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INTRODUCTION

Dimerization of G-Protein Coupled Receptors

Protein-protein interactions govern a large number of biological processes. A number of cell surface and nuclear receptors mediate their actions via dimerization. While this has been well established, the dimerization of G-protein coupled receptors (GPCRs) (that represents ~1% of the genes present in a mammalian genome) has not been well documented until recently. It was generally assumed that these receptors existed as monomers and coupled to G proteins in a 1:1 stoichiometric ratio. However, these classical models of receptor/G protein coupling may be oversimplified since a number of studies report not only the presence but also a role for GPCR multimers in modulating receptor function.

Early evidence for the interaction between GPCRs came from cross-linking experiments, radiation inactivation experiments, and hydrodynamic studies. Analysis of complex binding data from early pharmacological studies provided indirect evidence for the existence of GPCR dimers. Radiation inactivation (target size analysis), a technique based on the inverse relationship between the dose-dependent inactivation of a macromolecule by ionizing radiation and the size of that macromolecule, showed that a functional unit of GPCR is a complex of greater molecular mass than the size predicted by denaturing techniques. Cross-linking studies to visualize the receptors revealed multiple sizes of proteins. In some cases, agonists selectively bound to the higher molecular weight form, consistent with the notion that the functional unit of GPCR is a dimer/oligomer.

The isolation of cDNA clones encoding the receptors has enabled the critical evaluation of GPCR dimerization. Antisera against epitope tagged or native receptors have been used to visualize the receptor in heterologous cells or in native tissue. Differential epitope tagging of the receptor cDNA followed by selective immunoprecipitation of the dimer has been used to confirm the ability of members of the rhodopsin, secretin, and calcium sensing receptor family of GPCRs to dimerize.

A number of studies have explored the effect of agonists on the level of receptor dimers and found that, in some cases, they stabilize and/or increase the level of dimers, whereas in others, they have no effect nor decrease the level of dimers. The function of GPCR dimerization/oligomerization explored using peptides that block dimerization or mutant receptors that do not dimerize support a role for dimerization in the modulation of signal transduction, in addition to influencing agonist affinity and agonist-mediated endocytosis.

Several lines of evidence suggest the existence of GPCR heterodimers. An early study with chimeric receptors between the alpha2c-adrenergic receptor and m3-muscarinic receptor suggested the possible heterodimerization between GPCR types. Direct evidence for GPCR heterodimerization came from studies with non-functional $GABA_B$ receptors that dimerize to generate a functional receptor. Recent studies have also provided direct evidence for the heterodimerization between fully functional GPCRs. These studies have shown that heterodimers have distinct physical and functional properties and that heterodimerization leads to modulation of receptor function by regulating ligand binding properties, signaling, as well as receptor trafficking properties.

Receptor dimerization, brought about by the association of two monomers, could be mediated by covalent (disulfide) and/or non-covalent interactions; these could be association of the extracellular domains, transmembrane domains, and/or C-terminal tail. Several studies with a number of GPCRs have suggested that a combination of the above mentioned interactions between multiple domains occur during dimerization. Two mechanisms have been proposed for GPCR dimerization/oligomerization. One involves the association of 1:1 stoichiometric molecular complexes of receptors and

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the other involves the swapping of domains between two distinct receptor molecules resulting in a single or dual binding pockets. Further studies are required to delineate the molecular mechanism of GPCR dimerization.

Although several lines of evidence suggest that GPCRs can dimerize, it remains to be established whether this phenomenon is a general characteristic of these receptors and whether it is essential for receptor function. Also, studies need to be carried out to understand whether these receptors dimerize prior to targeting to the plasma membrane and to what extent GPCRs dimerize *in vivo.* The use of sensitive biophysical techniques such as, fluorescence resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET) have been helpful in answering some of these questions.

Development of tools that will allow selective detection and/or activation of dimers are required to examine the role of GPCR dimers *in vivo.* A complete understanding of the mechanisms of heterodimerization and its functional implications has enormous clinical significance as well as a high impact on GPCR pharmacology since it represents another mechanism that could modulate receptor function and thus provides a new strategy for the development of novel therapeutic drugs.

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