obesity, stress and osteoporosis [5,6].

All GPCRs share a common molecular architecture consisting of a 7 transmembrane (TM) helical bundle and common signaling mechanisms via G protein-dependent or independent pathways [7,8]. Recent crystallization of GPCRs in the presence of pharmacologically diverse ligands (with and without G protein or G protein mimics) provided structures of inactive or active forms for many class A GPCRs [9,10]. Recently, two research groups have elucidated the crystal structures of two members of the secretin family, the corticotropin releasing factor 1 (CRF_1R) [11] and the glucagon receptor (GCGR) [12], which add to the previously available (and growing) pool of class A structures. These new class B structures showed a remarkable structural similarity with class A despite the low degree of sequence homology. Importantly, they

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resolved the previous challenging task of aligning the sequences of the two families, facilitating the comparison between them. In this context, these structures enable to progress in the understanding of structure–function relationships within class B GPCRs.

The glucose-dependent insulinotropic polypeptide (GIP), a hormone that is released by the entero-endocrine K cells from the proximal duodenum, binds the class B1 GIP receptor (GIPR) to trigger Gs-mediated cAMP production and subsequent signaling cascades. GIPR stimulates insulin secretion from pancreatic β-cells after ingestion of nutrients and plays a role in lipid metabolism and fat deposition by increasing lipoprotein lipase activity, lipogenesis, fatty acid and glucose uptake in adipocytes [13–15]. These physiological actions make GIPR a potential pharmacological target in several metabolic diseases.

Here, we combine molecular modeling and mutagenesis experiments to identify functional motifs in GIPR as a model receptor of the secretin family. Because we have previously explored the determinants of GIP binding to GIPR [16], we focused on residues presumably involved in receptor activation and G protein binding. We assumed that the structural similarity between GPCRs belonging to classes A and B increases toward the cytoplasmic regions. For this purpose, we have modeled inactive and active states of GIPR and used them to predict residues and motifs spanning from the region close to the ligand binding pocket to the Gs binding domain. On the basis of these modeling predictions, single and double mutants of GIPR were constructed and functionally characterized. These results permitted us to identify several functional domains in GIPR, some of which are analogous to known class A functional domains. Overall, this study shows that despite the low degree of sequence similarity between class A and B GPCRs, the two families share conserved elements in their activation mechanisms.

2. Materials and methods

2.1. Chemicals

Fragment 1–30 of human GIP (termed GIP) and fragment 1–30 of human GIP having Tyr^1 substituted by Phe (termed Phe^1-GIP) was provided from Millegen (Toulouse, France). Fluorescent chemical DY647P1 dye was obtained from Dyomics GmbH Jena (Germany). DY647P1 labeled-GIP(1–30) (termed DY647-GIP) was obtained according to the procedure previously described [16]. Radio-labeled GIP was obtained by radio-iodination of Phe^1-GIP with ^{125}I-Na (Perkin Elmer, France) in the presence of chloramine T and was HPLC purified on a C-18 column as previously described for other labels [17].

2.2. Molecular models of the GIPR receptor

The inactive conformation of the human GIPR was modeled using the crystal structure of GCGR (PDB id 4L6R) [12] as a template, a secretin-like GPCR with 59% amino acid similarity within the TM domains. The active conformation of GIPR bound to the C-terminus of Gαs, its cognate-coupled G-protein, was modeled using both GCGR and the crystal structure of β_2-AR in complex with Gαs (PDB id 3SN6) [18], as templates. β_2-AR was used to model Gαs and the cytoplasmic half of TM 6, whereas the other elements of the receptor were modeled using GCGR. Modeller v9.13 [19] was used to build the initial homology models. Subsequently, these initial models were energy-minimized and subjected to molecular dynamics simulations in an explicit lipid bilayer. We used GROMACS software [20] for this purpose with the parameters and conditions described elsewhere [21]. Each system was simulated for 100 ns after an equilibration time of 21 ns.

2.3. Site-directed mutagenesis, cell culture and transfections

cDNA encoding short variant of the human GIPR cloned in the pcDNA3 vector was derived from a plasmid kindly given by Professor Bernard Thorens (Lausanne, Switzerland)[15]. All GIPR mutants were constructed by oligonucleotide-directed PCR based mutagenesis. The presence of desired mutation and the absence of undesired mutation were confirmed by automated sequencing of the complete GIPR coding sequence. HEKT 293 cells were maintained in Dulbecco's Modified Eagle's medium supplemented with 10% of FBS, in a humidified atmosphere at 95% air and 5% CO_2. Transfections were performed using polyethylenimine (PEI) transfection reagent (1 mg/mL, pH 7.4) (Polyplus). Plasmids were diluted in DMEM without FBS (ratio DNA (μg)/PEI (μL) 1:3). The mixture was incubated for 25 min at room temperature and then deposited on the cells.

2.4. Functional characterization of GIPR mutants

2.4.1. Cyclic AMP assay

cAMP production following GIPR activation was measured by BRET using EPAC BRET sensor (kindly given by Marc Caron, Dule University, NC, USA). HEKT 293 were plated onto 10-cm culture dishes. After overnight growth, cells were co-transfected with 5 μg of Epac biosensor and 1 μg of GIP receptors (wild type or mutants). 24 h after transfection cells were plated in 96-wells clear bottom plates (Corning) at a density of 100,000 cells per well in phenol red free DMEM. 24 h later, the medium was removed and replaced by Phosphate Buffer Saline (PBS) calcium and magnesium free. BRET assay was initiated by adding 10 μl of coelenterazine h to the wells (final concentration 5 μM). After 5 min incubation with coelenterazine h, GIP was added. After an additional 5 min-period, recordings were performed on a Mithras LB940 instrument (Berthold) that allows the sequential integration of signals detected in the 465–505 nm and 515–555 nm windows. Dose–response curves for cAMP production yielded EC_{50}, concentration of GIP producing 50% of cAMP maximum, termed potency. Mutation factors (F_{mut}) were calculated as EC_{50} (mutated GIPR)/EC_{50} (wild type GIPR). Moreover, E_{max}, maximum production of cAMP, termed efficacy, was obtained and expressed as % of maximum cAMP production achieved with the wild type GIPR. Since for the GIPR no pharmacological tool is available to accurately quantify active and inactive GIPR expression at the cell surface, the importance of any amino acid as functional structural element was submitted to tight comparison between functional, binding and molecular modeling data.

2.5. Receptor binding assays

GIP radiolabel was generated through radio-iodination of Phe^1-GIP. Phe^1-GIP corresponds to GIP(1–30) in which Tyr^1 was replaced by a Phe. This peptide enabled radio-iodination on a unique Tyr at position 10 which is outside the activation domain of GIP. ^{125}I-Phe^1-GIP bound to GIP expressed in HEKT 293 cells with a Kd of 75.7 ± 8.4 nM and a B_{max} of 1.8 ± 0.4 pM/10^6 cells (6 separated determinations, not illustrated). Competition binding with GIP(1–30) provided a value for GIP concentration inhibiting 50% of specific binding (IC_{50}) of 17.5 ± 3.7 nM (7 separated determinations) which was identical to calculated inhibition constant, K_i: 17.5 ± 3.6 nM. Therefore, for pharmacological analysis of all mutants, IC_{50} were considered as representative of affinity of GIP for the mutants.

For binding experiments, cells grown overnight onto 10-cm culture dishes were transfected as indicated for cAMP production measurements. 24 h later, cells were transferred to 24-well plates. Approximately 24 h later, binding assays were performed using ^{125}I-Phe^1-GIP according to the protocol previously described in detail [22].