Structure-activity characterization of glucagon receptor, an example of using HTRF® cAMP assay

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Abstract

The glucagon receptor (GLR), one of the 15 members of the class B family of G protein-coupled receptors (GPCRs), presents a potential target for treatment of diabetes. Despite a large body of information regarding the ligand binding and signaling characteristics of GPCRs in general, the molecular basis by which GLR selectively recognizes and binds its endogenous ligand - glucagon is still largely unknown. To better understand GLR-glucagon interactions, we performed a comprehensive mutagenesis study of GLR at 89 different residue positions. The mutants were expressed in CHO-K1 cells and assessed for expression levels, binding of radiolabeled glucagon and intracellular cAMP levels. A total of 128 mutants were tested and of which, 51 mutations were found to play important roles in ligand binding and cAMP accumulation. The results are consistent with the three-dimensional model of the glucagon bound GLR structure, based on the recently solved seven-transmembrane domain crystal and the previously reported GLR extracellular domain structures.

Introduction

One of the class B G protein-coupled receptors (GPCRs), glucagon receptor (GLR), is activated by the 29 amino acid hormonal peptide – glucagon and is a drug target for type 2 diabetes. During fasting, the pancreas dispatches glucagon to activate GLR in the liver causing the release of glucose into the blood. The desired anti-diabetic activity for GLR is via an antagonist that reduces blood glucose levels in diabetics.

Unlike class A, all class B GPCRs contain a globular N-terminal extracellular domain (ECD). They are activated by hormonal peptides, which bind to both the ECD and the seven-transmembrane (7TM) domains. Structural details of soluble ECDs, including the ECD of GLR, and their role in selective recognition of peptide hormones’ C-termin have been revealed for several class B receptors by X-ray crystallography and NMR studies. However, in the absence of a class B 7TM domain structure, understanding of the ligand-receptor interactions and the mechanism of signal transduction remains very limited. Here, we report an extensive mutagenesis study aimed at better understanding of glucagon interactions and functional features in the context of full-length GLR.

Methods

Construction of GLR mutants and cell culture transfection

The cDNA-encoding the human GLR was cloned into the expression vector pcDNA3.1/V5-His TOPO at the Hind III and EcoRI sites. The single and double mutants were constructed by PCR-based site directed mutagenesis. CHO-K1 cells were seeded onto 96-well poly-D-lysine treated cell culture plates at a density of 2.7 × 10^4 per well. After overnight culture, the cells were transiently transfected with wild-type or mutant GLR DNA using Lipofectamine 2000 transfection reagent.

Expression level quantitation of constructed GLR in cells by flow cytometry

Whole-cell glucagon binding assay

Cells were harvested 24 h after transfections and incubated with blocking buffer for 2 h at 37°C. For homogeneous binding, the cells were incubated in binding buffer with constant concentration of 125I-glucagon (40 pM) and different concentrations of unlabeled glucagon (0.02 nM ~ 500 nM) for 30 min at room temperature. Cells were lysed, and the signal intensity was measured using the cAMP-Dynamic HTRF detection kit (CisBio, France).

Results

Figure 1. Principle of the HTRF® cAMP assay.

Conclusions

The results of these GLR mutation studies illustrate that the residues that play an important role in glucagon binding line a binding site that covers parts of ECL1, ECL2, ECL3, and helices I, II, III, V, VI, and VII. Most importantly, the binding site extends deep into the 7TM cavity.

Figure 2. Cell surface expression profiles of human GLR mutants with significantly reduced specific 125I-glucagon binding.

Figure 3. Representative binding and cAMP accumulation curves of GLR mutants with glucagon. Data are expressed as a percentage of specific 125I-glucagon binding in the absence of unlabeled peptide (a) or as a percentage of the highest glucagon stimulated cAMP accumulation (b).

Figure 4. Effects of mutation studies plotted onto GLR snake plot. Mutagenesis study of GLR binding to glucagon mapped onto GLR snake plot. Residues that show <4-fold, 4-10-fold, and >10-fold changes of IC50 and EC50 values for glucagon binding and cAMP accumulation are colored purple, orange, and red, respectively.

References