

S012 Phenotypic classification of mutants: A tool for understanding ligand binding and activation of muscarinic acetylcholine receptors

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GPCRs such as the M_1 muscarinic receptor have so far proved recalcitrant to direct structure determination. Nevertheless systematic mutagenesis, particularly alanine-scanning, has advanced our understanding of their structure-function relationships. GPCRs exhibit multiple conformational states with different affinities for and abilities to activate their cognate G proteins. Ligand binding alters these conformational equilibria thus promoting or inhibiting signalling. Alanine-substitution mutagenesis probes the relative contributions of a particular amino acid side chain to the stability of the ground and activated states of the receptor and its complexes. These determine the phenotype of the mutant receptor. Classification of the phenotypes suggests functional roles for particular amino acid side chains allowing us to group them accordingly. From a rhodopsin-based homology model of the M_1 mAChR a coherent view emerges of how these clusters of residues function in ligand anchoring, transduction of binding energy, global structural stabilization, and selective stabilization of the ground state or the activated state of the receptor. We can identify differences in ligand binding modes, and suggest inter and intramolecular interactions that are weakened or broken, or formed or intensified during acetylcholine-induced activation. In due course, we may be able to extend these insights to activation by unconventional agonists.