Investigations of Interactions of G Protein-Coupled Receptors With Their Ligands and G Proteins Based on Homologous Receptor Subtypes With Different Biological Functions

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II. Abbreviations

3D	Three-dimensional
7-TM receptor	Receptor with 7 transmembran (TM) regions, GPCR
AC	Adenylyl cyclase
Acifran	4,5-Dihydro-5-methyl-4-oxo-5-phenyl-2-furan-carboxylic acid
Acipimox	5-Methylpyrazine-carboxylic acid-4-oxide
cAMP	cyclic adenosine monophosphate
C-terminus	Carboxy-terminus
Ctt	C-terminal tail, Carboxy-terminal tail
DAG	Diacyl glycerol
DNA	Deoxyribonucleic acid
ECL	Extracellular loop
ET-1	Endothelin-1
ET-2	Endothelin-2
ET-3	Endothelin-3
ETA	Endothelin receptor, subtype A
ETB	Endothelin receptor, subtype B
Gα	α-subunit of trimeric G protein
Gβ	β-subunit of trimeric G protein
Gγ	γ-subunit of trimeric G protein
GDP	Guanosine diphosphate
GPCR	G protein-coupled receptor
G protein	Guanine nucleotide binding protein
GPR109A	High affinity nicotinic acid receptor (also HM74A, PUMA-G)
GPR109B	Low affinity nicotinic acid receptor (also HM74)
GTP	Guanosine triphosphate
hETA	Human endothelin receptor, subtype A
hETB	Human endothelin receptor, subtype B
HM74	*see* GPR109B
HM74A	*see* GPR109A
ICL	Intracellular loop
IP3	Inositol-3-phosphate
MD	Molecular Dynamics
MPS	Mastoparan-S
MPX	Mastoparan-X

Niacin	*see* Nicotinic Acid
Nicotinic Acid	Pyridine-3-carboxylic acid
N-terminus	Amino-terminus
Ntt	N-terminal tail, Amino-terminal tail
PDB	Protein Data Base
P_i	Phosphate, ionic
PKA	Proteinkinase A
PKC	Proteinkinase C
PLC	Phospholipase C
PTX	Pertussis toxin
PUMA-G	Mice variant of GPR109A
RMP	Receptor mimetic peptide
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
Sf6a	Sarafotoxin 6a from *Atractaspis engaddensis*
Sf6b	Sarafotoxin 6b from *Atractaspis engaddensis*
Sf6c	Sarafotoxin 6c from *Atractaspis engaddensis*
Sf6d	Sarafotoxin 6d from *Atractaspis engaddensis*
TMH	Transmembrane helix

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VI. Abstract

G protein-coupled receptors (GPCRs) are important in current pharmacological research, being the target of about 50% of today's drugs. Due to the missing structural data on all GPCRs but rhodopsin, three problems occur in investigations of these receptors. The molecular basis of ligand-binding mechanisms, the intramolecular signal transduction, and the intracellular G protein-interactions are often poorly understood. Due to this, we have studied the interactions of particular GPCRs with their ligands and with their G proteins utilizing homologous receptor subtypes with close sequence similarity but different biological functions.

Delineating ligand-binding mechanisms of GPCRs, we focused in the first example on the endothelin receptor subtypes ETA and ETB, which differ in ligand and G protein-selectivity. Due to the apparent binding affinity of ET-1 (0.01-0.06 nM) that is an order of magnitude higher compared to small molecules such as bosentan (4.7 nM), comprehensive knowledge of peptide binding and initial receptor activation is of importance for understanding receptor mechanism and for designing new ligands. We studied a collection of 15 endothelin receptor peptide ligands regarding agonism, antagonism, and receptor subtype selectivity. The ligands were dissected into 4 regions: addressor, hook, core and modulator. The addressor is a selectivity filter optimized for ETA. Negatively charged residues within the hook region characterize agonistic peptides. While the hydrophobic core seems to be necessary for high affinity binding, the modulator region is the true endothelin receptor ligand.
Ligand binding studies on homology models of ETA and ETB identified 4 ligand-complementary epitopes (gateway, edge, neck, binding cleft) at each receptor. The pairwise interactions of addressor and gateway, hook and edge, core and neck, as well as modulator and binding cleft accurately explain the peptide ligands' selectivity for endothelin receptors and endothelin receptor subtypes. We demonstrated this by the design of a new ETB-selective agonist.

As a second example, the nicotinic acid receptor GPR109A and its homologue GPR109B, which is not binding nicotinic acid, were studied. Residues, being critically involved in binding of nicotinic acid to GPR109A, were identified based on homology models of this receptor as well as known nicotinic acid-protein complexes from the PDB (*e.g.* ferric soybean leghemoglobin). The binding site was predicted to be located between transmembrane helices

TMH2, TMH3 and TMH7. This interaction site is different from most other rhodopsin-like GPCRs, where the binding site is located between TMH4, TMH5 and TMH6. Experimental data on mutations of aromatic residues within TMH5, TMH7 and extracellular loop ECL2, which have been predicted by our models, confirm this result. Comparison of the binding sites of nicotinic acid receptor GPR109A and its homologue GPR109B explains the differences in ligand selectivity. As a result, 2-oxo-octanoic acid was proposed as selective ligand for GPR109B but not GPR109A, which was experimentally confirmed.

Unraveling the unidentified mechanism of GPCR-G protein interactions, we investigated ligands of small, medium and large size directly interacting with the G protein-subtypes. A common pattern, consisting of negative charges on G proteins and positive charges on G protein-ligands, was identified to be important in recognition and/or interaction. Investigations of four different G protein-subtypes (Gαi, Gαo, Gαq, Gαs) clearly showed different localizations and, therefore, varying distances of negatively charged residues in close proximity to the G proteins' C-termini (below 12 Å for Gαi, around 15 Å for Gαq, and greater than 18 Å for Gαs). As a result, it was possible to design a new compound (FU244) that exclusively interacts with Gαi but not with Gαq or Gαs. Additionally, two residues within the intracellular loop ICL2 of ETA and ETB have been identified and experimentally verified as necessary for interaction with Gαi.